

***TREVESIA PALMATA* ALLEVIATES INFLAMMATION AND
GUT DYSBIOSIS IN ULCERATIVE COLITIS RAT MODEL**

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
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PHILOSOPHY**

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Submitted

**In partial fulfillment of the requirement of the Degree of Doctor of Philosophy
in Zoology of Mizoram University, Aizawl.**



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I **SAEED AHMED LASKAR**, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/ Institute.

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Aizawl, Mizoram

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	TPF10: <i>Trevesia palmata</i> flower bud methanolic extract (10 mg/kg, p.o.); TPF20: <i>Trevesia palmata</i> flower bud methanolic extract (20 mg/kg, p.o.).
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Figure 5.10	Histopathological alterations in colon sections of TNBS-induced ulcerative colitis rats treated with different therapeutic regimens. (A) Control group: Normal arrangement of mucosa (mu), submucosa (sm), muscularis (m), numerous goblet cells (*) and thin lamina propria (Lp). (B) TNBS group: Abnormal crypts (arrows), diffusion inflammatory cell infiltration (#) and severe damage of tissue architecture. (C) TNBS with sulfasalazine (50 mg/kg) group: preserved crypts (arrows), and goblet cells (*). (D, E) TNBS + TPF 10 and TPF 20 group: Protection of the colon from structural damage by <i>Trevesia palmata</i> flower bud methanolic extract (TPF10 and TPF20, 10 and 20 mg/kg). H&E: Hematoxylin and eosin staining, scale bar = 50 μ m for 10X

	magnification
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	%) and saline (0.9%) TNBS: Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); SPS50: Sulfasalazine in DMSO) (50 mg/kg, p.o.); TPF10: <i>Trevesia palmata</i> flower bud methanolic extract (10 mg/kg, p.o.); TPF20: <i>Trevesia palmata</i> flower bud methanolic extract (20 mg/kg, p.o.). Scale bar = 50 μ m for 10X magnification
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	using one-way ANOVA followed by Tukey's multiple comparison tests. Bar graph with different letter means statistically significant difference at $p < 0.05$ and similar letters are not significant ($p > 0.05$). Control: Dimethyl sulfoxide (1 %) and saline (0.9%) TNBS: Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); SPS50: Sulfasalazine in DMSO (50 mg/kg, p.o.); TP10: <i>Trevesia palmata</i> flower bud methanolic extract (10 mg/kg, p.o.); TP20: <i>Trevesia palmata</i> flower bud methanolic extract (20 mg/kg, p.o.). Scale bar = 50 μm for 10X magnification
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	Photomicrograph of the immunohistochemical studied protein expression of active caspase-3 in the colons of the (A) control, (B) Trinitrobenzene sulfonic acid (TNBS), (C) TNBS + Sulfasalazine 50 mg/kg (TNBS +SPS50), TNBS + methanolic extract of (D and E) <i>T. palmata</i> flower buds (10, 20) mg/kg (TNBS +TPF10; TNBS + TPF20). The bar graph (F) representing the expression area of each group. Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison tests. Bar graph with different letter means statistically significant difference at $p < 0.05$ and similar letters are not significant ($p > 0.05$). Control: Dimethyl sulfoxide (1 %) and saline (0.9%) TNBS: Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); SPS50: Sulfasalazine in DMSO) (50 mg/kg, p.o.); TP10: <i>Trevesia palmata</i> flower bud methanolic extract (10 mg/kg, p.o.); TP20: <i>Trevesia palmata</i> flower bud methanolic extract (20 mg/kg, p.o.). Scale bar = 50 μm for 10X magnification
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Figure 5.21	Effects of <i>Trevesia palmata</i> flower bud methanolic extract on the expression pattern of inflammation, oxidative stress, and cell signalling markers in colon tissue of TNBS-induced ulcerative colitis. The markers evaluated included (A) Interlukin-6, (B)) NOX-2 (NADPH Oxidase 2), and (C) p53 (Tumor Protein 53) all normalised to β-tubulin. Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison tests. Bar graph with different letter means statistically significant difference at $p < 0.05$, and similar letters are not significant ($p > 0.05$). Control: Dimethyl sulfoxide (1 %) and saline (0.9%) TNBS: Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); SPS50: Sulfasalazine in DMSO) (50 mg/kg, p.o.); TP10: <i>Trevesia palmata</i> flower bud methanolic extract (10 mg/kg, p.o.); TP20: <i>Trevesia palmata</i> flower bud methanolic extract (20 mg/kg, p.o.).
Figure 5.22	Effects of <i>Trevesia palmata</i> flower bud methanolic extract on the expression pattern of inflammation, and apoptotic markers in

	colon tissue of TNBS-induced ulcerative colitis. . The markers evaluated included (A) PPAR- γ (Peroxisome Proliferator-Activated Receptor Gamma), (B)) BAX (Bcl-2-associated X protein), and (C) Bcl-2 (B-cell lymphoma 2) all normalised to β -tubulin. Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison tests. Bar graph with different letter means statistically significant difference at $p < 0.05$, and similar letters are not significant ($p > 0.05$). Control: Dimethyl sulfoxide (1 %) and saline (0.9%) TNBS: Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); SPS50: Sulfasalazine in DMSO) (50 mg/kg, p.o.); TP10: <i>Trevesia palmata</i> flower bud methanolic extract (10 mg/kg, p.o.); TP20: <i>Trevesia palmata</i> flower bud methanolic extract (20 mg/kg, p.o.).
Figure 6.1	MultiQC report on the fecal composition of swiss albino female rats. A (Sequence count), B (Mean Quality Score), C (Per sequence quality score), D (Per sequence GC content), E (Per base N content), F (Sequence duplication level), G (Overrepresented sequences), H (Adapter content), I (Status check).
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Figure 6.10	Venn Diagram operational taxonomic unit (OUT) of Microbial Overlap Across Experimental Groups

ABBREVIATION

16S rRNA	16S ribosomal RNA
5-ASA	5-Aminosalicylic Acid
ABTS	2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
ACE	Abundance-based Coverage Estimator
ADMET	Absorption, Distribution, Metabolism, Excretion, Toxicity
AhR	Aryl Hydrocarbon Receptor
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
API	Active Pharmaceutical Ingredient
ARE	Antioxidant response element
ARRIVE	Animal Research: Reporting of In Vivo Experiments
ASA	Ascorbic acid
AST	Aspartate aminotransferase
ASV	Amplicon Sequence Variants
AutoDock Vina	Molecular docking tool
bp	Base pairs
BSH	Bile Salt Hydrolase
BTB	Broad Complex, Tramtrack, and Bric à brac domain
BUN	Blood Urea Nitrogen
Ca	Calcium
CBD	Convention on Biological Diversity
CD	Crohn's Disease

CHARMM27	Chemistry at HARvard Macromolecular Mechanics force field 27
Co	Cobalt
COX-2	Cyclooxygenase-2
CSs	Corticosteroids
Cu	Copper
CUL3	Cullin 3
DADA2	Divisive Amplicon Denoising Algorithm 2
DLG	D ⁶ L ⁷ G ⁸ motif
DMSO	Dimethyl sulphoxide
DPPH	1,1-diphenyl-2-picrylhydrazyl
DPX	Dibutylphthalate polystyrene xylene
EDTA	Ethylenediamine tetra acetic acid
EI	Electron Ionization
ETGE	E ⁷ G ⁸ E ⁹ motif
FC-Ext	Ferula communis extract
FCR	Folin-Ciocalteu Reagent
Fe	Iron
FeCl₂	Ferrous Chloride
FMT	Fecal microbiota transplantation
FTIR	Fourier-Transform Infrared Spectroscopy
FTIR	Fourier Transform Infrared
GAE	Gallic Acid Equivalent

GC	Guanine-Cytosine content
GCLC	Glutamate-cysteine ligase catalytic subunit
GC-MS	Gas Chromatography-Mass Spectrometry
GC-MS	Gas Chromatography-Mass Spectrometry
GGT	Gamma-glutamyl transferase
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GPR109A	G Protein-Coupled Receptor 109A
GPR41	G Protein-Coupled Receptor 41
GPR43	G Protein-Coupled Receptor 43
GROMACS	GRoningen Machine for Chemical Simulations
H₂SO₄	Sulfuric Acid
HALERI	Hydro-alcoholic leaf extract of Reinwardtia indica
HCl	Hydrochloric Acid
HClO₄	Perchloric Acid
HCT	Hematocrit
HGB	Hemoglobin
HLA-B27	Human Leukocyte Antigen B27
HNO₃	Nitric Acid
HO-1	Heme oxygenase-1
HPLC	High-Performance Liquid Chromatography
HPTLC	High-Performance Thin Layer Chromatography
HS	High Sensitivity

IAEC	Institutional Animal Ethical Committee
IBD	Inflammatory Bowel Disorders
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
IC50	Half Maximal Inhibitory Concentration
IL-10	Interleukin-10
IL-13	Interleukin-13
IL-1β	Interleukin-1 beta
IL-23Th17	Interleukin-23/ T helper 17 cells
IL-6	Interleukin-6
IVR	Intervening region
K	Potassium
kb	Kilobases
kcal	Kilocalories
KEAP1	Kelch-like ECH-associated protein 1
LBP s	Live-biotherapeutic products
LC-MS	Liquid Chromatography-Mass Spectrometry
LD50	Lethal Dose 50%
LINCS	LINear Constraint Solver
LOAEL	Lowest Observed Deleterious Effect Level
LR	Lower Risk
MAE	Microwave-Assisted Extraction
MCHC	Mean Corpuscular Hemoglobin Concentration

MeOH	Methanol
METP	Methanolic Extract of <i>T. palmata</i> flower buds
Mg	Magnesium
MGL	Molecular Graphics Laboratory
MMFF94	Merck Molecular Force Field 1994
MM-PBSA	Molecular Mechanics Poisson-Boltzmann Surface Area
Mn	Manganese
MS	Mass Spectrometry
MSMS	Tandem Mass Spectrometry
MUC2	Mucin 2
MultiQC	Multi-Sample Quality Control
Na	Sodium
Na₂HPO₄	Disodium Hydrogen Phosphate
NADH	Nicotinamide adenine dinucleotide
NaH₂PO₄·H₂O	Sodium Dihydrogen Phosphate Monohydrate
NaNO₂	Sodium Nitrite
NaOH	Sodium Hydroxide
NBT	Nitro Blue Tetrazolium
NEBNext	New England BioLabs Next
NF-κB	Nuclear Factor-kappa B
NMR	Nuclear Magnetic Resonance
NMR	Nuclear Magnetic Resonance
NOAEL	No Observed Adverse Effect Level

NPT	Constant number of particles, pressure, and temperature ensemble
NQO1	NAD(P)H quinone dehydrogenase 1
Nrf2	Nuclear factor erythroid 2 related factor 2
NSAIDs	Non-steroidal anti-inflammatory drugs
NVT	Constant number of particles, volume, and temperature ensemble
OECD	Organization for Economic Co-operation and Development
P	Phosphorus
P	Prostaglandin
Pa	Probability of activity
PAMPs	Pathogen-associated molecular patterns
PASS	Prediction of Activity Spectra for Substances
PCR	Polymerase Chain Reaction
PDB	Protein Data Bank
PDBQT	Protein Data Bank, Partial Charge, & Atom Type
PGE2	Prostaglandin E2
PhiX	Control DNA used in sequencing
Pi	Probability of inactivity
PME	Particle Mesh Ewald
PMS	Phenazine Methosulfate
PPI	Protein-Protein Interaction
ProTox-II	Protein Toxicity II

PRRs	Pattern-recognition receptors
ps	Picoseconds
QE	Quercetin Equivalent
QIIME2	Quantitative Insights into Microbial Ecology 2
QSAR	Quantitative Structure-Activity Relationship
QSAR	Quantitative Structure Activity Relationships
RBC	Red Blood Cell
RMS	Root Mean Square
RMSD	Root Mean Square Deviation
RMSF	Root Mean Square Fluctuation
ROS	Reactive Oxygen Species
SAR	Structure-Activity Relationship
SCFA	Short Chain Fatty Acids
Se	Selenium
SFE	Supercritical Fluid Extraction
SGOT	Serum Glutamic-Oxaloacetic Transaminase
SGPT	Serum Glutamic-Pyruvic Transaminase
SMEs	Small and Medium-sized Enterprises
SMILES	Simplified Molecular Input Line Entry System
SwissADME	Swiss Absorption, Distribution, Metabolism, Excretion
TAE	Tannic Acid Equivalent
TCA	Trichloroacetic Acid
TCM	Traditional Chinese Medicine

TNBS	2,4,6-Trinitrobenzenesulfonic acid
TNF-α	Tumor necrosis factor-alpha
TPF10	<i>Trevesia palmata</i> flowers 10 mg/kg (dose)
TPF20	<i>Trevesia palmata</i> flowers 20 mg/kg (dose)
TPFME	<i>T. palmata</i> Flower Bud Methanolic Extract
UAE	Ultrasound-Assisted Extraction
UC	Ulcerative Colitis
UDG	Uracil DNA Glycosylase
UPLC	Ultra Performance Liquid Chromatography
USER	Uracil-Specific Excision Reagent
V3-V4	Variable regions 3 and 4
WBC	White Blood Cell
Zn	Zinc

Chapter 1

1.1 ULCERATIVE COLITIS

1.1.1 Epidemiology, distribution and global burden

Ulcerative colitis (UC) is one of the two chronic, idiopathic inflammatory bowel disorders (IBD) that primarily impact the colon and rectum, the other being Crohn's disease. This debilitating condition, with its relapsing and remitting inflammation of the intestinal mucosa, has become a worldwide health concern **(Lynch and Hsu, 2023; Le Berre and Honap, 2023)**. According to the latest available statistics, 5 million UC patients globally were reported in 2023 **(Le Berre and Honap, 2023)**.

The highest incidence of UC is reported in the West, especially in North America, Northern Europe and Oceania. The high-income countries, mainly Norway, US, and Finland display higher prevalence than others, ranging from 32.5 to 44 cases per 1,00,000 population. However, incidence has been increasing in Asia, Africa, and South America as well in modern times, with Japan and parts of Korea nearing Western levels, an epidemiological transition linked to changing environmental factors, urbanisation, westernisation of lifestyle and dietary changes **(Hendrickson et al., 2002)**. Recent statistics from India and China report a lower base, but it is still alarming. Moreover, the data understates the true prevalence of UC in the face of data gaps and rapid urbanisation, and the actual values are around 43-44 UC patients per 1,00,000 individuals **(Chiba et al., 2021; Lee and Eun, 2022; Yang et al., 2022)**.

1.1.2 Pathophysiology and clinical manifestations

The pathophysiology of UC occurs in genetically susceptible individuals after exposure to environmental factors that disrupt the epithelial barrier, produce mucin, and alter the intestinal microbiota in a manner that activates the dysregulated mucosal immunity with aberrant stimulation of T-helper cells, especially Th2 and Th17 subsets. These three axes, i.e., barrier integrity, microbial ecology, and immune control, interact in a two-way fashion. Barrier breaches expose lamina propria immune cells to luminal antigens, dysbiosis reduces protective metabolites, including

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short-chain fatty acids (SCFA), and innate/adaptive responses promote inflammation through cytokine pathways, including tumour necrosis factor-alpha (TNF-alpha), interleukin-1-beta (IL-1-beta), and IL-6, IL-13, IL-23/Th17, that play important roles in perpetuating the inflammatory cascade. Contemporary reviews converge on this integrated model focusing on epithelial junctional defects, alterations in mucus (MUC2) biology, and aberrant host-microbe crosstalk (**Bullard et al., 2022; Feng et al., 2022; Le Berre and Honap, 2023**). The UC histopathology is thus characterised by infiltration of neutrophilic and lympho-plasma cellular elements in the mucosa and the distortion of the epithelial crypts, cryptitis, crypt abscesses and widespread mucosal erosions. Oxidative stress is another important aspect of UC pathophysiology. Increased production of reactive oxygen species (ROS) and a reduced efficiency of the antioxidant mechanism result in cell damage, lipid peroxidation (LPO), and DNA damage in the colonic mucosa. This oxidative burden not only contributes to tissue damage but also perpetuates the inflammatory response through the activation of nuclear factor-kappa B (NF-kB) and other pro-inflammatory signalling pathways (**Touny et al., 2024**).

The clinical manifestation of UC is usually a constellation of gastrointestinal symptoms that may vary in severity and extent. The hallmark symptom is bloody diarrhoea, often with mucus, that distinguishes UC from other types of IBD. Other symptoms include a sense of urgency, tenesmus, rectal bleeding, and abdominal cramping, especially in the left lower quadrant. UC affects the distal portion of the colon in most cases, i.e., between 60-85% of diagnosed cases (**Vecchi et al 2003**). The severity of any symptom typically corresponds to the extent and intensity of inflammation of the mucosa (**Salvarani and Fries, 2009; Lynch and Hsu, 2023; McDowell et al., 2025**).

The onset age is measured on average between the third and fourth decades of life, after which the disease takes a chronic, relapsing course (**Hoie O et al., 2007**). A minority of patients have a single episode; however, 67% have at least one relapse in a 10-year timeframe. The symptoms severely affect the quality of life of the patients, leading to loss of productivity (**Pastorelli et al., 2009**).

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1.1.3 Existing therapy approaches and limitations

The main goal in treating UC has been to induce and maintain clinical and endoscopic remission and decrease the incidence of complications such as dysplasia and colorectal carcinoma. Among the conventional non-biological therapies, salicylates such as 5-aminosalicylic acid (5-ASA) plays a pivotal and first-line role for both the induction and maintenance of remission. Another one, corticosteroids (CSs), is the recommended therapy in the induction of remission in moderate to severe active UC patients and in mild to moderate UC patients who do not respond to 5-ASA (Ferretti et al., 2020). There are other immunosuppressive agents too, especially azathioprine or methotrexate, 6-mercaptopurine and steroid-sparing maintenance therapies for patients with refractory disease. The advent of biological therapies has also revolutionized the treatment of UC with anti-TNF- α (infliximab, adalimumab, golimumab), integrin inhibitors (vedolizumab) and other targeted therapies, giving new hope for patients with moderate to severe disease (**Lynch and Hsu 2023**).

However, the focus is still on symptom control, rather than developing a curative treatment of UC, despite substantial progress in the understanding of the disease and the development of new therapies with a specific targeted pathophysiological mechanism (**Ferretti et al., 2020**). Recently, there has been a growing realisation that personalised medicine is an utmost necessity to treat UC. Well-designed comparative trials and improved disease models are essential for the same. Importantly, even with all these advancements, unmet needs have persisted, such as higher rates of adverse effects caused by medication, incomplete response in some patients and continued risk of long-term complications, including colorectal cancer (**Zhang et al., 2017**). Failure rates for advanced therapy as the first line remain significant, with 68-84% of patients not achieving clinical remission. Failure rates for advanced therapy as the second-line treatment remain at >75%. Numerous people eventually require numerous doses to achieve results. Furthermore, access is hindered by the costs associated with biological medicines, especially in low- and middle-income nations (**Lee et al., 2024; Karami et al., 2025; Solitano et al., 2025**).

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1.2 GUT MICROBIOTA AND ULCERATIVE COLITIS

1.2.1 Composition and role of healthy gut microbiota

The human gut microbiota is one of the most complex and dynamic ecosystems in the body, consisting of trillions of microorganisms that exert fundamental effects on physiology, immunity and disease susceptibility. This complex microbial community, made up mostly of bacteria, as well as fungi, viruses, archaea and protozoa, has developed over thousands of years to develop a mutually beneficial relationship with the human body. In healthy people, the gut flora is dominated by four major phyla of bacteria, Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria, with the first two phyla, Firmicutes and Bacteroidetes, constituting about 90% of the total microbial population.

This versatile ecosystem ensures the autonomic maintenance of homeostasis in the intestine through many different pathways, such as competitive exclusion of pathogens, production of antimicrobial products, modulation of epithelial barrier functions, activation of immune reactions, etc. The gut microbiota is also responsible for converting primary bile acids to secondary bile acids in a process mediated by bacterial bile salt hydrolase (BSH) activity. Another function is to metabolise dietary tryptophan to indole derivatives, such as indole-3-propionic acid and indole-3-aldehyde, which activate the aryl hydrocarbon receptor (AhR) and mediate anti-inflammatory responses. The microbiota also participates in the production of vitamins, and fermentation of dietary fibre and resistant starches to short-chain fatty acids (SCFAs), mainly acetate, propionate and butyrate (mostly by the Clostridiales order of the Bacteroidetes phylum), etc. (Zheng and Wen, 2021; Qiu et al., 2022; Santana et al., 2022). These metabolites are the major energy supply for colonic epithelial cells and have profound anti-inflammatory effects through several pathways such as inhibition of histone deacetylases, activation of G protein-coupled receptors (GPR41, GPR43, GPR109A), and promotion of differentiation of regulatory T cells (Caetano and Castelucci, 2022; Shin et al., 2023; Ning et al., 2023; Bu et al., 2025).

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1.2.2 Gut dysbiosis in ulcerative colitis

When the delicate ecological balance of the gut microbiota is broken by a condition termed dysbiosis, the ramifications are extensive, contributing to the onset of numerous inflammatory illnesses such as UC (**Zheng and Wen, 2021; Qiu et al., 2022; Santana et al., 2022; Ren et al., 2025**). The gut bacteria of UC patients show consistent and characteristic changes, significantly different from healthy individuals. These modifications, collectively called dysbiosis, include a decrease in microbial diversity (alpha diversity), characterised by reduced Shannon-Weiner diversity indices and species richness as compared to healthy controls, and a modification in community structure (beta diversity), as well as functional metabolic disturbances. This is linked to loss of colonisation resistance and enhanced infection susceptibility to pathogenic bacterial overgrowth (**Ryan et al., 2020; Zakerska-Banaszak et al., 2021; Santana et al., 2022; Ren et al., 2025**).

Taxonomic changes in UC are described in terms of specific patterns of microbial abundance changes. At the phylum level, UC patients mainly have lower levels of Firmicutes and Bacteroidetes, and increased levels of Proteobacteria and Actinobacteria. More specifically, UC patients exhibit a major depletion of beneficial bacteria such as *Faecalibacterium prausnitzii*, *Roseburia intestinalis*, *Eubacterium rectale*, *Bifidobacterium longum* or *Akkermansia muciniphila*, *Asaccharobacter celatus* and *Gemmiger formicilis*,. On the other hand, potentially pathogenic bacteria, such as adherent-invasive *Escherichia coli*, *Fusobacterium nucleatum* and various Enterobacteriaceae species, are frequently enriched in UC patients (**Zheng and Wen, 2021; Zakerska-Banaszak et al., 2021; Santana et al., 2022; Ning et al., 2023; Ju et al., 2024; Ren et al., 2025**).

1.2.3 Functional consequences of ulcerative colitis-related dysbiosis

The functional consequences of microbial dysbiosis in UC include metabolic disruptions, manifested by considerable changes in the production of SCFAs, and the reduction of the concentration in faeces of butyrate, propionate and acetate in UC patients. This reduction is associated with reduced abundances of SCFA-producing bacteria and directly contributes to impaired barrier function of the epithelium and

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enhanced inflammation (Caetano and Castelucci, 2022; Shin et al., 2023; Ning et al., 2023; Ju et al., 2024). Disturbed bile acid metabolism is also another key functional outcome of gut dysbiosis related to UC. Altered microbial communities translate to altered bile acid profiles that are characterised by increased conjugated and sulphated bile acids, and reduced levels of secondary bile acids. These alterations again influence host lipid metabolism, immune responses and intestinal barrier function (Zheng and Wen 2021; Vargas et al. 2025). Disrupted amino acid metabolism, especially tryptophan metabolism, is another significant outcome of UC-related gut dysbiosis. A reduction of tryptophan metabolising bacteria leads to a reduction in the use of these protective metabolites and a loss of immune tolerance (Bu et al., 2025; Jing et al., 2025; Vargas et al., 2025).

1.3 TRADITIONAL MEDICINAL PLANTS AND ULCERATIVE COLITIS

1.3.1 Brief overview of medicinal plants and traditional knowledge system

For decades, traditional medicinal plants deeply rooted in world folklore and healing traditions have been a great source for both curative and preventive health actions. In India, Ayurvedic and Unani systems highly depend on plants such as Neem (*Azadirachta indica*), Turmeric (*Curcuma longa*) and Tulsi (*Ocimum sanctum*) for their therapeutic value. Traditional Chinese Medicine (TCM) uses ginseng (*Panax ginseng*), astragalus (*Astragalus membranaceus*) and goji berries (*Lycium barbarum*) to boost equilibrium and energy (Li et al., 2008). African traditional medicinal plants such as rooibos (*Aspalathus linearis*) and the devil's claw (*Harpagophytum procumbens*) are plants with curative qualities (van Wyk, 2011). The therapeutic potential of such plants is attributed to their bioactive constituents, such as alkaloids, flavonoids, tannins, saponins, and essential oils. These compounds, isolated from various plant organs, such as roots, leaves, bark, seeds and flowers, etc., have a broad range of pharmacological activities including anti-inflammatory, antibacterial, antioxidant and analgesic activities (Gurib-Fakim, 2006).

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Medicinal plants are crucial for biodiversity, ecosystem health, cultural attribution, public health and welfare. Environmental degradation, unsustainable harvesting of monoculture crops, and climate change are threats to all plant species globally. Hence, sustainable farming, establishment of genetic banks, and community-driven conservation projects are the need of the hour. Notably, the Convention on Biological Diversity (CBD) has stressed the importance of sustainable utilisation and equitable distribution of the benefits from plant resources. These endeavours are essential to the preservation of medicinal plants for future generations and the continuation of their contribution to the world's healthcare systems **(Hamilton, 2004)**. Documentation and preservation of traditional knowledge systems globally are also crucial for equitable sharing of benefits. Ethical issues such as preservation of intellectual property rights and fair pay must also be stressed to empower local people and sustain their traditional proficiency **(Posey & Dutfield, 1996)**.

1.3.2 Traditional medicinal plants and modern medicine

Modern medicine is significantly influenced by traditional systems **(Ekor, 2014)**. A large percentage of pharmaceutical compounds are either extracted directly from plants or synthesised based on their chemical structures. For example, the development of aspirin was predicated on salicylic acid, a compound found in willow bark (*Salix* spp.), and artemisinin, which is utilised to combat malaria, is derived from *Artemisia annua* **(Cragg & Newman, 2013)**. Traditional medicinal plant research has re-emerged in the last few decades due to concerns over side-effects of synthetic pharmaceuticals, antibiotic-resistant infections and the challenge of long-term healthcare. Modern scientific approaches are being applied to assess the efficacy of these herbs, confirming wisdom passed on through the ages, and uncovering new medicinal potential. Advances in biotechnology and analytical chemistry are useful in the identification, extraction and synthesis of bioactive phytochemicals **(WHO, 2019)**. Collaborative efforts among healers, researchers and healthcare professionals are required to develop a comprehensive and inclusive approach to health **(Tilburt and Kaptchuk 2008)**.

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1.3.3. Traditional medicinal plants in the treatment of ulcerative colitis

Ulcerative colitis and other inflammatory bowel conditions have traditionally been treated using medicinal plants. Their anti-inflammatory, antioxidant, immunomodulatory, and mucosal-healing qualities are the key reasons why contemporary research is progressively supporting their medicinal promise. Numerous Traditional therapeutic herbs such as *Glycyrrhiza glabra* (liquorice) limit COX-2 and iNOS activity, lower pro-inflammatory cytokines, and inhibit NF- κ B signalling. Flavonoids and phenolic acids are examples of phytochemicals that improve endogenous antioxidant enzymes (SOD, catalase, GPx) and scavenge free radicals. Tight-junction proteins are upregulated, mucus production is increased, and epithelium healing is encouraged by compounds found in plants like *Plantago ovata* and Aloe vera. *Punica granatum* and *Andrographis paniculata* are two examples of plants that regulate T-cell responses and assist in rebalancing the immune system away from excessive Th1/Th17 activity. Polyphenols and prebiotic fibres encourage the growth of helpful bacteria like *Lactobacillus* and Bifidobacterium while lowering the numbers of harmful microorganisms. *Curcuma longa* (curcumin) is effective as adjunct therapy as it reduces relapse rates. *Boswellia serrata* (boswellic acids) reduce leukotriene-mediated inflammation (Tilburt and Kaptchuk 2008).

1.4 *Trevesia palmata* (SNOWFLAKE ARALIA)

1.4.1 Taxonomic classification, morphology and distribution

Trevesia palmata (Roxburgh ex Lindley) Visiani, 1842, commonly known as the Snowflake Aralia in English and Kawhtebel in Mizo, a plant of the Araliaceae family, is an evergreen arboreal species native to a wide variety of territories in Asia, especially Bangladesh, Bhutan, Cambodia, China, India, Laos, Myanmar, Nepal, Thailand and Vietnam (Rahman et al., 2014; Khomdram et al., 2019). In India, it is found mainly in the northeast. The plant grows to a height of 15-20 feet and is characterised by its peculiar architectural shape, with scarce to zero lateral branches that lead to a terminal foliar cluster. The leaves with 1-2 feet breadth are supported by long petioles displaying deep lobes that create an intricate pattern, similar to a snowflake (Victoria et al., 2019; De Tommasi et al., 2000).

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The systematic position of *Trevesia palmata* is given below (**Bentham & Hooker, 1883**).

KINGDOM: Plantae

DIVISION : Dicotyledones

SERIES : Polypetalae

SUBCLASS: Calyciflorae

ORDER : Umbellales

FAMILY : Araliaceae

GENUS : *Trevesia*

SPECIES : *palmata* (Roxb. ex Lindl.) Vis. 1842

1.4.2 Significance in traditional medicine

The *T. palmata* flower buds are used as a nutritious edible flower, and the leaf extract is used as a leafy vegetable and as a therapeutic agent for dyspepsia in the Mizo community of northeast India (**Khomdram et al., 2019; Lalfakzuala et al. 2007; Angami et al. 2006**). **Rahman, et al. (2007)** reported that the *T. palmata* species is used in traditional medicine for the treatment of venereal diseases and contusions. The plant is widely utilised in folk medicine as a general tonic (**De Tommasi et al., 2000**). In Thailand, Mien (Yao) people use it as part of their postpartum herbal bath practices. The roots and fruit extracts are used for promotion of uterine contraction during delivery, recovery after perineal delivery, lactation stimulation, release of gastrointestinal discomfort, and the treatment of snake envenomation injuries, by the Kry ethnic group of Laos (**Arifin et al. 2017**). In the Chittagong Hill Tracts area, root-based paste is given as a therapeutic intervention for paediatric patients suffering from penile oedema. In Peninsular Malaysia, the plant is used for the treatment of bone fractures and dermatological diseases (**Lamxay et al., 2011**). In traditional Vietnamese medicine, leaves and fruits of the plant are used as antipyretics and to relieve severe inflammatory conditions.

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1.4.3 Significance in modern medicine

Rahman et al. (2014) have documented the capacity of *T. palmata* leaves and fruits methanolic extract to potentially reduce blood sugar levels, as well as mitigate gastrointestinal discomfort and hypertension. The plant has analgesic properties that are effective in alleviating pain caused by both chronic and acute diseases like rheumatoid arthritis, cancer, sprains, and trauma (**Manoharan et al., 2023**). Anti-thrombolytic and anti-arthritic properties of the plant are reported by **Rahman et al., 2014** and **Sayeed et al., 2014**.

T. palmata contains a large diversity of primary and secondary metabolites such as polyphenols and alkaloids, terpenes, phytosterols, and organosulfur compounds. Scientific investigations have proved that triterpenoid saponins and lactone glycosides isolated from this plant species have antiproliferative properties toward different lines of neoplastic cells (**Sayeed et al., 2014**).

1.5 TOXICITY STUDIES IN PHARMACO-THERAPEUTIC RESEARCH

Toxicology is one of the sciences that studies the properties, effects, identification, diagnosis and management of potential toxins. Every pharmaceutical drug or commercial food item needs to undergo necessary toxicological tests, such as the establishment of a dose-response curve, analysis of safety profile, and evaluation of its absorption, distribution, metabolism, and excretion (ADME) in the human body, to be deemed suitable for consumption (**Hodgson, 2011; Agrawal and Paridhavi, 2007**). The obtained data may be used to classify and name commercial chemicals according to their level of hazards. Toxicology studies are classified as systemic, reproductive, local tolerance, hypersensitivity, genotoxicity and carcinogenicity studies. Systematic investigations include single and multiple dosages, while reproductive research focuses on the fertility rate, offspring sterility and health, etc. (**Gassama, 2021**). The investigations are carried out through three methodologies: in vivo (on animals), in vitro (on isolated cells or tissue, or compositional and functional analysis of the compound) and in silico (computer simulation).

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In vitro toxicity assays evaluate the constitutional and functional properties of the compounds, drugs or extracts in a laboratory, such as evaluation of antioxidant and anti-microbial properties, membrane integrity, total bio-active compounds, nutritional composition analysis, etc. They provide rapid, mechanistic toxicity insights while reducing the need for animal experimentation (**Agrawal and Paridhavi, 2007**).

In-vivo toxicity studies include acute toxicity, sub-acute toxicity and sub-chronic toxicity. Acute toxicity studies assess the adverse effects that occur when substances that are inhaled, absorbed or eaten are ingested in a short period of time. In order to determine dosage levels for ensuing sub-acute and chronic toxicity studies, sub-acute toxicity studies look at possible adverse effects following a two to four-week treatment period. These tests help to ascertain the speedy negative impacts of new treatments. Sub-acute toxicity test examines animals through a continuous dose-period of 28-30 days, while sub-chronic toxicity test is a medium-term study, usually of 90 days, that assesses cumulative toxic effects, target-organ toxicity, and no-observed-adverse-effect levels (NOAELs) from repeated exposure. The tests are used to analyse continuous physical and behavioural changes of sample animals, as well as post-mortem examination to identify histological and biochemical abnormalities, and establish dosage response curves (**Klaassen, 2008; Woolley and Woolley, 2008**).

In-silico techniques utilise the power of computers to efficiently and on a high-throughput basis predict a huge number of compounds. The methods include computer-aided molecular drug designing, Quantitative Structure Activity Relationships (QSAR), use of computer-assisted learning, microfluidic chips, DNA chips, and computational or mathematical analysis (**Parasuraman, 2011**).

1.6. RATIONALE OF THE STUDY

Ulcerative colitis is recognised as a disease of the mucosal interface in which three axes, epithelial-barrier integrity, intestinal microbial ecology, and mucosal immune control interact bidirectionally to maintain inflammation. Despite expanded

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pharmacotherapy (5-ASA, corticosteroids, immunomodulators, biologics, and targeted small molecules), treatment procedures of UC are still considered a medical research failure due to incomplete or loss of response, adverse effects and cost/access barriers. Contemporary guidance focuses on individualised care, yet even the first-line advanced therapies have significant failure rates, and many patients need dose escalation or a switch. These limitations demand complementary strategies that are safe, affordable and coherent **(Zhang et al., 2017; Ferretti et al., 2020; Lynch and Hsu, 2023)**. Traditional medicinal plants rich in bioactive compounds (alkaloids, flavonoids, tannins, saponins, essential oils, etc.) possess anti-inflammatory, antioxidant, antibacterial and analgesic activities, important for the healing of mucosa. However, integration into modern care requires determination of safety profile, dose standardisation and quality control to assure safety, utility and reproducibility **(Gurib-Fakim, 2006; Tilburt and Kaptchuk, 2008; Cragg & Newman, 2013)**.

Within this context, *Trevesia palmata* (Araliaceae) is an underexplored example of an ethnomedicinal plant species that is used throughout South and Southeast Asia for gastrointestinal discomfort (including dyspepsia) and other ailments; the edible parts of the palm are part of the local cuisine. Reports document diverse secondary metabolites (e.g. oleanane-type saponins, polyphenols, terpenes, phytosterols) and pharmacological signals (analgesic, antihyperglycemic, anti-thrombolytic, anti-arthritic; antiproliferative triterpenoid saponins and lactone glycosides). Yet the scientific literature is scarce, and a glaring knowledge gap is evident **(Rahman et al., 2014; Sayeed et al., 2014; Manoharan et al., 2023)**.

The central rationale in this thesis is that a chemically standardised extract of *T. palmata* can target key pathogenic nodes in UC by: (i) ameliorating oxidative and cytokine-driven inflammation (consistent with saponin/polyphenol pharmacology); (ii) reinforcing epithelial barrier structure as well as function, and (iii) modulating gut microbial functions implicated in UC. These targets are in congruence with documented dysbiosis and barrier deficits in UC, and with the known activity spectrum of medicinal-plant constituents. A rat model of UC provides a suitable

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platform to address this translational hypothesis by integrating clinical and histological activity, barrier markers, inflammatory and oxidative stress indices, and microbiome/metabolite profiling while allowing dose-response and formulation assessment. In parallel, toxicity and standardisation considerations meet modern expectations for botanical development.

In summary, evaluation of *Trevesia palmata* in a UC rat model is scientifically defensible and motivated to address limitations of existing treatments, as well as to fill an identified evidence gap in this species, thus providing a safe, low-cost adjuvant treatment, consistent with UC's eco-immune pathophysiology.

1.7. OBJECTIVES

The objectives of the study are:

- I. Phytochemical characterisation and evaluation of in-vitro antioxidant property of methanolic extract of *Trevesia palmata* (Roxb. ex Lindl.) Vis. 1842 flower buds.
- II. Computational screening of *Trevesia palmata* flower bud phytochemicals for anti-inflammatory potential, targeting COX-2 and KEAP1 through PASS, molecular docking, molecular simulation, and integrated ADMET profiling.
- III. Evaluation of acute and sub-acute toxicity of methanolic extract of *Trevesia palmata* flower buds in Wistar albino rat model.
- IV. Evaluation of ameliorative potential of methanolic extract of *Trevesia palmata* flower buds against ulcerative colitis in TNBS-induced rat model.
- V. Metagenomics analysis of gut microbial composition to demonstrate the ameliorative potential of methanolic extract of *Trevesia palmata* flower buds in a rat model with TNBS-induced ulcerative colitis.

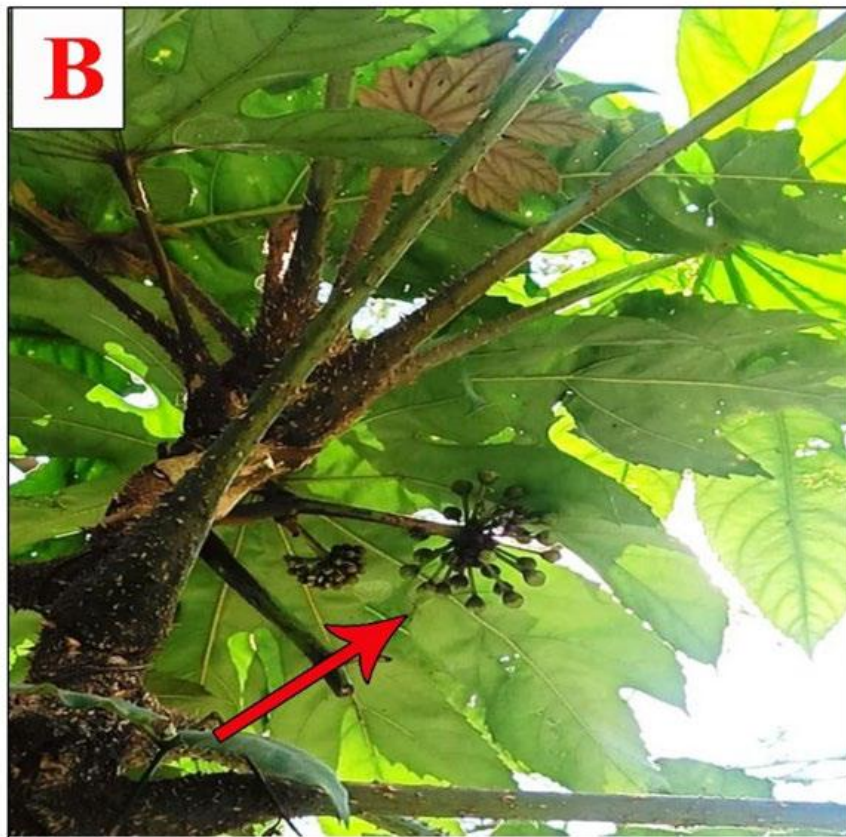


Figure 1.1 A photograph of the *Trevesia palmata* (Roxb. ex Lindl.) Vis. 1842 plant:
(A) Whole plant; (B) Flower buds

2.1. INTRODUCTION

2.1.1 Pharmacological properties of plants

For ages, plants have served as an integral source of medicinal compounds, opening door to development of conventional and alternative medicine, through their unique pharmacological properties (**Newman and Cragg, 2020; Atanasov et al., 2015**). Two-thirds of the plant species of the world have been found to possess therapeutic values (**Pharmawati and Wrsiati, 2020**). Among such properties are the hepatoprotective, cardioprotective, anti-inflammatory, antibacterial, anticancer, antidiabetic and antioxidant properties (**Ekor, 2014; Pan et al., 2013**). Bioactive phytocompounds such as alkaloids, flavonoids, terpenoids, tannins, glycosides, and saponins, have diverse molecular targets within the body, to influence biochemical and physiological processes. E.g., *Papaver somniferum* alkaloids act as a potent analgesic (**Atanasov et al., 2015**). About 80% of the world's population uses traditional medicine for public and livestock healthcare (**Venugopal and Liu, 2012; Auwal et al., 2014**).

Medicinal plants have emerged to be of major relevance in modern medicine too, as they facilitate the development of innovative drugs. Contemporary pharmaceutical products, such as artemisinin from *Artemisia annua* for the treatment of malaria and paclitaxel from *Taxus brevifolia* for the treatment of cancer are obtained from natural plant sources (**Newman and Cragg, 2020**). A large variety of bioactive substances in plants possess potent antioxidant and free radical scavenging activity (e.g., flavonoids of citrus fruits), which is extremely important for maintaining cellular homeostasis and preventing diseases associated with oxidative stress. Reactive oxygen species and other free radicals can cause lipid peroxidation, DNA damage, and protein breakdown with long-term consequences such as cancer, heart disease, and neurological disorders (**Valko et al., 2007**). By donating their electrons, phytochemicals, such as flavonoids, phenolic acids and tannins, neutralise ROS and lower oxidative damage (**Pisoschi and Pop, 2015**). As a result, there has been an increasing attention from the food, nutraceutical and pharmaceutical sectors to the exploration of plants as natural potential sources of antioxidants, since metabolic diseases associated with oxidative stress is one of the most critical new-

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age problem. To ensure the usefulness, efficacy and potential incorporation of traditional remedies into established evidence-based healthcare practices, pharmacological studies are becoming more necessary because of increased interest in natural and alternative medicines (Ekor, 2014).

2.1.2 *Trevesia palmata* (Roxb. ex Lindl.) Vis. 1842: A traditional medicinal plant

Trevesia. palmata, a plant belonging to the Araliaceae family, is a traditionally important medicinal plant native to Southeast Asia. Commonly known as the Snowflake Tree or False Castor Oil Plant, *T. palmata* is a deciduous to semi-evergreen tree or large shrub growing to 3 to 10 meters. Because it is made up of 7 to 11 segments and has large, extensively lobed, hand-shaped palmate leaves, its distinctive form articulates a snowflake-like appearance. Small, yellowish flowers produced by the plant are clustered in big terminal umbels, which become globose, producing dense fruits (Li et al., 2003). The species is widely used in indigenous medical practices, particularly in China, Vietnam and the surrounding regions (Rahman et al., 2014; Sayeed et al., 2014). Ethnobotanical records have identified various uses of different parts of the plant in traditional medicine, some of which are documented below.

- Rheumatic disorders: Decoctions made from the roots are used to reduce pains and stiffness in the joints (Manoharan et al., 2023; Chen et al., 2025)
- Dermatological conditions: Poultices prepared from the crushed leaves are externally applied to treat boils, ulcers and wounds (Lamxay et al., 2011; Chen et al., 2025).
- Respiratory problems: Infusions or decoctions of the leaves are used for the relief of coughs, asthma, and inflammation of the bronchi (Chen et al., 2025).

These applications are part of long-standing traditional knowledge handed down through their art, passing through generations of rural and indigenous applications. However, despite such usage, there are scientific studies on its pharmacological potential (Victoria et al., 2019; De Tommasi et al., 2000).

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2.1.3 Extraction of plant material

Compounds of plant origin have tremendous utility in different industries such as dietary, cosmetics and pharmaceuticals. Isolated bioactive molecules are precursors in laboratory synthesis of drugs and are models for the preparation of physiologically active chemicals. The phytochemical processing of plant materials is important for the optimisation of the concentration of recognised components and preserving their activities (**Dhanani et al., 2013**). The first stage of phytochemical analysis is extraction, a procedure in which chemicals are isolated using an appropriate solution. An effective standardised extraction procedure is used to solubilise all elements without altering their nature, which facilitates additional analysis (**Dhanani et al., 2013; Gahlot et al., 2018; Azwanida, 2015**). Products procured from plants are relatively impure, and the extract obtained, either in liquid infusion, semi-gel or powdered form, after necessary toxicological and clinical evaluation, is ready for use as a medicinal agent (**Handa et al, 2008; Auwal et al, 2014**).

Extraction of the medicinally active portions of plants, such as secondary metabolites and essential oils, is conducted using universal solvents (distilled water, selective solvents such as saline solutions and alcohols) using standard procedures (**Perez-Vila et al, 2022**). Maceration and Soxhlet extraction may be used in small research settings or in small and medium-sized enterprises (SMEs). Innovations in extraction techniques like microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) and supercritical fluid extraction (SFE) have made an enormous contribution to enhancing the science of phytochemistry (**Kapadia et al., 2022**).

2.1.4 Phyto-elements and phytocompounds

A wide variety of important elements are needed by plants to complete their life cycles, carry out metabolic processes and promote growth and development. According to **Marschner (2012)**, these elements are classified as macronutrients (including nitrogen, phosphorus and potassium) and micronutrients (including iron, zinc and manganese). They are engaged in activities such as photosynthesis, enzyme activation, and nucleic acid synthesis (**Marschner et al., 2012**). According to

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Nadeem et al. (2018), plants mainly obtain these elements from the soil, where all microbial interactions, pH, and organic matter affect their bioavailability (**Nadeem et al., 2018**).

Phytochemicals are active biomolecules found in plants that contain significant health benefits. They are located in bark, roots, leaves, flowers, stems, fruits and seeds (**Cragg and Newman, 2001**). These components are classified into numerous classes, such as polyphenols, carotenoids, alkaloids, etc., with each of these components impacting the taste, colour, odour, and potential health benefits of the plant (**Rudzinska et al., 2023**). Polyphenols are an important class of bioactive plant chemicals, including several subclasses such as flavonoids, phenolic acids, stilbenes, and lignans (**Manach et al., 2005**). Each of these subclasses is again classified into a number of groups and subgroups, e.g., anthocyanins, flavan-3-ols, flavanones, flavones and flavanols are subgroups under the flavonoids subclass (**Corcoran et al., 2012; Butiuk et al., 2016**).

The potential of phytochemicals to prevent illness and promote health has been well investigated. Such compounds display several biological properties (**Yang and Ling 2024**). E.g., through the manipulation of metabolic pathways and the reduction of oxidative stress, they have been found to prevent chronic illnesses such as diabetes and cardiovascular disorders (**Kumar et al., 2023; Dillard and German, 2000**). Phytochemicals are known to modulate the growth of tumour cells by inducing pro-autophagic and pro-apoptotic processes, which are vital in the treatment of cancer (**Bonam et al., 2018; Braicu et al., 2020**). A Mediterranean diet rich in phytochemicals, consisting of berries, honey and olive oil, is associated with a reduced risk of cardiovascular disease and cancer (**Xiao and Bai, 2019**). Additionally, phytochemicals possess antibacterial properties, e.g., they damage bacterial membranes and inhibit the formation of biofilms, which are prerequisites in the fight against antibiotic resistance; hence, they are a suitable alternative to conventional clinical antibiotics (**Barbieri et al., 2017**). They also may affect ion channels in the transmission of pain, and this may lead to the development of novel analgesics (**Kim et al., 2025**).

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2.1.5 In vitro screening of phytocompounds and physicochemical characterisation of plant extract

The complex chemical nature of phytocompounds requires multiple analytical approaches for their identification, quantification, and elucidation of their chemical structure **(Federica et al, 2018; Ingle et al., 2022)**. These methods can be broadly classified as qualitative and quantitative methods and they range from the more classical methods of colorimetric assay to highly sophisticated chromatographic and spectroscopic methods such as nuclear magnetic resonance (NMR) spectroscopy, gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and Fourier-Transform Infrared Spectroscopy (FTIR). In phytochemical research, each approach provides unique information on the existence, concentration, and molecular composition of bioactive chemicals **(Harborne, 1998; Sasidharan et al., 2011)**.

The first stage of phytochemical analysis is qualitative and quantitative screening of phytocompounds. Qualitative tests include several biochemical tests which rely on recognisable colour changes or precipitate formation in response to selected reagents. E.g., alkaloids are usually tested by Mayer's, Wagner's and Dragendorff's tests, flavonoids are tested using Shinoda and lead acetate tests, whereas phenolic compounds and tannins are indicated by the ferric chloride and gelatin tests respectively **(Trease and Evans, 2002)**. Quantitative methods estimate the concentration of some groups or single molecules of phytocompounds. E.g., the aluminium chloride colorimetric test is used to check total flavonoid content (TFC) while the Folin-Ciocalteu method is conducted to estimate the total phenolic content (TPC) **(Singleton et al., 1999)**.

Polar or thermally labile phytocompounds are detected through LC-MS analysis. It profiles complicated plant matrices through sensitive, accurate methods by fusing the mass analysis capabilities of mass spectrometry (MS) with the physical separation capabilities of liquid chromatography (LC). While MS provides information on molecular weight, fragmentation patterns, and structural features, LC helps in classification of constituent compounds based on their polarity and

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interaction with the stationary phase. Compounds in trace amounts can be identified with very high mass accuracy using high-resolution approaches (HR-LC-MS). Further advances in LC-MS techniques include HR-LC-Electrospray Ionisation Quadrupole Time-of-Flight-MS (HR-LC- ESI-QTOF-MS), HR-LC-MS Orbitrap etc. (Fernie et al., 2007; Duan et al., 2014; Jorge et al., 2016). Another basic quantitative technique used in phytochemical analysis is GC-MS (Gas chromatography- Mass spectroscopy), which is particularly useful for identification of volatile, non-polar and thermally stable compounds such as terpenes, fatty acids, essential oils and aromatic hydrocarbons, due to its sensitivity, repeatability and availability of extensive spectrum libraries (like Nist) for compounds matching. This is a method that works by vaporising plant extracts and then separating these extracts using a gas chromatograph based on plants' boiling temperatures and their interactions with the GC column's stationary phase. The MS ionises and analyses the separated molecules, creating exact fragmentation patterns, which are used to identify the substances (Zhang et al., 2012; Balasubramanian et al., 2014).

Infrared spectroscopy is a non-destructive qualitative and quantitative method for the location of functional groups of phytochemicals is FTIR spectroscopy. It analyses the vibrational transition through chemical connexions, for absorption of infrared, by producing a spectrum. The types of chemicals or classes of compounds represented in such a sample are identified by comparing the data with databases or standards. FTIR is especially useful in the characterization of crude mixes, evaluating the purity of extracts, and extract fingerprinting. For a thorough phytochemical profiling and herbal product verification, it is often used in combination with chromatographic procedures (Pharmawati and Wrsiati, 2020; Junaedi 2021). In plant metabolomics, Nuclear magnetic resonance (NMR) method is a specialised tool for structure determination, e.g., three-dimensional structure of proteins or other macromolecules (Kim et al., 2011; Malet-Martino & Holzgrabe, 2011).

2.2 REVIEW OF LITERATURE

2.2.1 *Trevesia palmata* in traditional healing practices

Several studies have documented the long-standing use of medicinal plants in traditional healthcare systems across diverse regions of the world. **Chitme et al. (2003)** reported that such plants are the primary source of medication in many rural areas of developing nations even in modern times. Aiming at preserving, investigating, advancing and promoting the medicinal plants industry in the state, the State Medicinal Plants Board of Mizoram, a state in northeast India, has listed down several indigenous medicinal plants. Amongst those, *Trevesia palmata* has been listed to be a significant ethnomedicinal plant in the region. It is classified in the lower health risk/conservation dependent (LR) category (**State Medicinal Plants Board- Mizoram, 2001**). The local people of Lunglei district in Mizoram use the juice from the leaves in the treatment of dyspepsia, stomach-aches and high blood pressure (**Chen et al., 2025**).

Ethnobotanical studies highlight the diverse medicinal significance of the plant throughout South and Southeast Asia. According to **Loi (2004)**, **Thao et al. (2018)**, and **Ha & Bach (2023)**, the species is widely incorporated into traditional therapeutic practices for conditions such as fever, inflammation, and diabetes. In India, it has been documented as a general restorative tonic and a remedy for bone fractures (**De Tommasi et al., 2000**). **Lamxay et al. (2011)** reported its application in Laos for postpartum recovery, management of lochia, abdominal pain, muscle restoration, and as a lactagogue. Ethnomedicinal surveys in the Chittagong Hill Tracts of Bangladesh describe the use of root and fruit paste in treating snake bites and penile pain (**Arifin et al., 2017**). **Thao et al. (2018)** documented its application in eastern Indonesia for the treatment of urinary tract disorders, while historical and ethnobotanical records from Peninsular Malaysia indicate its long-standing use in managing bone fractures and various dermatological conditions (**Gardens, 1913; Burkill, 1966**). Nutritional analyses report a protein content of 40.47%, a total carotenoid level of 0.023%, and a carbohydrate content of 7.64% (**NyoTin, 2008; Kumar et al., 2023; Johnson and Grivetti, 2002; Chaudhry and Murtem, 2016**) in the plant, because of which it is incorporated in Hmong cuisines (**Lalmuanpuui et**

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al., 2020; Nguanchoo et al., 2014). Phytochemical investigations reveal the presence of various secondary metabolites in the plant, including saponins, flavonoids, tannins, and terpenoids; compounds widely known for their anti-inflammatory, antibacterial, and antioxidant activities (**Xiao et al., 2010; Chen et al., 2025**).

2.2.2 Features of elements in medicinal plants

Analysing the elemental make-up of plants provides insight into their therapeutic role, nutritional significance and possible health threats, since a synergy of phytochemicals with essential elements increases the antioxidant, anti-inflammatory and metabolic regulatory effects of the plant (**Watzl & Leitzmann, 2012; Gupta et al., 2015**). Essential elements reported in medicinal plants till date include macro elements such as calcium (Ca), potassium (K), magnesium (Mg) and phosphorus (P), and trace elements such as iron (Fe), zinc (Zn), manganese (Mn), selenium (Se) and copper (Cu) (**Gopalakrishnan et al., 2016**). Macro-elements, which are required in relatively large quantities, play essential roles in maintaining bone integrity, muscular function, and metabolic processes (**Combs, 2012**). In contrast, micro-elements or trace elements, though needed in smaller amounts, are critical for enzymatic activity and antioxidant defence mechanisms (**Gupta et al., 2014**).

Moringa oleifera has been reported to contain high levels of calcium, iron, and potassium, contributing to its effectiveness in addressing malnutrition and anaemia. Iron-rich species such as *Embllica officinalis* have been shown to enhance haemoglobin synthesis and alleviate anaemic conditions (**Akter et al., 2020**). Zinc present in *Ocimum sanctum* (Tulsi) has been associated with immunomodulation and wound-healing properties (**Wakchaure et al., 2017**), while magnesium in leafy herbs like *Coriandrum sativum* has been linked to neuromuscular and cardiovascular functions (**Mahleyuddin et al., 2021**).

Studies further indicate that the mineral composition of medicinal plants is strongly influenced by external environmental factors, particularly soil characteristics and agronomic conditions. For example, the selenium content in *Allium sativum*

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(garlic) has been shown to vary directly with selenium concentrations in the soil (Fairweather-Tait et al., 2011). As a result, plants of the same species collected from different geographic regions have exhibited considerable variation in their therapeutic efficacy and safety profiles (Fairweather-Tait et al., 2012).

2.2.3 Physicochemical properties of phytochemicals

A wide range of phytochemicals have been identified in plants, with carotenoids, phytosterols, saponins, glucosinolates, polyphenols, protease inhibitors, monoterpenes, phytoestrogens, and sulphides reported as the most significant classes (Watzl and Leitzmann, 2012). Rahman et al. (2014) carried out a preliminary phytochemical analysis on methanolic leaf extract of *T. palamata* (METP) to determine the presence of saponins, tannins, alkaloids and flavonoids (Rahman et al., 2014).

Carotenoids, abundant in red and yellow fruits and vegetables dependent on carotenoid pigments for photosynthesis, have been extensively studied for their nutritional relevance. A diverse range of naturally occurring carotenoids have been identified, of which approximately 50 are considered nutritionally significant, including several with provitamin A activity. According to Leitzmann (2016), human serum contains both oxygen-free carotenoids and xanthophylls, with β -carotene accounting for up to 30% of total serum carotenoids. Research further indicates notable differences in thermal stability among carotenoids; β -carotene is comparatively heat stable, while lycopene and xanthophylls present in tomatoes, carrots, and green leafy vegetables exhibit greater sensitivity to heat.

Phytosterols occurring in nuts, seeds, and plant oils are structurally analogous to cholesterol but display much lower absorption efficiency. Their limited bioavailability, due to active transport back into the intestinal lumen, has been widely documented. Research indicates that phytosterols reduce cholesterol absorption, forming the basis for functional foods such as phytosterol-enriched margarines developed since 2001 (Leitzmann, 2016). Saponins, an important polysterol, has been described as surface-active phytochemical that readily form complexes with

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fats and proteins, and occur abundantly in legumes. Despite their haemolytic properties, studies indicate that their biological activity is largely confined to the digestive tract due to slow absorption rates. Research on members of the genus *Brassica* further demonstrates that these plants are important sources of glucosinolates, which degrade into indoles, thiocyanates, and isothiocyanates; metabolites associated with the characteristic flavours of mustard, horseradish, and broccoli. The stability of glucosinolates has been shown to be influenced by processing conditions; mechanical disruption through chopping or chewing affects enzymatic interactions, while thermal treatment can reduce glucosinolate content by approximately 50% (**Dinkova-Kostova and Kostov, 2012**).

Polyphenols, including phenolic acids and flavonoids, have been reported in high concentrations in red wine, tea, dark chocolate, and apples. Their levels generally increase during ripening. Flavonoids, commonly located in the outer tissues of fruits and vegetables, show higher concentrations in field-grown plants, with quercetin identified as the predominant compound. Studies have reported that legumes and grains are important sources of protease inhibitors, which function by suppressing endogenous proteases and consequently stimulating the production of digestive enzymes. Research on aromatic plant constituents further identifies monoterpenes such as limonene, carvone, and menthol as common metabolites in herbs and spices. **Tetens et al. (2013)** also noted the presence of phytoestrogens, including lignans and isoflavones, which share structural similarities with endogenous oestrogens despite their comparatively weak biological potency. Lignans have been found predominantly in flax seeds and whole grains, whereas soybeans and soy-derived products have been reported to constitute the principal dietary sources of isoflavones.

Sulphides, particularly the organosulfur compounds abundant in bulbous plants like garlic, have also been intensively investigated. Allicin, the principal bioactive component of garlic, is reported to be produced when tissue damage activates alliinase, which converts alliin into allicin (**Borlinghaus et al., 2014**).

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Overall, medicinal plants contain a rich diversity of bioactive molecules that have contributed to modern drug discovery (**Kim, 2005**). Given that only about 1% of the world's estimated 500,000 plant species have been phytochemically examined, the literature emphasizes a substantial untapped potential for identifying novel anti-infective compounds, particularly in the context of rising antimicrobial resistance.

2.2.4 Analytical techniques in phytochemical studies

In the past, plant components were used directly in therapy; but, with the development of modern technology, the active ingredients are now extracted and synthesised in pure form (**Kocabas, 2017**). The phytoconstituents offer the chemical structures which are utilised as models for the development of synthetic medications (**Raaman, 2006**). Numerous biochemical methods such as a maceration, percolation, infusion, digesting, decoction and hot continuous extraction have been used for extracting bioactive compounds from plant material (**Silva et al., 2017; Ezeonu and Ejikeme, 2016**). Maceration extraction is one of the main techniques to obtain natural materials with universal solvents such as ethanol and methanol. The endogenous components of the plants get dissolved by the extraction processes and solvents. There is the possibility of both polar and non-polar plant components. Eco-friendly techniques like Accelerated Solvent Extraction (ASE), Supercritical Fluid Extractions (SFE), Microwave Assisted Extraction (MAE), and Ultrasound-Assisted Extraction (UAE) are also widely used in modern times (**Azwanida, 2015; Kumar et al., 2011; Ezeonu and Ejikeme, 2016**).

Altemimi et al. (2017) stated that researchers employ different methods of analysis such as chromatography, sensors, spectroscopy, and spectrometry to discover the most important bioactive compounds in the extracted component (**Altemimi et al. 2017; Lozada-Ramirez et al., 2021**). An analytical method that is frequently used for the detection and quantification of therapeutically relevant analytes is mass spectrometry (MS). Its analytical powers are extended to a variety of therapeutic uses by its use with gas or liquid chromatographs (GC-MS and (LC-MS) (**Montano-Perez and Yanez-Ibarra, 2025**). **Fathalla et al. (2019)** investigated the active compounds of the ethyl acetate fraction of the seeds of *Senna tora* which are

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known for their biological effects. They confirmed the existence of phenolic compounds: 10-hydroxy-5-methoxy-2-methyl-1, Chrysarobin, Isotorachrysone, Parietin, Rubrofusarin, Cumbiasin B, Griseoxanthone-B, 4-anthracenedione and Chrysophanol, using GC-MS and LC-MS (**Fathalla et al., 2015**). *Alternanthera brasiliensis* or "penicillin," is a subject of a phytochemical study by **Alencar Filho et al. (2020)** where they determined its anti-inflammatory and wound healing properties, by identifying 27 chemicals such as flavonoids, hydrocarbons, terpenes, vitamins, phytosterols and carotenoids using LC-MS/MS and GC-MS. The research greatly contributed to the knowledge of the chemical composition of this species, since 23 previously unknown chemicals were discovered in the study (**Alencar Filho et al., 2020**). Other sophisticated techniques include high-performance liquid chromatography (HPLC) and high-performance thin layer chromatography (HPTLC) (**Silva et al., 2017**). Infrared spectroscopy (IR) is a fast, efficient, cost-effective, accurate and non-destructive method used in the qualitative and quantitative analysis of compounds in complex matrices, including plant-based medicines and supplements, when combined with chemometric methods (**Junaedi et al., 2021**).

Studies on *Meyna spinosa* leaf methanol extracts by **Sen et al. (2013)** have demonstrated that polyphenolic concentration is the most prominent phytochemical component influencing antioxidant properties of the plant. **Truong et al. (2019)** concluded that solvents have a significant effect on the phytochemicals, and in the antioxidant, anti-inflammatory activities, and extraction efficiency of *Severinia buxifolia*. Methanol was shown to be the best solvent and yielded the highest amounts of phenolic, flavonoid, terpenoid and alkaloid chemicals (**Truong et al., 2019**). The methanolic extract showed potent anti-inflammatory and antioxidant effects that were thrice higher than ascorbic acid regarding and 3.2-fold higher than aspirin, which make it an optimal solvent for phytochemicals and antioxidant compounds extraction.

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2.3 MATERIALS AND METHODS

2.3.1 Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Dimethyl sulphoxide (DMSO), Ethylenediamine tetra acetic acid (EDTA), Methanol (MeOH), Ferric chloride, Ferrozine, Folin–Ciocalteu’s reagent, Glacial acetic acid, Nicotinamide adenine dinucleotide (NADH), Nitro blue tetrazolium (NBT), Nitric acid (HNO₃), Perchloric acid (HClO₄), Potassium persulfate, Phenazine methosulfate(PMS), Potassium ferricyanide, Potassium iodide, Sodium hydroxide (NaOH), Sodium nitrite (NaNO₂), Trichloroacetic acid (TCA), 2-deoxyribose, Disodium hydrogen phosphate (Na₂HPO₄), Sodium dihydrogen phosphate monohydrate (NaH₂PO₄.H₂O), Sodium acetate, and Sodium nitroprusside were obtained from HiMedia Laboratories Pvt., Ltd. (Mumbai, India). Ascorbic acid (ASA) Sulfuric acid (H₂SO₄), was obtained from Merck Specialties Pvt., Ltd. (Mumbai, India). Hydrochloric acid (HCl), Griess reagent was obtained from Sigma Aldrich Inc (Louis, Germany). Bismuth nitrate, Quercetin dihydrate, Gallic acid, Tannic acid, Ferrous chloride (FeCl₂) was obtained from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India).

2.3.2 Collection of plant material

Unopened *T. palmata* flower buds were collected from Tanhril village located in the Aizawl District of Mizoram, a state in northeast India. According to the accession number 1543 (3575), the plant sample has been recognised, validated and sent to the herbarium at the Department of Environmental Science of Mizoram University, Aizwal, Mizoram, India.

2.3.3 Preparation of plant extract

The flower buds of *T. palmata* were plugged fresh, thoroughly washed under tap water, rinsed in distilled water, spread in buds into smaller single flowers and further dried in a well-ventilated place in the dark for 3 weeks at room temperature. The dried flower buds were pulverised in a mechanical grinder. Extraction procedure was followed by application of the cold maceration method (Kuate et al., 2006), wherein about 100 g coarsely powdered *T. palmata* flower buds was dipped in 400

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mL of methanol (100%) at a ratio of 1:4 (w: v) in a clean beaker. The mixed powder solution was then left at room temperature for 72 h, after which it was stirred for 6-7 h. The solution was then filtered using Whatman filter paper (Grade 1 circles-125 mm) to obtain the crude extract, i.e., *Trevesia palmata* flower-bud methanolic extract (TPFME). The filtered solution was evaporated at 37°C in the oven to obtain a solid texture (Figure 2.1). For further study the resultant substance was stored at -20 °C (Gahlot et al., 2018).

2.3.4 Estimation of extraction yield

The extraction yield (%), was calculated by dividing the mass of the extract by the mass of the dry matter of the extract and multiplying into 100 to convert the value to a percentage (Dhanani et al., 2017).

2.3.5 Phytochemical analysis

2.3.5.1 Qualitative analysis

Standard procedures were employed to perform qualitative phytochemical analysis of *T. plamata* flower buds, as described in previous studies (Bargah, 2015; Gangwar et al., 2014; Auwal et al., 2014; Muthukrishnan and Manogaran, 2018).

2.3.5.1.1 Test for Alkaloids (Dragendroff's Test)

Approximately 0.1 g crude extract of TPFME was dissolved in 1 mL of distilled water, and Dragendroff's reagent (0.5 mL) was added. The formation of a reddish-brown precipitate would indicate the presence of alkaloids.

2.3.5.1.2 Test for Cardiac glycosides (Keller-Kiliani Test)

A mixture of 2 mL glacial acetic acid, a single drop of ferric chloride (FeCl₃) solution, and 0.1 g TPFME crude extract dissolved in 1 mL distilled water was thoroughly homogenized. Subsequently, 1 mL concentrated H₂SO₄ was added. The formation of a brown ring at the interface would indicate the presence of deoxy sugar, a characteristic of cardenolides, thereby confirming the positive detection of cardiac glycosides.

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2.3.5.1.3 Test for Steroids (Salkowski's Test)

0.1 g crude extract of TPFME was dissolved in the respective solvents (1% DMSO), and a few drops of concentrated H_2SO_4 were added. The development of a red colour in the lower portion would indicate the presence of steroids.

2.3.5.1.4 Test for Saponins (Frothing Test)

0.1 g crude TPFME extract was combined with 1 mL of distilled water. Three drops of olive oil were added to the solution. The mixture was vigorously agitated for several minutes. The formation of a relatively stable emulsion would indicate the presence of saponins.

2.3.5.1.5 Test for Tannins (Ferric Chloride Test)

Tannin indication was determined by a few drops of ferric chloride (0.1%) with 0.1 g of crude extract of TPFME dissolved in 1 mL of the respective solvent (1% DMSO). The presence of a brownish green or blue-black colour would confirm the presence of tannins.

2.3.5.1.6 Test for Terpenoids (Salkowski's Test)

5 mL (0.1g/mL) of crude TPFME extract was combined with 2 mL of methanol. Subsequently, the mixture was evaporated in a water bath and heated with 3 mL concentrated H_2SO_4 . This process facilitates the formation of distinct layers. The presence of terpenoids is indicated by the formation of a reddish-brown colour at the interface.

2.3.5.1.7 Test for Phlobatannins (HCL Test)

The presence of phlobatannins was investigated by subjecting a 2 mL sample of TPFME crude extract (0.1g/mL) to thermal treatment in aqueous HCl solutions of 0.1% or 1% concentration. The emergence of a red precipitate during this analytical process would serve as an indicator of the presence of the compound.

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2.3.5.2 Quantitative analysis

2.3.5.2.1 Estimation of total phenolic content

Total phenolic content was quantified using a standard method (Sharma and Agarwal, 2015). This procedure involved the combination of 1 mL of TPFME crude extract (1 mg/mL) or gallic acid standard (20-120 µg/mL) with 5 mL of Folin-Ciocalteu reagent (FCR) diluted to 1:10 (w/w). After a 5-minute incubation at 25 °C, 4 mL of 7% sodium carbonate (Na₂CO₃) solution was added. The resulting mixture was incubated for 2 h at room temperature in the dark. The absorbance was measured at 765 nm. The results were expressed as mg gallic acid equivalent (GAE) per gram of plant material. Quantification was performed using a standard curve with the equation $y=0.008x-0.0396$ and coefficient of determination ($R^2=0.9968$), $y=0.003x+0.1345$ and coefficient of determination ($R^2=0.9884$) for total flavonoid content, and $y=0.0274x+0.062$ and coefficient of determination ($R^2=0.9964$) for total tannin.

2.3.5.2.2 Estimation of total flavonoid content

Total flavonoid content was determined using a modified version of the method described by (Khan et al., 2012). Briefly, 1.5 mL of TPFME crude extract (1 mg/mL) and quercetin standard solution (50-400 µg/mL) were combined with 75 µL of a 5% (w/v) sodium nitrite (NaNO₂) solution. Following a 6-minute incubation period, 150 µL of 10% (w/v) aluminium chloride (AlCl₃) solution was added, and the mixture was allowed to stand for an additional 6 min. Subsequently, 0.5 mL (1M) sodium hydroxide (NaOH) was added to this reaction. The final volume was adjusted to 2.5 mL by incorporating 275 µL of distilled water and thoroughly homogenizing the mixture. The absorbance was measured immediately at 510 nm. Total flavonoid content was expressed as mg quercetin equivalent (QE) per gram of plant sample. Quantification was performed using a standard curve with the equation $y=0.003x+0.1345$ and coefficient of determination ($R^2=0.9884$) for total flavonoid content.

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2.3.5.2.3 Estimation of total tannin content

The total tannin content was measured according to the standard protocol described by **(Dakhli et al., 2023)**. The procedure entailed combining 0.5 mL of TPFME crude extract (1 mg/mL) and tannic acid (10-60 µg/mL) with an equivalent volume of 50% Folin-Ciocalteu reagents (FCR). The mixture was allowed to stand for 2-5 minutes. Subsequently, 1 mL 20% sodium carbonate (NaCO₃) was added. The resulting solution was incubated at room temperature for 10 min. Following incubation, the mixture was centrifuged for 5 minutes at 1000 x g. The absorbance of the supernatant was measured at 730 nm wavelength. The final tannin content was expressed in terms of milligram of tannic acid equivalents per gram of plant sample. Quantification was performed using a standard curve with the equation $y=0.0274x+0.062$ and coefficient of determination ($R^2=0.9964$) for total tannin.

2.3.6 Micro-nutrient analysis

The micronutrient content was quantified using the standard calibration curve method described by **Auwal et al., 2014**. To assess trace elements, 500 mg of air-dried plant samples was accurately weighed. The pulverized sample was subjected to digestion in a 500 mL flask containing 10 mL of a di-acid mixture comprising nitric acid (HNO₃) and 70% perchloric acid (HClO₄) in a 4:1 ratio. After incorporation, the solution was left undisturbed overnight. Subsequent controlled heating on a hot plate was applied until the emission of crimson NO₂ vapours ceased, resulting in a colourless solution. The digested sample was then transferred to a 50 mL volumetric flask, filtered through a syringe filter, and brought to volume using distilled water. A blank sample was prepared without the addition of plant samples. Prior to the analysis, all samples were stored in glass containers at 4 °C in a refrigerator. The prepared samples were employed for the determination of potassium (K), magnesium (Mg), calcium (Ca), manganese (Mn), sodium (Na), zinc (Zn), iron (Fe), copper (Cu), and cobalt (Co). Quantification was performed using an atomic absorption spectrometer (Model: PinAAcle 900F; Perkin Elmer, Waltham, Massachusetts, US), with element-specific wavelengths, temperatures, and lamp currents applied.

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2.3.7 Assessment of free radical scavenging and antioxidant activity

2.3.7.1 DPPH radical scavenging activity

The DPPH radical model is a common method used for the effective evaluation of antioxidant activities of extracts (Gulcin et al., 2005). The radical has a deep violet colour in solution which changes to colourless or pale yellow when it is neutralised and converted to DPPH-H (Goldschmidt and Renn IV, 1922). The DPPH radical scavenging activity of methanolic extract of *T. palmata* flower buds was evaluated using a previously described method (Lalhminghlui and Jagetia, 2018), with minor modifications. This methodology entailed the combination of 0.5 mL of TPFME extracts (25-750 µg/mL) with 1 mL DPPH (0.1 mM) in methanol, which was subsequently incubated for 30 min in the dark. Following incubation, the absorbance of the mixture was measured at 523 nm and compared to that of the control. The efficacy of the extract in neutralizing DPPH radicals was quantified as IC₅₀, representing the concentration necessary to scavenge 50% of DPPH radicals. ASA served as the reference standard, and each sample concentration was analysed in triplicate. Scavenging activity was determined by calculating the percentage of neutralized DPPH radicals using a previously described formula.

$$\text{Scavenging (\%)} = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$$

In this formula, A_{blank} denotes the absorbance measured for the control solution, which comprises all the requisite reagents but lacks plant extracts. Conversely, A_{sample} represents the absorbance of the solution containing the plant extract. To find the 50% inhibitory concentration (IC₅₀), DPPH % inhibition was plotted against a range of concentrations of the TPFME extract and standard ASA.

2.3.7.2 ABTS radicals scavenging activity

The ABTS•⁺ chromophore is produced by the reaction of ABTS with potassium persulfate that leads to the conversion of ABTS to its radical cation. The radical cation has a blue colour and absorbs light of a wavelength of 734 nm. The ABTS•⁺ shows reactivity against various antioxidants such as phenols, thiols and vitamin C (Lalhminghlui and Jagetia, 2018). The ABTS radical scavenging activity

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of methanolic extract of *T. palmata* flower buds was evaluated using the protocol described by (Lalremruati et al., 2019). Equal volumes (5 ml) of 7 mM ABTS and 2.45 mM potassium persulfate were combined to prepare the stock solution. The mixture was subsequently incubated in the dark at room temperature for 12 h to generate a dark solution containing ABTS^{•+} radicals. A working solution was prepared by diluting the stock solution with 50% methanol to achieve an initial absorbance of 0.700 (± 0.02) at 745 nm. ABTS^{•+} radical scavenging activity was assessed by combining 150 μ l of *T. palmata* extract fractions (25-750 μ g/mL) with 1.5 ml of ABTS working solution. The reduction in the absorbance was measured immediately at 745 nm. Each sample concentration was analyzed in triplicate. ASA was used as the standard reference. The scavenging activity of the plant extract was then calculated using the formula:

$$\text{Scavenging (\%)} = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$$

In this formula, A_{blank} denotes the absorbance measured for the control solution, which comprises all the requisite reagents but lacks plant extracts. Conversely, A_{sample} represents the absorbance of the solution containing the plant extract. To calculate the IC₅₀ value, the ABTS^{•+} inhibition percent was plotted with different concentrations of the TPFME extract and ASA standard.

2.3.7.3 Superoxide radical scavenging activity

The extract of *T. palmata* flower buds in methanol was tested for superoxide anion (O₂^{•-}) scavenging activity by a PMS-NADH-NBT method reduction method by Lalhminghlui and Jagetia, 2018. This is a method that allows O₂^{•-} production from oxygen through PMS-NADH coupling, which reduces NBT. The experimental procedure involved creating a reaction mixture containing 0.2 ml of NBT (1 mg/ml dissolved in DMSO) and 0.6 ml the plant extract (ranging from 25 to 750 μ g/mL). This mixture was then diluted to 2.8 ml by introducing 2 ml of alkaline DMSO (prepared by combining 1 ml DMSO with 5 mM NaOH). Spectrophotometric measurements were performed at 560 nm using pure DMSO as the reference blank.

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ASA was used as the standard, and the superoxide radical scavenging potential of the methanolic extract of *T. palmata* flower buds was subsequently determined.

$$\text{Superoxide \% scavenging} = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100$$

Here, A_{control} denotes the absorbance without plant extract and A_{sample} denotes the absorbance with the extract. The IC₅₀ was calculated by plotting the percentage of inhibition as a function of the of TPFME extract concentration and standard ASA.

2.3.7.4 Scavenging activity against nitric oxide

The reaction of the nitric oxide (NO) produced by sodium nitroprusside with oxygen yields stable products such as nitrite and nitrate. Scavengers of nitric oxide compete with oxygen, leading to a reduced production of nitrite. The concentration of nitrite in aqueous solution was assayed spectrophotometrically by using the Greiss reagent, with which nitrite reacts to give a stable product absorbing at 546 nm (Preethi et al., 2010). Nitric oxide radicals were generated from sodium nitroprusside (SNP) solution at physiological pH. Sodium nitroprusside (1 ml of 10 mM) was mixed with 1 ml various concentrations of *T. palmata* extract (25-750 µg/mL) in phosphate buffer (pH-7.4). The mixture was incubated at 25°C for 150 min. To 1 ml of the incubated solution, 1 ml of Greiss reagent (1% sulphanilamide, 2% ortho phosphoric acid and 0.1 % naphthyl ethylene diamine dihydrochloride) was added. Absorbance was read at 546 nm and percentage inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100.$$

Here, A_{control} denotes the absorbance without plant extract and A_{sample} denotes the absorbance with the plant extract. To calculate the IC₅₀ value, a graph of NO inhibition percentage with different concentrations of the TPFME extract and that of standard ASA was plotted.

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2.3.7.5 Reducing power

The method described by **Adjimani and Asare, 2015** was used to evaluate the reducing capacity of methanolic extract of *T. palmata* flower buds. Reducing power, which serves as an indicator of antioxidant capacity, is directly proportional to the concentration of the extract. An increase in extract concentration corresponded to an enhancement in its reducing power. The process involved combining 2.5 ml of 0.2 M phosphate buffer (pH-6.6) and 1% potassium ferricyanide solution with plant extract (25-750 µg/mL; dissolved in their respective solvent). The mixture was incubated at 50°C for 20 min, and then 10% TCA (2.5 mL) was added. After centrifugation at 3,000 rpm for 10 min, an equal volume of distilled water was mixed with the supernatant, along with 0.5 mL of 1% ferric chloride solution. The absorbance of the resulting mixture was measured at 700 nm wavelength. A higher absorbance reading indicates a greater reducing power of the extract.

2.3.8 Metal Chelating power

2.3.8.1 Iron chelating activity

The chelating power of ferrous iron was determined according to the method of Carter (**Ouahhoud et al., 2022**). This test is used to assess the Fe²⁺ chelating activity by the formation of a red colour complex between Fe²⁺ and ferrozine. When other chelating agents are present, they interfere in complex formation which results in decrease in intensity of the red colour. The iron chelating capacity was estimated using the percentage of inhibition. The experimental procedure entailed combining 250 mL of sample or reference solution (at varying concentrations) with 1 mL of acetate buffer (0.1 M, pH 4.9) and 25 mL of FeCl₂ (2 mM). The mixture was subjected to a 30-minute incubation period at ambient temperature. Subsequently, 0.1 mL of ferrozine (5 mM) was introduced, followed by an additional 30-minute incubation at room temperature. This process facilitated the formation of a purple-hued Fe²⁺–ferrozine complex, which exhibited maximum absorption at 562 nm. The negative control was prepared by substituting the sample with an equal volume of distilled water. The absorbance was measured at 562 nm using distilled water as the blank. The degree of inhibition of Fe²⁺–ferrozine complex formation was calculated using the following equation:

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$$\text{Percentage inhibition} = [(A_0 - A_1)/A_0] 100$$

Here, A₀ denotes absorbance of the negative control and A₁ denotes absorbance of the sample or reference. To determine the IC₅₀, different concentrations of TPFME extract ranging from 200-1000 micrograms per millilitre were plotted with the percentage of iron chelation.

2.3.9 Gas chromatography-mass spectrometry (GC-MS) analysis

A GC-MS-2010 instrument (Shimadzu, Japan) was used to analyse the TPFME extract. The analysis was conducted in the EI mode with an electron ionization energy of 70 eV. The separation was performed on a Restek-5MS column (30 x 0.25 mm, 0.25 m film thickness). High-purity helium (99.99%) was used as the carrier gas at a constant flow rate of 1 mL/min. For GC-MS spectrum detection, 70 eV ionization energy, 0.2 s scan period, and fragment range of 40-650 m/z were utilized. The injector temperature was maintained at 260 °C, with a 2 µL injection volume and a 10:1 split ratio. Linear flow control was observed at 99.3 kPa pressure, with column and total flow rates of 1.21 mL/min and 16.3 mL/min, respectively. The column oven temperature program was initiated at 120 °C (2 min hold), increased at 10 °C/min to 280 °C, then to 300 °C, and was maintained for 20 min. Phytochemical identification was accomplished by comparing the retention times (min), mass spectral patterns, peak heights, and areas with those in the NIST library (containing over 62,000 patterns) and Dr. Duke's ethnobotanical phytochemical database (**Duke, 2000**).

2.3.10 Liquid chromatography-mass spectrometry (LC-MS) analysis

The investigation utilized an Acquity UPLC H-Class system, equipped with an auto sampler and binary pump (Water, USA), featuring a 10 L loop. Researchers have explored diverse chromatographic parameters for phytochemical separation, including mobile phase composition, injection volume, flow rate, and gradient programs. Several mobile phase combinations were tested in the gradient program at 1.5 mL/min, including as methanol-water, acetonitrile-water, and acetonitrile-0.1%

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(v/v) formic acid aqueous solution. The final mobile phase was composed of 0.1% (v/v) aqueous formic acid solution (A) and acetonitrile (B) at 1.5 mL/min. The optimal separation conditions were determined to be a column pressure of 0-300 bar and temperature of 30 °C. Compound separation was achieved using an Acquity CSH C18 column (2.1 mm x 100 mm, 1.7 µm particle size) at 30 °C. Gradient elution at 1.5 mL/min was developed using two solvents: 0.1% (v/v) formic acid in water (A) and methanol (B).

The gradient program began with 5% B for 1 min, progressed to 30% B between 6 and 12 min, and reached 60% B between 26 and 30 min. In contrast, component D started at 95%, remained constant for 1 min, decreased to 70% between 6 and 12 min, decreased to 40% from 16 to 20 min, and returned to 95% after 26–30 min. The sample injection volume was 10 µl. MS analysis was performed using a Water UPLC-TQD Mass Spectrometer (XEVOTQD#QCA1232) employing data-dependent automated switching between MS and MS/MS collection modes. Spectra were acquired in both positive and negative ionization modes with a mass acquisition range of 150-2000 m/z.

2.3.11 FTIR and NMR analysis

FTIR (Fourier transform infrared spectrophotometer) analysis (Shimadzu, Kyoto, Japan, IR Affinity1) was employed to identify functional groups. The scan range was 400–4000 cm⁻¹ with a resolution of 4 cm⁻¹ (Pharmawati et al., 2020). ¹H NMR spectra of the methanolic seed extract were obtained using a JEOL AL300 FTNMR spectrometer (JEOL Ltd., Tokyo, Japan) at 300 MHz TMS was used as an internal reference, and the chemical shift values were expressed in δ, ppm units.

2.3.12 Statistical analysis

Data of all results expressed as mean value ± standard error of the mean. Unpaired t-test was performed in IC₅₀ values of antioxidant capacities assays to test the significant difference between standard chemical and plant extract of different assay respectively. The IC₅₀ calculation were determined by the inhibition % of respective radicals against the concentration with help of statistical software

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GraphPad Prism Version 8.0.2. The p- values less than *p<0.05, **p<0.01, ***p<0.001 was considered statistically significant.

2.4 RESULTS

2.4.1 Determination of extraction yield

The yield of crude methanolic extract of *T. palmata* flower buds (TPFME) was 8.4% using the cold maceration method, using 100% methanol (MeOH), at a ratio of 1:4. Additionally there are no significant changes in appearance of the extract.

2.4.2 Qualitative analysis

The methanolic extract of *T. palmata* flower buds has shown the presence of various naturally occurring compounds, such as alkaloids, cardiac glycosides, steroids, saponins, tannins and terpenoids (**Figure 2.2**). Table 2.1 shows the presence (+) and absence (-) of the phytochemicals corresponding to the colour indication for each test.

2.4.3 Quantitative analysis: Total phenolic, flavonoid and tannin content

The methanolic extract of *T. palmata* flower buds shows total phenolic content of 21.51 ± 4.25 mg GAE/g of extract (Gallic Acid Equivalent/grams), total flavonoid content of 251.92 ± 13.77 mg QE/g of extract (Quercetin Equivalent/grams), and total tannin content of 13.58 ± 0.35 mg TAE/g of extract (Tannic acid Equivalent/grams) (**Table 2.2; Figure 2.3**).

2.4.4 GC-MS analysis

The GC-MS analysis identified seven phytochemicals from the methanolic extract of *T. palmata* flower buds. Palmitic acid and 1-Octadecanol, were detected as TMS derivative; Oleic acid, was detected as trimethylsilyl ester; Stigmasterol, Eicosanoic acid, tetradecyl ester, Hentetracontanoic acid, was detected as methyl ester; and Eicosanoic acid. These compounds belong to different classes or types of phytochemicals such as saturated long-chain fatty acid, monounsaturated long-

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chain fatty acid, triterpenoid sterol, long-chain wax ester and very-long-chain saturated fatty acid (**Table 2.3; Figure 2.4**)

2.4.5 LC-MS analysis

A total of fifteen phytocompounds were detected by LC-MS analysis; five through positive (Figure 2.5A) and eight through negative (Figure 2.5B) total ion modes. Ten identified phytocompounds were flavonoids, which were Kaempferol-3-O-rutinoside, Apigenin 6-C-glucoside 8-C-arabinoside, Luteolin-6-C-glucoside 8-C-arabinoside, Procyanidin B1, Vicenin-2, Luteolin-6-C-glucoside, Apigenin-8-C-glucoside, Quercetin-3-O-galactoside, Isorhamnetin, Kaempferol. The methanolic extract of *T. palmata* flower buds contained two Carotenoid, zeta-carotene, and zeaxanthin. In addition, the presence one phytocompound Procyanidin C1, condensed tannin was also demonstrated (**Table 2.4**).

2.4.6 FTIR and NMR analysis

A prominent absorption band observed at 3276.33 cm⁻¹ was indicative of the presence of –OH functional groups. Additionally, the determined absorption bands at 2924.21 cm⁻¹ and 2856.08 cm⁻¹ are associated with CH₂ and C–H stretching. The bands observed at 1698.76 cm⁻¹ and 1593.70 cm⁻¹ are indicative of C=C stretching and N-H bonding, while the absorption band ranging from 1720 to 1580 cm⁻¹ suggests the potential presence of ketonic, ester, carboxylic, alkene, and amine groups. The observed band between 1440-1465 cm⁻¹, 1370-1390 cm⁻¹, and 1200-1300 cm⁻¹ can be attributed to the presence of C-H deformation (weak to strong). Furthermore, the absorption band observed at 1021.61 cm⁻¹ is indicative of the pronounced stretching associated with the C-O bond within the ether group. The absorption band at 810.67 cm⁻¹ corresponds to the C-H out-of-plane bending (oop) vibration observed in substituted benzene rings of benzenoid compounds (**Table 2.5; Figure 2.6**)

The observed peaks in the NMR spectrum provided valuable insights into the types of protons present in the sample. The region between 0.5 and 2 ppm predominantly indicated the presence of aliphatic protons, such as CH₃, CH₂, and CH

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groups in the alkyl chains. These peaks suggested the presence of saturated hydrocarbon structures. Chemical shifts in the range of 2–3 ppm typically corresponds to protons adjacent to electronegative atoms such as CH groups bonded to carbonyl (C=O) or oxygen atoms. Additionally, this region may also include singlets arising from protons attached to sp-hybridized carbons in the alkyne groups, as observed in the NMR results. Signals in the 3–4 ppm region indicate protons in environments near oxygen or nitrogen, such as the CH groups in ethers, alcohols, or amines. This suggested the presence of functional groups associated with these elements. Chemical shifts ranging from 4 to 6 ppm are indicative of vinyl protons, such as those in alkenes. The δ 7–8 ppm region was characteristic of aromatic protons, indicating the presence of benzene-like structures in the sample. Peaks at approximately 2.5 ppm and 3.3 ppm, labeled as "DMSO" and "H₂O," are attributed to the solvent (DMSO-d₆) and residual water. The presence of multiplets in the spectrum suggests intricate coupling between nonequivalent protons, indicating complex molecular environments. The lack of strong signals in the 9–12 ppm region indicated the absence of aldehyde (R-CHO) or carboxylic acid (-COOH) groups in the sample (Table 2.6; Figure 2.7).

2.4.7 Micro-nutrient analysis

Of the 9 micronutrients tested, potassium (K) was present in a concentration of 251.070 ± 0.447 ppm, which is the highest among others, followed by magnesium (Mg), 11.41 ± 0.115 and calcium 8.984 ± 0.050 ppm, in decreasing order of abundance. Notably, several micronutrients, such as manganese (Mn), sodium (Na), zinc (Zn), iron (Fe), copper (Cu), cobalt (Co) were found to have concentration less than 1 ppm. (Table 2.7).

2.4.8 Assessment of free radical scavenging and antioxidant activity

2.4.8.1 DPPH radical scavenging activity

A dose dependent increase in DPPH radical scavenging activity was observed in the methanolic extract of *T. palmata* flower buds, which was indicated by the changes in the coloration of the test tube. The extract had the highest free radical-scavenging activity at 750 (ug/mL) (Figure 2.8A). The IC₅₀ value of the *T. palmata*

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flower bud methanolic extract was calculated as 161.286 ± 0.450 ug/mL and standard ASA 142.642 ± 3.210 ug/mL (**Table 2.8**).

2.4.8.2 ABTS $\cdot^+$ radicals scavenging activity

ABTS $\cdot^+$ radical scavenging capacity test of the methanolic extract of *T. palmata* flower buds showed decolourization of ABTS $\cdot^+$ radicals. The extract showed the highest radical scavenging activity of 750 micrograms per millilitre (Figure 2.8B). The analysis showed that the methanolic extract of *T. palmata* flower buds had an IC₅₀ (186.423 ± 1.230 and standard ASA 80.908 ± 0.950 ug/mL) (**Table 2.8**).

2.4.8.3 Superoxide radical scavenging activity

Through superoxide radical scavenging activity test, the extract showed an IC₅₀ value of 127.947 ± 1.251 µg/mL and standard ASA 86.749 ± 1.789 µg/mL (**Table 2.8; Figure 2.8C**).

2.4.8.4 Nitric oxide radical scavenging activity

The results showed that the extracts inhibited the formation of nitrites by direct competition with oxygen in their reaction with nitric oxide, with a concentration-dependent effect. Maximum radical scavenging activity was found at concentration 750 micrograms per ml (Figure 2.8D). The calculated IC₅₀ for NO scavenging activity of *T. palmata* flower buds was 631.472 ± 4.276 µg/mL and standard ASA 523.774 ± 4.930 µg/mL (**Table 2.8**).

2.4.8.5 Reducing power

At concentration 750 µg/mL, the methanolic extract of *T. palmata* flower buds exhibited a reducing power of 0.456 ± 0.002 , and is plotted along with standard ASA (**Table 2.8; Figure 2.8E**).

2.4.8.6 Iron chelating activity

The IC₅₀ value of iron chelating activity of TPFME was found to be 777.80 ± 1.94 (**Table 2.8; Figure 2.8F**).

2.5 DISCUSSION

2.5.1 Extraction and yield of extract

Several variables could influence the yield results of plant extract, such as the extraction technique used, the stirring process of maceration, the type of solvent, and the time and temperature of the extraction (**Zlotek et al., 2016**). The extraction result of the cold maceration method in this study showed a high percentage yield (8.4%) when compared to the yield percentage of the same by **Manoharan et al., 2023**, through hot percolation method using low to high polarity solvents, and *Paederia foetida* leaf extract by inundation method by **Yunita et al., 2023**. The two different solvents, aqueous and ethyl acetate extracts of *T. palmata* leaf displayed a yield percentage of 8.17% and 1.9% respectively, in the study by **Manoharan et al., 2023**. Higher yields are a good indication of the treatment method used during the extraction process, while preserving the integrity of other properties. The extract of the leaf had a higher concentration of bioactive ingredients than the leaf infusion. This is in line with the findings of **Dhanani et al. (2017)** which suggested that a high yield value indicated higher concentration of bioactive ingredients.

2.5.2 Phytochemical characterisation

The phytochemical analysis of the methanolic extract of the flowers of *T. palmata* showed the presence of alkaloids, cardiac glycosides, steroids, saponins, tannins and terpenoids and absence of phlobatannins. Similar studies conducted by **Rahman et al. (2014)** also confirmed the presence of alkaloids, flavonoids, saponins, and tannins. Each of those compounds is a member of the polyphenolic group and have been proven to have several medicinal uses.

Alkaloids have been associated with medicinal uses for centuries, a prominent biological feature being their cytotoxicity (**Nobori et al., 1994**). Their absence in this plant minimizes the risk of poisoning. Flavonoids have a health-promoting function because of their anion radicals (**Havsteen, 1983**). They exert their activities through changing membrane permeability and inhibiting membrane bound enzymes such as ATPase and phospholipase A2 (**Li et al., 2003**), which may explain the antioxidative processes of *T. palmata*. The study thus supports the

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utilization of the plant for its efficacy in traditional medicines for stress related conditions and applications for wounds commonly associated with circumcision rituals, contusions, lacerations, and lesions. This assessment is backed by many scientific literatures (**Mathekga, 2004; Lourens, 2004; Ferguson, 2001; Grierson and Afolayan, 1999**). In addition to possessing outstanding anticancer properties, tannins are widely known to heal irritated or ulcerated tissues (**Ruch et al., 1989; Olajide et al., 2004**). Therefore, *T. palmata* with this molecule may be a source of bioactive chemicals for ulcerative colitis therapy. The use of *T. palmata* in traditional medicine is also backed by the finding that the plant extract contains saponins, which are important components in traditional Chinese medicine and which are known to have an inhibitory effect on inflammation (**Just et al., 1998**). These saponins are also responsible for most of the observed beneficial biological effects (**Liu and Henkel, 2002**). The extract of the plant was found to test positive for steroids, which are very important substances because of their link to other substances such as sex hormone, as shown by **Okwu, 2001**. Besides, plant extract was also tested positive for terpenoid, an anti-inflammatory and anti-oxidant bioactive phytochemical. It has been revealed that terpenoids in medicinal plants contain pharmacological qualities to improve human health. While ginkgolides are naturally occurring platelet-activation inhibitors with neuroprotective and reparative properties, artemisinin is a common anti-malarial drug. Since ancient times, cannabinoids have been used by people for their effects as an aesthetics and anxiety-reducing agents. An effective anti-cancer natural medication that induces oxidative stress is paclitaxel. All these drugs are constituted of terpenoids (**Wang et al., 2023**).

As nutritional supplements, plants which have coumarins, glycosides and carbohydrates are important since they are known to strengthen the body and improve the immune system. Because coumarins have antibacterial and anti-inflammatory properties, they may be helpful for hyperproliferative skin conditions (**Theis and Lerdau, 2003**). Because they can be present in nearly all medicinal plants, glycosides also deliver a diverse range of therapeutic benefits (**Yadav et al., 2014**). The presence of these phenolic chemicals in the studied plant extract enhances its antioxidative effects, hence increasing its use in traditional herbal medicine.

2.5.3 Total phenol, flavonoid, and tannin content

According to the study, the methanolic extract of *T. palmata* flower buds included a significant quantity of total phenolic total tannin, and total flavonoids. These characteristics directs to the usage of this plant in several traditional herbal remedies. The antioxidant qualities of every plant are closely correlated with its phenolic content. Free radicals may be scavenged by phenolic compounds, which can function as hydrogen donors and reducing agents (**Phuyal et al., 2020**). It prevents fats from breaking down, improves the nutritional content and quality of food, stabilizes electrons, chelates metal ions, and protects against microbial diseases (**Singh et al., 2015**). Phenolic compounds have been shown to have anti-inflammatory, anti-allergenic, anti-thrombotic, anti-atherogenic, anti-microbial, cardio-protective, hepato-protective, and vasodilatory properties (**Lalremruati et al., 2019**). According to **Cook and Samman (1996)**, flavonoids possess distinct structural conformations that provide antioxidant, free radical scavenging, anti-inflammatory, metal chelating, and lipid peroxidation inhibitory qualities (**Cook and Samman, 1996**). Flavonoids have been described as a health-promoting molecule (**Havsteen, 1983**) that protects people against several ailments, including thrombosis, hepatotoxicity, carcinogenesis, and atherogenesis (**Tiwari, 2001**), because of their scavenging or chelating properties. Additionally, tannins are phenolic compounds that have antibacterial and antioxidant qualities. They may also form complexes with metal ions and macromolecules (**Sharma and Agarwal, 2015**).

2.5.4 Characterisation of plant minerals

Inorganic chemical elements are a significant source of nutrition, playing their role in cellular functions (**Willis et al., 1996**). The current study is the first of its kind to assess the micro-nutrient elements in *T. palmata*. TPFME was found to contain high levels of K, Mg and Ca, which have been reported to be tremendously beneficial for human and animal health (**Auwal et al., 2014**). Therefore, the present data justifies the mineral richness of *T. palmata* flower buds.

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2.5.5 Free radical scavenging activity, antioxidant and metal chelating power

While free radicals are linked to oxidative damage, antioxidants are the reducing agents that neutralizes and transfers electrons to the free radicals to reduce oxidative damage. Free radicals are produced when some chemicals react with oxygen in the body, potentially damaging biological materials such as proteins, DNA and cell membranes. Secondary metabolites present in plants tend to act as antioxidants that neutralise such free radicals (**Lalhminghlui and Jagetia, 2018**).

The extract of TPFME showed a concentration-dependent DPPH and ABTS radicals scavenging effect. A similar study on *T. palmata* leaf extracts performed by **Manoharan et al. (2023)** showed that out of five different solvents used, ethyl acetate showed the lowest IC₅₀ value (4.72 ± 0.81 mg/mL), indicating the highest DPPH radical scavenging property. Additionally, the ABTS cation radical scavenging capacity of ethyl acetate was determined to be 242.33 ± 6.81 mM TE/g extract, which shows significant radical scavenging capacity (**Manoharan et al., 2023**). The O₂•⁻ are generated in biological systems during cellular respiration and as such they are less toxic, however, they are converted into highly reactive OH radical in the presence of iron. In addition, superoxide anion generated because of incomplete metabolism of oxygen directly or indirectly by the formation of H₂O₂, •OH and peroxynitrite or singlet oxygen (**Kirkinezos and Moraes, 2001**). Thus, scavenging of O₂•⁻ could lead to inhibition of other ROS generation and protect the cells from oxidative damage. In this investigation, TPFME extract inhibited formation of O₂ •⁻ in concentration-dependent fashion. Kaempferol has been reported to scavenge O₂ •⁻ in an earlier report. Other plant extracts and some plant flavonoids such as mangiferin, naringin, quercetin, myricetin and rutin have also been found to scavenge superoxide free radical in a concentration dependent manner (**Lalhminghlui and Jagetia, 2018**).

The reducing power of compounds is an indication of their antioxidant properties (**Meir et al., 1995**). The enhanced absorbance at higher concentrations shows a highly reducing power potential of the extracts in a concentration-dependent way. The high reducing potential of TPFME in comparison with the standard ASA

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reflects the high reducing power of the extract. The observed graded increase in percentage inhibition with increasing concentrations of plant extracts suggests a strong dependence of scavenging effectiveness on the concentration of extracts.

Nitric oxide is an important cellular signaling molecule, and has a significant role in many physiological and pathological processes. This compound is a powerful vasodilator that has a short half-life of several seconds in the bloodstream (**Lushchak et al., 2014**). The nitric oxide radical (NO•) turns toxic after reaction with oxygen or superoxide anion radicals. The TPFME extracts in this study showed concentration-dependent decreased generation of NO•. Kaempferol, myricetin, epigallocatechin gallate, catechin, epicatechin, and resveratrol have been found to scavenge nitric oxide radicals (**Hou et al., 1999**). Several flavonoids, including delphinidin, pelargonidin, malvin, mangiferin and naringin, have shown NO radical neutralization ability in the prior studies (**Lalhminghlui and Jagetia, 2018**).

A study carried out by **Sanchez-Vioque et al. (2012)** evaluated the metal chelating capacity of by-products of saffron. The results showed that the highest Fe²⁺ chelating activity was observed for the leaf extract with a maximum of 31% at concentration of 490ug/mL, whereas the tepals extract showed minimal Fe²⁺ chelating activity. Our study has shown that the methanolic extract of *T. palamata* flower buds possesses iron metal chelating ability which is concentration-dependent. The extract showed the best Fe²⁺ chelating activity and maximum of 52.49% at a concentration of 1000 ug/mL.

2.5.6 LC-MS, GC-MS, FTIR and NMR analysis

Current literature shows a lack of analysis of phytochemicals associated species from the genus *Trevesia* using advanced analytical methods such as LC-MS, GC-MS, NMR and FTIR. However, the use of column chromatography (CC) and thin layer chromatography (TLC) followed by droplet counter current chromatography (DCCC) with solvent system of n-BuOH-EtOH-AcOH-H₂O (8:4:2:1) with combination of reverse-phase high performance liquid chromatography (RPHPLC) is reported, and have resulted in isolation of six novel

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bis desmosidic saponin compounds (i.e., triterpene saponins) and some known triterpenoid saponins from the aerial parts of *T. sundaica*, a similar species under the same genus. The chemical structures of these compounds were found to be determined by 1-D and 2-D NMR spectra and mass spectrometric analyses (**De Tommasi et al., 1997**). Some studies have been performed on the aerial parts of *T. palmata* too, resulting in identification of lupane and ursane-type triterpenoid saponins, and acetylated saponins. The chemical structure of these compounds was analyzed by HR-ESI-MS and NMR spectroscopy (**Thao et al., 2018; Yen et al., 2021**).

A recent study by **Manoharan et al. (2023)** analysed the activity of *T. palmata* leaf extract on MG 63 cell lines in arthropathy induced animal models to test the potential of *T. palmata* leaf extract. Polyphenolic compounds were detected using the chromatographic technique of High-Performance Liquids (HPLC). They found six significant compounds in the ethyl acetate extract. The present study on TPFME phytocompound analysis by LC-MS and GC-MS techniques identified a total 20 major compounds of which 7 compounds were detected by GC-MS technique and 13 compounds were detected by LC-MS technique. The methanolic extract of *T. palmata* seed flower buds mostly contains flavonoids, fatty acids, tannin and carotenoid. Previous research on *T. palmata* have shown similar bioactive compounds such as catechin, naringenin, kaempferol and quercetin (**Manoharan et al. 2023**). The variation of chemical composition can be explained by either species-specific variation of the phytochemical substances or by variation of the extraction method used. The chemical composition of plants is characterized by a high variability, even for those of the same species (**Koutsoukis et al., 2019**).

FTIR was used to estimate chemical bonding in the dried powdered methanolic extract of *T. palmata* flower buds. Strong bonds were found at 2924.21 cm^{-1} , 1698.76 cm^{-1} , 1021.61 cm^{-1} , 810.67 cm^{-1} , and 769.63 cm^{-1} in the infrared absorption spectra, whereas other bonds ranged from strong to medium, weak to medium, and medium. Numerous chemicals, including hydroxyl groups, lipids, alkanes, membrane lipids, fatty acids, amines, and R-CH₃ were reported. These

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included alkanes, secondary alcohol-free, alkyl-aryl ethers, benzonoid, 1,2-disubstituted, monosubstituted, and benzoene derivatives. The presence of phenols and flavonoids in the crude *T. palmata* methanolic extract was shown by the FTIR, which revealed that the flower buds extract had a high C-H out-of-plane bending (oop bend) vibration for the substituted benzene ring. Flavonoids are polyphenols that have a linear carbon chain connecting two benzene rings (**Corcoran et al., 2012**). FTIR spectrophotometry was used to identify benzenoid chemicals, which confirmed the results of the phytochemical screening that found flavonoids and phenols. The main functional groups of bioactive compounds were thought to be the amines, imines, alkanes, and phenols (**Pratheeba et al., 2015**). C–O stretching vibrations are indicated by peaks that occur at 1021.61 cm^{-1} (**Naseem et al., 2020**). Additionally, the existence of terpenes owing to the C-H group at 1442.68 cm^{-1} , polyphenols and flavonoids due to the O-H stretching at 3276.33 cm^{-1} , and alkaloids due to the N-H bonding at 1593.70 cm^{-1} (**Kale and Arvind, 2023**). The peak in this investigation was located at 1698.76 cm^{-1} . The TPFME powder of *T. palmata* was expected to contain associated carotenoids. According to reports, the structure of carotenoids responsible for light absorption is the conjugated double bond (**Mezzomo and Ferreira, 2016**). Additionally, the TPFME extract included several alkanes, including alcohol, esters, ether groups, aromatic compounds (Ar-H), and carbonyl compounds (O=C-C-H), according to the NMR spectra. A similar study by **Marak et al., (2022)** reported presence of alkyls, alkenes, alkynes, R–OH, R–NH₂, and –OCH₃, deficiency of aromatic protons, carboxylic acids, and aldehydic hydrogens in the extract.

2.6 KEY FINDINGS

- The yield of crude methanolic extract of *T. palmata* flower buds (TPFME) was estimated to be 8.4% with no significant changes in appearance of the extract.
- Preliminary phytochemical screening test of TPFM observed the presence of various naturally occurring compounds, including alkaloids, cardiac glycosides, steroids, saponins, tannins, and terpenoids.

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- Total phenol content was found to be 21.51 ± 4.25 mg GAE/g of extract, total flavonoid content 251.92 ± 13.77 mg QE/g of extract and total tannin content was measured to be 13.58 ± 0.35 mg TAE/g of extract.
- Micro-nutrient analysis showed highest concentration of potassium (K)- 251.070 ± 0.447 ppm, magnesium (Mg)- 11.41 ± 0.115 and calcium 8.984 ± 0.050 ppm and concentration of less than 1 ppm found in other micronutrients such as manganese (Mn), sodium (Na), zinc (Zn), iron (Fe), copper (Cu), and cobalt (Co).
- Assessment of free radical scavenging, antioxidant and metal chelating power activity of TPFME demonstrated concentration-dependent scavenging effect through DPPH, ABTS $\bullet +$, SOD, NO, iron chelating activity and reducing power activity tests, and IC_{50} were estimated at 161.286 ± 0.450 for DPPH; 186.423 ± 1.230 for ABTS $\bullet +$; 127.947 ± 1.251 for SOD; 631.472 ± 4.276 for NO and 777.807 ± 1.945 for Iron chelating activity. At concentration 750 $\mu\text{g/mL}$, the TPFME exhibited the highest reducing power of 0.456 ± 0.002 .
- Phytocompound analysis utilizing LC-MS and GC-MS techniques identified a total of 21 bioactive compounds, with 7 compounds detected by GC-MS and 13 compounds detected by LC-MS. The extract was found to primarily contain flavonoids, fatty acids, and carotenoids.
- The FTIR spectral peak analysis of the TPFME identified number of functional groups in strong to weak bonding such as O-H, CH_2 , C-H, C=C, N-H and C-O, and predicted presence of compounds such as hydroxyl groups, lipids, alkanes, membrane lipids, fatty acids, amines, and R- CH_3 . These included alkanes, secondary alcohol-free, alkyl-aryl ethers, benzonoid, 1,2-disubstituted, monosubstituted, and benzoene derivatives.
- NMR spectrum analysis of TPFME indicted predominated presence of alkanes, alkenes alcohol, esters, ether groups and carbonyl compounds and absence of aldehyde (R-CHO) or carboxylic acid (-COOH) groups.

Table 2.1 Preliminary phytochemical screening of methanolic extract of *T. palmata* flower buds (TPFME) (“+” indicates presence of phytochemical and “-” indicates absence of phytochemical).

Phytochemicals	Reagent	Colour Indication	TPFME (+/-)
Alkaloids	Dragendorff’s reagent	Redish brown	+
Cardiac glycosides	Glacial acetic acid Ferric chloride Sulphuric acid	Brown ring	+
Steroids	Sulphuric acid	Red colour	+
Saponins	Olive oil	Whitish emulsion	+
Tannins	Ferric chloride	Brownish green/ Blue-black	+
Terpenoids	Sulphuric acid	Reddish brown	+
Phlobatannins	Hydrochloric acid	Red precipitate	-

Table 2.2 Physiochemical quantitative assays of TPFME. Values are expressed as Means $\pm$ Standard error of the mean (SEM) (n = 3). The units of expression are GAE: gallic acid equivalent for TPC, QE: Quercetin equivalent for TFC, and TAE: Tannic acid equivalent for TTC.

Parameter analysed	Concentration
Total Phenolic Content (TPC) (mg GAE/g of extract)	21.51 $\pm$ 4.25
Total Flavonoids Content (TFC) (mg QE/g of extract)	251.92 $\pm$ 13.77
Total Tannin Content (TTC) (mg TAE/g of extract)	13.58 $\pm$ 0.35

Table 2.3 Mineral composition of TPFME. Values are expressed as Mean $\pm$ SEM (n = 3). The unit of expression of concentration is milligram decilitre-1 or parts per million (mg dL-1 or ppm).

Component	Concentration (mg dL-1 or ppm)
Potassium (K)	251.070 $\pm$ 0.4470
Magnesium (Mg)	11.410 $\pm$ 0.1150
Calcium (Ca)	8.984 $\pm$ 0.0500
Manganese (Mn)	0.808 $\pm$ 0.0003
Sodium (Na)	0.506 $\pm$ 0.0020
Zinc (Zn)	0.259 $\pm$ 0.0040
Iron (Fe)	0.140 $\pm$ 0.0020
Copper (Cu)	0.089 $\pm$ 0.0010
Cobalt (Co)	0.017 $\pm$ 0.0040

Table 2.4 Phytochemicals detected from TPFME, using gas chromatography-mass spectrometry (GC-MS). Initial temperature 120 degree Celsius (°C) for 2 minutes (mins), ramp at 10 °C/min to 300 °C, hold for 20 mins; Injection temperature: 260 °C; Split ratio: 10:1; Ion Source Temp: 220 °C; Interface temperature: 270.00 °C; Scan: 40 – 650 mass per charge (m/z). (RT: Retention Time; A: Area; MS: Mass spectrum; MW: Molecular Weight)

Sl. No.	Compound Name	Formula	Class	MW	RT	MS (m/z)	A (%)
1.	Palmitic acid†	C ₁₆ H ₃₂ O ₂	Long-chain lipid (fatty acid)	256.4	16.628	117	7.97
2.	1-Octadecanol†	C ₁₈ H ₃₈ O	Long-chain alcohol	270.5	17.562	327.25	0.49
3.	Oleic acid‡	C ₁₈ H ₃₄ O ₂	Long-chain lipid (fatty acid)	282.5	18.178	73.05	17.24
4.	Stigmasterol	C ₂₉ H ₄₈ O	Sterol	412.7	18.601	55.05	0.60
5.	Eicosanoic acid, tetradecyl ester	C ₃₄ H ₆₈ O ₂	Long-chain ester	508.9	23.968	43.05	46.63
6.	Hentetracontanoic acid†‡	C ₄₁ H ₈₂ O ₂	Very long-chain fatty acid	607.1	25.517	43.05	11.50
7.	Eicosanoic acid	C ₂₀ H ₄₀ O ₂	Long-chain lipid (fatty acid)	312.5	27.422	43.05	0.81

†Detected as TMS derivative; ‡detected as trimethylsilyl ester; †‡Detected as methyl ester

Table 2.5 Phytochemicals detected from TPFME through liquid chromatography-mass spectrometry (LC-MS) analysis. Flow: 1.500 millilitre per minute (mL/min); Stop Time: 5.0 mins; Column Temperature: 30-degree Celsius (°C); Min Pressure: 0.0 Bar; Max Pressure: 300.0 Bar; Acquisition: spectra were recorded in positive (+) and negative (-) ionization mode between mass per charge (m/z) 150 and 2000. (RT: Retention Time; A: Area; MS: Mass spectrum; MW: Molecular Weight).

Sl. No.	Compound Name	Formula	Class/Type	MW	RT	MS (m/z)	Mode
1.	zeta-Carotene	C ₄₀ H ₆₀	Carotenoid	540.90	15.39	264.4707	+
2.	Kaempferol-3-O-rutinoside	C ₂₇ H ₃₀ O ₁₅	Flavonoid glycoside	594.50	15.63	264.3967	+
3.	Apigenin 6-C-glucoside 8-C-arabinoside	C ₂₇ H ₃₀ O ₁₅	Flavonoid C-glycoside disaccharide	564.50	22.30	279.4566	+
4.	Zeaxanthin	C ₄₀ H ₅₆ O ₂	Carotenoid	568.90	20.38	279.2716	+
5.	Luteolin-6-C-glucoside 8-C-arabinoside	C ₂₇ H ₃₀ O ₁₆	Flavonoid C-glycoside	610.50	23.28	403.3389	+
6.	Procyanidin B1	C ₃₀ H ₂₆ O ₁₂	Procyanidin (dimer)	578.50	1.15	401.2669	-
7.	Procyanidin C1	C ₄₅ H ₃₈ O ₁₈	Procyanidin (trimer)	866.80	8.09	1039.8745	-
8.	Vicenin-2	C ₂₇ H ₃₀ O ₁₅	Flavonoid C-glycoside	594.50	16.99	299.4008	-
9.	Luteolin-6-C-glucoside	C ₂₁ H ₂₀ O ₁₁	Flavonoid C-glycoside	448.40	17.04	299.5488	-
10.	Apigenin-8-C-glucoside	C ₂₁ H ₂₀ O ₁₀	Flavonoid C/O-glycoside	432.40	23.74	578.9843	-
11.	Quercetin-3-O-galactoside	C ₂₁ H ₂₀ O ₁₂	Flavonoid O-glycoside	464.40	25.30	255.4421	-
12.	Isorhamnetin	C ₁₆ H ₁₂ O ₇	Flavonoid (aglycone, methoxy)	316.26	25.53	255.4051	-
13.	Kaempferol	C ₁₅ H ₁₀ O ₆	Flavonoid (aglycone)	286.24	25.98	281.4178	-

Table 2.6 FTIR spectral peak values and functional groups of TPFME. (w: weak; m: medium; s: strong; cm: centimetre).

Spectrum No.	Wave No. (cm ⁻¹)	Wave No. (cm ⁻¹)	Functional Group Assignment	Predicted Compound
1	3276.33	3130-3500	v; O-H stretching vibration (associated)	Hydroxyl group
2	2924.21	2920 - 2930	s; CH ₂ asymmetry stretching vibration	Lipid
3	2856.08	2840-2870	m; C-H stretching vibration- symmetry	Alkane
4	1698.76	1645-1700	s; C=C stretching vibration	Membrane lipid, fatty acid
5	1593.70	1580-1660	m; N-H bonding vibration	Amine
6	1442.68	1440-1465	m; C-H deformation vibration-asymmetry	R-CH ₃ ; Alkane
7	1373.73	1370-1390	s-m; C-H deformation vibration symmetry	Secondary alcohol-free
8	1268.67	1200-1300	w; C-H deformation vibration- symmetry	Secondary alcohol-free
9	1021.61	1020-1120	s; C-O stretching vibration-symmetry	Alkyl-aryl ethers
10	810.67	700-900	s; C-H out-of-plane bending (oop) vibration for substituted benzenes ring	Benzenoid
11	769.63	765-870	s; CH ₂ rocking vibration	1,2-disubstituted
12	737.61	725-735	w-m; C-C skeleton vibration (rocking)	Monosubstituted
13	641.58	625-640	w-m; Ring deformation	Benzene derivative

Table 2.7 Compounds name and parts per million (ppm) analysis of ¹H-NMR of TPFME (DMSO: Di-methyl sulfonate)

PPM	Compounds
0.5-2	Alkanes (C-C-H)
2-3	Carbonyl compounds (O=C-C-H)
3-4	Alcohol, esters, ethers (O-C-H)
4-6	Alkenes (C=C-H)
7-8	Aromatic compounds (Ar-H)
2.5-3.3	DMSO, H ₂ O

Table 2.8 Antioxidant potential of TPFME. Values are represented as Mean $\pm$ SEM (n = 3). Unpaired t-test was performed, where * = p<0.05, ** = p<0.01, *** = p<0.001 significantly different from standard was observed. The unit of expression is microgram per millilitre (μ g/mL) (ND = Not Determined, TPFME = methanolic extract of *T. palmata* flower buds, ASA = Ascorbic acid, EDTA = Ethylenediaminetetraacetic acid).

Assays	IC ₅₀ (μ g/mL)	
	Standard (ASA/EDTA)	Plant extract (TPFME)
DPPH	142.642 $\pm$ 3.210	161.286 $\pm$ 0.450**
ABTS $\cdot^+$	80.908 $\pm$ 0.950	186.423 $\pm$ 1.230***
SOD	86.749 $\pm$ 1.789	127.947 $\pm$ 1.251***
NO	523.774 $\pm$ 4.930	631.472 $\pm$ 4.276***
Iron chelating activity	ND	777.807 $\pm$ 1.945



Figure 2.1 Preparation of crude methanolic extract of *Trevesia palmata* flower buds (TPFME).

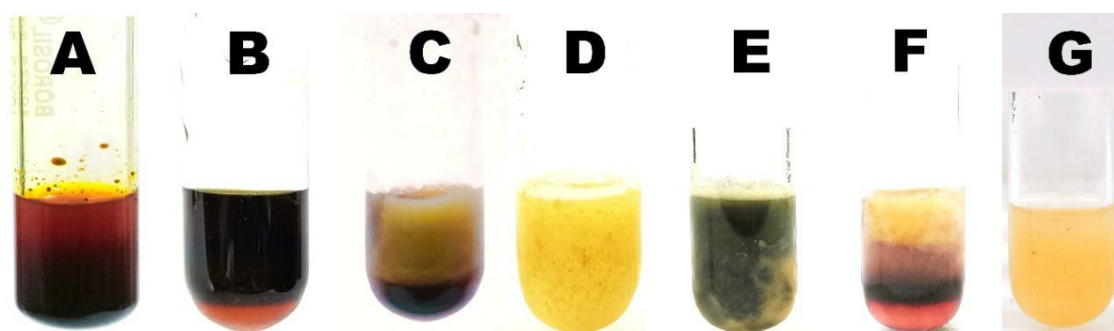


Figure 2.2 Result of phytochemical compounds screening of the TPFME: **A.** Alkaloid, **B.** Cardiac glycosides, **C.** Steroids, **D.** Saponins, **E.** Tannins, **F.** Terpenoids, **G.** Phlobatannins

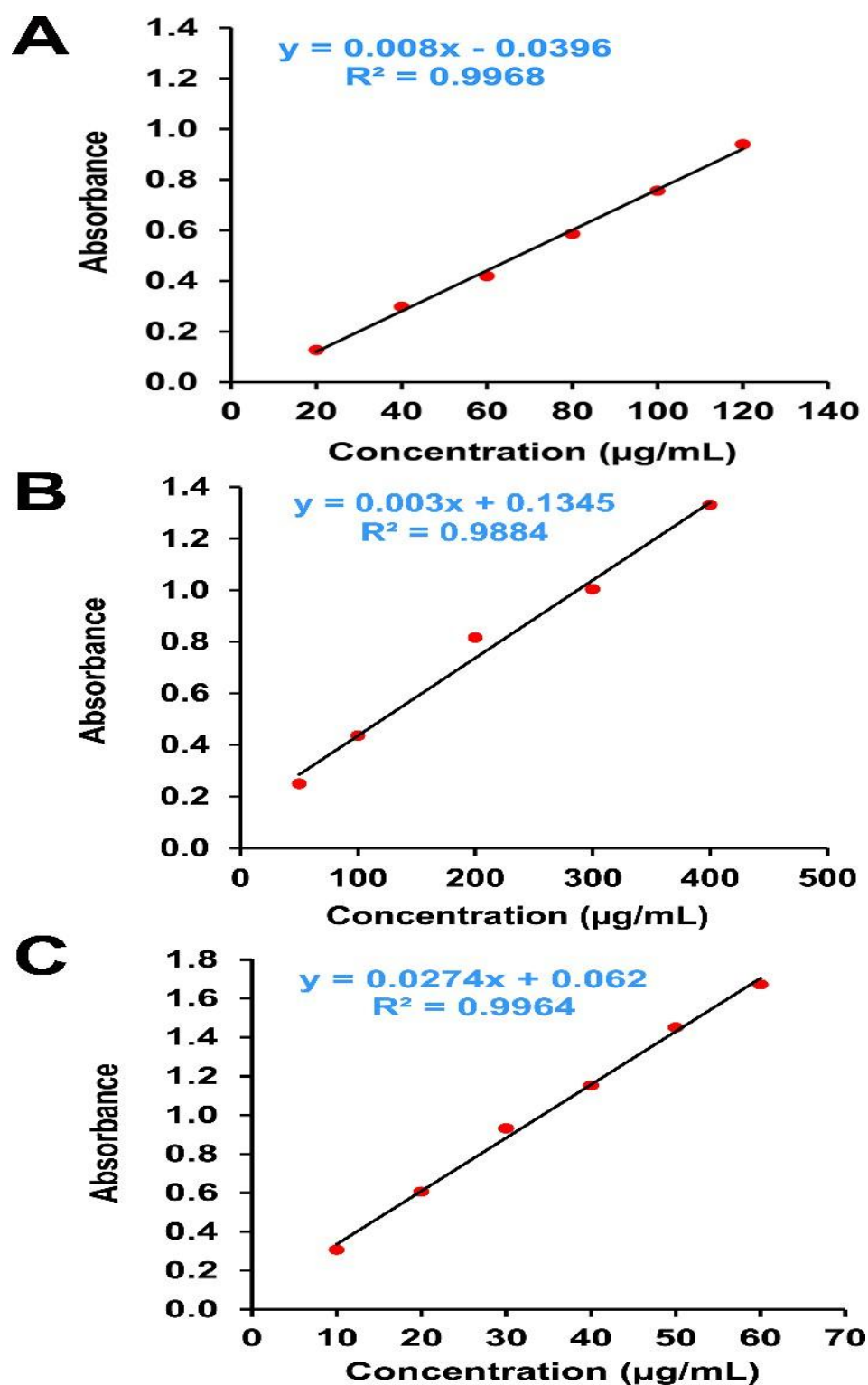


Figure 2.3 Quantitative phytochemical analysis of TPFME: (A) Gallic acid standard curve of total phenolic content (mg GAE/g of extract), (B) Quercetin standard curve of total flavonoids content (mg QE/g of extract), (C) Tannic acid standard curve of total tannin content (mg TAE/g of extract).

GC-MS Chromatogram of *Trevesia palmata* flower buds phytocompounds

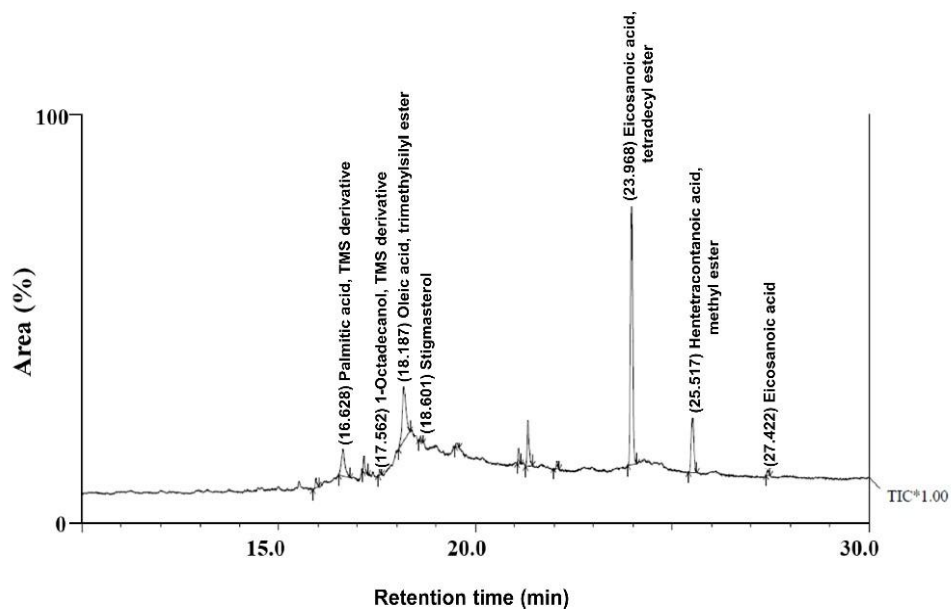


Figure 2.4 The chromatogram displays the results of the GC-MS (gas chromatography-mass spectrometry) analysis of TPFME.

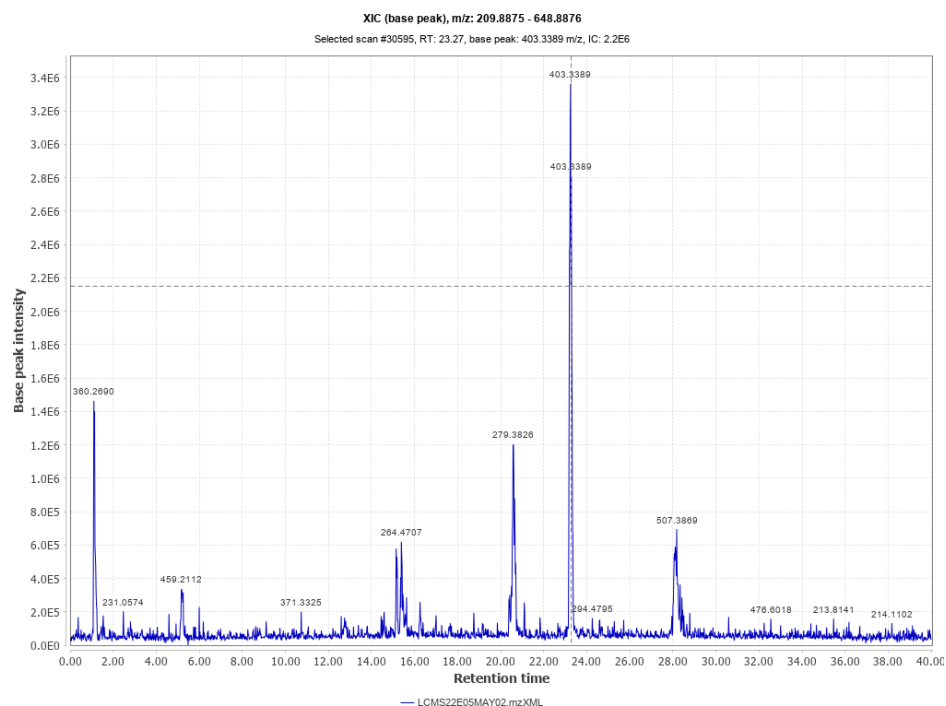
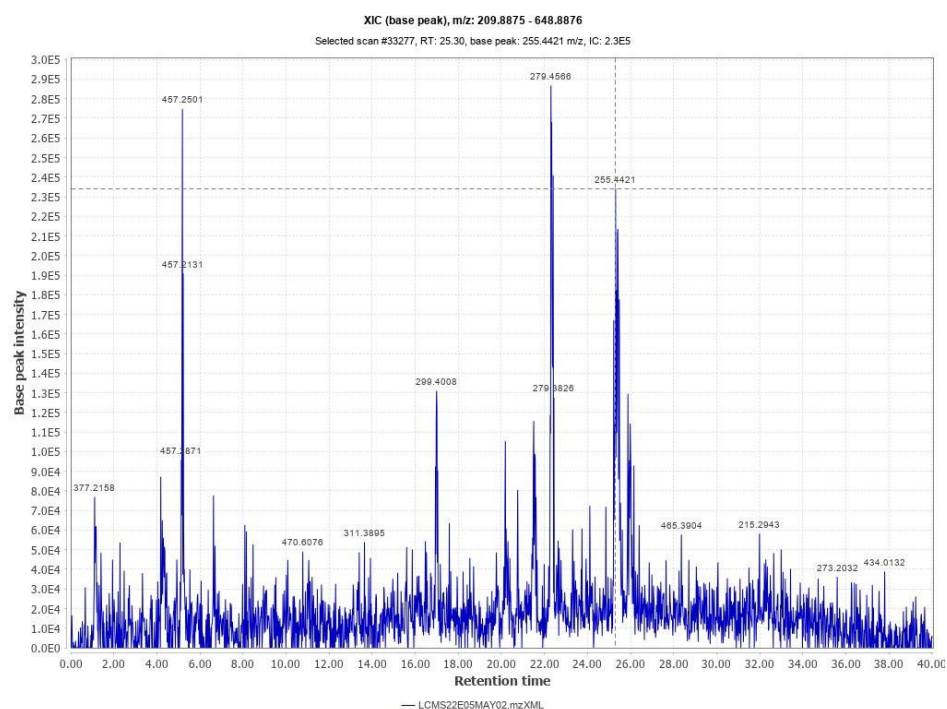
A**B**

Figure 2.5 LC-MS (liquid chromatography-mass spectrometry) chromatogram peaks of TPFME: **A**: positive ion mode; **B**: negative ion mode

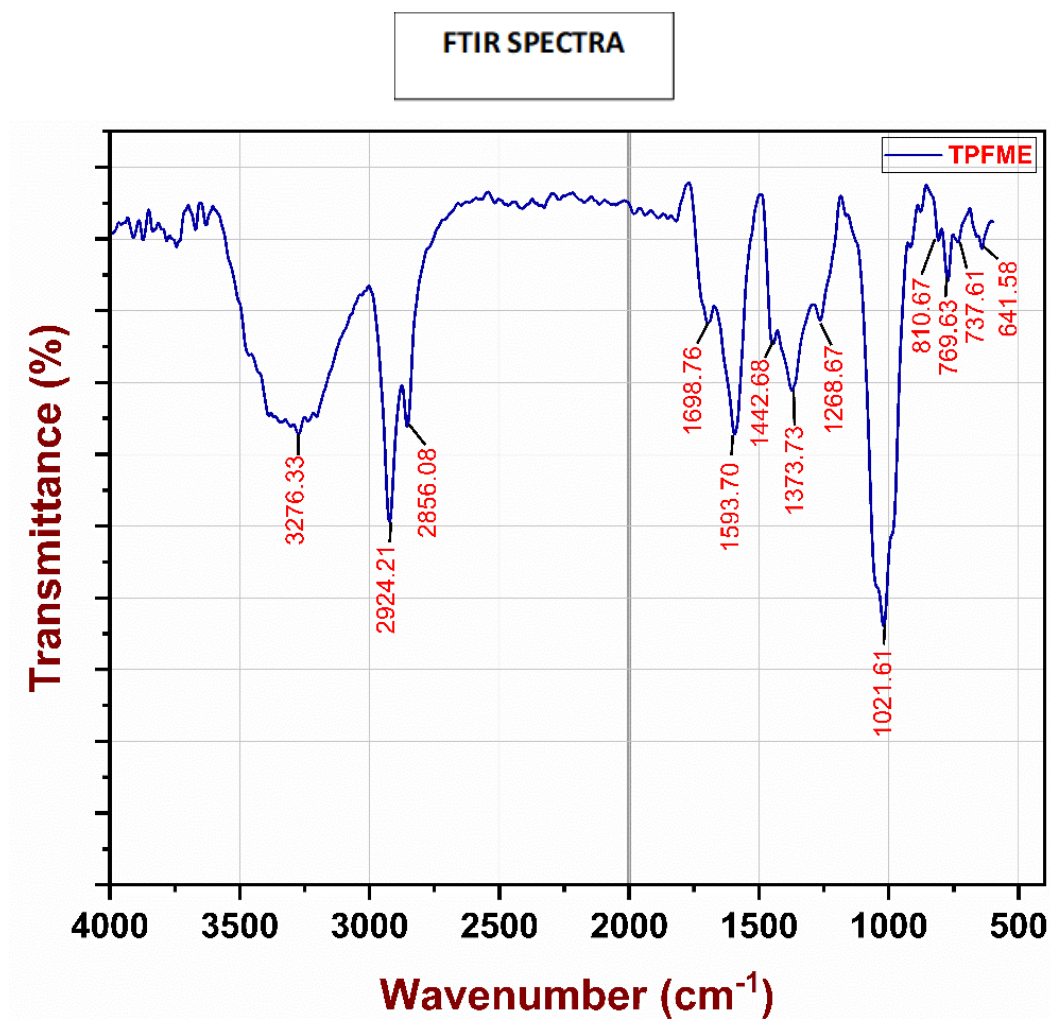


Figure 2.6 Fourier Transform Infrared spectroscopy (FTIR) spectra of TPFME display transmittance percentage (%) against wave number (cm^{-1}).

NMR SPECTRA

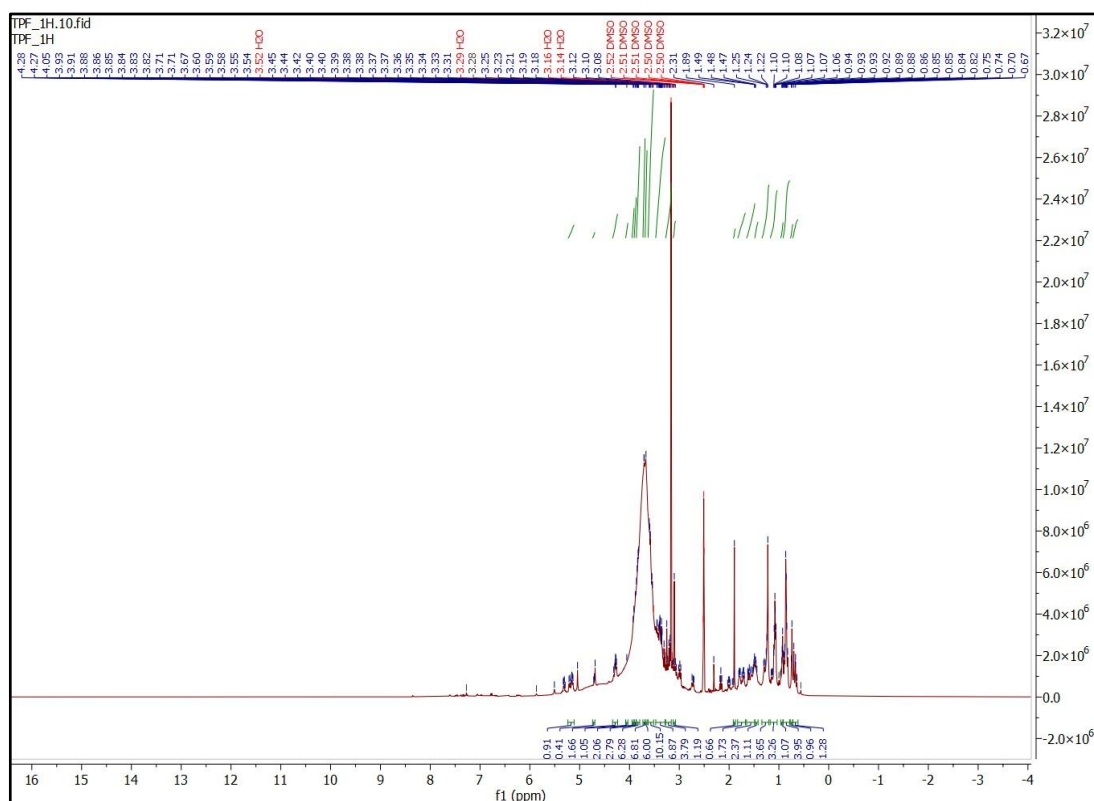


Figure 2.7 Nuclear magnetic resonance (NMR) spectroscopy spectra displaying the observed peaks at parts per million (ppm) provide valuable insight into the types of protons present in TPFME.

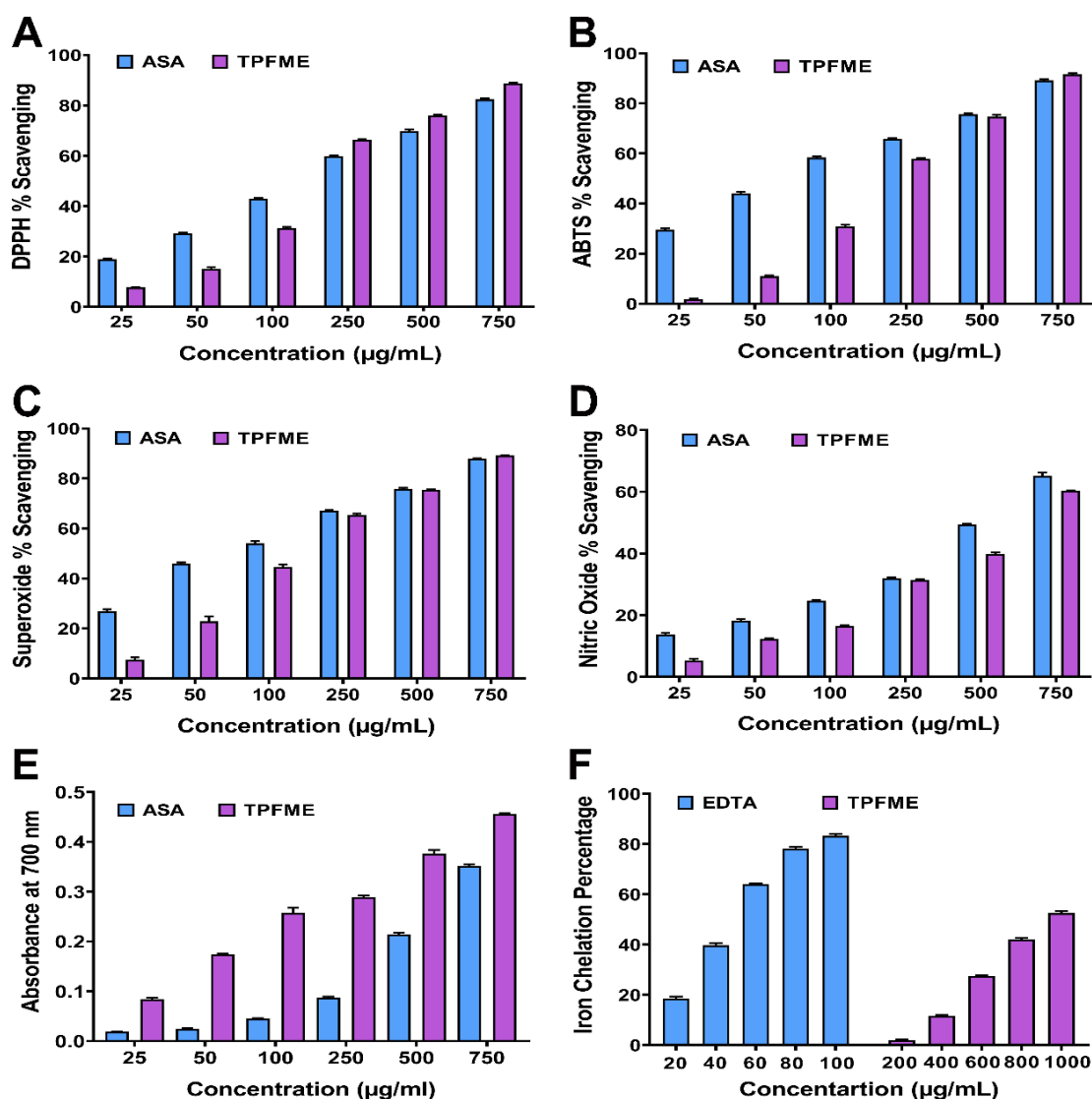


Figure 2.8 Free radical scavenging activity and antioxidant activity of TPFME for the calculation of IC₅₀: (A) DPPH· free radical scavenging activity; (B) ABTS•+ free radical scavenging activity; (C) Superoxide free radical scavenging activity; (D) Nitric oxide free radical scavenging activity; (E) Reducing power; (F) Iron chelation percentage. The unit of expression is microgram per millilitre (µg/mL).

Chapter 3

3.1 INTRODUCTION

3.1.1 Inflammation and its molecular targets COX-2 and KEAP1

Inflammation represents a complex and very orchestrated biological response which involves the interplay of innate and adaptive immune mechanisms, initiated by different stimuli such as invasion by pathogens, tissue injury or chemical insult. The acute inflammatory phase is a protective process to destroy the harmful agents, remove the necrotic debris, and repair the tissues to restore the physiological homeostasis. However, if acute inflammation is not properly resolved, a low-grade, chronic inflammatory state in which there is invariant infiltration of immune cells, aberrant cytokine production, and oxidative stress occurs. This chronic inflammatory environment is involved in the development and pathogenesis of many pathological conditions such as metabolic syndrome, atherosclerosis, neurodegenerative disorders and cancer. Listed as, proinflammatory cytokines like tumor necrosis factor-alpha (TNF- α), interleukin-1 beta (IL-1 β) and interleukin-6 (IL-6) are downstream upregulated upon activation of pattern-recognition receptors (PRRs), namely nuclear factor kappa B (NF- κ B) mediated signaling pathway is the major downstream effector of PRRs. These mediators create a feed forward loop of immune cell activation, endothelial adhesion and acute-phase protein synthesis that ultimately perpetuates tissue injury and maladaptive remodeling (**Bennett et al., 2018; Chopra et al., 2024; Parvin et al., 2025**).

Besides cytokine and chemokine signaling, the lipid mediator and redox regulatory pathways are proven modulators of the inflammatory network and such other pathways are tractable therapeutic targets. The cyclooxygenase-2 (COX-2)-prostaglandin cascade is a key lipid-signaling axis that involves catalysis of arachidonic acid to generate diversified prostaglandins, particularly prostaglandin E2 (PGE2) by the inducible isoform of COX-2 that modulates pain, fever, vascular dynamics and immune cell functionality. Selective pharmacological inhibition of COX-2 is effective in reducing inflammatory prostaglandin synthesis and is a proven anti-inflammatory approach, finding an increasing number of applications in chronic

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inflammatory diseases and neoplastic diseases (**Ricciotti and FitzGerald, 2011; Ju et al., 2022**)

Another important pathway that integrates redox and inflammatory signals is the KEAP1-Nrf2 oxidative stress axis. Under physiological conditions, nuclear factor erythroid 2 related factor 2 (Nrf2) is targeted to ubiquitin-dependent proteasomal degradation by Kelch-like ECH-associated protein 1 (KEAP1). Upon oxidative/electrophilic challenge this interaction is disrupted, which allows Nrf2 translocation into nucleus where it drives the expression of antioxidant response element (ARE) driven genes comprising heme oxygenase-1 (HO-1), NAD(P)H quinone dehydrogenase 1 (NQO1) and glutamate-cysteine ligase catalytic subunit (GCLC) in order to restore redox balance and limit inflammatory processes. Pharmacological approaches to interfere with Nrf2 up-regulation or direct Keap1 blocking become more and more discussed for buffering reactive oxygen species (ROS) induced damage and cytokines enhancement (**Saha et al., 2020; Ngo and Duennwald, 2022**).

Mechanistically, these pathways intersect the NF- κ B and cytokine regulatory network to have profound impacts in disruptive importance as to inflammatory outcomes. COX-2 generated PG may enhance stimulation of NF- κ B and proinflammatory signaling to sustain an elevated proinflammatory environment. On the other hand, the activation of Nrf2 has an antagonistic effect on NF- κ B, which leads to the limitation of its transcriptional activity by antioxidant genes induction and redox buffering to provide a negative feedback mechanism to reduce the inflammation. Hence, the interaction of proinflammatory COX-2 signaling and cytoprotective Nrf2 activation is a factor deciding pro-resolution and increased development of inflammation and justifies combinatorial therapeutic approaches targeting both oxidative and cytokine signaling-related axes in chronic inflammatory disease (**Saha et al., 2020; Jin et al., 2023**).

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3.1.2 Plant-based drug discovery

Distance therapeutic development strategies are permitted and common in the device sector against the backdrop of the fact that plants constitute a long-standing and abundant reserve of bioactive molecular frames for medicinal development, and for many decades, regulatory approvals, of petrochemical small-molecule medications, reflect this feature. Seminal analyses by Newman & Cragg show systematically and evidence that a significant proportion of approved drugs - in the fields of anti-infective, oncological, cardiometabolic and neuropsychiatric indications - originate as natural products or direct semi-synthetic derivatives from nature, which confirms the translational continuous power of plant chemistry in modern pharmacotherapy (**Furst and Zundorf, 2014; Nisar et al., 2023**)

Good examples throughout history include artemisinins (isolated from *Artemisia annua*), considered to be a paradigm-shifting antimalarial agent and acknowledged by the Nobel Committee in 2015, and paclitaxel (Taxol, from *Taxus* spp.), which continues to form the backbone of oncologic regimens. Each agent overcame significant challenges for mechanism elucidation, sustainable production and that of scaling plant sourced compounds for global therapeutic applications that not only proved therapeutic efficacy but also the feasibility of production at scales allowing for the use of plant sourced compounds and their derivatives in human therapies (**Furst and Zundorf, 2014; Davila and Papada, 2023**)

From the public health perspective, the generalized application of the traditional and herbal remedies in the diverse healthcare environments strengthens the need for continued bioprospecting and for the strategic incorporation of the culturally-deployed pharmacopoeias in drug discovery pipelines. Methodologically, the recent progress in metabolomics, dereplication, high-throughput bioactivity screening, and computational biology such as network pharmacology, docking, molecular dynamics, and in-silico ADMET profiling, has increased the potential to rationally explore the domain of plant secondary metabolites, navigate through their chemical complexity, and prospect for novel anti-inflammatory leads to a great extent. Such innovations are very relevant in understanding mechanisms at validated

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anti-inflammatory targets, including COX-2 and KEAP1-Nrf2, where plant-derived chemotypes are mechanistically tractability discernible for downstream optimization via structure-based strategies (**Samiappan and Chalakoth, 2025**).

Important inflammatory drugs, like polyphenols, alkaloids, terpenoids, and saponins are examples of the fruitful interplay of organic product chemistry and molecular pharmacology governing eicosanoid synthesis, cytokine signaling, antioxidant pathways of importance to immune homeostasis and inflammatory diseases (**Khayatan et al., 2024**).

3.1.3 Computational approach of drug discovery

Computational drug discovery uses various in silico approaches to optimise and rationalise the creation of new drugs. These approaches encompass prediction of biological activity, molecular docking, molecular dynamics simulation, ADME characterization and computational toxicity to accelerate drug design and optimize attrition rates during preclinical development (**Leelananda and Lindert, 2016; Gupta et al., 2024**).

3.1.3.1 Predication of biological activities

Computational prediction of biological activity, often with the help of Quantitative Structure-Activity Relationship (QSAR) models and machine learning, enables the prediction of the efficacy of compounds gathered in this way to be quickly evaluated using information on the molecular descriptors and correlations with the experimental results. These models are then used to prioritize which chemical scaffolds to use and optimize the lead compounds before they are synthesized and tested on animals. Recent improvements of deep learning methods have further unsheathed the potential for the prediction accuracy and scope for virtual screening strategies (**Novic 2023**).

3.1.3.2 Molecular docking

Molecular docking is a center structure-based drug discovery technique that simulates the binding of small-diameter molecules to a target protein and estimates

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the mode and energies of binding. Docking enables much high-throughput screening of chemical libraries for potential ligands thereby identifying hits and optimizing them to leads. Common docking platforms like AutoDock Vina, Glide and GOLD are commonly integrated with ADME and toxicity modules for multi-functional evaluation of the candidate **(Enni and Maraj, 2022)**.

3.1.3.3 Molecular dynamics simulation

Molecular dynamics (MD) simulation is a way to gain atomistic knowledge of the dynamic behavior and stability of ligand-protein complex. MD allows the flexibility of structures to be explored, understanding of binding mechanisms, and cryptic binding sites can be discovered that may not be available using static docking protocols alone. The incorporation of MD combined with docking in the Relaxed Complex Method is currently used extensively for structure-based drug design **(Wei and McCammon, 2024)**.

3.1.3.4 ADME and computational toxicity assessment

ADME (Absorption, Distribution, Metabolism, Excretion) prediction is used to evaluate pharmacokinetic properties important for the in vivo efficacy and clinical safety of drugs. In silico ADME screening, which is implemented in platforms like SwissADME, ADMETlab and ADMET-AI, can effectively filter molecules which are having adverse or suboptimal pharmacokinetic profile thereby reducing the risk of failure of drug candidates at later stages of development **(Zhang et al., 2025)**

Early toxicity prediction with computational models (rule-based, statistical and deep learning models) focuses on the main reasons for drug attrition such as organ-specific toxicities, genotoxicity and cardiotoxicity. In vitro and in vivo endpoint integration improves safety profiles and helps promote a fast and ethical drug development **(Naithani and Guleria, 2024)**

3.1.4 Computational tools

Computational pipelines now form the basis of early-stage drug discovery, through the rapid narrowing of enormous chemical spaces to a tractable set of

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bioactive, developable and safe drug candidates. In a typical in-silico workflow, orthogonal tools will be orchestrated to (i) hypothesize biological activities, (ii) evaluate drug-likeness and ADME/T risks, (iii) estimate ligand-receptor binding modes and affinities at target proteins, and (iv) understand the stability of ligand-receptor complexes under near-physiological dynamics. Together, these efforts decrease the weight of experiment and enhance the quality and interpretability of subsequent assays.

To obtain early hypotheses of pharmacological effects that are based directly on structure, this work uses PASS (Prediction of Activity Spectra for Substances). PASS provides the inference of probabilities of activity/inactivity at hundreds of pharmacological mechanisms given a compound's structural formula, resulting in a rapid triage of chemotypes and the repositioning of ideas prior to the liabilities involved in modeling or wet-lab work **(Lagunin et al., 2000)**.

Drug-likeness & developability are profiled in using SWISSADME and ADMETlab 3.0. SwissADME offers fast validated predictors and visual diagnostics (e.g. by the use of BOILED-Egg, iLOGP, Bioavailability Radar) for physicochemical properties, absorption and medicinal chemistry "friendliness", helping to flag liabilities (e.g. poor permeability or rule-of-five violations). This can be complemented by ADMETlab 3.0 which provides a wide range of sensitized, modern-day ADMET endpoints with better coverage, performance, and access for API and batch evaluation and decision support for modern screening campaigns **(Duot et al., 2017; Dutlat et al., 2024)**.

Since scale is important in the modern prioritization process, this study also employs ADMET-AI (sometimes formatted as ADMET-AI/ADME-AI), a machine learning platform in the form of a web service and Python package. It provides - with large libraries - fast and large library capabilities in the standard ADMET tasks and has been shown with excellent top average rankings on community leaderboards in order to provide the ability to iterate rapidly when candidates in the thousands to millions range must be scored **(Swanson et al., 2024)**.

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Safety-related risks are further examined using ProTox-II, a freely available toxicity prediction server which combines a number of approaches and knowledge bases to estimate outcomes such as LD50, toxicity classes and specific toxicological endpoints (e.g. hepatotoxicity, immunotoxicity, mutagenicity). Early in silico toxicity information to ProTox-II facilitate deprioritization of problematic scaffolds before docking or dynamics (**Banerjee et al., 2018**).

For the structure-based assessment at the protein targets, the pipeline uses AutoDock Tools (preparation of the receptor/ligand and generation of PDBQT) as well as AutoDock Vina for docking and virtual screening. Vina's scoring and efficient multi-threading speed pose prediction while retaining competitive accuracy, while ADT simplifies the setup of the grid, conversion of files, and checking binding hypotheses before execution of simulations (**Morris et al 2009, Trott and Olson 2010**).

Finally, molecular dynamics (MD) simulation using GROMACS is performed to give test of the physical realism and stability of docked complexes under an explicit solvent. GROMACS offers the finest performance and multi-level parallelism from workstations to HPC clusters, allowing the routine simulation of trajectories with microsecond time steps, as well as an efficient analysis of the interaction persistence, conformational drift, and free-energy-associated observables (**Abraham et al., 2015**).

In combination, PASS-guided activity hypotheses, Swiss ADME or admetlab or admet-AI developability screen, ProTox-II safety filters, Vina docking and GROMACS simulation form a coherent and reproducible in-silico workflow for selecting and justifying lead compounds for experimental validation. This integrated approach is designed to blur between breadth and making the top-down mechanistic depth to increase the efficiency and transitional relevance of the candidates moved forward for laboratory testing.

3.2 REVIEW OF LITERATURE

3.2.1 Molecular targets: COX-2 and KEAP1

COX-2 is an inducible isoform of prostaglandin G/H synthase that converts arachidonic acid to prostaglandin H₂ precursor to diverse prostanoids (notably PGE₂) that determine pain, fever, vascular tone, and leukocyte function. COX-2 expression is highly induced by cytokines and pattern-recognition signaling, and in epithelial dysfunction and therapy non-response in enteritis, COX-2-PGE₂ signaling is involved in UC (Rouzer et al., 2020; Li et al., 2018), highlighting its pathological significance. COX-2 cyclooxygenase channel is very similar to COX-1 but is different at critical positions making an auxiliary "side-pocket" and larger effective volume, allowing for coxib selectivity. Classical crystallographic and structural work has identified Val523 (Ile523 in COX-1) and Arg513 (His513 in COX-1) as key predictors of the COX-2-specific pocket situated distal to the Arg120/Tyr355/Glu524 bottleneck, whereas other substitutions (Val434) further expand the channel. These characteristics justify the high selectivity of di-aryl heterocycles in a time-dependent manner. (Kurumbail et al., 1996; Blobaum and Manrnett, 2007; Limnogelli et al., 2010).

Human COX-2 is a popular subject for inhibitor bound structures defining the pocket, confirming docking grids, in particular the bifuran compound 5KIR (rofecoxib/Vioxx; 2.7 Å) and the sulfonamide 5IKR (mefenamic acid). These provide an atomistic location of side-pocket pharmacophore and channel-mouth interactions that can be exploited using your screen (Orlando & Malkowski, 2016a; Orlando & Malkowski, 2016b). Selective COX-2 inhibition is an established approach to inflammation but, withdrawal of rofecoxib following trials (VIGOR/APPROVe), revealed the dose- and duration-related cardiovascular risk associated with this class of drug in the context of novel scaffold development that provides the impetus for careful in-silico triage of novel scaffolds (Sibbald, 2004; Solomon et al., 2005).

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In basal conditions, NRF2 is ubiquitinated and degraded via the proteasome following its identification as a substrate by the CUL3-RING E3 ligase, which is the substrate adaptor Keap1 (KEAP1). Thus, extracellular oxidant and/or electrophilic stress, through modification of several KEAP1 reactive cysteines and/or disrupting KEAP1-NRF2 binding, permits accumulation of NRF2, nuclear import, and expression of ARE-dependent antioxidant/phase-II genes (e.g., HO-1, NQO1, GCLC). In colitis models and clinical tissue studies, activation of NRF2 is protective against mucosal oxidative injury and inhibits pro-inflammatory signaling and epithelial NRF2 loss also exacerbates disease supporting KEAP1/NRF2 as a protective axis in UC **(Crisman et al., 2023; Saha et al., 2020; Peng et al., 2023)**

KEAP1 has an N-terminal BTB (homodimerization/CUL3 binding) domain, an intervening region (IVR) with redox-sensitive cysteine residues, and a C-terminal Kelch (b-propeller) domain that binds two degrons present in the NRF2 Neh2 region, a high-affinity ETGE and a low-affinity DLG motif. Co-crystal structures with ETGE peptides revealed the canonical binding mode and subdivision of the Kelch pocket into P1-P5 sub pockets with the polar hot-spot near Arg415/Arg483/Ser508 with surrounding polar residues (e.g. Ser363, Asn382). These properties form the basis of both peptide and small molecule PPI antagonists **(Lo et al., 2006 and Canning et al., 2015; Yurchenko et al., 2023)**.

In addition to electrophilic NRF2 activators, such as fumarates and triterpenoids, that alter KEAP1 cysteines, a class of expanding non-covalent inhibitors target the Kelch pocket and imitate ETGE interactions. Recent co-structures (e.g., PDB 7OFE and related Kelch-ligand complexes) and fragment-to-lead campaigns map ligand ability across the P4-P5 and polar P1-P2 between them and clarify the residues those must be able to recognize (Arg415/Arg483/Ser508 and hydrophobic anchors like Tyr334/Tyr525/Tyr572) **(Li et al., 2004; Narayanan et al., 2022)**.

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As reviewed across models of inflammatory insult and drug toxicity, NRF2 activation antagonises NF- κ B driven cytokine programmes through redox buffering and direct transcriptional cross-regulation, in the GIT resulting in barrier maintained and diminished cytokine burden. The therapeutic relevance of KEAP1 for UC-like pathology is further supported by its activation via NRF2 by direct KEAP1-NRF2 PPI inhibitors (e.g., CPUY192018) or several natural products which improve the readouts of DSS induced colitis (Lu et al., 2016; Lin et al., 2020; Gao et al., 2022).

Furthermore, the cross-talk between the above-mentioned targets exists within pathophysiological settings: The COX-2 axis is pro-inflammatory in that PGE2 production boosts pro-inflammatory signaling, whereas Nrf2 activation limits oxidative stress and suppresses NF- κ B transcriptional output. Literature in IBD and related epithelial injuries related that the downregulation of COX-2 activity whereas improving NRF2-ARE defense could be complementary to reduce inflammatory mediator production and ROS-induced tissue damage. Natural products (flavonoids, phenolics, terpenoids) are often shown to exhibit this duality directly targeting the active-site residues of COX-2 and/or regulating KEAP1-NRF2 (e.g. greenhouse *Trevesia palmata* flower-bud constituents) providing a rational space for computational screening (Li et al., 2018; Saha et al., 2020; Liu et al., 2021).

3.2.2 Computational approaches of drug discovery

PASS (Prediction of Activity Spectra for Substances) is commonly employed to quickly scan for potential phytochemicals and prioritize screening of those compounds whose predicted probability of activity (P_a) is higher than that of being inactive (P_i): $P_a > 0.5$ -0.7 is often chosen as a practical threshold before further validation of the identified potential candidates. Methodologically, PASS is a structure to activity prediction system which reads structure and derives activity spectra by Multilevel Neighborhoods of Atoms (MNA)-type descriptors, which were trained on large-amount historical SAR sets, and is accessible as a PASS Online server (Lagunin et al., 2000) reporting P_a or P_i for thousands of pharmacological effects or mechanisms. Plants have found Good concordance between Pass outcomes and the experiments: *Pairchym sellvicum* leaf constituents (piperine,

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piperlonguminine, etc.) exhibited P_a is greater than P_i for antibacterial and anthelmintic activities in terms of in vivo & in vitro results (Chy er al., 2019); the metabolites of Sterculia varieties detected by GC-MS using PASS prediction showed antipyretic, anti-inflammatory, thrombolytic, analgesic, and highly cytotoxic actions (Alam et al., 2021); Marak et el. (2023) used Cycas pectinata methanolic extract characterized by LC-MS, FTIR, NMR yielded thirty two phytochemicals with PASS tool guided antioxidant, antineoplastic, steroid modulator and CYP related activities (Marak et al., 2023).

Molecular docking has been extensively used in the priority of phytochemicals as COX-2 modulators. Most of the studies on the COX-2 channel related to flavonoids have identified the key contacts of the COX-2 channel entrance and the selectivity side pocket (Arg120, Tyr355, Tyr385, Val523) of the COX-2 channel as predicted by the cocrystal structure, for example, the binding mode of COX-2 selectivity identified by the docking studies of natural flavones or flavanols frequently recovered H-bonds to Tyr385/Tyr355 and hydrophobic space near Val349/Val523, constituting the COX- The quest to discover alternatives to the classical NSAIDs or coxibs is still alive in recent in vitro research, in silico screening for COX-2 coupled with ADMET triage for botanicals (Tendulkar and Mahajan, 2014; Levita et al., 2017; Abdollahi et al., 2025; Reheim et al., 2025).

In case of KEAP1, modern screens are against the Kelch domain pockets (P4/P5) that bind to the ETGE/DLG motifs on Nrf2 to interrupt the PPI/stabilize Nrf2. Multiple reviews/primary screen Natural Product Chemotypes (Alkaloids, Phenolics, Terpenoids) Association for the Arginine-rich Hot spots in the Kelch groove: Recent in silico campaigns using Glide/other gave virtual screening of natural product libraries against KEAP1, Tractable binders for follow-up. Methodologically these studies put the use of Kelch structures at high-resolution and filters that are site-specific of ETGE first; similar to your study (Culletta et al., 2024; Brogi et al., 2023; Huang et al., 2025)

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Recent plant-centric computational ones, mainly computationally do printed libraries were combined with LC/GC-MS-guiding, COX-2 (sometimes dual COX1/COX2) docking, KEAP1- Kelch screening and ADME-theory screening to triage extract constituents before dry-lab testing occurs. Examples of these are screens of natural products covering COX-2 specific docking as well as pharmacokinetics and screens of natural compounds and approved drugs to find Nrf2 Stabilizers, but new studies also bring together docking with MD to strengthen stability. These trends are in great agreement with PASS-ADMET-COX-2/KEAP1 docking-MD workflow (Aliawobaei et al., 2025; Mathe and Karlapudi, 2025; Brogi et al., 2023).

State of the science reviews position Nrf2 protective in UC, of reduction of oxidative injury and readouts of inflammation, offers mechanistic rationale for pairing COX-2 suppression (decreased prostaglandin drive) with Nrf2 activation (cytoprotection). (Peng et al., 2023).

Nowadays, many papers on the discovery of COX-2 are prolonging the docking using 100-ns long MD and MM/PB(GB)SA, pose stability (RMSD/RMSF), stable H-bonding with gate/side pocket residues (e.g. Arg120, Tyr355, Tyr385, Val523), and favorable binding free energies. For instance, Leao et al. (rofecoxib analogs; PDB 5KIR) and MD-MM-PBSA in combination docking has also been employed to cover the structure activity around the COX-2 channel or side-pocket, a workflow that is directly comparable to yours. More recent studies have also made measurements of MD of COX-2 inhibitor complexes (100ns) to validate dynamic compatibility of hits generated by docking/pharmacophore screens (Leao et al., 2020; Moussa et al., 2021; Bhattacharjee et al., 2025).

Furthermore, for KEAP1, MD is typically used for validation of the docking poses in the Kelch ETGE/DLG binding groove, and to explore the P4/P5 sub pocket engagement resulting in Keap1-Nrf2 PPI disruption. MD and umbrella sampling by Londhe et al. was used to dissect the atomistic determinants of small-molecule Keap1 binders, which highlighted the Arg rich hot-spots in the groove (Londhe et al., 2019); more recent natural-product SBDD screens also go for both docking -MD for

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stabilized P4/P5 pharmacophores (**Alzain et al., 2023**). The molecular simulation and experimental evidence of Kyoto Encyclopedia of Genes and Genomes (KEG) which are observed for KEAP1-Nrf2 interaction and is supported by CPUY192018 a Keap1-Nrf2 noncovalent protein-protein interaction (PPI) inhibitor which activates Nrf2 and defends against DSS in colon cells and mice (**Lu et al., 2016**).

Contemporary natural product screens routinely combine such docking with in-silico ADMET to help de-risk absorption, distribution, CYP liabilities and toxicity before performing MD or wet lab work. Recent in-silico anti-inflammatory research has shown preference and progression for phytochemicals (flavonoids, terpenoids) which fulfill the criteria of acceptable ADMET flags in comparison to standardization (e.g. diclofenac/rofecoxib) and your approach is follow due to these possibilities (**Chibuye et al., 2024**). Most recent phytochemicals use SwissADME/BOILED-Egg and ADMET-AI in the screening of oral absorption, bioavailability, metabolism & clearance, and ProTox-II in the prediction of acute oral toxicity (LD50 class I-VI), hERG, DILI, mutagenicity & anticancer potential to predict the lead selection before docking/MD (**Wahnou et al., 2024**).

The reason is converging in the literature as because a dual axis of rationale: one is enzymatic target, COX-2 is a validated show-holder whose inducible enzymatic channel (Tyr355/Arg120 gate) COX-2-specific side pocket (Arg120/Tyr355 gate; His90/Arg513/Gln192 region) makes it one of selective inhibition of prostaglandin signaling and the other (KEAP1-NRF2) is a tractable Kelch-domain PPI target wherein ETGE/DLG binding (P1/P5 sub pockets; Arg415/Arg483/ Recently The key gap is the evaluation of one integrated approach of using transparent site filters and ADMET gatekeeping of exactly what your study is contributing on plant parts/design single (e.g. flower buds) target.

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3.3 MATERIAL AND METHODS

3.3.1 *Trevesia palmata* phyto-ligands

The phyto-ligands used for this computational work were obtained from the methanolic extract of flower buds of *Trevesia palmata*. A total of 21 phyto-compounds were identified and characterized by liquid chromatography / gas chromatography - mass spectrometry (LC/GC - MS) from the crude methanolic extract. Anti-inflammatory drug sulfasalazine approved by FDA was used as standard ligand. The identified phyto-compounds and standard drug, 2D structure (**Figure 3.1**) and canonical simplified molecular input line entry system (SMILES) strings were retrieved by using PubChem database under the National Center for Biotechnology Information (NCBI) in .sdf (structure data file) file format (<https://pubchem.ncbi.nlm.nih.gov/>). The list of all phyto-compounds with PubChem CID, molecular formula, canonical SMILES and biological activity from the published literature is given in **Table 3.1**.

3.3.2 Structure retrieval of target protein

The 3D crystal structures of the target proteins resolved by X-ray diffraction, COX-2 (PDB ID: 5KIR) and Keap1 (PDB ID: 7OFE), with resolution 2.70 Å and 1.19 Å respectively (Figure 12) were retrieved from a database that includes experimentally-determined 3D structures of biological macromolecules including proteins and nucleic acids, the RCSB Protein Data Bank (PDB) (<https://www.rcsb.org/>). The Protein human cyclooxygenase-2 (COX – 2) was having two identical chains (A, B) of 551 sequence length with no mutation similarly kelch domain Keap1 protein have single chain (A) of 291 with no mutation in structure (**Figure 3.3**)

3.3.3 PASS prediction

PASS was used for assessment of prospective anti-inflammatory and anti-ulcerative activity of *T. palmata* phyto-compounds and sulfasalazine, anti-inflammatory standard drug. In order to predict activities in biology, the retrieved

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canonical SMILES strings were used as input to the PASS online server (<https://way2drug.com/PassOnline/>), for predicting biological activities. The PASS server predicts two conditions of probabilities, activity probability (Pa) and inactivity probability (Pi). The probability values vary between 0 and 1. The probability of activities (Pa) values that were higher than the corresponding probability of activities (Pi) values were calculated as potential biological effects for compounds. Pa predictions with Pa greater than or equal to 0.7 were taken as indicating a high probability of experimental confirmation for the respective activities. Values between $0.5 \leq Pa < 0.7$ were considered to decrease the probability of biological activity. On the other hand, values of $Pa < 0.5$, indicate as low probability of experimental detection or may also signify the possibility of identification of novel or unreported biological properties for that particular compound. This tool was served as for initial computational screening for compounds to guide the downstream computational workflow (Pravin et al., 2025).

3.3.4 Physiochemical properties of phyto-ligands

The physiochemical properties of phyto-ligands from *T. palmata* were assessed using the SwissADME web server, which is operated by the Swiss Institute of Bioinformatics (SIB), based in Lausanne, Switzerland (<https://www.swissadme.ch/>), with the tool's default settings applied. Multiple descriptors were measured for the physiochemical properties of each phyto-ligand, including molecular weight (MW), number of heavy (nHA) atoms, number of aromatic heavy atoms (nAHA), number of rotatable bonds (nROT), H-bond acceptors and donors (HBA and HBD), fraction of sp³-hybridized carbons (Fraction Csp³), molar refractivity (MR), topological polar surface area (TPSA), consensus Log Po/w (calculated as the arithmetic mean of IlogP, XLOGP3, WLOGP, MLOGP, and SILICOS-IT values (Daina et al., 2017), and aqueous solubility ESOL logS (Delaney, 2004; Daina et al., 2017). These properties were used to characterize the Lipinski rule of five along with other drug likeness criteria.

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3.3.5 Drug likeness and medicinal chemistry

By conducting a physiochemical property assessment in a single batch session, SwissADME exported drug-likeness and medicinal-chemistry values; the web server evaluated several rule sets—including Lipinski rule (**Lipinski et al., 1997**), Ghose rule (**Ghose et al., 1999**), Veber rule (**Veber et al., 2002**), Egan rule (**Egan et al., 2000**), and Muegge rule (**Muegge et al., 2001**) using YES/NO decisions, violation thresholds, and the Martin bioavailability score (**Martin, 2005**); it also flagged PAINS alerts (Pan Assay Interference Compounds or frequent hitters or promiscuous compounds) (**Baell and Holloway, 2010**), Brenk alerts (**Brenk et al., 2008**), and assessed lead-likeness (**Daina et al., 2017**).

In this study, we applied a drug-likeness ruleset—particularly the Lipinski rule to filter phyto-ligands before the computationally intensive molecular-docking stage (**Sarmah et al., 2024**). This stepwise filtering strategy concentrates structure-based investigations on phyto-ligands that are more likely to exhibit favorable oral bioavailability and drug-likeness properties. Moreover, we noted medicinal-chemistry features such as reactive groups, drug-like scaffolds, and common substructures found in bioactive molecules. By applying these filters, the compounds become pre-docking drug candidates, allowing us to focus on phyto-compounds with a higher chance of clinical validation.

3.3.6 Active site of target protein

The distinguishing and depiction of binding pockets as well as active site in a protein structure is a pivotal step in a molecular docking study (**Le Guilloux et al., 2009**). The targeted protein of human cyclooxygenase-2 (COX-2) complex bound with specific inhibitor rofecoxib (RCX) (**Orlando and Malkowski et al., 2016**) and, Keap1 bound to inhibitor 2-[(9-oxidanylidene)fluoren-4-yl] carbonylamino] ethanoic acid was used to selected active site for molecular docking (**Narayanan et al., 2022**) (Figure 12).

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For COX-2 active site in which RCX anchored the binding cavity was comprises of the binding pocket of catalytic and selective residues such as His90, Arg120, Try355, Arg513, Val523, Gln192, Ser530 and Met522 at as per reported by Suleman et al. (2025). These amino acids residues were applied to select center and size of docking grid parameters, so that all important residues interaction within the RCX binding area were covered (Suleman et al., 2025). Furthermore, For Keap1, the ligand-binding region was selected from the inhibitor bound Kelch domain part. The co-crystallized ligand 2-[(9-oxidanylidene fluorene-4-yl) carbonylamino] ethanoic acid occupies the ETGE-recognition cavity of Kelch β - propeller, which further subdivided into five sub-pockets such as P1-P5 and this pockets regions cover positively charged and polar amino acids residue such as Arg415, Arg483, Arg485, Ser508, Ser555, Tyr334 and Tyr525 which make the canonical binding grooves including the ETGE motif of Nrf2 and are frequently targeted by small-molecule Keap1 inhibitors (Londhe et al., 2019; Cores et al., 2020) (Figure 12).

3.3.7 Molecular docking

The phyto-ligands that have successfully passed drug likeness criteria were chosen for molecular docking with the targeted proteins. The docking was performed by using command-based software AutoDock Vina (v1.1.2) to better efficiency of scoring as well as algorithm in ligand-protein docking work (Trott and Olson, 2010).

3.3.7.1 Ligand preparation

All retrieved canonical SMILES strings of phyto-ligand from PubChem were further used for drawing 2D (Figure 3.1) and 3D (Figure 3.2) by the use by using chemical drawing software ChemDraw (Version 22.0.0). Before making 2D to 3D all structure were cleaned for adjust standard bond length and angles. Then further all phyto-ligand converted into 3D structure were geometry, energy minimized by employing the MMFF94 (Merck Molecular Force Field 1994) with a maximum steepest descent of 2000 steps and RMS (root mean square) gradient 0.010 kcal/mol Å and checked the rotatable bonds then saved as PDB format (Halgren, 1996). After

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that the minimized structured were converted PDB to PDBQT format, which involves employing polar hydrogen, partial charges and fixing torsion degree of freedom as well as root. To performed this AutoDockTools (ADT) from MGL Tool 1.5.6 was utilize (Morris et al., 2008; Halder et al., 2023).

3.3.7.2 Protein preparation

After retrieval of the preferred crystal protein structure of COX-2 and Keap1 the structure was visualized as well as filtered by removing water molecules, all non-protein, co-crystal ligands, and metal ions in BIOVIA Discovery Studio (v21.1.0.20298) (<https://discover.3ds.com/discovery-studio-visualizer-download>) (Biovia 2015) to isolate protein. The isolating protein structure PDB file converted in to PDBQT format, which involves checking missing atoms as well as adding polar hydrogen, partial charges such as Kollman charges was applied for consequential docking study.

3.3.7.3 Grid box preparation

For grid box preparation the binding site was selected based on there co-crystal native ligand inhibitor of the protein respectively. Based on their inhibitor, for COX-2 receptor, attributes of grid box size, the center dimensions 22 x 22 x 22 Å was created and centered at co-ordinates (x = 23.206, y = 1.318, z = 34.259 Å) and for Keap1 receptor, grid box size 22 x 22 x 22 Å and center co-ordinates (x = -43.0999, y = 20.7678, z = -9.8692 Å) with 0.375Å spacing. After the box preparation, the docking runs at exhaustiveness of 16 with maximum of 20 binding modes and an energy range of 4 kcal/mol. This are parameters information were used to make configuration file.

3.3.7.4 Binding affinity and interaction

Following grid box generation, docking simulations were performed in AutoDock Vina. For each ligand, up to 200 binding poses were produced and subsequently grouped into clusters using a root mean square deviation (RMSD) cutoff of 1.0 Å, in accordance with Nisa et al. (2023). The clusters were then ordered

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based on their predicted binding energies, with the most negative value taken to indicate the most stable protein–ligand complex. The numerical docking outputs were saved in text format for further processing.

For each ligand, the pose exhibiting the lowest binding affinity (kcal/mol) was selected as the representative binding conformation and used for detailed interaction analysis. The corresponding protein–ligand contacts, including hydrogen bonding, hydrophobic interactions, and π –cation interactions, were analyzed and visualized using BIOVIA Discovery Studio Visualizer and PyMOL (Schrödinger and DeLano, 2020).

3.3.8 Molecular dynamics simulations

The results of best docking pose of molecular docking studies were further carried out for molecular dynamics (MD) simulation by using GRONingen Machine for Chemical Simulations (GROMACS) version 2024 was applied to check the dynamic behavior, stability of complexes as well as changes of conformation of the protein-ligand complexes over time (Abraham et al., 2015; Berendsen et al., 1995).

Whole system was energy minimized by utilized the CHARMM27 all-atom force field (Bjellmar et al., 2010). In process of simulation, protein-ligand complex was placed with a triclinic (dodecahedral) where simulation of box was maintaining 1.0 nm distance from the edges of the box and three-point water model (TIP3P) was utilized. To achieve system neutralization, sodium ion (Na^+) and Chlorine (Cl^-) ions were added (Sharma et al., 2020).

Furthermore, the entire system underwent energy minimization and equilibration prior to the production molecular dynamics simulation. Energy minimization of the prepared complex was performed for up to 50,000 steps using the steepest descent algorithm, until the maximum force was reduced below 1000 kJ/mol/nm, to eliminate steric clashes (Singh et al., 2024). Under periodic boundary conditions, a two-step equilibration protocol was then applied. First, an NVT (constant number of particles, volume, and temperature) ensemble equilibration of 100 ps at 300 K was conducted using a modified Berendsen (V-rescale) thermostat

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with a coupling constant of 0.1 ps, during which the heavy atoms of both proteins were position-restrained (Berendsen et al., 1984). This was followed by an NPT (constant number of particles, pressure, and temperature) ensemble equilibration of 100 ps at 1 atm using the Parrinello–Rahman barostat with a coupling constant of 2.0 ps to achieve pressure and thermal stability (Parrinello and Rahman, 1981). Long-range electrostatic interactions were treated using the Particle Mesh Ewald (PME) method, and all bond lengths were constrained employing the LINear Constraint Solver (LINCS) algorithm (Hess et al., 1998; Sharma et al., 2020).

Production MD simulations were subsequently performed for 100 ns with a 2 fs time step under NPT conditions. The resulting trajectories were analyzed using standard GROMACS analysis tools and in-house scripts to characterize the structural dynamics. Specifically, root mean square deviation (RMSD; *gmx rms*) was calculated to evaluate global conformational stability, root mean square fluctuation (RMSF; *gmx rmsf*) to determine residue-level flexibility, radius of gyration (Rg; *gmx gyrate*) to assess overall structural compactness, solvent-accessible surface area (SASA; *gmx sasa*) and hydrogen bonds (H-bond; *gmx hbond*) to examine solvent exposure. Simulation data were further processed and visualized using Microsoft Excel spreadsheet software (<https://www.microsoft.com/en-in/microsoft-365/>), and the resulting plots were exported in .tif format for inclusion in the results section.

3.3.9 ADME and toxicity prediction

Following molecular docking and MD simulation to characterize the pharmacokinetic and safety profile of phytocompounds of the crude methanolic extract from *T. palmata* flower buds, comprehensive prediction of ADME and toxicity analysis were performed. These properties were evaluated for all 20 identified compounds as well as 1 reference drug sulfasalazine. ADME prediction comprises of absorption, distribution, metabolism and excretion which is predicted by integrated of multiple online ADME server which are SwissADME (<https://www.swissadme.ch/>) (accessed, on 20 September 2025) and ADMET-AI (<https://admet.ai.greenstonebio.com/>) (accessed on 25 October 2025) (Wahnou et al., 2024)

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Initially, the phytochemicals were filtered out by drug-likeness criteria but ADMET profile was done to estimation of pharmacokinetics across the whole chemical space, revealing of potential compounds that have been showed violation under Lipinski rule of five and other drug likeness criteria. There are number of drugs violate one or more Lipinski parameters, especially natural bioactive products absorbed by mechanism of active transport in to body system. The ADME properties showed the permeability into cell plasma membrane, affinity of transporter as well as role of enzyme in absorption and elimination of drug and also stability of metabolic pathways

Moreover, the toxicity prediction for safety of drug molecule was evaluated by using ADMET-AI (<https://admet.ai.greenstonebio.com/>) (accessed on 28 October 2025) and Pro-Tox-3.0 (<https://tox.charite.de/protox3/>) (accessed on 29 October 2025). ADMET-AI platforms worked as comparing query compounds with a set off 2,579 reference molecules which is incorporated with ADMET-AI machine learning algorithm.

3.4 RESULT

3.4.1 PASS-Predicted Anti-inflammatory and Anti-ulcerative Activities of *Trevesia palmata* Phytocompounds

PASS analysis of the selected phytochemicals from *T. palmata* (flower buds) revealed that several constituents exhibited promising probabilities for anti-inflammatory and anti-ulcerative activities. Compounds with $P_a > P_i$ were considered potentially active, while $P_a > 0.70$ was regarded as highly predictive of experimental activity, and $0.5 < P_a < 0.7$ as moderately predictive.

For anti-inflammatory activity, the highest predicted potential was observed for zeta-Carotene ($P_a = 0.754$), followed by Oleic acid ($P_a = 0.614$) and Eicosanoic acid, tetradecyl ester ($P_a = 0.641$), all of which showed P_a – P_i differences supportive of strong activity. Moderate activity (P_a between 0.5–0.7) was predicted for several compounds including Palmitic acid ($P_a = 0.515$), Henetracontanoic acid ($P_a = 0.515$), Apigenin-6-C-glucoside ($P_a = 0.606$), Apigenin-6-C-arabinoside ($P_a =$

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0.675), Kaempferol-3-O-rutinoside ($P_a = 0.743$), Luteolin-6-C-glucoside ($P_a = 0.496$) and Kaempferol ($P_a = 0.530$). Flavonoids generally exhibited favorable P_a - P_i differences, indicating a consistent anti-inflammatory potential. Regarding anti-ulcerative activity, several compounds demonstrated high predictive values, particularly zeta-Carotene ($P_a = 0.688$) and Oleic acid ($P_a = 0.567$). Other notable compounds with moderate predicted activity included Palmitic acid ($P_a = 0.525$), 1-Octadecanol ($P_a = 0.476$), Henetracontanoic acid ($P_a = 0.525$) and Apigenin-6-C-arabinoside ($P_a = 0.724$). Flavonoids such as Quercetin-3-O-galactoside ($P_a = 0.537$) and Isorhamnetin ($P_a = 0.533$) also showed promising anti-ulcerative potential.

Overall, PASS predictions suggest that carotenoids (zeta-Carotene), fatty acids (Oleic acid, Eicosanoic derivatives), and flavonoids (Apigenin, Kaempferol, Quercetin derivatives) are among the most promising candidates for anti-inflammatory and anti-ulcerative properties in *T. palmata*. Compared to the reference drug Sulfasalazine, which showed lower activity probabilities ($P_a = 0.404$ for anti-inflammatory and $P_a = 0.451$ for anti-ulcerative), several natural compounds exhibited stronger predicted activities, highlighting their therapeutic potential (**Table 3.2**)

3.4.2 Physicochemical Properties of *Trevesia palmata* Phytochemicals

The physicochemical profiling of the *T. palmata* phytochemicals using SwissADME revealed notable variability in molecular weight, polarity, flexibility, and lipophilicity, all of which influence their potential as drug-like molecules. Most compounds exhibited molecular weights within or slightly above the acceptable range for oral drug candidates (≤ 500 g/mol), with simpler lipophilic molecules such as palmitic acid, oleic acid, stigmasterol, isorhamnetin, and kaempferol falling well within this limit, whereas several glycosylated flavonoids (e.g., rutin derivatives, vicianin-2, luteolin-6-C-glucoside) and procyanidin C1 exceeded 600–800 g/mol, suggesting limited oral bioavailability. The number of hydrogen bond acceptors (HBA) and donors (HBD) varied widely, with aglycone flavonoids such as isorhamnetin (HBA: 7; HBD: 4) and kaempferol (HBA: 6; HBD: 4) showing favorable profiles, while glycosylated flavonoids presented substantially higher

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values (HBA 10–18; HBD 7–15), indicating increased polarity and reduced permeability.

Topological polar surface area (TPSA) also showed considerable differences among the compounds. Lipophilic terpenoids (stigmasterol, eicosanoic acid derivatives) exhibited low TPSA (20–40 Å²), which is conducive to membrane permeability, whereas glycosylated flavonoids showed very high TPSA values (200–330 Å²), exceeding the ideal threshold (<140 Å²) for effective intestinal absorption. The number of rotatable bonds (nROT) ranged from 0 to 39, with more flexible long-chain fatty acids and ester derivatives showing high rotatability, while flavonoids such as isorhamnetin and kaempferol remained structurally rigid with fewer than seven rotatable bonds, supporting stronger, more stable target interactions. Lipophilicity (Consensus LogP) values indicated that sterols and fatty acid derivatives were highly lipophilic (LogP 5–14), which may hinder solubility but enhance membrane affinity, whereas flavonoids displayed moderate LogP values between –2.3 and 1.65, reflecting balanced hydrophilic–lipophilic characteristics. Water solubility (LogS ESOL) ranged widely, with many terpenoids and long-chain fatty acids showing poor solubility (LogS –5.0 to –14.0), while certain flavonoids demonstrated comparatively higher solubility, with values between –1.9 and –3.4. Notably, both isorhamnetin (LogS –3.36) and kaempferol (–3.31) showed moderate solubility favorable for drug development (**Table 3.3**)

3.4.3 Druglikeness of *Trevesia palmata* phytochemicals

The Swiss-ADME evaluation of the *T. palmata* phytochemicals revealed substantial variability in their drug-likeness and predicted oral bioavailability, reflecting the structural diversity of the metabolites. Fatty acids such as palmitic acid, oleic acid, and 1-octadecanol displayed favorable drug-likeness profiles with minimal violations across Lipinski, Ghose, Veber, Egan, and Muegge criteria, and correspondingly high bioavailability scores (0.55–0.85), indicating strong potential for oral absorption. In contrast, larger and more structurally complex flavonoid glycosides—including luteolin-C-glucoside derivatives, vicenin-2, procyanidin B1/C1, and various glycosylated apigenin and quercetin forms—showed multiple

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violations across all drug-likeness rules, largely due to high molecular weight, elevated topological polar surface area, and excessive hydrogen-bonding capacity. These features were reflected in their uniformly low bioavailability scores (0.17), suggesting limited suitability for oral drug development without structural modification. Aglycone flavonoids such as isorhamnetin and kaempferol performed markedly better, exhibiting no violations across all rules and moderate bioavailability scores (0.55), aligning them more closely with the reference drug sulfasalazine (bioavailability 0.56). Collectively, the findings indicate that while several *T. palmata* phytochemicals possess physicochemical attributes compatible with oral drug candidates, others—particularly the glycosylated polyphenols—may require formulation strategies or derivatization to overcome poor ADME characteristics (Table 3.4)

3.4.4 Medicinal chemistry of *Trevesia palmata* phytochemicals

The medicinal chemistry profiling of *T. palmata* phytochemicals using SwissADME revealed notable differences in PAINS alerts, Brenk structural alerts, lead-likeness, and synthetic accessibility among the evaluated metabolites. Most compounds demonstrated clean PAINS profiles, with the exception of several polyphenolic constituents—specifically luteolin-6-C-glucoside, procyanidins B1 and C1, quercetin-3-O-galactoside, and apigenin-6-C-glucoside—which all triggered catechol-type alerts, consistent with the known reactivity of catechol motifs. Brenk alerts were minimal across the dataset; only oleic acid and stigmasterol displayed isolated alkene alerts, and the reference drug sulfasalazine exhibited a diazo group alert, reflecting its established structural liability. Lead-likeness assessment showed that most simple fatty acids and sterols failed to comply due to violations in physicochemical constraints, whereas some flavonoid derivatives, including isorhamnetin and kaempferol, satisfied lead-likeness criteria with only minor deviations. Synthetic accessibility scores ranged from 2.31 to 6.95, indicating substantial variability in the ease of chemical synthesis. Simple lipid-based molecules (e.g., palmitic acid, oleic acid) exhibited low accessibility scores, denoting ease of synthesis, while complex glycosylated flavonoids and procyanidins presented

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higher scores (>6.0), signifying considerable synthetic difficulty. These medicinal chemistry parameters collectively highlight that, although many *T. palmata* constituents are structurally complex and present potential reactivity alerts, several possess favorable chemical tractability and may serve as promising leads following structural optimization (**Table 3.5**).

3.4.5 Molecular docking and interaction profiling of *T. palmata* bioactive compounds

3.4.5.1 Interaction profile of COX-2–ligand complexes

Docking simulations revealed that the three lead compounds i.e isorhamnetin, kaempferol, and sulfasalazine interact with the COX-2 catalytic domain through a combination of hydrogen bonding, hydrophobic contacts, and van der Waals stabilisation. The interaction density and residue specificity varied substantially between the flavonoids and the reference drug.

Among the compounds, isorhamnetin demonstrated the strongest and most cohesive interaction pattern among the tested ligands. It formed hydrogen bonds with HIS90, TYR355, PHE518, GLN192, and MET522, complemented by extensive hydrophobic contacts with VAL349, VAL523, LEU352, TRP387, and LEU384. These residues lie within, or immediately adjacent to, the COX-2 active cleft, indicating a high degree of pocket complementarity. The interaction map showed a dense network of van der Waals contributions surrounding the flavonoid scaffold, suggesting stable ligand anchoring and strong predicted affinity. Kaempferol displayed a similar but slightly reduced interaction footprint compared with isorhamnetin. Key hydrogen bonds were formed with HIS90, TYR355, PHE518, and GLN192, accompanied by hydrophobic contacts involving VAL523, LEU352, TRP387, and VAL349. Although the overall binding orientation paralleled that of isorhamnetin, the reduced interaction density indicates moderately lower COX-2 affinity.

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The reference drug sulfasalazine exhibited fewer and more dispersed interactions. Hydrogen bonding mainly involved SER119, SER530, and TYR355, with hydrophobic contacts from LEU93, VAL116, VAL349, LEU531, and VAL523. While the ligand occupied the catalytic region, its alignment and interaction richness were weaker relative to the flavonoids, suggesting reduced stabilisation within the COX-2 pocket (**Figure 3.4, Table 3.10 and Table 3.12**)

3.4.5.2 Interaction profile of KEAP1–ligand complexes

All ligands bound to the KEAP1 Kelch domain, which mediates Nrf2 recognition and degradation. However, only the flavonoids showed strong interactions with the canonical Nrf2-binding groove, indicating their potential to interfere with KEAP1-mediated repression. Isorhamnetin exhibited a robust interaction network involving key residues required for Nrf2 engagement. Hydrogen bonds and stabilising contacts were observed with ARG483, ARG415, GLN530, SER555, SER363, and TYR525. These residues cluster around the Nrf2-binding interface, demonstrating the ligand's high structural compatibility with the Kelch β -propeller pocket. The radial interaction diagram revealed a compact, well-stabilised binding pose, indicating strong potential for disrupting KEAP1–Nrf2 association. Kaempferol showed a similar binding orientation, interacting with ARG483, ARG415, SER555, GLN528, and ALA556, along with π -alkyl and van der Waals contributions from adjacent Kelch residues. Although the interaction density was slightly lower than that of isorhamnetin, kaempferol still demonstrated substantial affinity toward the Nrf2-binding hotspot.

Sulfasalazine established moderate interactions within the Kelch pocket, primarily through SER508, ARG483, ARG415, and hydrophobic contacts with TYR525 and PHE478. The reduced number and distribution of stabilising interactions indicate comparatively weaker binding and limited capacity to compete with Nrf2 for KEAP1 recognition (**Figure 3.5, Table 3.11 and Table 3.12**).

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3.4.7 Molecular dynamics simulation analysis

Molecular dynamics (MD) simulations were conducted for 100 ns to evaluate the structural stability, conformational flexibility, and dynamic behavior of the top-ranking *T. palmata* bioactive compounds isorhamnetin and kaempferol in complex with COX-2 and KEAP1, in comparison with the reference drug sulfasalazine. The RMSD and RMSF trajectories generated during the simulations provided insights into the time-dependent stability of each complex and the residue-level motions influencing ligand accommodation within the binding pockets.

3.4.7.1 RMSD analysis of COX-2 and KEAP1 complexes

The RMSD analysis of both COX-2 and KEAP1 complexes revealed consistently stable binding behavior for all three ligands (isorhamnetin, kaempferol, and sulfasalazine) throughout the 100 ns simulation, despite minor variations in their dynamic profiles. For the COX-2 complexes, all systems underwent an initial equilibration phase within the first 10–15 ns before stabilizing for the remainder of the trajectory. Among them, the COX-2–isorhamnetin complex demonstrated the highest stability, maintaining RMSD values between 0.20 and 0.28 nm, reflecting a firmly anchored ligand and minimal structural deviation. The COX-2–kaempferol system also achieved equilibrium but showed slightly broader fluctuations, reaching up to ~0.30 nm, indicative of moderate conformational adjustments at the binding interface. Sulfasalazine exhibited RMSD values ranging from 0.15 to 0.27 nm, suggesting a stable yet somewhat more dynamic interaction. In parallel, the KEAP1 complexes stabilized even earlier, within the first 8 ns, and displayed lower overall RMSD amplitudes. The KEAP1–isorhamnetin complex again showed the greatest structural stability, fluctuating only between 0.06 and 0.11 nm, whereas kaempferol induced slightly higher variability (0.07–0.13 nm) and sulfasalazine demonstrated the most dynamic motion among the three (0.08–0.14 nm). Collectively, the RMSD results indicate that isorhamnetin forms the most stable complexes with both COX-2 and KEAP1, corroborating the strong binding affinities predicted from molecular docking (**Figure 3.6**)

3.4.7.2 RMSF Analysis of COX-2 and KEAP1 Residues

The RMSF analysis of both COX-2 and KEAP1 residues revealed that ligand binding produced distinct yet consistently stabilizing effects across the two protein systems, with isorhamnetin exhibiting the strongest influence on residue-level mobility in both cases. For COX-2, the RMSF profiles showed that the N-terminal segments and flexible loop regions displayed comparatively higher fluctuations, reflecting their inherent structural mobility and minimal involvement in ligand binding. In contrast, residues within and surrounding the catalytic pocket remained highly stabilized, with RMSF values generally below 0.15 nm. Among the three ligands, isorhamnetin induced the lowest fluctuations in key active-site residues such as HIS90, TYR355, and PHE518, indicating tighter structural packing and enhanced rigidity in the binding environment. Kaempferol produced moderate stabilization, while sulfasalazine allowed slightly greater mobility within the active-site region. Similarly, the KEAP1 RMSF patterns demonstrated uniformly low flexibility across residues 300–650 of the Kelch domain, which mediates Nrf2 binding. The KEAP1–isorhamnetin complex again showed the smallest fluctuation amplitudes—typically below 0.10 nm—indicating highly stable interactions within the ligand-binding pocket. Kaempferol caused marginally higher fluctuations, particularly in certain loop segments where RMSF values reached 0.12–0.15 nm, while sulfasalazine showed slightly elevated motions, especially around Nrf2-associated residues such as ARG483 and SER508. Overall, the combined RMSF analysis clearly demonstrates that isorhamnetin most effectively stabilizes critical functional regions of both COX-2 and KEAP1, supporting its strong binding affinity and superior structural compatibility relative to kaempferol and sulfasalazine (**Figure 3.6**)

3.4.7.3 Structural Compactness, Solvent Exposure, and Interaction Profile Analysis of Ligand–Protein Complexes

The R_g profiles of the COX-2 complexes showed stable compactness throughout the 100 ns simulation (Figure A). All three ligands—isorhamnetin, kaempferol, and sulfasalazine—maintained R_g values within a narrow range of 2.38–2.48 nm, indicating that ligand binding did not induce major structural fluctuations in the overall protein fold. Among the complexes, COX-2–isorhamnetin displayed the lowest variation in R_g , suggesting relatively stable protein compactness during the simulation. A similar pattern was observed for the KEAP1 complexes (Figure B), where R_g values remained consistent between 1.69 and 1.76 nm, indicating that none of the ligands caused extensive structural expansion or contraction of the Kelch domain. The KEAP1–isorhamnetin complex exhibited the smallest fluctuations in R_g , demonstrating greater structural stability (**Figure 3.7**)

SASA values remained stable throughout the simulation for all COX-2 complexes (**Figure 3.7**), displaying values between 292 and 312 nm². Minor fluctuations were noted but did not indicate any significant expansion or collapse of the protein surface. The COX-2–isorhamnetin complex exhibited slightly lower SASA values compared to kaempferol and sulfasalazine, reflecting less solvent exposure. For KEAP1 (**Figure 3.7**), all ligand complexes maintained SASA values ranging from 136 to 148 nm², with no major deviations over the 100 ns trajectory. As observed in COX-2, the isorhamnetin complex consistently exhibited slightly reduced SASA values relative to the other ligands. The number of hydrogen bonds formed between COX-2 and the ligands varied throughout the simulation (**Figure 3.7**), with fluctuations ranging from 0 to 6 bonds. Isorhamnetin maintained a higher frequency of hydrogen-bonding events compared to kaempferol and sulfasalazine, indicating more frequent intermolecular contacts. In the KEAP1 complexes (**Figure 3.7**), hydrogen-bond formation ranged from 0 to 7, with isorhamnetin again showing recurrent hydrogen-bond interactions throughout the trajectory. Kaempferol and sulfasalazine exhibited more intermittent bonding patterns, reflecting variations in ligand–protein contact stability.

3.4.7.4 Time-Resolved Structural Evolution of Protein–Ligand Complexes (COX-2 and KEAP1)

The time-resolved structural snapshots of both COX-2 and KEAP1 complexes across the 20–100 ns simulation window revealed distinct yet consistent stabilization patterns that reflect the differential binding behaviors of isorhamnetin, kaempferol, and sulfasalazine. In all complexes, the ligands remained bound throughout the simulation; however, the degree of positional mobility and protein conformational accommodation varied notably between the phytochemicals and the reference drug. Isorhamnetin consistently demonstrated the most stable interaction profile in both COX-2 and KEAP1, maintaining a fixed orientation within the respective binding cavities with minimal displacement over time. The surrounding secondary structural elements of both proteins exhibited only subtle adjustments in its presence, indicating strong anchoring of the ligand and reduced dynamic perturbation of the binding site. Kaempferol, while also stably retained in both proteins, showed moderate internal reorientation during intermediate simulation stages, reflected in slight rotation of its aromatic rings and localized movements in adjacent loops, suggesting a dynamic yet persistent binding mode. Sulfasalazine, by contrast, displayed the highest degree of conformational flexibility, particularly evident through its shifting poses at 40–80 ns in COX-2 and more pronounced ligand repositioning in KEAP1 during the later simulation stages. These movements were accompanied by mild expansions or fluctuations in nearby loop regions, indicative of greater structural adaptability required to accommodate its bulkier and more flexible scaffold. Despite this increased motion, sulfasalazine remained within the binding pockets of both proteins throughout the simulation. Collectively, the temporal structural analyses reinforce the superior stabilizing influence of isorhamnetin on both COX-2 and KEAP1, in agreement with the RMSD/RMSF trends, while highlighting the comparatively dynamic interaction landscapes associated with kaempferol and sulfasalazine (**Figure 3.8 and Figure 3.9**)

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3.4.8 ADMET Profile

3.4.8.1 Absorption and distribution

In silico ADMET analysis of the major phytoconstituents identified from *T. palmata* revealed substantial variability in their absorption, distribution, and permeability characteristics. Most compounds demonstrated high human intestinal absorption (HIA; 70–100%), with several—such as palmitic acid, stigmasterol, isohamnetin, and kaempferol—exhibiting optimal oral bioavailability ($OB > 0.3$). Caco-2 permeability values indicated moderate to low membrane permeability across the dataset, with the lowest transport observed for glycosylated flavonoids such as apigenin-6-C-glucoside-8-C-arabinoside and luteolin-6-C-glucoside-8-C-arabinoside ($\log P_{app} < -6.7$), consistent with their higher polarity and molecular weight. Conversely, non-polar terpenoids and fatty acid derivatives displayed comparatively better permeability profiles. PAMPA predictions showed that most compounds possess acceptable passive membrane diffusion, with permeability ranging between 0.09 and 0.97. P-gp inhibition analysis identified several strong inhibitors—particularly eicosanoic acid and stigmasterol—suggesting a potential role in modulating efflux transporter activity and enhancing intracellular drug retention. Distribution predictions revealed favorable blood–brain barrier (BBB) penetration for lipophilic constituents ($BBB > 0.7$), whereas flavonoid glycosides demonstrated limited CNS access ($BBB < 0.1$). Plasma protein binding (PPBR) was notably high across all compounds ($\geq 76\%$), indicating strong affinity to serum proteins, while volume of distribution (VDss) values suggested moderate tissue distribution for most phytochemicals. Compared with sulfasalazine, the reference drug, several phytoconstituents—particularly kaempferol, isorhamnetin, and zeaxanthin—exhibited superior absorption and distribution properties, supporting their potential contribution to the pharmacological efficacy of *T. palmata* extract (**Table 3.6**).

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3.4.8.2 Metabolism and Excretion

The metabolism assessment of *Trevesia palmata* phytoconstituents revealed differential interactions with major cytochrome P450 (CYP) isoenzymes, with several compounds displaying low inhibition values across CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4, indicating a minimal risk of metabolic interference or drug–drug interactions. Lipophilic constituents such as isorhamnetin and kaempferol exhibited relatively higher inhibition of CYP1A2, CYP2C19, and CYP3A4, suggesting their potential involvement in phase I biotransformation pathways. Only a few molecules, including kaempferol-3-O-rutinoside, were predicted as substrates for CYP2C9 and CYP3A4, indicating selective enzymatic susceptibility within the extract. Flavonoid glycosides demonstrated the lowest metabolic liability, consistent with their hydrophilic nature and reduced affinity for hepatic microsomal enzymes.

Excretion profiles revealed substantial variability in elimination characteristics across the compounds. Predicted half-life values ranged widely, with the longest persistence observed for long-chain fatty acids (e.g., eicosanoic and hentetracontanoic acids; $t_{1/2} > 80$ h), indicating slow clearance and extended systemic retention. In contrast, glycosylated flavonoids exhibited shorter half-lives and higher clearance rates, reflecting rapid elimination due to their polarity. Drug clearance parameters supported this trend, with minimal clearance for highly lipophilic molecules and enhanced renal or biliary elimination for polar constituents. Notably, kaempferol and isorhamnetin displayed moderate half-lives and clearance values, aligning with their balanced lipophilicity and metabolic stability. Compared to sulfasalazine, which showed moderate half-life and low clearance consistent with its colon-targeted delivery, several *T. palmata* phytochemicals demonstrated superior metabolic stability and prolonged systemic retention, reinforcing their potential to contribute to sustained pharmacodynamic effects in vivo (**Table 3.7**).

3.4.8.3 Acute, Organ, and End-Point Toxicity of *T. palmata* Phytoconstituents

The comprehensive toxicity profiling of *T. palmata* phytoconstituents using ProTox-3 revealed an overall favorable safety landscape with minimal acute, organ-specific, and mechanistic endpoint toxicities. Acute toxicity (oral LD₅₀) values varied substantially across compounds, clustering predominantly within toxicity classes IV and V, indicative of low acute toxicity. Long-chain fatty acids and glycosylated flavonoids such as quercetin-3-O-galactoside, isorhamnetin, kaempferol-3-O-rutinoside, and several procyanidins exhibited LD₅₀ values ≥ 5000 mg/kg (Class V), supporting their relatively high safety margin. Moderate toxicity (Class III–IV) was observed in palmitic acid, 1-octadecanol, and eicosanoic acid, whereas zeaxanthin (LD₅₀ = 10 mg/kg, Class II) emerged as the only highly toxic component, although still lacking substantial organ toxicity alerts.

Organ toxicity predictions demonstrated that most phytoconstituents were inactive in hepatotoxicity, nephrotoxicity, cardiotoxicity, and respiratory toxicity, underscoring the extract's systemic tolerability. A few flavonoid glycosides and procyanidin derivatives displayed isolated “active” alerts, particularly for nephrotoxicity and respiratory toxicity, but these remained sporadic and not consistently observed across compounds. Neurotoxicity activity was minimal, with only a subset showing weak predictive activation. When compared to sulfasalazine, *T. palmata* compounds demonstrated comparable or substantially better organ safety, as sulfasalazine showed multiple organ toxicity alerts despite its higher LD₅₀.

Mechanistic toxicity endpoints further validated the benign toxicological nature of the phytoconstituents. Carcinogenicity, mutagenicity, cytotoxicity, and endocrine disruption were predominantly inactive across all compounds, with only a few instances of low-intensity activation, especially in sterol or carotenoid derivatives such as stigmasterol and zeaxanthin. Immunotoxicity signals were similarly scarce, suggesting limited risk of immune-mediated adverse effects. Kaempferol, isorhamnetin, vicianin-2, and most glycosides were consistently inactive across all endpoints, reinforcing their suitability as safe therapeutic candidates.

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Overall, the integration of acute toxicity, organ-specific toxicity, and mechanistic endpoints strongly indicates that *T. palmata* phytocompounds possess a high safety margin, with negligible risk of acute poisoning, organ dysfunction, carcinogenicity, or mutagenicity. This robust safety profile provides strong justification for their further in vivo pharmacological evaluation, particularly in long-term or high-dose applications such as anti-inflammatory or ulcerative colitis studies (Table 3.8).

3.4.8.4 Toxicity and Nuclear Receptor Interaction Analysis

The toxicity-ADMET-AI predictions for the major phytocompounds identified from *T. palmata* flower buds revealed compound-specific variations across multiple toxicity endpoints and nuclear receptor signaling pathways. Most compounds showed low predicted brRGS blocking, DILI, skin reaction, and aromatase inhibition (AR/AI) scores, indicating minimal toxicity tendencies. Palmitic acid, 1-octadecanol, and oleic acid exhibited particularly low toxicity scores across the majority of endpoints. In contrast, compounds such as stemonoside, visoamericanoside, and β -sitosterol integrated ester showed comparatively higher DILI and skin reaction values, although still within low-risk probability ranges. Nuclear receptor pathway predictions showed negligible activation potential for androgen receptor (AR), androgen receptor ligand-binding domain (AR-LBD), estrogen receptor (ER), estrogen receptor ligand-binding domain (ER-LBD), and PPAR-gamma for most metabolites. The recorded values were consistently below 0.40. Kaempferol, luteolin-C-glucoside, apigenin-C-glucoside, and quercetin-3-O-galactoside showed slightly higher scores (0.20–0.30 range) across ER and ER-LBD pathways but remained non-critical. The reference drug sulfasalazine displayed higher activity values for AR-LBD (0.11), ER (0.14), and PPAR-gamma (0.004), confirming the presence of known receptor-interaction liabilities compared to the plant-derived metabolites (Table 3.9).

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3.4.8.5 Stress Response Pathway–Related Toxicity

The Tox21 stress-response predictions demonstrated distinct variation among the compounds in assays related to nr/ARE, ATAD5, HSE, MMP, and p53 pathways. The majority of fatty acids (palmitic acid, 1-octadecanol, oleic acid) exhibited very low stress response activity, with scores below 0.20 across all pathways. Carotenoid β -carotene showed modest activation in the HSE pathway (0.74), indicating mild heat-shock response potential.

Among the flavonoids, kaempferol-3-O-rutinoside, apigenin-C-glucoside, luteolin-C-glucoside, and quercetin-3-O-galactoside showed moderate activation in the p53 and HSE pathways (0.10–0.32 range), suggesting mild genotoxic or stress-signaling sensitivity typical of polyphenolic structures. Zearalenone showed the highest predicted p53 activation (0.42) and ATAD5 score (0.38) among all metabolites. Compounds including raucaffricine B1, proconvallarin C1, and visenoside displayed slightly elevated MMP and p53 scores, but none of these values exceeded 0.50, indicating low to moderate mitochondrial and genotoxic stress probabilities.

The reference anti-colitis drug sulfasalazine showed noticeably higher ATAD5 (0.06), HSE (0.06), and MMP (0.06) activity compared with most phytochemicals, supporting the relatively safer stress-response profile of *T. palmata* metabolites (Table 3.9).

3.5 DISCUSSION

The present study provides a comprehensive computational evaluation of *T. palmata* bioactive compounds, focusing on their anti-inflammatory, antioxidant, and anti-ulcerative potential. In silico methods including PASS prediction, physicochemical and ADMET profiling, toxicity modeling, molecular docking, and molecular dynamics simulations were used to identify isorhamnetin and kaempferol as leading phytochemicals with strong promise for therapeutic development. The PASS-predicted biological activities of *T. palmata* phytochemicals align with the traditional use of the plant in managing inflammatory conditions and ulcers.

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PASS prediction analysis revealed that compounds such as zeta-carotene, kaempferol, and several flavonoid derivatives (apigenin and quercetin derivatives) displayed Pa values substantially higher than Pi (predicted inactivity), indicating a high likelihood of manifesting significant anti-inflammatory and anti-ulcerative activity. This finding strongly supports the therapeutic potential of *T. palmata* compounds in these disease pathways. A clear distinction emerged between different compound classes, with carotenoids, fatty acids, and flavonoids exhibiting the highest predicted activity. This highlights a likely synergistic pharmacological profile, where a combination of compounds contributes to the overall effect, rather than relying on a single “active principle” (Parvin et al., 2025).

Interestingly, many *T. palmata* compounds outperformed the reference drug sulfasalazine in PASS predictions, particularly in anti-inflammatory and anti-ulcerative endpoints. This finding reinforces the concept that plant-based multi-component mixtures may sometimes offer more versatile therapeutic benefits than conventional single-target drugs. Our observation resonates with the approach demonstrated in Parvin et al. (2025), where phytochemicals were computationally screened against both 5-LOX and COX-2 pathways to identify multi-target anti-inflammatory candidates (Parvin et al., 2025). This dual-target approach, as demonstrated by *T. palmata* compounds, could offer more comprehensive therapeutic effects with fewer side effects than traditional single-target therapies.

Flavonoids, such as kaempferol and isorhamnetin, have long been recognized for their ability to modulate inflammation by downregulating pro-inflammatory cytokines, enhancing antioxidant defenses, and promoting mucosal healing. These activities are consistent with their strong predictive activity in the PASS model, reinforcing their central role in the medicinal potential of *T. palmata*. These compounds also align with established research demonstrating the anti-inflammatory benefits of flavonoids in chronic conditions like ulcerative colitis and arthritis.

The physicochemical evaluation (via SwissADME) revealed substantial structural diversity within *T. palmata* metabolites, spanning from simple fatty acids and terpenoids to complex glycosylated flavonoids and oligomeric procyanidins.

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Notably, aglycone flavonoids such as isorhamnetin and kaempferol display molecular weights below 350 g/mol, moderate lipophilicity, balanced polarity, acceptable solubility, and limited rotatable bonds, features consistent with established drug-likeness criteria (**Lipinski et al., 2001**). These properties imply favorable passive membrane permeability, metabolic stability, and potential for adequate oral bioavailability.

In contrast, glycosylated flavonoids and procyanidins, mainly due to their high molecular weights (450–866 g/mol), large TPSA values, and numerous hydrogen bond donors/acceptors, fall outside standard drug-like thresholds, predicting poor gastrointestinal absorption. This pattern mirrors common limitations in natural-product pharmacokinetics (**Wang et al., 2020**). However, such compounds often exert local effects in the gastrointestinal tract or may undergo microbial or enzymatic transformation to release more bioavailable aglycones, a phenomenon frequently documented in flavonoid pharmacology (**Yuan et al., 2017**). Future research could explore prodrug strategies or formulation techniques, such as nanoencapsulation, to enhance the pharmacokinetic profiles of these compounds.

The high lipophilicity and poor solubility predicted for terpenoids and fatty acid derivatives (e.g., stigmasterol, oleic acid) present a different challenge: although these molecules may readily cross lipid membranes or integrate into cell bilayers, their systemic distribution and formulation stability may be compromised without suitable delivery strategies (**Borah et al., 2021**).

Collectively, these observations suggest that while the *T. palmata* metabolite pool is chemically rich, only a subset, mainly the aglycone flavonoids, possesses natural structural features conducive to systemic bioavailability and drug development. The remainder may still contribute to therapeutic effects via local action or could serve as scaffolds for structural optimization (e.g., via deglycosylation, prodrug formation, or semi-synthetic modifications).

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The toxicity assessment of *T. palmata* phytochemicals demonstrated a generally favorable safety profile. Most metabolites showed minimal predicted risks across critical toxicity endpoints (carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity, and hepatotoxicity) suggesting they are biologically safe at theoretical exposure levels. Fatty acids, carotenoids, and flavonoid glycosides exhibited largely inactive predictions. Importantly, kaempferol and isorhamnetin, despite their pronounced predicted bioactivity, remained inactive across nearly all toxicity categories. This finding reinforces their suitability for further drug development. Some compounds, such as certain sterol derivatives and procyanidins, did show isolated toxicity "alerts" (e.g., mild hepatotoxicity or cytotoxicity). These results, while not disqualifying, highlight the need for careful downstream validation (e.g., in vitro cytotoxicity assays, metabolic stability testing, and dose-dependent safety profiling). Relative to sulfasalazine, *T. palmata* metabolites display a much milder predicted toxicity spectrum, underscoring their potential as safer alternatives for chronic inflammatory and ulcer management.

Molecular docking studies of COX-2 and KEAP1, two therapeutically relevant targets in inflammation and oxidative stress, revealed that isorhamnetin and kaempferol exhibited stronger binding interactions compared to sulfasalazine. Isorhamnetin, in particular, formed a dense network of hydrogen bonds and hydrophobic contacts with critical residues in both the COX-2 and KEAP1 active sites, suggesting potent inhibitory potential. Such dual-target docking aligns with the growing recognition that simultaneous modulation of inflammatory (COX-2) and oxidative stress (KEAP1/Nrf2) pathways can offer superior therapeutic benefit compared to single-target strategies (Singh et al., 2019). Indeed, recent in silico and experimental studies have prioritized dual COX/LOX inhibitors or combined anti-inflammatory/antioxidant approaches to achieve broader efficacy with fewer side effects (Wang et al., 2020). This approach, as demonstrated by *T. palmata* compounds, supports the concept of multi-target drug design. Moreover, the reference paper by Parvin et al. (2025) employed a similar dual-target computational framework (COX-2 and 5-LOX) combined with PASS prediction and ADMET

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evaluation, highlighting the validity and growing acceptance of such multi-target screening strategies in phytochemical research.

Molecular dynamics (MD) simulations further substantiated the docking results. The isorhamnetin–COX-2 and isorhamnetin–KEAP1 complexes demonstrated low RMSD and RMSF fluctuations over 100 ns, indicating stable binding throughout the simulation period. The ligand remained firmly anchored with persistent hydrogen bonds and favourable hydrophobic packing. In comparison, kaempferol displayed moderate stability, while sulfasalazine exhibited greater structural fluctuation, suggesting weaker binding and less stable inhibitory potential. These dynamic stability results strengthen the case for isorhamnetin (and to a lesser extent kaempferol) as robust dual-target modulators capable of maintaining functional interaction under physiological conditions.

3.6 KEY FINDINGS:

- Isorhamnetin and kaempferol emerged as the most promising bioactive compounds with strong anti-inflammatory, antioxidant, and anti-ulcerative potential.
- Carotenoids, fatty acids, and flavonoids showed the highest predicted biological activity.
- Zeta-carotene, kaempferol, apigenin derivatives, and quercetin derivatives had significantly higher Pa values than Pi, indicating a strong likelihood of exhibiting biological activity, particularly in anti-inflammatory and anti-ulcer effects.
- Several *T. palmata* compounds outperformed the reference drug sulfasalazine in PASS prediction models, especially for anti-inflammatory and anti-ulcerative endpoints.
- Flavonoids played a major role in modulating inflammation, enhancing antioxidant defenses, and promoting mucosal healing, supporting their central role in *T. palmata*'s medicinal properties.

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- Aglycone flavonoids (e.g., isorhamnetin, kaempferol) exhibited favorable drug-like properties such as moderate lipophilicity, balanced polarity, and good solubility.
- Glycosylated flavonoids and procyanidins were predicted to have poor gastrointestinal absorption, which limits their systemic bioavailability but suggests potential local effects or prodrug formulations.
- Most metabolites, including kaempferol and isorhamnetin, demonstrated minimal predicted toxicity across key endpoints (carcinogenicity, mutagenicity, hepatotoxicity), indicating good safety profiles.
- Some sterol derivatives and procyanidins showed isolated toxicity alerts, highlighting the need for further validation in in vitro studies.
- Isorhamnetin and kaempferol exhibited stronger binding interactions with COX-2 and KEAP1, key targets in inflammation and oxidative stress, suggesting potent inhibitory potential.
- Isorhamnetin showed stable binding to COX-2 and KEAP1 with low RMSD and RMSF fluctuations in molecular dynamics simulations, indicating robust dual-target modulation.
- Dual-target docking and molecular dynamics results emphasize the potential of isorhamnetin and kaempferol as multi-target modulators of inflammation and oxidative stress, providing a broader therapeutic benefit compared to single-target drugs.

Table 3.1. Phytocompounds from methanolic extract of *Trevesia palmata* flower-bud (LC-MS and GC-MS) used as ligand candidates against COX2 and KEAP1 for inflammation and ulcerative colitis disease.

Sl. No.	Ligand	PubChem CID	Molecular formula	Canonical SMILE	Biological activity	References
1.	Palmitic acid	985	C ₁₆ H ₃₂ O ₂	CCCCCCCCCCCCCCCC(=O)O	Anti-bacterial, Anti-fungal, Anti-inflammatory, Anti-oxidant, Antitumor, Immune-enhancer	Casillas-Vargas G et al. (2021); Prasath et al. (2020); Wang et al. (2023)
2.	1-Octadecanol	8221	C ₁₈ H ₃₈ O	CCCCCCCCCCCCCCCCCO	Anti-bacterial, Anti-fungal, Anti-larva	Gnanashree et al. (2018);
3.	Oleic acid	445639	C ₁₈ H ₃₄ O ₂	CCCCCCCC/C=C\CCCCCCC(=O)O	Anti-inflammatory, Anti-fungal, Anti-oxidant, Anti-bacterial, Wound healer, Immunomodulator and Antitumor	Santa-María et al. (2023); Ramadan et al. (2024); Katariya et al. (2021); Carrillo Pérez et al. (2012)
4.	Stigmasterol	5280794	C ₂₉ H ₄₈ O	CC[C@H](/C=C/[C@@H](C)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CC=C4[C@@]3(CC[C@@H](C4)O)C)C(C)C	Anticancer, Anti-osteoarthritis, Anti-inflammatory, Anti-diabetic, Immunomodulatory, Anti-parasitic, Anti-fungal, Anti-bacterial, Anti-oxidant, Neuroprotective	Bakrim et al. (2022)

					Neuroprotective,	(2020)
12.	Luteolin-6-C-glucoside 8-C-arabioside	3549960	C ₂₇ H ₃₀ O ₁₆	<chem>C1=CC(=C(C=C1C2=CC(=O)C3=C(C(=C(C(=C3O2)C4C(C(C(C(O4)CO)O)O)O)O)C5C(C(C(C(O5)CO)O)O)O)O)O</chem>	Hepatoprotective, Anti-inflammatory, Anti-oxidant,	Kundu et al. (2011); Costa et al. (2016); Ghozzi et al. (2024)
13.	Procyanidin B1	11250133	C ₃₀ H ₂₆ O ₁₂	<chem>C1[C@@H]([C@H](OC2=C1C(=CC(=C2[C@@H]3[C@H]([C@H](OC4=CC(=CC(=C34)O)O)C5=CC(=C(C=C5)O)O)O)O)C6=CC(=C(C=C6)O)O)O</chem>	Anti-cancer, Anti-inflammatory Anti-oxidant	Na et al. (2020); Feng et al. (2025) Dasiman et al. (2022)
14.	Procyanidin C1	169853	C ₄₅ H ₃₈ O ₁₈	<chem>C1[C@H]([C@H](OC2=C1C(=CC(=C2[C@@H]3[C@H]([C@H](OC4=C(C(=CC(=C34)O)O)[C@@H]5[C@H]([C@H](OC6=CC(=CC(=C56)O)O)C7=CC(=C(C=C7)O)O)O)C8=C(C(=C(C=C8)O)O)O)O)O)C9=CC(=C(C=C9)O)O)O</chem>	Anti-inflammatory, Anti-oxidant, Antiaging, Ant-cancer	Dasiman et al. (2022); Xu et al. (2021) Yu et al. (2025)
15.	Vicenin-2	3084407	C ₂₇ H ₃₀ O ₁₅	<chem>C1=CC(=CC=C1C2=CC(=O)C3=C(C(=C(C(=C3O2)C4C(C(C(C(O4)CO)O)O)O)O)C5C(C(C(C(O5)CO)O)O)O)O</chem>	Anti-inflammatory, Anti-oxidant, Wound healer, Anticancer Anti-thrombotic and antiplatelet, Ant-photoaging, Antispasmodic	Marrassini et al. (2011); Silva et al. (2014); Tan et al. (2020); Yang et al. (2018); Lee and Bae

						(2015); Duan et al. (2019); Fujii and Buchwald- Werner (2015)
16.	Luteolin-6-C- glucoside	114776	C ₂₁ H ₂₀ O ₁₁	<chem>C1=CC(=C(C=C1C2=CC(=O)C3=C(O2)C=C(C(=C3O)[C@H]4[C@@H]([C@H]([C@@H]([C@H](O4)CO)O)O)O)O)O</chem>	Anticancer, Anti-diabetic, Anti-osteoporotic, Anti-oxidant, Antibacterial Anti-inflammatory	Ye et al. (2016); Ziqubu et al. (2020); Zhang et al. (2024); Cao et al. (2022); Wang et al. (2021); Li et al. (2020);
17.	Apigenin-8-C- glucoside	5280441	C ₂₁ H ₂₀ O ₁₀	<chem>C1=CC(=CC=C1C2=CC(=O)C3=C(O2)C(=C(C=C3O)O)[C@H]4[C@@H]([C@H]([C@@H]([C@H](O4)CO)O)O)O)O</chem>	Anti-oxidant, Anti-inflammatory, Anticancer, Cardioprotective, Hepato-pancreatic protective, Neuroprotective,	Babaei et al. (2020); Ranjan et al. (2023); Li et al. (2021);

18.	Quercetin-3-O-galactoside	5281643	C ₂₁ H ₂₀ O ₁₂	<chem>C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O[C@H]4[C@@H]([C@H]([C@H]([C@H]([C@H](O4)CO)O)O)O)O</chem>	Antioxidant, Anti-inflammatory, Anti-arthritic, Anti-cancer, Antiviral, Antisteatotic, Antifibrotic Hepatoprotective Cardioprotective, Neuroprotective	Zhang et al. (2017); Jin et al. (2016); Fu et al. (2016); Jang (2022); Hou et al. (2016); Xu et al. (2023)
19.	Isorhamnetin	5281654	C ₁₆ H ₁₂ O ₇	<chem>COC1=C(C=CC(=C1)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O</chem>	Anti-inflammatory, Anti-cancer, Antioxidant, Cardiovascular and cerebrovascular,	Kim et al. (2019); Mei et al. (2025); Lri et al. (2024)
20.	Kaempferol	5280863	C ₁₅ H ₁₀ O ₆	<chem>C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O</chem>	Anti-inflammatory, Antioxidant, Anti-diabetic, Anticancer and anti-angiogenic, Antiviral, Cardioprotective, Neuroprotective	Herrera et al. (2025); Moore et al. (2023); Kim (2017); Gao et al. (2023); Zhou et al. (2015); Chaubey and Singh, (2025)
21.	Sulfasalazine (Reference drug)	5339	C ₁₈ H ₁₄ N ₄ O ₅ S	<chem>C1=CC=NC(=C1)NS(=O)(=O)C2=CC=C(C=C2)N=NC3=CC(=C(C=C3)O)C(=O)O</chem>	Drug (Antibiotic and anti-inflammatory)	Wahl et al. (1998)

Table 3.2 Biological activities of *Trevesia palmata* (flower buds) selected phytochemicals predicted using PASS (Prediction of Activity Spectra for Substances) tool <http://www.way2drug.com/passonline/>

SL. No	Phytochemicals (<i>Trevesia palmata</i>)	Anti-inflammatory		Anti-ulcerative	
		Pa	Pi	Pa	Pi
1.	Palmitic acid	0.515	0.052	0.525	0.019
2.	1-Octadecanol	0.498	0.058	0.476	0.028
3.	Oleic acid	0.614	0.029	0.635	0.008
4.	Stigmasterol	0.542	0.045	-	-
5.	Eicosanoic acid, tetradecyl ester	0.641	0.024	0.567	0.014
6.	Hentetracontanoic acid	0.515	0.052	0.525	0.019
7.	Eicosanoic acid	0.515	0.052	0.525	0.019
8.	zeta-Carotene	0.754	0.010	0.712	0.005
9.	Kaempferol-3-O-rutinoside	0.743	0.011	0.586	0.012
10.	Apigenin 6-C-glucoside 8-C-arabinoside	0.510	0.054	0.292	0.105
11.	Zeaxanthin	0.675	0.019	0.781	0.005
12.	Luteolin-6-C-glucoside 8-C-arabinoside	0.533	0.047	0.292	0.105
13.	Procyanidin B1	0.430	0.081	0.403	0.044
14.	Procyanidin C1	0.430	0.081	0.403	0.044
15.	Vicenin-2	0.510	0.054	0.292	0.105
16.	Luteolin-6-C-glucoside	0.496	0.058	0.367	0.060
17.	Apigenin-8-C-glucoside	0.606	0.030	0.437	0.035
18.	Quercetin-3-O-galactoside	0.739	0.011	0.519	0.020
19.	Isorhamnetin	0.663	0.021	0.477	0.028
20.	Kaempferol	0.676	0.019	0.487	0.026
21.	Sulfasalazine (Reference Drug)	0.404	0.093	0.551	0.016

Pa: Probability "to be active"; Pi: Probability "to be inactive"; $P_a > P_i$: activity manifest experimentally active compound; $P_a > 0.7$: indicate highly probable for the activity to manifest experimentally; $0.5 < P_a < 0.7$: indicates moderate probability; $P_a < 0.5$: the chance of finding the activity experimentally is less, but it may indicate a chance of finding a new compound; Lower Pi values indicate stronger confidence in activity; $P_i < 0.05$: is generally desirable.

Table 3.3. SwissADME-predicted physicochemical properties of selected phyto-ligands, including molecular weight (MW, g/mol), number of heavy atoms (nHA), number of aromatic heavy atoms (nAHA), number of rotatable bonds (nROT), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), fraction of sp³-hybridized carbons (F. Csp3), molar refractivity (MR), topological polar surface area (TPSA, Å²), consensus octanol/water partition coefficient (Con. LogP o/w) and ESOL-predicted aqueous solubility (Log S, ESOL).

Sl. No.	Phyto-ligands	Swiss ADME-Physicochemical Properties										
		MW (g/mol)	nHA	nAHA	nROT	HBA	HBD	F. Csp3	MR	TPSA (Å ²)	Con. LogP _{o/w}	Log S (ESOL)
1.	Palmitic acid	256.4	18	0	14	2	1	0.94	80.80	37.3	5.2	-5.02
2.	1-Octadecanol	270.4	19	0	16	1	1	1.00	89.80	20.2	6.15	-5.63
3.	Oleic acid	282.4	20	0	15	2	1	0.83	89.94	37.3	5.65	-5.41
4.	Stigmasterol	412.6	30	0	5	1	1	0.86	132.75	20.2	6.98	-7.46
5.	Eicosanoic acid, tetradecyl ester	508.9	36	0	32	2	0	0.97	166.84	26.3	11.67	-11.13
6.	Hentetracontanoic acid	607.0	43	0	39	2	1	0.98	200.97	37.3	14.16	-14.05
7.	Eicosanoic acid	312.5	22	0	18	2	1	0.95	100.03	37.3	6.62	-6.44
8.	zeta-Carotene	540.9	40	0	18	0	0	0.45	189.18	0	6.62	-6.44
9.	Kaempferol-3-O-rutinoside	594.5	42	16	6	15	9	0.44	139.36	249.2	-1.13	-3.42

10.	Apigenin 6-C-glucoside 8-C-arabinosideis	564.4	40	16	5	14	10	0.42	133.26	250.9	-1.54	-2.27
11.	Zeaxanthin	568.8	42	0	10	2	2	0.45	186.76	40.4	-1.54	-2.27
12.	Luteolin-6-C-glucoside 8-C-arabinoside	610.5	43	16	5	16	12	0.44	141.25	291.4	-2.3	-1.93
13.	Procyanidin B1	578.5	42	24	3	12	10	0.20	146.71	220.7	1.37	-5.14
14.	Procyanidin C1	866.7	63	36	5	18	15	0.20	219.09	331.1	1.9	-7.39
15.	Vicenin-2	594.5	42	16	5	15	11	0.44	139.23	271.2	-2.07	-2.05
16.	Luteolin-6-C-glucoside	448.3	32	16	3	11	8	0.29	108.63	201.2	-0.34	-2.7
17.	Apigenin-8-C-glucoside	432.3	31	16	3	10	7	0.29	106.61	181.05	-0.02	-2.84
18.	Quercetin-3-O-galactoside	464.3	33	16	4	12	8	0.29	110.16	210.5	-0.38	-3.04
19.	Isorhamnetin	316.2	23	16	2	7	4	0.06	82.50	120.3	1.65	-3.36
20.	Kaempferol	286.2	21	16	1	6	4	0.00	76.01	111.1	1.58	-3.31
21.	Sulfasalazine (Reference Drug)	398.3	28	18	6	8	3	0.00	100.95	149.6	2.3	-3.76

Table 3.4 SwissADME-predicted drug-likeness profile of *Trevesia palmata* phytochemicals showing compliance with major oral drug-likeness filters: Lipinski’s Rule of Five (Lipinski), Ghose filter (Ghose), Veber rule for oral bioavailability (Veber), Egan rule for passive membrane permeability (Egan), and Muegge filter for drug-like chemical space (Muegge), together with the predicted oral Bioavailability Score. “Yes/No; *n* violation(s)” indicates whether a compound satisfies each rule and, if not, the number of violated criteria; sulfasalazine is included as a reference drug.

Sl. No.	Phytochemicals (<i>Trevesia palmata</i>)	Swiss ADME-Drug likeness					
		Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability Score
1.	Palmitic acid	Yes; 1 violation	Yes; 0 violation	No; 1 violation	Yes; 0 violation	No; 1 violation	0.85
2.	1-Octadecanol	Yes; 1 violation	No; 1 violation	No; 1 violation	No; 1 violation	No; 3 violations	0.55
3.	Oleic acid	Yes; 1 violation	No; 1 violation	No; 1 violation	No; 1 violation	No; 1 violation	0.85
4.	Stigmasterol	Yes; 1 violation	No; 3 violations	Yes; 0 violation	No; 1 violation	No; 2 violations	0.55
5.	Eicosanoic acid, tetradecyl ester	No; 2 violations	No; 4 violations	No; 1 violation	No; 1 violation	No; 2 violations	0.17
6.	Hentetracontanoic acid	No; 2 violations	No; 4 violations	No; 1 violation	No; 1 violation	No; 3 violations	0.85
7.	Eicosanoic acid	Yes; 1 violation	No; 1 violation	No; 1 violation	No; 1 violation	No; 2 violations	0.85
8.	zeta-Carotene	Yes; 1 violation	No; 1 violation	No; 1 violation	No; 1 violation	No; 2 violations	0.85
9.	Kaempferol-3-O-rutinoside	No 3 violations	No 4 violations	No 1 violation	No 1 violation	No 3 violations	0.17
10.	Apigenin 6-C-glucoside 8-C-	No	No	No	No	No	0.17

	arabinosideis	3 violations	3 violations	1 violation	1 violation	3 violations	
11.	Zeaxanthin	No 3 violations	No 3 violations	No 1 violation	No 1 violation	No 3 violations	0.17
12.	Luteolin-6-C-glucoside 8-C-arabinoside	No 3 violations	No 4 violations	No 1 violation	No 1 violation	No 5 violations	0.17
13.	Procyanidin B1	No 3 violations	No 2 violations	No 1 violation	No 1 violation	No 3 violations	0.17
14.	Procyanidin C1	No 3 violations	No 3 violations	No 1 violation	No 1 violation	No 5 violations	0.17
15.	Vicenin-2	No 3 violations	No 4 violations	No 1 violation	No 1 violation	No 4 violations	0.17
16.	Luteolin-6-C-glucoside	No 2 violations	No 1 violation	No 1 violation	No 1 violation	No 3 violations	0.17
17.	Apigenin-8-C-glucoside	Yes 1 violation	Yes 0 violation	No 1 violation	No 1 violation	No 2 violations	0.55
18.	Quercetin-3-O-galactoside	No 2 violations	No 1 violation	No 1 violation	No 1 violation	No 3 violations	0.17
19.	Isorhamnetin	Yes 0 violation	Yes 0 violation	Yes 0 violation	Yes 0 violation	Yes 0 violation	0.55
20.	Kaempferol	Yes 0 violation	Yes 0 violation	Yes 0 violation	Yes 0 violation	Yes 0 violation	0.55
21.	Sulfasalazine (Reference Drug)	Yes 0 violation	Yes 0 violation	No 1 violation	No 1 violation	Yes 0 violation	0.56

Table 3.5 SwissADME-predicted medicinal chemistry properties of *Trevesia palmata* phytochemicals, including Pan-Assay Interference Structures alerts (PAINS), Brenk structural alert filter (Brenk), lead-likeness assessment (Lead likeness) and synthetic accessibility score (Synthetic accessibility). “0 alert / 1 alert” indicates the presence or absence of structural alerts for potential assay interference or toxicity, while “Yes/No; *n* violation(s)” under Lead likeness denotes compliance with lead-like criteria; sulfasalazine is included as a reference drug.

Sl. No.	Phytochemicals (<i>Trevesia palmata</i>)	Swiss ADME-Medicinal Chemistry			
		PAINS	Brenk	Lead likeness	Synthetic accessibility
1.	Palmitic acid	0 alert	0 alert	No; 2 violations	2.31
2.	1-Octadecanol	0 alert	0 alert	No; 2 violations	2.52
3.	Oleic acid	0 alert	1 alert: isolated_alkene	No; 2 violations	3.07
4.	Stigmasterol	0 alert	1 alert: isolated_alkene	No; 2 violations	6.21
5.	Eicosanoic acid, tetradecyl ester	0 alert	0 alert	No; 3 violations	4.77
6.	Hentetracontanoic acid	0 alert	0 alert	No; 3 violations	5.41
7.	Eicosanoic acid	0 alert	0 alert	No; 2 violations	2.77
8.	zeta-Carotene	0 alert	0 alert	No; 2 violations	2.77
9.	Kaempferol-3-O-rutinoside	0 alert	0 alert	No; 1 violation	6.48
10.	Apigenin 6-C-glucoside 8-C-arabinoside	0 alert	0 alert	No; 1 violation	6.18
11.	Zeaxanthin	0 alert	0 alert	No; 1 violation	6.18
12.	Luteolin-6-C-glucoside 8-C-arabinoside	1 alert: catechol_A	1 alert: catechol	No; 1 violation	6.44
13.	Procyanidin B1	1 alert: catechol_A	1 alert: catechol	No; 1 violation	5.32
14.	Procyanidin C1	1 alert: catechol_A	1 alert: catechol	No; 1 violation	6.95
15.	Vicenin-2	0 alert	0 alert	No; 1 violation	6.4
16.	Luteolin-6-C-glucoside	1 alert: catechol_A	1 alert: catechol	No; 1 violation	5.04
17.	Apigenin-8-C-glucoside	0 alert	0 alert	No; 1 violation	5.12

18.	Quercetin-3-O-galactoside	1 alert: catechol_A	1 alert: catechol	No; 1 violation	5.32
19.	Isorhamnetin	0 alert	0 alert	Yes; 0 violation	3.26
20.	Kaempferol	0 alert	0 alert	Yes; 0 violation	3.14
21.	Sulfasalazine (Reference Drug)	1 alert: azo_A	1 alert: diazo_group	No; 1 violation	3.07

Table 3.6 ADMET-AI-predicted absorption and distribution parameters of *Trevesia palmata* phytochemicals, including human intestinal absorption (HIA), oral bioavailability (OB), Caco-2 cell permeability Caco-2 log (10⁻⁶ cm/s), PAMPA (parallel artificial membrane permeability assay) permeability, P-glycoprotein inhibition (P-gp inhibition), blood–brain barrier penetration (BBB), plasma protein binding rate (PPBR, %) and steady-state volume of distribution (VD_{ss}, L/kg); sulfasalazine is included as a reference drug.

SL. No.	Phytochemicals (<i>Trevesia palmata</i>)	Absorption					Distribution		
		HIA	OB	Caco-2 log (10 ⁻⁶ cm/s)	PAMPA Permeability	P-gp inhibition	BBB	PPBR (%)	VD _{ss} (L/kg)
1.	Palmitic acid	1.00	0.66	-4.82	0.62	0.25	0.77	100	10.39
2.	1-Octadecanol	1.00	0.50	-4.33	0.91	0.86	0.96	100	61.31
3.	Oleic acid	1.00	0.66	-5.07	0.69	0.36	0.82	100	5.33
4.	Stigmasterol	1.00	0.79	-5.07	0.97	0.93	0.79	100	7.09
5.	Eicosanoic acid, tetradecyl ester	1.00	0.31	-4.91	0.88	0.98	0.84	100	17.55
6.	Hentetracontanoic acid	0.99	0.41	-5.56	0.52	0.94	0.44	100	14.76
7.	Eicosanoic acid	1.00	0.58	-5.09	0.62	0.50	0.69	100	13.99
8.	zeta-Carotene	0.71	0.34	-5.09	0.84	0.93	0.71	100	18.96
9.	Kaempferol-3-O- rutoside	0.09	0.18	-6.73	0.09	0.14	0.06	85.68	6.37
10.	Apigenin 6-C-glucoside 8-C-arabinoside	0.12	0.25	-6.75	0.09	0.20	0.04	79.34	4.47
11.	Zeaxanthin	0.91	0.41	-5.16	0.80	0.90	0.44	100	5.46
12.	Luteolin-6-C-glucoside 8- C-arabinoside	0.25	0.32	-6.80	0.09	0.27	0.04	79.49	5.65
13.	Procyanidin B1	0.61	0.27	-6.59	0.16	0.68	0.04	81.29	0.78

14.	Procyanidin C1	0.67	0.29	-6.54	0.16	0.83	0.03	84.79	0.00
15.	Vicenin-2	0.26	0.32	-6.76	0.09	0.29	0.03	80.15	5.88
16.	Luteolin-6-C-glucoside	0.33	0.24	-6.47	0.10	0.16	0.06	77.41	2.31
17.	Apigenin-8-C-glucoside	0.37	0.26	-6.38	0.11	0.17	0.07	76.88	1.28
18.	Quercetin-3-O-galactoside	0.26	0.23	-6.51	0.10	0.12	0.05	78.05	6.38
19.	Isorhamnetin	1.00	0.43	-5.25	0.71	0.48	0.13	89.85	2.57
20.	Kaempferol	1.00	0.36	-5.20	0.66	0.26	0.07	90.58	1.92
21.	Sulfasalazine (Reference Drug)	0.82	0.89	-5.58	0.10	0.03	0.05	100	1.55

Table 3.7 ADMET-AI–predicted metabolism and excretion parameters of *Trevesia palmata* phytochemicals, including inhibition of cytochrome P450 isoforms CYP1A2 (CYP1A2 inhibition), CYP2C19 (CYP2C19 inhibition), CYP2C9 (CYP2C9 inhibition), CYP2D6 (CYP2D6 inhibition) and CYP3A4 (CYP3A4 inhibition), as well as substrate probability for CYP2C9 (CYP2C9 substrate), CYP2D6 (CYP2D6 substrate) and CYP3A4 (CYP3A4 substrate). Excretion-related descriptors comprise elimination half-life (Half-life, hr), intrinsic drug clearance in hepatocytes (Drug clearance – hepatocyte, $\mu\text{L}/\text{min}/10^6$ cells) and intrinsic drug clearance in liver microsomes (Drug clearance – microsome, $\mu\text{L}/\text{min}/\text{mg}$ protein); sulfasalazine is included as a reference drug.

SL. No.	Phytochemicals (<i>Trevesia palmata</i>)	Metabolism								Excretion		
		CYP1A2 Inhibition	CYP2C19 Inhibition	CYP2C9 Inhibition	CYP2C9 Substrate	CYP2D6 Inhibition	CYP2D6 Substrate	CYP3A4 Inhibition	CYP3A4 Substrate	Half Life (hr.)	Drug Clearance Hepatocyte ($\mu\text{L}/\text{min}/10^6$ cells)	Drug Clearance (Microsome) $\mu\text{L}/\text{min}/\text{mg}$
1.	Palmitic acid	0.40	0.03	0.04	0.37	0.02	0.17	2.10e-03	0.26	36.10	106.77	31.77
2.	1-Octadecanol	0.61	0.10	0.02	0.09	0.04	0.30	3.84e-03	0.29	154.69	105.28	69.77
3.	Oleic acid	0.52	0.11	0.11	0.32	0.07	0.16	0.01	0.37	26.80	98.21	28.69
4.	Sigmastrol	0.01	0.09	0.04	0.16	0.05	0.21	0.12	0.72	14.81	76.03	54.24
5.	Eicosanoic acid, tetradecyl ester	0.09	0.02	0.01	0.07	0.02	0.17	0.03	0.55	82.18	147.77	150.51
6.	Hentetracontanoic acid	0.05	3.52e-03	0.01	0.13	0.01	0.08	4.70e-03	0.38	48.27	75.94	62.73
7.	Eicosanoic acid	0.36	0.02	0.03	0.31	0.03	0.16	3.73e-03	0.33	44.96	99.78	36.58
8.	zeta-Carotene	0.01	0.09	0.03	0.05	0.03	0.06	0.08	0.70	43.16	63.62	66.09

9.	Kaempferol-3-O-rutinoside	0.01	0.03	0.01	0.03	0.03	0.02	0.01	0.41	43.84	29.40	37.07
10.	Apigenin 6-C-glucoside 8-C-arabinosideis	0.01	0.04	0.02	0.04	0.01	0.02	0.01	0.39	62.96	12.36	25.62
11.	Zeaxanthin	0.01	0.10	0.09	0.03	0.02	0.02	0.25	0.77	16.59	36.58	72.22
12.	Luteolin-6-C-glucoside 8-C-arabinoside	0.02	0.08	0.06	0.06	0.01	0.02	0.02	0.40	70.07	3.95	27.78
13.	Procyanidin B1	0.07	0.17	0.17	0.08	0.06	0.05	0.11	0.62	38.72	0.00	22.12
14.	Procyanidin C1	0.07	0.19	0.22	0.08	0.05	0.04	0.15	0.68	73.59	0.00	25.72
15.	Vicenin-2	0.02	0.09	0.06	0.07	0.01	0.02	0.02	0.40	63.81	6.08	26.66
16.	Luteolin-6-C-glucoside	0.07	0.06	0.04	0.07	0.03	0.03	0.01	0.30	50.03	24.22	4.26
17.	Apigenin-8-C-glucoside	0.07	0.08	0.04	0.08	0.03	0.04	0.01	0.31	49.81	28.99	1.93
18.	Quercetin-3-O-galactoside	0.04	0.05	0.03	0.06	0.04	0.03	0.02	0.32	47.70	30.42	12.97
19.	Isorhamnetin	0.92	0.32	0.34	0.07	0.29	0.08	0.77	0.49	5.35	86.09	7.20
20.	Kaempferol	0.90	0.30	0.30	0.07	0.34	0.07	0.65	0.52	8.70	86.24	6.22
21.	Sulfasalazine (Reference Drug)	0.07	0.03	0.10	0.44	0.01	0.03	3.36e-03	0.10	37.98	31.11	0.00

Table 3.8 ProTox-3 predicted acute oral toxicity and organ-/system-specific toxicity endpoints of *Trevesia palmata* phytocompounds, including median lethal dose after oral administration in rats (LD₅₀, mg/kg), acute toxicity class (Toxicity Class, GHS I–VI), and probabilities for hepatotoxicity (Hepatotoxicity), neurotoxicity (Neurotoxicity), nephrotoxicity (Nephrotoxicity), respiratory toxicity (Respiratory toxicity) and cardiotoxicity (Cardiotoxicity). The table further extends to global toxicity endpoints comprising carcinogenicity (Carcinogenicity), immunotoxicity (Immunotoxicity), mutagenicity (Mutagenicity), cytotoxicity (Cytotoxicity), environmental toxicity (Ecotoxicity), clinical toxicity (Clinical toxicity) and nutrition-related toxicity (Nutritional toxicity). Values are given as probability scores accompanied by categorical predictions (“Active”/“Inactive”); sulfasalazine is included as a reference drug.

SL. No.	Phytocompounds (<i>Trevesia palmata</i>)	Acute Toxicity (Oral)		Organ Toxicity				
		LD ₅₀ (mg/kg)	Toxicity Class	Hepato- toxicity	Neuro- toxicity	Nephro- toxicity	Respiratory Toxicity	Cardio- toxicity
1.	Palmitic acid	900	IV	0.52; Inactive	0.92; Inactive	0.53; Inactive	0.85; Inactive	0.99; Inactive
2.	1-Octadecanol	1000	IV	0.90; Inactive	0.90; Inactive	0.67; Inactive	0.93; Inactive	1.0; Inactive
3.	Oleic acid	48	II	0.55; Inactive	0.91; Inactive	0.55; Inactive	0.84; Inactive	0.99; Inactive
4.	Stigmasterol	890	IV	0.87; Inactive	0.54; Active	0.89; Inactive	0.82; Active	0.85; Inactive
5.	Eicosanoic acid, tetradecyl ester	5000	V	0.76; Inactive	0.87; Inactive	0.57; Inactive	0.99; Inactive	0.79; Inactive
6.	Hentetracontanoic acid	900	IV	0.52; Inactive	0.92; Inactive	0.53; Inactive	0.85; Inactive	0.99; Inactive
7.	Eicosanoic acid	900	IV	0.52; Inactive	0.92; Inactive	0.53; Inactive	0.85; Inactive	0.99; Inactive

8.	zeta-Carotene	5700	V	0.79; Inactive	0.65; Inactive	0.88; Inactive	0.59; Inactive	0.79; Inactive
9.	Kaempferol-3-O-rutinoside	5000	V	0.80; Inactive	0.89; Inactive	0.77; Active	0.63; Active	0.98; Inactive
10.	Apigenin 6-C-glucoside 8-C-arabinosideis	832	IV	0.81; Inactive	0.89; Inactive	0.67; Active	0.69; Active	0.54; Inactive
11.	Zeaxanthin	10	II	0.79; Inactive	0.59; Inactive	0.83; Inactive	0.61; Inactive	0.76; Inactive
12.	Luteolin-6-C-glucoside 8-C-arabinoside	1213	IV	0.83; Inactive	0.89; Inactive	0.70; Active	0.68; Active	0.55; Inactive
13.	Procyanidin B1	2500	V	0.74; Inactive	0.90; Inactive	0.59; Active	0.78; Active	0.99; Inactive
14.	Procyanidin C1	2500	V	0.74; Inactive	0.90; Inactive	0.59; Active	0.78; Active	0.99; Inactive
15.	Vicenin-2	832	IV	0.83; Inactive	0.89; Inactive	0.70; Active	0.68; Active	0.55; Inactive
16.	Luteolin-6-C-glucoside	159	III	0.81; Inactive	0.88; Inactive	0.67; Active	0.66; Active	0.63; Inactive
17.	Apigenin-8-C-glucoside	832	IV	0.81; Inactive	0.88; Inactive	0.67; Active	0.66; Active	0.63; Inactive
18.	Quercetin-3-O-galactoside	5000	V	0.82; Inactive	0.88; Inactive	0.76; Active	0.61; Active	0.67; Inactive
19.	Isorhamnetin	5000	V	0.72; Inactive	0.88; Inactive	0.64; Active	0.85; Active	0.82; Active
20.	Kaempferol	3919	V	0.68; Inactive	0.89; Inactive	0.62; Active	0.83; Active	0.91; Inactive
21.	Sulfasalazine (Reference Drug)	12500	VI	0.65; Active	0.82; Inactive	0.54; Active	0.63; Active	0.89; Inactive

Continued.

SL. No.	Phytocompounds (<i>Trevesia palmata</i>)	Toxicity end points						Nutritional toxicity
		Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity	Ecotoxicity	Clinical toxicity	
1.	Palmitic acid	0.63; Inactive	0.99; Inactive	1.0; Inactive	0.74; Inactive	0.63; Active	0.64; Inactive	0.93; Inactive
2.	1-Octadecanol	0.50; Inactive	0.97; Inactive	1.0; Inactive	0.83; Inactive	0.65; Active	0.67; Inactive	0.86; Inactive
3.	Oleic acid	0.64; Inactive	0.99; Inactive	1.0; Inactive	0.71; Inactive	0.65; Active	0.61; Inactive	0.89; Inactive
4.	Stigmasterol	0.60; Inactive	0.99; Active	0.98; Inactive	0.94; Inactive	0.65; Active	0.52; Inactive	0.66; Active
5.	Eicosanoic acid, tetradecyl ester	0.56; Active	0.96; Inactive	0.99; Inactive	0.78; Inactive	0.79; Active	0.81; Inactive	0.97; Inactive
6.	Hentetracontanoic acid	0.63; Inactive	0.99; Inactive	1.0; Inactive	0.74; Inactive	0.63; Active	0.64; Inactive	0.93; Inactive
7.	Eicosanoic acid	0.63; Inactive	0.99; Inactive	1.0; Inactive	0.74; Inactive	0.63; Active	0.64; Inactive	0.93; Inactive
8.	zeta-Carotene	0.76; Inactive	0.97; Inactive	0.98; Inactive	0.81; Inactive	0.62; Active	0.77; Inactive	0.83; Inactive
9.	Kaempferol-3-O-rutinoside	0.91; Inactive	0.95; Active	0.88; Inactive	0.64; Inactive	0.60; Inactive	0.52; Active	0.54; Active
10.	Apigenin 6-C-glucoside 8-C-	0.69; Inactive	0.85; Inactive	0.55; Inactive	0.81; Inactive	0.61; Inactive	0.50; Active	0.53; Active

	arabinosideis							
11.	Zeaxanthin	0.67; Inactive	0.92; Inactive	0.81; Inactive	0.89; Inactive	0.54; Active	0.52; Inactive	0.61; Inactive
12.	Luteolin-6-C- glucoside 8-C- arabinoside	0.72; Inactive	0.58; Active	0.50; Active	0.82; Inactive	0.60; Inactive	0.52; Active	0.52; Active
13.	Procyanidin B1	0.54; Inactive	0.92; Active	0.59; Inactive	0.80; Inactive	0.59; Active	0.52; Inactive	0.64; Active
14.	Procyanidin C1	0.54; Inactive	0.91; Active	0.59; Inactive	0.80; Inactive	0.59; Active	0.52; Inactive	0.64; Active
15.	Vicenin-2	0.72; Inactive	0.85; Inactive	0.50; Active	0.82; Inactive	0.60; Inactive	0.52; Active	0.52; Active
16.	Luteolin-6-C- glucoside	0.72; Inactive	0.65; Inactive	0.52; Active	0.87; Inactive	0.59; Inactive	0.51; Active	0.54; Active
17.	Apigenin-8-C- glucoside	0.72; Inactive	0.82; Inactive	0.52; Active	0.87; Inactive	0.59; Inactive	0.51; Active	0.54; Active
18.	Quercetin-3-O- galactoside	0.85; Inactive	0.66; Active	0.76; Inactive	0.69; Inactive	0.58; Inactive	0.51; Active	0.55; Active
19.	Isorhamnetin	0.68; Inactive	0.58; Active	0.94; Inactive	0.95; Inactive	0.55; Inactive	0.5; Inactive	0.57; Active
20.	Kaempferol	0.72; Inactive	0.96; Inactive	0.52; Inactive	0.98; Inactive	0.51; Inactive	0.54; Inactive	0.66; Active
21.	Sulfasalazine (Reference Drug)	0.77; Active	0.99; Inactive	0.86; Inactive	0.79; Inactive	0.85; Inactive	0.50; Active	0.71; Inactive

Table 3.9 Toxicity–ADMET-AI–based pathway toxicity profile of *Trevesia palmata* phytochemicals. The first part of the table (Toxicity–Tox21 nuclear receptor signaling pathways) summarizes predicted activities in the Tox21 nuclear receptor (NR) panel, encompassing major ligand-activated transcription factors. The continuation presented here (Toxicity–ADMET-AI, Tox21 stress response pathways) reports predicted probabilities of activation for nuclear factor erythroid 2–related factor 2/antioxidant response element signaling (nrf2/ARE), ATPase family AAA domain-containing protein 5 DNA-damage response reporter (ATAD5), heat shock response element (HSE), mitochondrial membrane potential disruption (MMP) and tumor protein p53–mediated stress response (p53). Values represent ADMET-AI–derived probabilities of pathway activation (0–1); sulfasalazine is included as a reference drug.

SL. No.	Phytochemicals (<i>Trevesia palmata</i>)	Toxicity-ADMET-AI									
		Toxicity			Tox21-Nuclear receptor signaling pathways						
		hrRG Blocking	DILI	Skin Reaction	AR	AR-LBD	AhR	Aro	ER	ER-LBD	PPAR-Gamma
1.	Palmitic acid	0.26	0.47	0.79	0.02	2.53e-04	1.44e-03	0.01	0.23	0.04	0.14
2.	1-Octadecanol	0.73	0.17	0.84	0.03	3.00e-04	2.27e-03	0.01	0.09	3.79e-03	2.06e-03
3.	Oleic acid	0.30	0.36	0.92	0.01	7.65e-04	0.01	0.02	0.12	0.03	0.33
4.	Stigmasterol	0.79	0.08	0.58	0.05	0.01	0.01	0.05	0.17	0.04	0.01
5.	Eicosanoic acid, tetradecyl ester	0.76	0.18	0.85	0.03	1.82e-03	1.37e-03	3.06e-03	0.09	4.51e-03	3.89e-03
6.	Hentetracontanoic acid	0.54	0.36	0.84	0.01	7.34e-04	1.28e-03	0.01	0.21	0.03	0.12
7.	Eicosanoic acid	0.40	0.42	0.84	0.02	4.11e-04	1.97e-03	0.01	0.26	0.05	0.17
8.	zeta-Carotene	0.63	0.22	0.94	0.01	0.11	4.55e-03	0.08	0.17	0.37	0.04
9.	Kaempferol-3-O-rutinoside	0.63	0.71	0.20	0.07	0.11	0.09	0.13	0.29	0.21	0.01
10.	Apigenin 6-C-glucoside 8-C-	0.59	0.76	0.19	0.05	0.06	0.12	0.13	0.20	0.14	0.01

	arabinosideis										
11.	Zeaxanthin	0.55	0.34	0.84	0.01	0.14	0.01	0.22	0.21	0.39	0.10
12.	Luteolin-6-C-glucoside 8-C-arabinoside	0.55	0.84	0.17	0.03	0.03	0.11	0.11	0.11	0.09	0.03
13.	Procyanidin B1	0.77	0.67	0.24	0.06	0.08	0.18	0.24	0.20	0.10	0.02
14.	Procyanidin C1	0.82	0.73	0.23	0.05	0.08	0.16	0.35	0.23	0.13	0.05
15.	Vicenin-2	0.51	0.82	0.15	0.03	0.03	0.09	0.10	0.11	0.09	0.02
16.	Luteolin-6-C-glucoside	0.58	0.79	0.34	0.07	0.10	0.29	0.18	0.25	0.21	0.02
17.	Apigenin-8-C-glucoside	0.55	0.75	0.32	0.07	0.10	0.25	0.17	0.29	0.22	0.02
18.	Quercetin-3-O-galactoside	0.59	0.81	0.31	0.09	0.14	0.22	0.17	0.26	0.22	0.01
19.	Isorhamnetin	0.52	0.92	0.76	0.05	0.04	0.88	0.26	0.54	0.56	0.04
20.	Kaempferol	0.45	0.90	0.84	0.08	0.04	0.88	0.25	0.75	0.71	0.03
21.	Sulfasalazine (Reference Drug)	0.09	0.98	0.08	0.08	0.01	0.11	4.52e-03	0.14	0.01	4.15e-03

Continued.

SL. No.	Phytochemicals (<i>Trevesia palmata</i>)	Toxicity-ADMET-AI				
		Tox21 Stress response pathways				
		nrf2/ARE	ATAD5	HSE	MMP	p53
1.	Palmitic acid	0.04	4.10e-04	0.02	0.02	4.62e-03
2.	1-Octadecanol	0.04	5.38e-05	0.03	0.02	4.97e-03
3.	Oleic acid	0.15	8.36e-04	0.20	0.07	0.01
4.	Stigmasterol	0.38	4.70e-04	0.21	0.39	0.01
5.	Eicosanoic acid, tetradecyl ester	0.04	6.93e-05	0.03	0.01	3.35e-03
6.	Hentetracontanoic acid	0.19	5.32e-05	0.05	0.01	0.01
7.	Eicosanoic acid	0.09	2.75e-04	0.03	0.02	0.01
8.	zeta-Carotene	0.95	0.03	0.74	0.10	0.08
9.	Kaempferol-3-O-rutinoside	0.15	0.06	0.02	0.11	0.19
10.	Apigenin 6-C-glucoside 8-C-arabinosideis	0.18	0.06	0.03	0.12	0.17
11.	Zeaxanthin	0.97	0.38	0.80	0.50	0.42
12.	Luteolin-6-C-glucoside 8-C-arabinoside	0.23	0.05	0.03	0.13	0.14
13.	Procyanidin B1	0.12	0.04	0.15	0.37	0.20
14.	Procyanidin C1	0.20	0.06	0.21	0.57	0.34
15.	Vicenin-2	0.20	0.04	0.04	0.11	0.11
16.	Luteolin-6-C-glucoside	0.21	0.09	0.04	0.19	0.26
17.	Apigenin-8-C-glucoside	0.18	0.07	0.04	0.18	0.21
18.	Quercetin-3-O-galactoside	0.22	0.09	0.03	0.18	0.29
19.	Isorhamnetin	0.71	0.28	0.16	0.82	0.40
20.	Kaempferol	0.77	0.21	0.11	0.89	0.24
21.	Sulfasalazine (Reference Drug)	0.09	0.02	4.35e-03	0.06	0.01

Table 3.10 Molecular docking-based evaluation of ligand with COX-2 receptor binding affinities using AutoDock Vina

(<http://vina.scripps.edu/>)

Rank	Phytocompounds	PubChem ID	Binding affinity (Kcal/mol)
1.	Kaempferol	5280863	-9.7
2.	Isorhamnetin	5281654	-9.5
3.	Sulfasalazine (Reference Drug)	5339	-7.8
4.	Zeaxanthin	5280899	-7.7
5.	Quercetin-3-O-galactoside	5281643	-7.7
6.	zeta-Carotene	5280788	-7.7
7.	Oleic acid	445639	-7.4
8.	Eicosanoic acid, tetradecyl ester	537381	-7.1
9.	Eicosanoic acid	10467	-6.8
10.	Luteolin-6-C-glucoside	114776	-6.7
11.	Palmitic acid	985	-6.3
12.	1-Octadecanol	8221	-6.3
13.	Apigenin-8-C-glucoside	5280441	-6.2
14.	Stigmasterol	5280794	-5.4
15.	Kaempferol-3-O-rutinoside	5318767	-4.6
16.	Apigenin 6-C-glucoside 8-C-arabinoside	131750832	-2.3
17.	Vicenin-2	3084407	-0.2
18.	Luteolin-6-C-glucoside 8-C-arabioside	3549960	0.0
19.	Procyanidin B1	11250133	8.6
20.	Hentetracontanoic acid	636113	13.9
21.	Procyanidin C1	169853	22.7

COX-2- Cyclooxygenase-2

Table 3.11 Molecular docking-based evaluation of ligand with KAEP1receptor binding affinities using AutoDock Vina (<http://vina.scripps.edu/>)

Rank	Phytochemicals	PubChem ID	Binding affinity (Kcal/mol)
1.	Sulfasalazine (Reference drug)	5339	-9.4
2.	Isorhamnetin	5281654	-8.8
3.	Kaempferol	5280863	-8.4
4.	Procyanidin B1	11250133	-8.2
5.	Procyanidin C1	169853	-8.1
6.	Kaempferol-3-O-rutinoside	5318767	-8.1
7.	Vicenin-2	3084407	-8.0
8.	Luteolin-6-C-glucoside 8-C-arabioside	3549960	-7.9
9.	Luteolin-6-C-glucoside	114776	-7.8
10.	Zeaxanthin	5280899	-7.8
11.	Apigenin-8-C-glucoside	5280441	-7.6
12.	Stigmasterol	5280794	-7.4
13.	Quercetin-3-O-galactoside	5281643	-7.2
14.	Apigenin 6-C-glucoside 8-C-arabinoside	131750832	-7.2
15.	zeta-Carotene	5280788	-7.1
16.	Oleic acid	445639	-6.8
17.	Palmitic acid	985	-6.7
18.	Eicosanoic acid	10467	-6.3
19.	1-Octadecanol	8221	-6.0
20.	Eicosanoic acid, tetradecyl ester	537381	-5.8
21.	Hentetracontanoic acid	636113	-3.2

KAEP1- Kelch-like ECH-associated protein 1

Table 3.12 Integrated molecular docking, interaction analysis assessment of the top-ranking bioactive compounds derived from *Trevesia palmata* complex with COX-2 and Keap1 target receptors.

Molecular docking and interaction analysis of COX2-ligand complex					
Sl. no	Ligand	Docked complex (COX2-ligand) interactions			
		Hydrogen bond	Distance(Å)	Hydrophibic bond	Distance(Å)
1.	Isorhamnetin	HIS 90	4.70	VAL 349	4.92
		TYR 355	5.32	VAL 523	4.98, 4.36
		PHE 518	4.05	LEU 352	4.33, 4.43
		GLN 192	5.24	LEU 384	5.24
		MET 522	5.21	MET 522	5.21
				TRP 387	6.42
2.	Kaempferol	HIS 90	4.67	VAL 349	5.03
		TYR 355	5.35	VAL 523	4.15, 4.15
		PHE 518	4.23	LEU 352	5.35, 4.45
		GLN 192	5.70		
3.	Sulfasalazine	SER 119	4.22	LEU 93	6.57
		SER 530	3.50, 4.21	VAL 116	3.93
		TYR 355	5.70	VAL 349	5.54
				LEU 531	6.80
				ALA 527	4.05, 3.89
				VAL 523	5.26
Molecular docking and interaction analysis of Keap1-ligand complex					
5.	Isorhamnetin	ARG 483	5.69	TYR 525	5.90
		ARG 415	7.73, 6.36	ALA 556	6.49
		GLN 530	5.08	ARG 415	4.83
		SER 555	4.25		

		SER 363	3.86, 4.01		
		ASN 414	5.13		
6.	Kaempferol	ARG 483	5.69	TYR 525	5.89
		ARG 415	7.64, 7.64	ALA 556	5.75
		SER 555	4.05		
7.	Sulfasalazine	GLN 528	4.97	TYR 525	4.27
		ASP 529	3.57	PHE 478	6.28
		SER 508	4.29		
		ARG 483	5.56, 6.85		
		ARG 415	6.22, 5.68, 5.20		

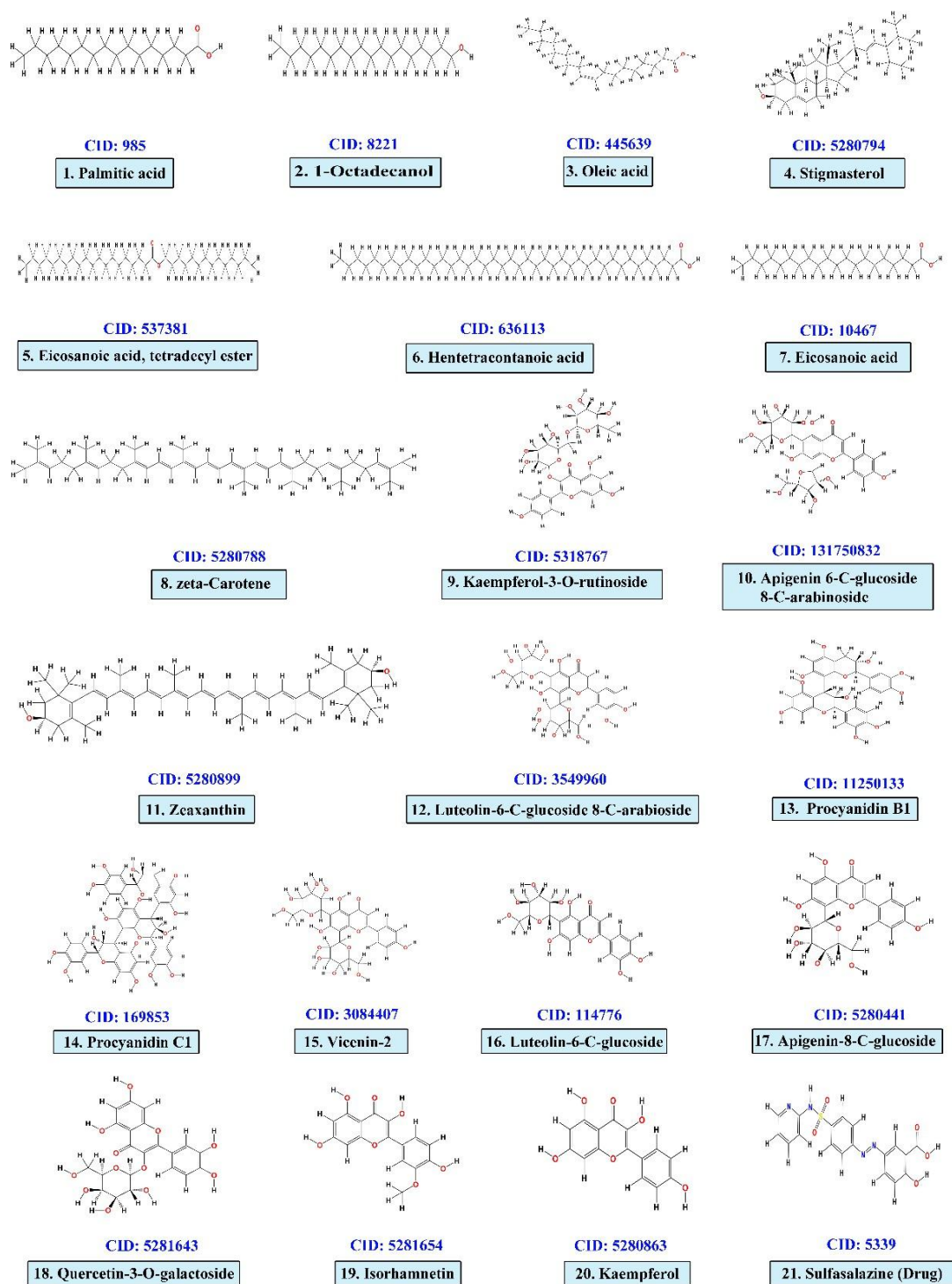


Figure 3.1 Two-dimensional chemical structures of the twenty selected phyto-ligands (1–20) derived from the methanolic flower bud extract of *Trevesia palmata* and the reference drug sulfasalazine (21), with corresponding PubChem CID numbers indicated below each structure.

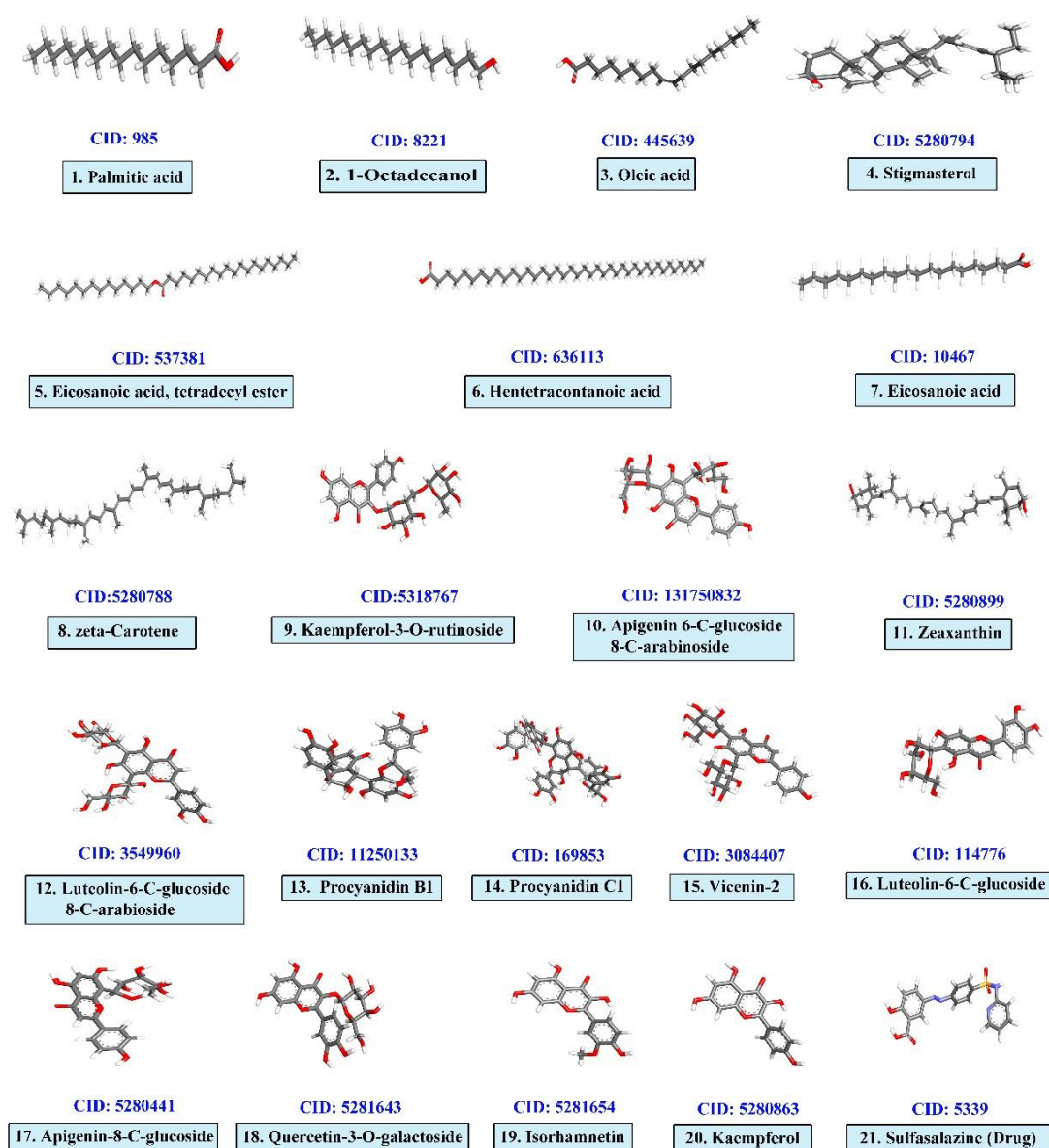


Figure 3.2 Three-dimensional structures of the twenty selected phyto-ligands (1–20) identified from the methanolic flower bud extract of *Trevesia palmata* and the reference drug sulfasalazine (21). The individual molecules are numbered and annotated with their corresponding PubChem CID identifiers as indicated beneath each structure.

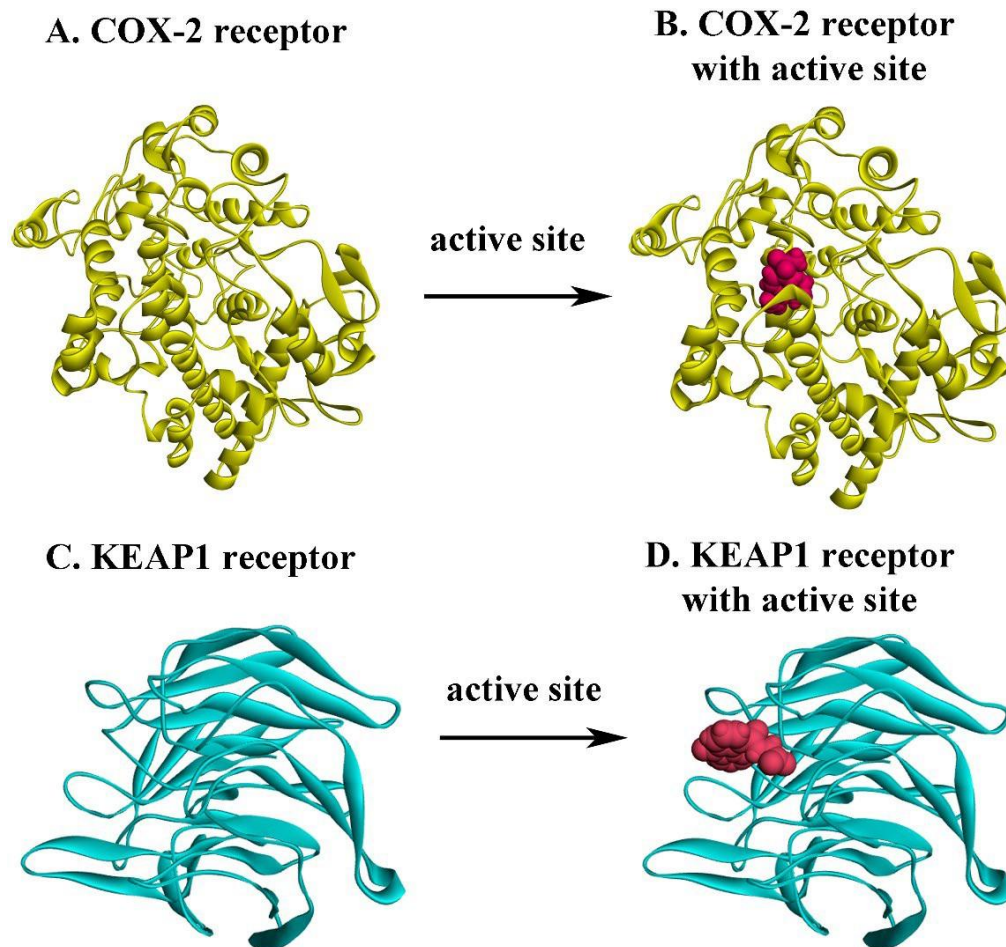


Figure 3.3 Three-dimensional representations of the target receptors and their native co-crystallized ligands used to define the docking active sites. (A) Cyclooxygenase-2 (COX-2) receptor. (B) COX-2 receptor with the native ligand occupying the catalytic pocket. (C) Kelch-like ECH-associated protein 1 (KEAP1) receptor. (D) KEAP1 receptor with the native ligand bound at the active site, delineating the binding region employed for molecular docking and simulation.

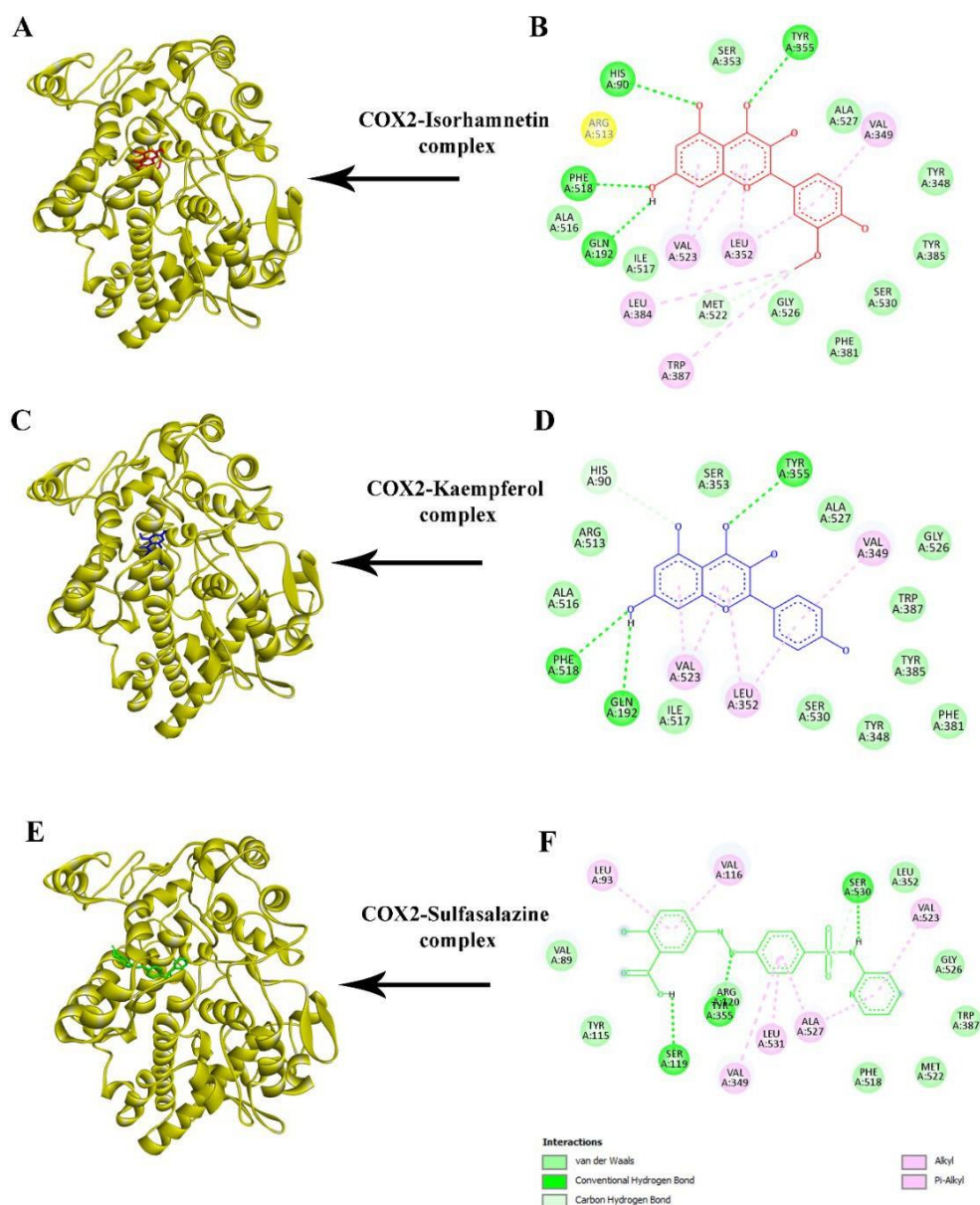


Figure 3.4 Molecular docking poses and ligand–receptor interaction profiles of *Trevesia palmata* phytoligands and the reference drug within the COX-2 active site. (A) Three-dimensional view of the COX-2–isorhamnetin complex. (B) Two-dimensional interaction diagram of isorhamnetin, illustrating contacts with key active-site residues. (C) Three-dimensional view of the COX-2–kaempferol complex. (D) Two-dimensional interaction map of kaempferol in the COX-2 binding pocket. (E) Three-dimensional view of the COX-2–sulfasalazine (reference drug) complex. (F) Two-dimensional interaction diagram of sulfasalazine. Interaction types are indicated by distinct color codes for van der Waals contacts, conventional hydrogen bonds, carbon–hydrogen bonds, alkyl interactions and π –alkyl interactions.

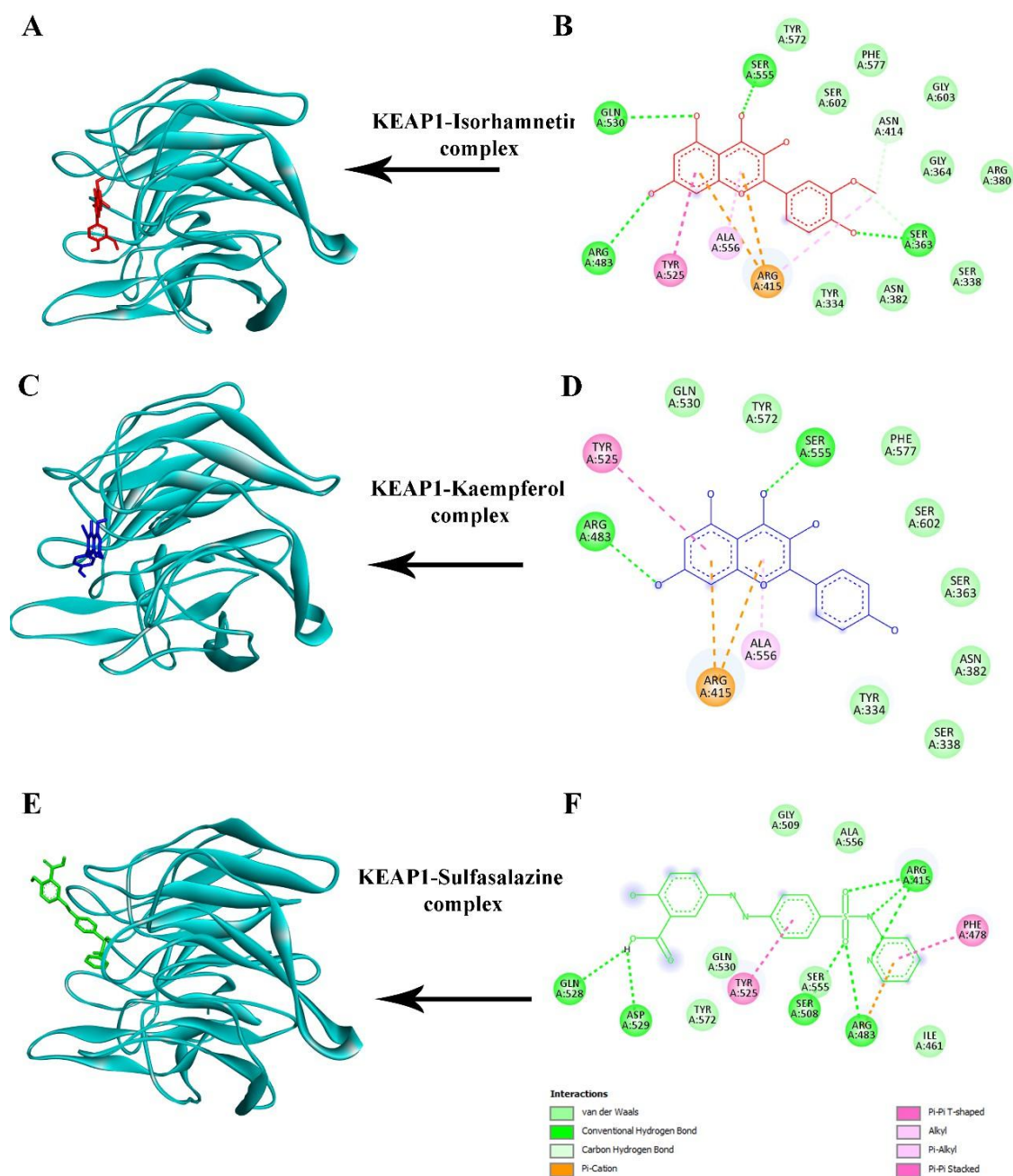


Figure 3.5 Docking poses and interaction profiles of *Trevesia palmata* phytoligands and the reference drug within the KEAP1 binding pocket. (A, C, E) Three-dimensional views of KEAP1 complexes with isorhamnetin, kaempferol and sulfasalazine, respectively. (B, D, F) Two-dimensional interaction diagrams highlighting key contacts between each ligand and Kelch-domain residues, with interaction types indicated by color-coded van der Waals, hydrogen-bond and hydrophobic/ π -interaction

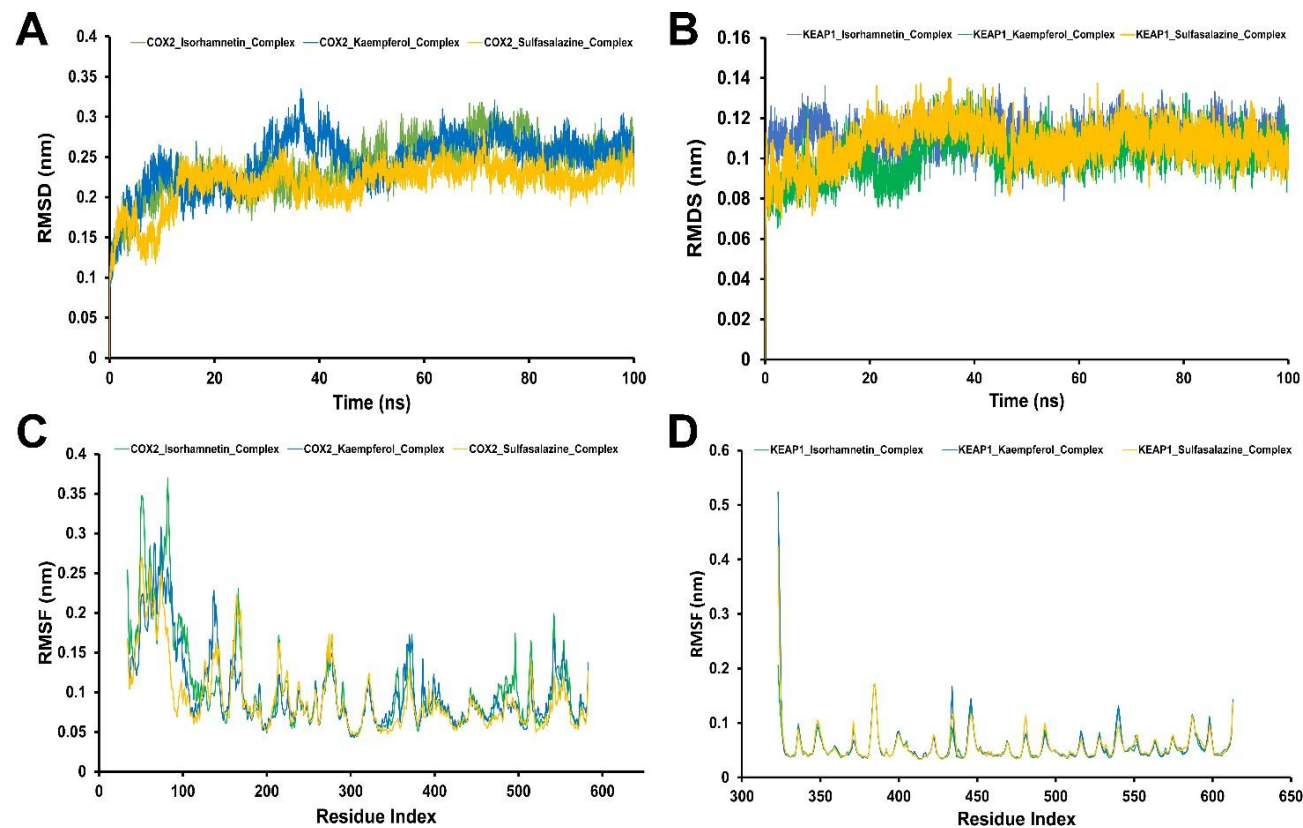


Figure 3.6 Molecular dynamics analysis of COX-2 and KEAP1 complexes with isorhamnetin, kaempferol and sulfasalazine. (A) Time evolution of backbone root mean square deviation (RMSD) for COX-2 complexes over 100 ns. (B) Backbone RMSD profiles of KEAP1 complexes over 100 ns. (C) Residue-wise root mean square fluctuation (RMSF) of COX-2 in complex with each ligand. (D) RMSF profiles of KEAP1 residues for the corresponding ligand-bound systems, illustrating local flexibility within the binding regions.

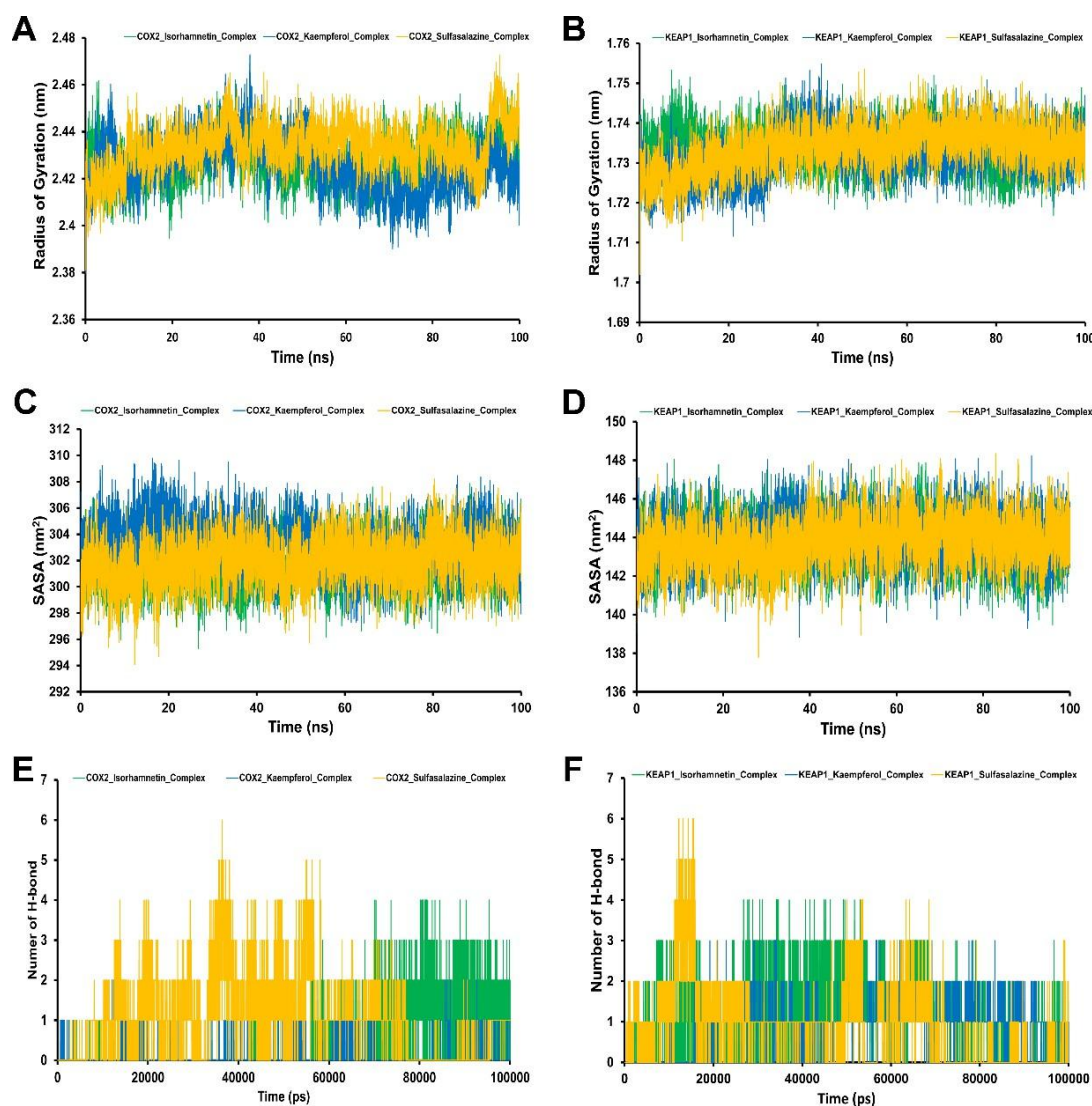


Figure 3.7 Time evolution of structural compactness, solvent exposure and hydrogen bonding in COX-2 and KEAP1 complexes with isorhamnetin, kaempferol and sulfasalazine. (A, B) Radius of gyration (Rg) profiles of COX-2 and KEAP1 complexes over 100 ns. (C, D) Solvent-accessible surface area (SASA) trajectories. (E, F) Time-dependent number of protein–ligand hydrogen bonds for COX-2 and KEAP1 complexes, respectively.

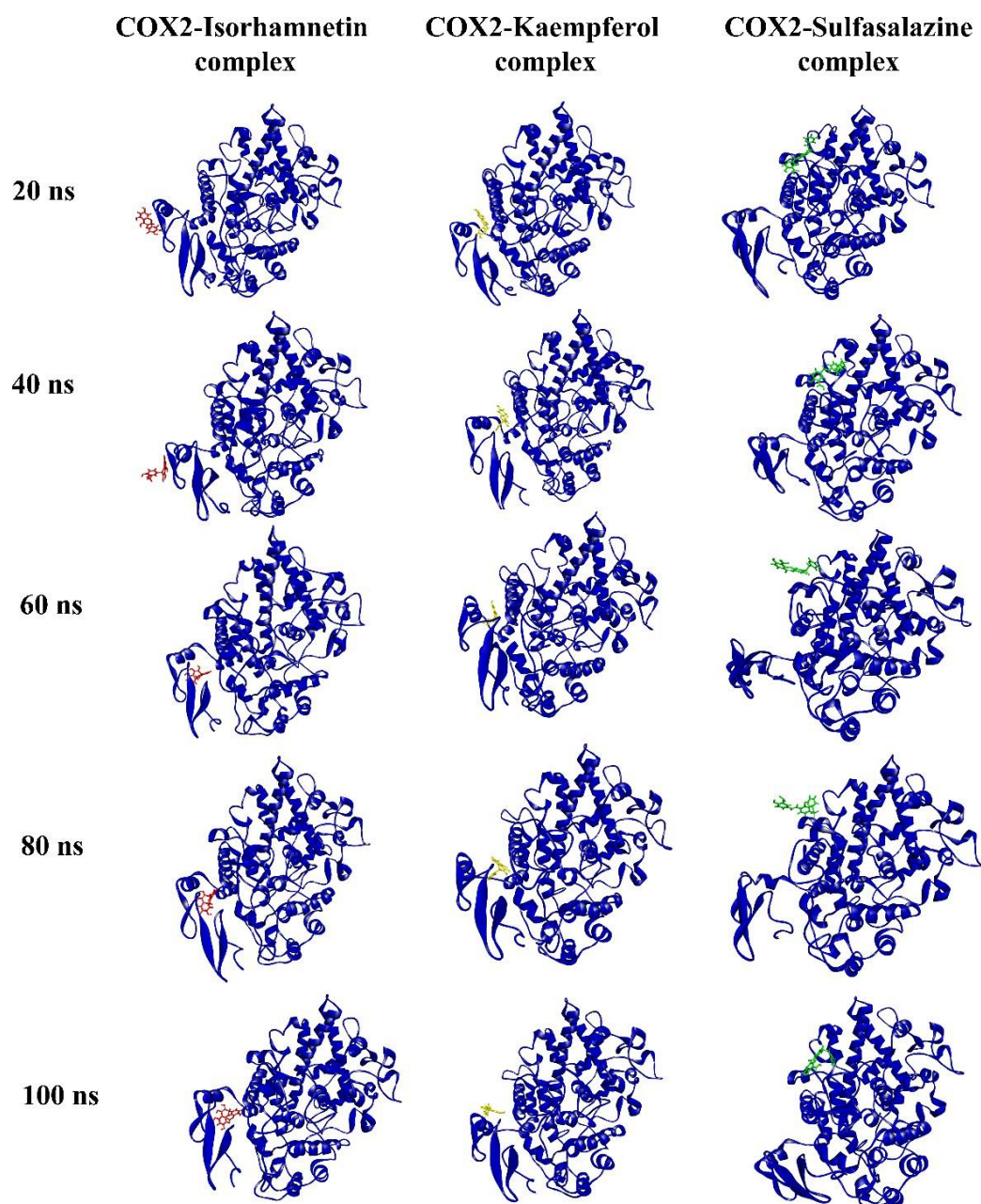


Figure 3.8 Time-resolved conformational snapshots of COX-2 complexes with isorhamnetin, kaempferol and sulfasalazine at 20, 40, 60, 80 and 100 ns of molecular dynamics simulation, illustrating the stability and persistence of ligand binding within the COX-2 active site over the 100-ns trajectory.

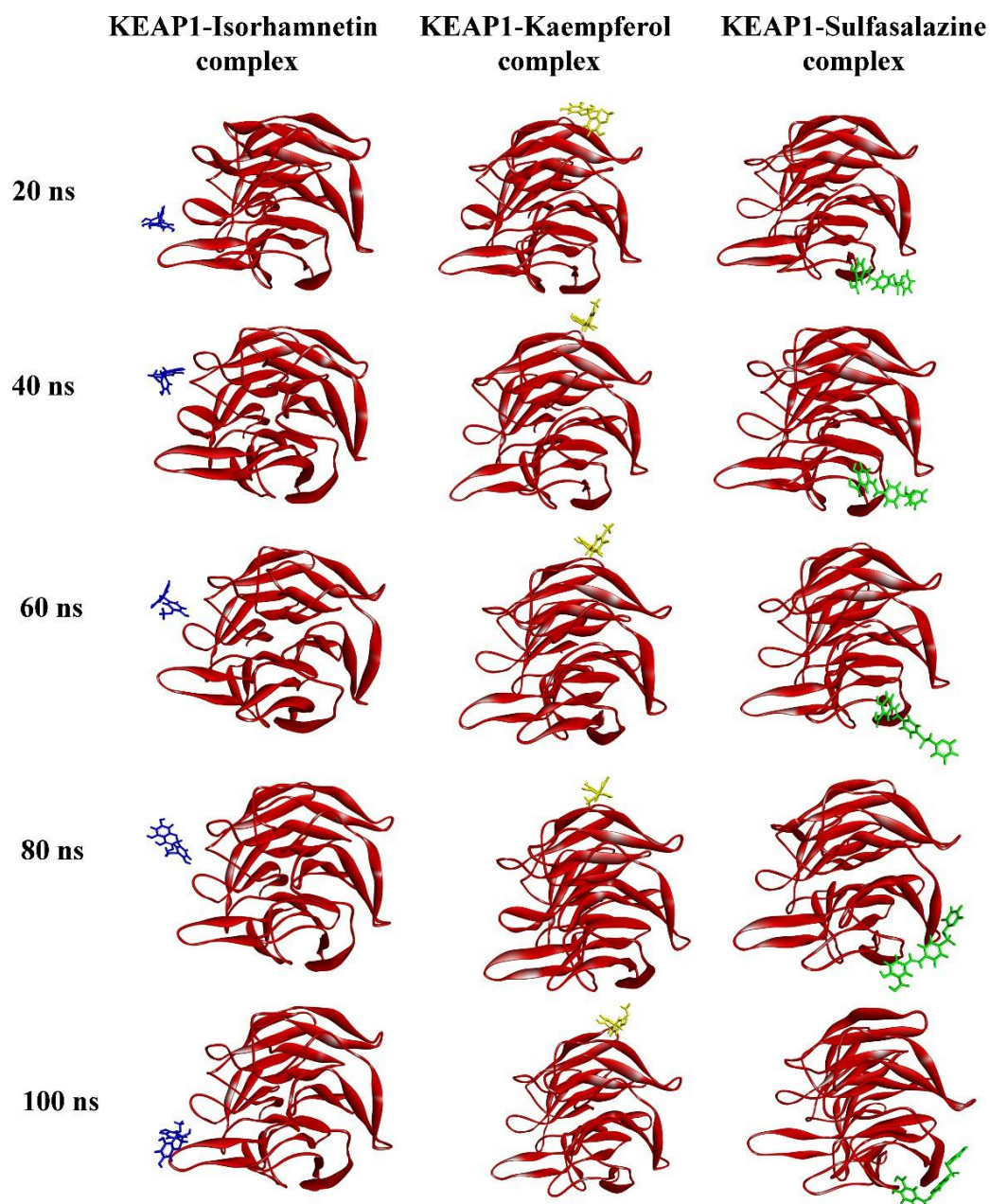


Figure 3.9 Time-resolved conformational snapshots of KEAP1 complexes with isorhamnetin, kaempferol and sulfasalazine at 20, 40, 60, 80 and 100 ns of molecular dynamics simulation, illustrating the persistence and orientation of ligand binding within the KEAP1 Kelch domain over the 100-ns trajectory.

Chapter 4

4.1 INTRODUCTION

Worldwide, healthcare systems rely heavily on medications derived from plants, but this is especially true in underdeveloped nations where traditional herbal remedies are widely used. Along with to being widely used and affordable, these treatments are frequently the sole option for people to keep themselves healthy. In order to cure illness and keep people well, traditional healers have long relied on medications produced from plants, minerals, and even animals. Many people in Asia, particularly in India and China, rely on these medications. Developed nations are increasingly turning to medications produced from plants because of the potential they provide to alleviate negative side effects and prevent drug resistance. But new research shows that many therapeutic plants have side effects as well. Chronic usage of certain medicinal plants may have harmful effects, which is a cause for caution. Any therapeutic plant extract that is to be utilized in clinical or preclinical settings must undergo a thorough evaluation of its toxicological consequences (**Porwal et al., 2017**).

4.1.1 Acute toxicity study

Acute toxicity is the term used to describe the harmful effects that happen promptly when a chemical or substances are exposed. It can also mean the negative symptoms that someone has just after taking a dose of a drug, whether it's one dose or many. Anything that compromises an organism's ability to adapt to new situations or reduces its overall performance is considered an unfavorable consequence. This includes both physiological and functional impairments (**Sass, 2000; Rhodes, 1993**). Acute toxicity studies seek to identify the dosage that induces mortality or significant toxicological consequences when delivered over a short duration (**OECD, 2001**). The conventional LD50 test, a statistically determined dosage anticipated to result in mortality for 50% of administered animals, has traditionally served as the foundation for the toxicological categorization of substances. Laboratory rodents, such as mice and rats, are the chosen species in regulatory testing (**Walum, 1998**). Acute toxicity studies may also evaluate clinical symptoms, alterations in body weight, pathology abnormalities, and organ toxicities to deliver a thorough assessment of probable

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adverse effects (**OECD, 2001**). Modern methodologies underscore the significance of monitoring animals for postponed mortality.

The area of acute toxicity testing has progressed in response to ethical considerations and the necessity to minimize animal utilization while preserving scientific integrity. Alternatives comprise the limit test, fixed-dose process, hazardous class method, and up-and-down procedures (**Bruce 1985; Walum, 1998; OECD, 2001**). The OECD Test Guideline 423 (Acute Toxic Class Method) employs three animals of the same sex for each phase, with future dose determinations reliant on death results. The Up-and-Down Procedure (OECD Test Guideline 425) administers doses to animals sequentially, adjusting the dosage upward or downward according to survival or mortality outcomes. These alternative methodologies have achieved worldwide regulatory approval and minimized animal utilization while delivering equivalent hazard categorization results (**Bruce 1985; OECD, 2001**).

The OECD has established three methodologies for evaluating acute oral toxicity: Guidelines 420, 423, and 425. These methodologies are integral to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), which classifies chemicals into various toxicity categories according to their LD50 values, with Category 1 denoting the most toxic substances ($LD50 \leq 5 \text{ mg/kg}$) and Category 5 indicating the least toxic ($2000 \text{ mg/kg} < LD50 < 5000 \text{ mg/kg}$) (**Bruce, 1985; Walum, 1998**). The harmonization initiatives have optimized international trade, instituted standardized safety protocols, and enhanced animal welfare via methodological improvements and decreased animal utilization. Regulatory authorities persist in enhancing these criteria in light of new scientific insights and validation studies to guarantee that acute toxicity testing is both scientifically rigorous and morally sound.

4.1.2 Sub-acute toxicity study

Sub-acute toxicity studies assess a substance's detrimental effects during repeated exposure over 14-28 days, yielding essential insights into target organ toxicity and dose-response correlations. The established methodology, as delineated

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in OECD Test Guideline 407, entails daily administration of the test chemical to rats at various dosage levels for a duration of 28 days. During the trial, animals are observed for clinical symptoms, mortality, food consumption, water intake, changes in body weight, and behavioral modifications. Upon research conclusion, thorough evaluations encompass hematology, clinical chemistry, gross pathology, organ weights, and histological analysis of tissues to ascertain possible target organs of toxicity (Gelbke et al., 2007; Ghauri et al., 2022; Nouioura et al., 2023). Data from acute toxicity studies, such as the usage of LD50 and the slope of the dose response curve, are often used to establish dosages (Agrawal and Paridhavi, 2007).

The sub-acute toxicity investigations produce comprehensive data sets that need meticulous evaluation to determine safety levels and detect possible issues. Two essential criteria generated from this research are the No Observed Adverse Effect Level (NOAEL), which denotes the maximum dosage that yields no identifiable adverse effects, and the Lowest Observed deleterious Effect Level (LOAEL), which indicates the minimum dose that produces noticeable deleterious effects (Colerangle, 2017).

4.1.3 Hepatic and renal function test

Since the liver is the primary site of drug metabolism and biotransformation, liver cells are an excellent candidate for toxicological and pharmacological testing (Soldatow et al., 2013). The combination of liver tissue-specific toxicity and general cell type-nonspecific cytotoxicity often leads to chemically induced liver damage (Tabernilla et al., 2021). The most common cause of liver dysfunction is drug-induced liver damage. It may result in a variety of symptoms, ranging from liver failure to mild, nonspecific ones including cholestasis, acute hepatitis, chronic hepatitis, and silent transaminitis (Aleempijevic et al., 2017). It may be triggered by a variety of pharmacological medications, herbal remedies, and nutritional supplements (Holt and Ju, 2010).

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Drug-induced liver injury is frequently indicated by an elevated liver function test result. In uncommon instances, it may result in fatalities (**García Rodríguez et al., 1997**). As an outcome, there is a possibility of increased levels of liver enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and total bilirubin (Ye et al., 2018). Symptoms of severe liver injury include abnormally elevated blood bilirubin levels, inadequate blood coagulation, and insufficient albumin levels (**Yu et al., 2017**). In instances of hepatocellular drug-induced liver injury, ALT levels are elevated in comparison to ALP levels. In cholestatic liver injury, **Katarey and Verma (2016)** observed that ALP levels are elevated in comparison to ALT levels (**Katarey and Verma 2016**).

The kidneys produce hormones like erythropoietin, 1,25 dihydroxy vitamin D, and renin, and they play a vital role in the removal of waste products and toxins like creatinine, urea, and uric acid. They also control the amount of fluid outside the cells, the concentration of electrolytes, and the osmolality of the blood serum (**Okoro and Farate, 2019; Damiati, 2019**). Kidney function may be investigated and evaluated using a variety of clinical laboratory tests. The most used endogenous measure for assessing glomerular function is creatinine. The body produces creatinine, a metabolic waste product, at a steady rate from creatine phosphate in muscle tissue. The primary function of the kidney is to eliminate creatinine from the circulation entirely. Elevated blood creatinine levels are facilitated by reduced renal clearance (**Gounden et al., 2023**). The nitrogenous chemical urea, known as BUN, is a byproduct of protein metabolism and the urea cycle that the liver produces. About 85% of urea is eliminated by the kidneys, whereas the other 15% is eliminated via the GI tract. Serum urea levels increased due to illnesses marked by impaired renal clearance (**Kamianowska et al., 2019**).

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4.1.4 Blood parameters

The assessment of hematological parameters can serve as a diagnostic tool for identifying the detrimental impacts of foreign substances on the blood components of an animal (**Ashafa et al., 2012**). Administering chemical substances at hazardous dosages can result in changes in blood parameters indicative of hematological disorders, such as anemia, characterized by reduced hemoglobin levels (**Price and Schrier, 2008**). A reduction in white blood cell generation, an increase in their use and destruction, or both are characteristics of neutropenia (**Bodey et al., 1982**). Reduced platelet generation, shortened platelet survival, and dilution of platelet counts by transfusion of low platelet count blood are the causes of thrombocytopenia, a disorder in which the number of platelets decreases. These disorders are also linked to cancers such myeloma, lymphoma, and leukemia (**Bradbury and Murray, 2013; Izak and Bussel, 2014**).

Blood parameters are essential indicators of an organism's actual physiological state. An organism has to keep its blood and its contents constant and consistent under normal conditions in order to function well (**Pankaj and Varma, 2013**). More accurate markers of human toxicity are hematological anomalies (**Yakubu et al., 2007**).

4.2 REVIEW OF LITERATURE

4.2.1 Acute and sub-acute toxicity study

The acute toxicity of *Trevesia palmata* extracts in rats has been assessed in a number of research (**Rahman et al., 2014; Victoria et al., 2019; Sayeed et al., 2014**). Usually, a single large dosage of the extract is given to the animals in these tests, and they are watched for symptoms of toxicity or death over a brief period of time typically 24 to 72 hours. According to the findings of these investigations, acute administration of methanolic extracts of *Trevesia palmata* leaves often has no notable negative effects, including behavioral, clinical, and biochemical abnormalities. However, depending on the dose, length of exposure, and endpoints assessed, sub-

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acute toxicity studies using leaf extract from *Trevesia palmata* (Victoria et al., 2019) have shown inconsistent findings.

Marak et al. (2023) evaluated the acute and sub-acute toxicity of the methanolic extract of *Cycas pectinate* fruit (CPFE) on mice through oral administration. The study acute toxicity was conducted using a single dosage of 1000, 3000, and 5000 mg/kg. while the sub-acute toxicity involved the daily administration of doses of 100, 300, and 500 mg/kg over a 28-day period, research on The LD50 was ascertained to be 4000 mg/kg. The results of the study showed that the levels of AST, ALT, ALP, and bilirubin were lower in the groups that received 1000 and 3000 mg/kg compared to the control group. Additionally, direct bilirubin was found to be diminished. The mice were found to be harmful at a dosage of 5000 mg/kg and 500 mg/kg in both the acute and sub-acute investigations. The acute toxicity findings indicated that CPFE had a minor toxic impact on the kidney at concentrations of 3000 and 5000 mg/kg. This was demonstrated by the observed deterioration of the kidney and an increase in creatinine levels. White blood cells (WBC) were elevated as a consequence of acute toxicity at a dosage of 3000 mg/kg. However, sub-acute toxicity assays do not suggest any adverse effects on hematological, renal, or hepatic markers. Consequently, it is possible to infer that CPFE would be deemed safe for ingestion when administered at a dosage of 100 and 300 mg/kg (Marak et al., 2023)

Porwal et al. (2017) examine the safety of the ethanolic extract of *Marsdenia tenacissima* leaves using acute and subacute oral toxicity assessments in albino rats. The study examines a deficiency of toxicological data for this historically used medicinal herb, with the objective of endorsing its safe application in medicines. In acute toxicity studies, rats were given a single high dosage (5000 mg/kg) of the extract and monitored for 14 days. Subacute toxicity was assessed in female rats with daily oral dosages of 250, 500, or 1000 mg/kg for a duration of 28 days. Measured parameters included body and organ weights, clinical manifestations, hematological and biochemical indicators, and histopathological examination of essential organs. The study findings demonstrate that there were no instances of mortality or toxic

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symptoms in either study, with no deaths or overt signs of toxicity observed, even at the highest dose administered. No notable differences were observed between the treated and control groups, indicating an absence of adverse effects on growth or organ health. Hematological and biochemical parameters indicated that all values remained within normal ranges, suggesting no effects on blood or major organ function. Histopathological examination of organs from treated rats revealed a normal microscopic structure, with only minor, non-significant alterations observed in a few specimens. The estimated lethal dose exceeds 5000 mg/kg, categorizing the extract within the lowest toxicity class as per OECD guidelines. The ethanolic extract of *Marsdenia tenacissima* leaves did not produce acute or subacute toxicity in rats at the administered doses. The research indicates the plant's safety for further pharmacological development; however, it suggests that additional studies, such as chronic toxicity and reproductive safety assessments, are necessary to comprehensively define its toxicological profile (Porwal et al., 2017).

Loha et al. (2019) assessed the safety of an 80% methanol extract of *Syzygium guineense* leaves in rats, concentrating on acute and subacute toxicity, including evaluations of organ histology, blood biochemistry, and hematology. The acute investigation demonstrated that doses of 2000 mg/kg and 5000 mg/kg resulted in no death, clinical toxicity, or aberrant behavior in rats. No notable alterations were seen in body weight or organ weights (liver and kidneys) relative to the control group. Both gross and microscopic examinations of the liver and kidneys revealed no abnormal alterations. The LD50 is expected to exceed 5000 mg/kg, signifying minimal acute toxicity. No fatalities or overt symptoms of toxicity were seen during the sub-acute trial period. Body weight and organ weights exhibited typical increases, with no significant deviations from the control group. Hematological markers (WBC, RBC, HGB, HCT, etc.) remained within normal limits and exhibited no significant differences compared to controls. Biochemical indicators of renal and hepatic function (urea, creatinine, ALT, AST, total protein) were stable, with the exception of a notable decrease in blood glucose levels in the treated groups. Histological examination of liver and kidney tissues revealed normal architecture, with no indications of damage or inflammation. In addition, the significant decrease

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in blood glucose indicates possible hypoglycemic effects, necessitating more pharmacological research. This work addresses a deficiency in the safety assessment of *S. guineense*, often used in traditional medicine (Loha et al., 2019).

Nouioura et al. (2023) examine the acute and subacute toxicity of an aqueous extract from the fruit of *Ferula communis* (FC-Ext), a plant commonly utilized in Moroccan traditional medicine, employing Swiss albino mice as the experimental paradigm. The acute toxicity investigation demonstrated that oral administration of FC-Ext up to 1.5 g/kg body weight did not elicit any discernible toxic effects in either sex, thereby designating this dosage as the No-Observed-Adverse-Effect Level (NOAEL). Toxic symptoms (e.g., anorexia, ataxia, hypoactivity, piloerection, syncope) and mortality were noted at elevated doses, particularly in females. The oral LD50 (lethal dose for 50% of subjects) was determined to be 3.6 g/kg body weight; for intraperitoneal treatment, the LD50 was 2.3 g/kg body weight. Intraperitoneal injection exhibited increased toxicity, resulting in the mortality of all animals at a dosage of 4 g/kg body weight. The sub-acute study (doses tested: 125, 250, and 500 mg/kg BW daily) found no significant alterations in body weight or overt indications of toxicity in either sex. In male mice, substantial increases in the weights of the liver, kidneys, and spleen were noted at the highest dosage (500 mg/kg), whereas females exhibited no such alterations. Hematological research indicated that in males, the highest dosage (500 mg/kg) resulted in significant reductions in red blood cells (RBC), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), and platelets. In females, reductions in hemoglobin (HGB), white blood cells (WBC), and certain subtypes of white cells were noted at the maximum dosage. A dosage of 250 mg/kg also decreased white blood cells and platelets in both genders. No notable alterations were observed in serum biochemical indicators of liver (ALT, AST) or kidney (urea, creatinine) function at any dosage. Histological analysis of the liver, kidney, and spleen demonstrated no notable tissue damage or abnormalities in any group (Nouioura et al., 2023).

The safety of hydro-alcoholic leaf extract of *Reinwardtia indica* (HALERI) in Charles Foster rats was examined by Upadhyaya et al. (2019), with an emphasis on

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both acute and sub-acute toxicity. Although *Reinwardtia indica* has long been used in folk medicine to treat a variety of illnesses, there was previously a lack of scientific evidence about its safety. Rats were given a single oral dosage of HALERI at 50, 250, 500, 1000, 2000, or 5000 mg/kg as part of the acute toxicity study. For seven days, observations were taken for any indications of toxicity or death. Sub-acute toxicity, for 28 days, rats received doses of 50, 250, 500, 1000, or 2000 mg/kg every day. For six weeks, a satellite group was given 2000 mg/kg. The results of the study showed that, up to 5000 mg/kg, no mortality or notable clinical symptoms of toxicity were seen in cases of acute toxicity. At the maximum dosage, no serious side effects were observed, only moderate sedation. Sub-acute toxicity, on the other hand, showed no discernible effects on appetite or growth in any group when compared to controls. There were no appreciable variations in the satellite group or in the red and white blood cell counts, hemoglobin, platelets, or other indices at 14 or 28 days. Tests of kidney (urea, creatinine, BUN) and liver (SGOT, SGPT, ALP) function revealed no appreciable changes at any dosage or time point. Analysis of the brain, liver, kidney, and heart showed no structural or cellular abnormalities in any of the groups. The hydro-alcoholic leaf extract of *Reinwardtia indica* exhibits non-toxicity at doses up to 5000 mg/kg in acute experiments and 2000 mg/kg in sub-acute (28-day) studies in rats. No notable detrimental effects were seen in behavior, metabolism, hematological parameters, organ function, or tissue architecture at these dosages (Upadhyaya et al., 2019).

4.3 MATERIALS AND METHODS

4.3.1 Collection, identification and extraction of *Trevesia palmata* flower buds

Flower buds of *Trevesia palmata* were collected from Tanhril village, Aizawl, Mizoram. The plant specimen associated with accession number 1016 was accepted by the Botanical Survey of India, Eastern Regional Centre, Shillong. The flower buds were thoroughly washed and air-dried at room temperature before being crushed into a fine powder using an electric grinder (Figure 1). The extraction process was followed by applying the cold maceration method (Kuate et al., 2006), in which approximately 100 g of coarsely powdered *T. palmata* flower buds was soaked in 400

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mL of methanol (100%) at a ratio of 1:4 (w: v) in a clean beaker. The mixed powder solution was then maintained at room temperature for 72 h. The solution was stirred for 6–7 h. Then, the solution was filtered using Whatman filter paper (Grade 1 circles-125 mm) to obtain the crude extract (TPFME) part of the solution. Finally, the filtered solution was evaporated in an oven at temperature 37°C to form a solid texture (**Figure 1**). For further study, the resulting substance was kept at -20°C in the freezer. (**Gahlot et al., 2018**).

4.3.2 Animal ethics

This study was conducted in accordance with the guiding principles established by the ARRIVE guidelines (**Du Sert et al., 2020**) regarding the care and use of laboratory animals. All experimental procedures involving animals were approved by the Institutional Animal Ethical Committee at Mizoram University, Aizawl, Mizoram, India (approval no. MZU/IAEC/2021-22/08, dated September 03, 2021). All necessary measures and care were implemented prior to the experiment to minimize the suffering and distress of the animals.

4.3.3 Animal maintenance

A total of 80 rats, male (N=40) and non-pregnant female adult (N=40) Wistar albino rats (*Rattus norvegicus*), aged 8-12 weeks and weighing 130-245 g, were obtained from the Animal Care and Housing Facility at Mizoram University, Aizawl, Mizoram. Male and female rats were housed separately, three per cage, in clean polypropylene rat cages measuring 421 × 290 × 190 mm. They were maintained in an environmentally controlled setting at a temperature of 20 ± 2 °C, with relative humidity between (50-70) %, and under a consistent photoperiod of 12 h of light and 12 h of darkness. The rats were provided a standard commercial rat pellet diet daily at regular intervals and ad libitum access to ultrapure water. The cages and housing areas were subjected to daily sanitation, and the health status of the rats was monitored regularly.

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4.3.4 In vivo toxicity study

4.3.4.1 Acute oral toxicity study

Acute oral toxicity of *T. palmata* methanolic extract (TPFME) was assessed in mature rats of both sexes in accordance with the OECD 423 guidelines (Acute Oral Toxicity-Acute Toxic Class Method) (OECD, 1996). This protocol implements a graduated approach to minimize animal utilization and distress. This method may preclude the administration of substances at levels known to induce severe pain or irritation due to their corrosive properties. Subjects displaying indicators of severe, persistent suffering, or excessive weight gain underwent humane euthanasia, with their outcomes interpreted equivalently to those of animals that perished during the trial. This procedure, which can be reiterated as required, yields a ranking of the test chemicals comparable to alternative acute toxicity testing protocols (OECD 420 and OECD 423; OECD 425). Following the administration of a single dose of the test compound, the investigators conducted methodical observations of the subjects and monitored the manifestations of discomfort, distress, behavioral alterations, or mortality.

4.3.4.1.1 Dosage selection, experimental groups and LD₅₀ calculation

In accordance with the OECD 423 Guidelines (OECD, 1996), the initial dosage was determined to be 2000 mg/kg body weight, which is one of the four predetermined dosages (5, 50, 300, and 2000 mg/kg body weight). Prior to the administration of the test substance, all experimental animals were fasted overnight, during which only food was withheld while water remained available. Animals were weighed individually and orally administered TPFME via ball-tipped gavage in accordance with their body weight. Animals received a single dose of TPFME in several groups, commencing with an initial low dose on day one, with subsequent doses increasing progressively based on observations from prior doses. All experimental animals were allocated to five distinct groups, comprising equal numbers of males and females (1:1), based on their average body weight, and were administered the following oral doses-

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GROUP	DOSE (p. o., 1 mL/100g b. w)	MALE + FEMALE = TOTAL (number; n)		
I (Control)	Vehicle only	5	5	20
II	TPFME 2000 mg/kg b. w	5	5	
III	TPFME 3000 mg/kg b. w	5	5	
IV	TPFME 5000 mg/kg b. w	5	5	

Following a single oral dose, the rats were subjected to a 3-hour fast, after which their general behavior and potential toxicity were monitored continuously for 30 min, intermittently for 4 h, and subsequently throughout a 24-hour period. The rats were examined daily for 14 days to observe any behavioral alterations, indications of toxicity, mortality, and latency of death. The dosages of TPFME were increased in response to the behavioral alterations and deteriorating conditions seen with successive lower doses. Dead or moribund rats were immediately taken out from the cages, followed by post-mortem inspection. Small portion of tissue samples were collected after death for histology study. The acute oral toxicity to determine the lethal dose 50 (LD50) was calculated using the arithmetic method (**Karber, 1931**).

$$LD50 = LD100 - \Sigma (a \times b) / n \quad (1)$$

where n is the total number of animals in a group,

a is the difference between two successive doses of administered

extract/substance,

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b is the average number of dead animals in two successive

doses, and LD_{100} is the lethal dose causing 100% death of all the test animals.

After calculation of LD_{50} value, the toxicity class was measured by using toxicity scale of Hodge and Sterner Scale (Ahmed, 2015).

4.3.4.2 Sub-acute oral toxicity study

The OECD 407 Guidelines (OECD, 2008) for a 28-Day Repeated Dose Oral Toxicity Study in rodents were adopted to conduct a sub-acute oral toxicity study. This 28-day study examined the effects of regular oral exposure, indicating the need for further investigation. It also facilitates the establishment of dose-response relationships and assists in determining the no-observed-adverse-effect level (NOAEL). The duration of the investigation enabled researchers to analyze the effects of sustained oral administration to evaluate the requirement for more comprehensive studies.

4.3.4.2.1 Dosage selection, experimental groups

In accordance with the acute toxicity study results and OECD 407 Guidelines (OECD, 2008), dosages for the sub-acute test were determined among four groups, including the control group. Each group comprised 10 adult Wistar albino rats of both sexes, consisting of five males and five females, in compliance with the established protocols. Animals received TPFME via oral gavage once daily for 28 days. Throughout the treatment period, the animals were monitored for physiological or behavioral alterations, consistent with acute toxicity assessment methodology. The rats were randomly divided into the following four groups:

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GROUP	DOSE (p.o., 1 mL/100g b.w)	MALE + FEMALE = TOTAL (number; n)		
I (Control)	Vehicle only	5	5	20
II (Low Dose)	TPFME 10 mg/kg b.w	5	5	
III (Moderate Dose)	TPFME 100 mg/kg b.w	5	5	
IV (High Dose)	TPFME 500 mg/kg b.w	5	5	

4.3.4.3 Sample collection and preservation

All items underwent a thorough cleaning process with detergent and a subsequent 95% ethanol rinse before sterilization. Following a 14-day feeding period, the rats were subjected to a 14-hour fast and then euthanized using mild chloroform-induced anesthesia (**Hasan et al., 2018**). By decapitation, and blood samples (2.5 3 mL per rat) were collected. A portion was placed in a 2.0 mL EDTA-coated tube for hematological analysis, while the remainder was transferred to a 1.5 mL Eppendorf tube without an anticoagulant (**Loha et al. 2019**). To allow for clotting, the samples were left undisturbed at room temperature for 20 min. An Eppendorf centrifuge was used to obtain serum by centrifugation at $3000 \times g$ for 15 min at 4 °C. The resulting serum was transferred to a sterile 1.5 mL Eppendorf tube and stored at -20 °C for biochemical analyses (**Hasan et al., 2018**). From each rat (both male and female), all vital organs, including the liver, kidneys, brain, heart, lungs, spleen, colon, ovaries, uterus, and testes, were swiftly extracted, individually weighed, and washed twice in ice-cold saline buffer (20 mM Tris-HCl, 0.14 M NaCl, pH 7.4). Certain tissue samples were preserved in Bouin's fixative, a mixture of saturated picric acid (75%), formaldehyde (25%), and glacial acetic acid (5%), while

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other portions were stored at -20 °C for the evaluation of oxidative stress antioxidant characteristics.

4.3.4.4 Daily monitoring-clinical signs and symptoms, body weight, food and water consumption, rectal temperature

The animals were thoroughly observed for 30 minutes after treatment, and then for 4 and 8 hours to look for any kind of anomalies. After then, throughout the final 14 days of acute and the final 28 days of sub-acute treatment, monitoring was conducted every 24 hours. Each group member's body weight (g), food consumption (g), water consumption (mL), and rectal temperature (°C) were recorded once a day between 6:00 AM and 6:00 PM during the treatment period. A digital probe attached to a lubricated metallic thermocouple (2 mm in diameter) was inserted approximately 2 cm below the rectum and held for one minute to get a steady reading for rectal temperature. At regular intervals throughout the day, any indications of discomfort, distress, or abnormal behavior were noted. Immediately after observation, every piece of data was recorded and saved for further examination.

Animals' body weight was measured every day, and the relationship between their final body weight (FW) and beginning body weight (IW) was used to determine their weight increase or loss status (%);

$$\text{Weight gain/loss (\%)} = (\text{FW}-\text{IW})/\text{IW} \times 100 \quad (\text{Bidanchi et al., 2022}).$$

4.3.4.5 Relative organ weight measurement

Following sacrifice of the rats on the 15th day for acute toxicity test and the 29th day for sub-acute toxicity, the vital organs including the liver, kidney, lungs, spleen etc. were removed, body fats were carefully removed from the organs and dried using absorbent tissues paper before being measured for weight. The relative organ's weight was determined and documented as a ration to the bodyweight using following formula (Nalimu et al., 2022).

$$\text{Relative organ weight (\%)} = (\text{Organ weight}/\text{Body weight}) \times 100$$

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4.3.4.6 Haematological analysis

The acid hematin method, employing Sahli's hemoglobinometer, was used to measure blood hemoglobin (Hb) concentration. A hemocytometer was used to quantify the total red blood cell (RBC) count, total white blood cell (WBC) count, and packed cell volume (PCV), as described by Thrall and Weiser, (2002). Moreover, RBC-related parameters such as mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) were derived using conventional formulas. To assess the differential leukocyte count (DLC), a blood film was prepared, stained with Leishman stain, and examined under a microscope hemocytometer (Thrall and Weiser, 2002).

4.3.4.7 Biochemical analysis of serum

Serum samples kept at -20 °C were utilized for the investigation of many indicators of hepatorenal function and lipid profiles. The samples were thawed to room temperature, and several serological tests were conducted individually for males and females in triplicate. Serum concentrations of total protein (Gornall et al., 1949), albumin (Doumas et al., 1971), alkaline phosphatase (ALP, Kind and King, 1954), alanine aminotransferase (ALT), aspartate aminotransferase (AST, s and Frankel, 1957; Mury, 1984), creatinine (Bonsnes and Taussky, 1945), urea (Berthelot, 1859), and uric acid (Yahia, 2019) were assessed using commercially available kits (Coral Clinical Systems, Goa, India; Euro Synergy Bio, Chennai, India), while total and direct bilirubin (Garber, 1981) were measured utilizing a diagnostic kit produced by Asritha Diotech India Pvt. Ltd (Euro Diagnostic Systems, Chennai, India), in accordance with the manufacturer's protocol. Blood urea nitrogen (BUN) and globulin levels were calculated using the formulae ($\text{BUN} = \text{urea} \times 0.467$) and ($\text{total protein (g/dL)} - \text{albumin}$), respectively. Correspondingly, the ratios of AST to ALT and albumin to globulin were calculated by dividing the former by the latter. The aforementioned parameters were assessed to determine the state of hepato-renal functioning across all treatment groups in comparison to the control group. The lipid profile indicators, including triglycerides (Trinder, 1969a; Bucolo and David, 1973), cholesterol (Trinder, 1952), and high-density lipoprotein (HDL) cholesterol

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(**Mire and Snow, 1986**), were assessed using commercial diagnostic kits from Coral Clinical Systems, Goa, India, whereas Low-density lipoprotein (LDL) cholesterol was determined using the formula established by Friedewald et al. (**1972**), whereby

$$\text{LDL cholesterol} = (\text{Total cholesterol}) - (\text{HDL cholesterol} + \text{Triglyceride}/5)$$

Blood glucose levels (**Trinder, 1969b**), calcium ions (**Gitelman, 1967**), sodium ions, potassium, and chloride ions (**Maruna and Tie, 1958**) were individually evaluated in all groups utilizing coral clinical kits (Coral Clinical Systems, Goa, India; Euro Synergy Bio, Chennai, India) in accordance with the manufacturer's protocol.

4.3.4.8 Oxidative stress and antioxidant enzyme status

4.3.4.8.1 Sample homogenate preparation

The tissue sample was homogenized in a 10% w/v homogenate using ice-cold phosphate buffer saline (PBS, 1X), which contained 137 mM sodium chloride (NaCl), 2.7 mM potassium chloride (KCl), 10 mM sodium phosphate dibasic (Na_2HPO_4), and 1.8 mM potassium phosphate monobasic (KH_2PO_4) dissolved in distilled water.

4.3.4.8.2 Quantification of protein in the tissue sample

Protein quantification was performed using Bradford's technique (**Bradford, 1976**). The experiment was conducted by diluting 1 μL of Bradford's reagent. The absorbance was measured promptly using a UV-VIS spectrophotometer (Eppendorf, BioPhotometer) at 595 nm, using a blank that included all components except for 1 μL of distilled water in place of the tissue sample. The protein content in the sample was expressed in micrograms (μg).

4.3.4.8.3 Measurement of tissue lipid peroxidation (LPO) and malondialdehyde (MDA) levels

MDA is the end point of lipid peroxidation at the cellular or tissue level; hence, the levels of lipid peroxidation are directly proportional to the concentration

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of MDA. MDA was identified following the methodology outlined by Ohkawa et al. (1979). The fundamental premise of the procedure involves permitting MDA to react with Thioburbituric acid (TBA) subsequent to the precipitation of the sample's protein using Trichloroacetic acid (TCA). A 10% tissue homogenate (in 1X PBS, w/v) was combined with newly made 15% TCA in a 1:1 ratio, resulting in the precipitation of the proteins in the sample. The combination underwent centrifugation at $10,000 \times g$ for 30 minutes, and the supernatant was meticulously collected. A new solution of 0.8% TBA was produced and combined with the supernatant in a 1:1 ratio, then heated to a boil at 95 °C for 25 minutes. The solution was let to cool to ambient temperature, and the optical density was measured at 532 nm against a blank, free of protein lysate. MDA concentration was quantified as nmol/mg of protein (Ohkawa et al., 1979).

4.3.4.8.4 Superoxide dismutase (SOD) assay

The tissue concentration of SOD was measured according to the methodology established by Kakkar et al. (1984). In summary, to 50 μL of 10% tissue homogenate (w/v), 60 μL of 25 mM sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7$) buffer (pH 8.3), 25 μL of 186 μM phenazine methosulphate (PMS), and 75 μL of 300 μM nitro blue tetrazolium (NBT) were included. The reaction commenced with the addition of 50 μL of 760 μM nicotinamide adenine dinucleotide (NADH), leading to the production of the NADH-phenazine methosulphate-nitro blue tetrazolium complex after a 90-second incubation at 30 °C. The reaction was promptly halted by the addition of 250 μL of acetic acid and 500 μL of n-butanol to the assay mixture. The mixture was centrifuged at $10,000 \times g$ for 10 minutes at room temperature, resulting in the extraction of the colored complex using n-butanol. An assay mixture lacking tissue homogenate served as the control for the experiment. The color intensity of the supernatant was quantified at 560 nm, utilizing n-butanol as a blank reference. The value of SOD was given as U/mg of protein (Kakkar et al., 1984).

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4.3.4.8.5 Catalase (CAT) assay

The catalase assay was conducted using the methodology and principles outlined by Hadwan (2018), with minor modifications from the approach of Aebi et al. (1984). Initially, hydrogen peroxide (H_2O_2) in the test mixture was decomposed by catalase (CAT) present in the tissue homogenate (10%, w/v). This occurred following the combination of 10 μL of tissue homogenate with 100 μL of 10 mM hydrogen peroxide (H_2O_2), followed by a 2-minute incubation at 37 °C. The working solution comprising 110 mM cobalt nitrate $[\text{Co}(\text{NO}_3)_2]$, 10 mM sodium hexametaphosphate $[(\text{Na}(\text{PO}_3)_6; \text{Graham salt}]$, and 2.14 M sodium bicarbonate (NaHCO_3) was introduced to the assay mixture and incubated for 10 minutes at ambient temperature in darkness, leading to the synthesis of carbonato-cobaltate $[\text{Co}(\text{CO}_3)_3\text{Co}]$ following the reaction of $\text{Co}(\text{NO}_3)_2$ with residual H_2O_2 . The solution had an olive-green hue, presenting a distinct absorbance peak at 440 nm, evaluated against pure water as a blank. A control without tissue lysate was also generated to measure the initial presence of H_2O_2 in the test mixture. CAT was quantified as U/mg of protein (Aebi et al., 1984).

4.3.4.8.6 Glutathione peroxidase (GPx) assay

The GPx test was conducted following a formerly established methodology (Paglia et al., 1967). The test combination was constituted by amalgamating 20 μL of a solution comprising 0.8 mM EDTA, 10 mM sodium azide, 4 mM reduced GSH, and 2.5 mM H_2O_2 , with 40 μL of phosphate buffer and 10 μL of homogenate. The mixture was incubated at 37 °C for 10 minutes, following which the reaction was halted by the addition of 50 μL of 10% trichloroacetic acid and subsequent centrifugation at 2000 rpm. A 50 μL aliquot of the resultant supernatant was combined with 300 μL of disodium phosphate (0.3M) and 100 μL of DTNB (4 mg in 10 ml of 1% sodium citrate). Absorbance was quantified at 412 M. A control blank including all reagents, excluding the tissue homogenate, was utilized for comparison. GPx activity was determined as $\mu\text{mol GSH oxidized/min/mg protein}$ utilizing the subsequent equation: GPx activity is calculated as the change in absorbance per unit time multiplied by the total reaction volume of the GSH standard, divided by the

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product of the standard absorbance and the molecular weight of GSH (307.3235 g/mol). Sample x protein concentration (mg). The GSH standard was formulated at a concentration of 20 mg per 100 ml.

4.3.4.9 Histopathological analysis

The tissue sample was prepared for the histopathological analysis by pre-fixing it in Bouin's fixative for 24 hours and preserving it in 70% alcohol (v/v). After being dehydrated using various alcohol grades, the liver, kidney, brain, heart, lung, spleen, colon, ovaries, uterus, and testis were cut into minute pieces, immersed in paraffin block, and then cut into sections that were 5 μ m thick (Leica, RM 2125 RTS, Leica systems, Mumbai, India). After allowing the cut tissue sections to stick to the pre-coated microscope slides, they were cleaned with xylene, dipped in progressively stronger alcohol, stained with hematoxylin solution, counterstained with eosin solution, and then appropriately dehydrated, cleaned with xylene, and mounted using DPX (dibutylphthalate polystyrene xylene) (Jensen, 2008). To determine whether there was any degree of organ damage in the treatment group, all tissue sections were examined under a microscope (Leica DM 2500, Leica Microsystems, Wetzlar, Germany) equipped with a computerized digital camera (DFC 450C, Leica Microsystems, Wetzlar, Germany). The results were compared to the control group.

4.3.4.10 Statistical analysis

All quantitative data are presented as the mean $\pm$ standard error of the mean (SEM). Statistical comparisons among experimental groups were carried out using one-way analysis of variance (one-way ANOVA), which was employed to determine whether there were overall significant differences between group means. A probability value (P) of less than 0.05 was considered to indicate statistical significance for all analyses, and only differences meeting this threshold were interpreted as meaningful in the context of the study.

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4.4 RESULTS

4.4.1 Acute toxicity study

4.4.1.1 Clinical signs, mortality, and LD₅₀ determination

A number of clinical signs were observed in each group for 14 days such as loose stool, red tear, change in skin, movement, ear bleeding, eye colour change, diarrhoea, coma drowsiness, tremor, salivation, sedation, swollen paws with haemorrhage paralysis and death listed according to dose level in **Table 4.1**. The extract of TPFME does not show any changes in clinical signs and death at dose of 2000 and 3000 mg/kg but dose of 5000 mg/kg treatment observed few clinical signs such as slow movement in both male and female rats, loose stool and diarrhoea only in female group of rats, respectively. Mortality was only observed in both male and female group of rats, at dose of 5000 mg/kg. The percentage of death in male was 60% and in female was 80%. Respective dose-wise, percentage of death was recorded and used to calculate the lethal dose 50 (LD₅₀) values for both male and female Wister albino rats. Following OECD 423 guidelines and performing by arithmetic method according to Karber (1931), LD₅₀ of 4400 mg/kg in male and 4200 mg/kg in female were determined (**Table 4.2**). The toxicity class was determined for treatment of TPFME as slightly toxic from the table (**Table 4.3**).

4.4.1.2 Body weight, food, water consumption and rectal temperature

In both male and female rat at the end of the experiment were observed no statistically significant variation changes in the body weight (g) (**Figure 4.1 A and B**), food consumption (g) (**Figure 4.1 C and D**), water consumption (mL) (**Figure 4.1 E and F**) and rectal temperature (C°) (**Figure 4.1 G and H**) compared with control group and treated group at the dose of 2000 mg/kg and 3000 mg/kg. Moreover, there were also no significant difference of the initial, final body weight and the percentage of weight gain/loss in both male and female rats in treated group compare to control (**Table 4.4**). All the measurement of different observation in male and female group were recorded and tabulated in the form of mean $\pm$ SEM. Statistical comparison was performed using one-way ANOVA followed by Tukey's

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post hoc tests for all pair-wise multiple comparisons. Different alphabets in **(Figure 4.1 A-H)** and **(Table 4.4)** indicate that treatment groups are significantly different at $p < 0.05$.

4.4.1.3 Absolute weight and relative organ ratio

Following to the sacrifices of animals from each group on the 15th day, in male and female rats, there were no statistically significant change ($p > 0.05$) in the absolute weight (g) and relative organ ratio (%) of organ such as liver, kidney, brain, heart, spleen, colon, stomach, pancreas, lungs, testis, seminal vesicle, ovaries and uterus when comparing the control group to the treated groups (2000 and 3000 mg/kg). All values are expressed in Mean $\pm$ SEM, $n = 5$ animals/group/sex. Statistical comparison was performed using one-way ANOVA followed by Tukey's post-hoc tests for all pair-wise multiple comparisons. Different alphabets in **(Table 4.5)** row wise indicate that treated and non-treated groups. similar alphabets indicate not statistically significant at $p > 0.05$.

4.4.1.4 Status of hematological parameters

At the end of treatment, the effects of a single dose of TPFME resulted in modifications to the hematological parameters of both male and female rats in the experimental and control groups, as shown in the tables, respectively. The total count of RBC, WBC, and percentage of NP cells increased considerably ($p < 0.05$) in both male and female rats at a dosage of 3000 mg/kg as compared to the control group. Hemoglobin (Hb.), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), lymphocytes (LC), monocytes (MC), eosinophils (EP), and platelets (PLT) did not differ significantly between the sexes when compared to the control group ($p > 0.05$). Additionally, the counts of all the hematological parameters were within the normal range **(Table 4.6)**

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4.4.1.5 Serum biochemical parameters

The examination of blood serum biochemical tests of various liver and kidney biomarkers and metabolites in both male and female rats showed that TPFME oral toxicity no changes in the liver except ALT level and no effect on the kidney function test. The levels of liver biomarkers ALT (**Figure 4.2 B**) concentration altered statistically significant ($p < 0.05$) at the dose of 3000 mg/kg when compared with control and 2000 mg/kg in both the sexes (**Figure 4.2**). The levels of AST, ALP (**Figure 4.2 C and F**); total protein; albumin, globulin; A/G ratio (**Figure 4.3 G and H**); Total bilirubin and Direct bilirubin (**Figure 4.4 A-D**); Creatinine and Uric acid (**Figure 4.5 A-D**); Urea and BUN (**Figure 4.6 A-D**) were no significant changes ($p > 0.05$) as compared between control and treatment group (**Table 4.7**)

The serum levels of lipid profiles, blood glucose, and electrolyte concentrations in male and female rats are presented in the appropriate tables. In male rats, a significant decrease ($p < 0.05$) in triglyceride levels was observed at a dose of 3000 mg/kg when compared to the control group, while no significant differences ($p > 0.05$) were noted in total cholesterol, HDL, or LDL levels. In female rats, a significant reduction ($p < 0.05$) in total cholesterol and triglyceride levels was observed at different doses; however, no significant differences ($p > 0.05$) were found in HDL and LDL levels compared to the control group. Nonetheless, a notable reduction in blood serum glucose levels was observed in both genders. Furthermore, the assessment of some vital ions or electrolyte concentrations demonstrated a significant rise ($p < 0.05$) in calcium and potassium ions in males, whereas a decrease in calcium ion concentration was observed in females within the treatment group compared to the control group (**Table 4.7**)

4.4.1.6 Oxidative stress and antioxidant status

The acute toxicity study (14 days) involving single-dosage administration of TPFME in male and female rats revealed no differences in oxidative stress indicators, including MDA levels, or in antioxidant enzymes such as SOD, GSH-Px, and CAT within the treatment group. No significant difference ($p > 0.05$) was seen

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when comparing the control group to the treated groups at dosages of 2000 and 3000 mg/kg in both male and female rats (**Table 4.8**).

4.4.1.7. Histopathological analysis

4.4.1.7.1 Histopathology of liver

Hematoxylin and eosin (H&E)-stained liver sections from both male and female rats in the control group (Figure X) revealed normal hepatic architecture. Widely spaced central veins (CV) were surrounded by anastomosing cords of polyhedral hepatocytes (HC) displaying compact cytoplasm, Kupffer cell (K), centrally located round nuclei (single or binucleated; BH), and prominent nucleoli. Distinct hepatic sinusoids (S) separating the hepatocytes were clearly visible in these sections.

Similarly, liver tissues from male and female rats administered with 2000 mg/kg and 3000 mg/kg of the plant extract exhibited preserved hepatic architecture comparable to the control group. Prominent central veins, hepatocytes, and hepatic sinusoids were histologically distinguishable. However, in females, hepatocytes demonstrated polyhedral morphology with slightly flocculent cytoplasm, while the overall parenchymal organization remained unaffected.

In contrast, liver sections from animals treated with the highest dose of 5000 mg/kg (Figure W) revealed marked histopathological alterations. These included extensive endothelial disruption, congested central veins (CCV), degenerative hepatocytes (DH), and pronounced vacuolar degeneration (V). Inflammatory cell infiltration (IF) was evident in male rats, whereas female rats displayed notably dilated hepatic sinusoids (DS). These findings indicate dose-dependent hepatic alterations, with significant tissue damage observed at the highest concentration of the extract (**Figure 4.7; Figure 4.8**)

4.4.1.7.2 Histopathology of kidney

Hematoxylin and eosin (H&E)-stained sections of renal cortex and medulla from both male and female control groups (Figure) revealed normal renal

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histoarchitecture. The Malpighian corpuscles exhibited glomeruli (G) with compact organization, surrounded by Bowman's capsule (BC) and distinct Bowman's space (BS). The proximal convoluted tubules (PCT), distal convoluted tubules (DCT), collecting tubules (CT), and medullary renal tubules (@) also appeared normal, with well-preserved vasculature. In the treatment groups receiving 2000 and 3000 mg/kg, renal sections maintained compact cortical and medullary organization; however, at 3000 mg/kg, mild congestion of the medullary tubules was observed in both males and females. In contrast, administration of 5000 mg/kg produced pronounced alterations in renal histology in both sexes, characterized by distorted Malpighian corpuscles with atrophied glomeruli (AG), congested collecting tubules (*), and evidence of hemorrhage (\$) along with congestion of the medullary rays (black arrow) (**Figure 4.7; Figure 4.8**)

4.4.2 Sub-acute toxicity study

4.4.2.1 Clinical signs and mortality

Following 28 days of repeated oral administration of the methanolic extract of *T. palmata* flower buds to Wistar albino rats, no abnormalities were observed in clinical signs related to physical parameters (including loose stools, chromodacryorrhea, changes in skin, ear bleeding, eye discoloration, diarrhoea, salivation, swollen paws with hemorrhage, or paralysis) or behavioural responses (such as altered movement, coma, drowsiness, tremors, or sedation) across any treatment group. Furthermore, no mortality was recorded throughout the duration of the study (**Table 4.9**)

4.4.2.2 Body weight, food, water consumption and rectal temperature

The mean body weight, food consumption, and water consumption of both male and female rats in the treatment groups showed a general decrease when compared with the control group (**Figure 4.9**). In males, no significant differences were observed at 10 and 100 mg/kg; however, a statistically significant reduction ($p < 0.05$) in mean body weight was recorded at 500 mg/kg. In females, a similar significant reduction ($p < 0.05$) in body weight was also observed at 500 mg/kg. Rectal temperature was

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elevated in both sexes across treatment groups relative to controls, with statistically significant increases ($p < 0.05$) noted at 100 and 500 mg/kg in males and at 500 mg/kg in females. Food consumption was significantly reduced ($p < 0.05$) at 500 mg/kg in both sexes, while water consumption was significantly reduced at 100 and 500 mg/kg in male and female groups, respectively. Furthermore, significant differences were observed in final day mean body weight and percentage body weight gain/loss between the treated and control groups in both sexes. In contrast, no significant differences were noted in initial mean body weight across groups (Table 4.10). Different alphabets in figures and tables denote statistically significant differences among groups at $p < 0.05$.

4.4.2.3 Absolute weight and relative organ ratio

Repeated 28-day oral administration of TPFME (10, 100, and 500 mg/kg) resulted in no statistically significant alterations ($p > 0.05$) in the absolute organ weights (liver, kidney, brain, heart, spleen, colon, stomach, pancreas, lungs, testes, seminal vesicles, ovaries, and uterus) or in their corresponding relative organ weight ratios in both male and female rats across treatment groups. In females, a statistically significant change ($p < 0.05$) was noted in the absolute weight of the colon at dosages of 10 and 100 mg/kg, and in the uterus at 500 mg/kg, relative to the control group. Different alphabets in table denote statistically significant differences among groups at $p < 0.05$ (Table 4.11)

4.4.2.4 Status of hematological parameters

Oral administration of TPFME for 28 days in Wistar albino rats of both sexes was evaluated for its effects on the hematological profile, as summarized in the table. Hematological parameters, including hemoglobin (Hb), total red blood cell count (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), total white blood cell count (WBC), differential leukocyte counts [neutrophils (NP), lymphocytes (LC), monocytes (MC), eosinophils (EP)], and platelet count (PLT), were generally within the normal physiological range across treatment groups (10, 100, and 500 mg/kg). However, a statistically

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significant increase ($p < 0.05$) in total WBC count was observed at the 500 mg/kg dose in both sexes compared with the control group. (**Table 4.12**)

4.4.2.5 Status of serum biochemical parameters

The biochemical evaluation of serum samples from Wistar albino rats following 28 days of oral administration of TPFME revealed no statistically significant alterations ($p > 0.05$) in hepatic function indices, including total protein, albumin, globulin, albumin/globulin ratio, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), and direct bilirubin (DB). Similarly, renal function biomarkers such as serum creatinine, uric acid, urea, and blood urea nitrogen (BUN) remained unaffected across all treatment groups when compared with control animals (**Figure 4.10; Figure 4.11; Figure 4.12, Figure 4.13; Figure 4.14 and Table 4.13**)

Assessment of lipid profile, blood glucose, and electrolyte concentrations demonstrated limited but significant changes. Specifically, treatment groups exhibited a significant ($p < 0.05$) reduction in serum triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), sodium, and chloride levels in both male and female rats relative to controls. In male rats, an additional decrease in total cholesterol (TC) and a concomitant elevation in low-density lipoprotein cholesterol (LDL-C) were observed, whereas in female rats, significant reductions were noted in serum calcium and potassium levels. Conversely, no significant differences were detected in blood glucose levels in either sex, as well as in LDL-C, chloride concentration in females, and calcium and potassium in males. Importantly, despite these statistically significant differences, the mean values of all biochemical and electrolyte parameters remained within the established physiological reference ranges for Wistar rats, suggesting that TPFME administration did not elicit adverse systemic effects under the experimental conditions (**Table 4.13**)

4.4.2.6 Oxidative stress and antioxidant status

Following 28 days of repeated oral administration of TPFME in both male and female Wistar albino rats, oxidative stress and antioxidant defense markers were

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evaluated in liver and kidney tissues. A marginal elevation in malondialdehyde (MDA), a well-established marker of lipid peroxidation, was observed statistically significant in the kidney tissues of treated animals compared with the control group. In contrast, liver tissues from both sexes did not show any appreciable changes in MDA levels.

Analysis of endogenous antioxidant defense enzymes revealed tissue- and sex-specific alterations. In male rats, a significant elevation ($p < 0.05$) of superoxide dismutase (SOD) activity was recorded in kidney tissue, while catalase (CAT) activity was markedly increased in the liver compared with control counterparts. These findings suggest a compensatory upregulation of specific enzymatic antioxidant defenses in response to mild oxidative stress in males. Conversely, no significant alterations were detected in glutathione peroxidase (GSH-Px) activity in either liver or kidney tissues of both sexes, nor in CAT activity in female liver and kidney tissues, or SOD activity in female kidney and liver tissues. Different alphabets in table denote statistically significant differences among groups at $p < 0.05$ (Table 4.14)

4.4.2.7. Histopathological analysis

4.4.2.7.1 Histopathology of liver

Photomicrographs of liver sections from both male and female control groups revealed normal hepatic architecture, characterized by well-organized central veins (CV), polyhedral hepatocytes (HC), binucleated hepatocytes (BH), Kupffer cells (K), and clearly discernible hepatic sinusoids (S). In the treatment groups (10 and 100 mg/kg in both sexes, and 500 mg/kg in males), the hepatic parenchyma appeared largely preserved, with visible hepatocytes (HC), sinusoids (S), and prominent portal triads consisting of the portal vein (P), hepatic artery (HA), and bile duct (BD), surrounded by interlobular connective tissue (&) and portal connective tissue (&&). In females administered 500 mg/kg, the liver sections showed widely spaced central veins encircled by anastomosing cords of polyhedral hepatocytes with compact cytoplasm, along with distinct hepatic sinusoids. In contrast, the male 100 mg/kg

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group exhibited mild degeneration of the interlobular connective tissue surrounding the portal vein, while at 500 mg/kg the portal vein and hepatic artery appeared relatively enlarged compared with the 10 and 100 mg/kg treatment groups. In females receiving 10 and 100 mg/kg, the portal triads appeared largely comparable across groups. Overall, both male and female control and treated groups displayed normal-appearing hepatic vasculature without marked differences (**Figure 4.15** and **Figure 4.16**).

4.4.2.7.2 Histopathology of kidney

Light microscopic examination of renal cortex sections from both male and female control groups (Figure) revealed normal histological organization, including compact glomeruli (G) encased within Bowman's capsule (BC), distinct Bowman's space (BS), proximal convoluted tubules (PCT), distal convoluted tubules (DCT), and collecting tubules (CT). The renal medulla also appeared normal, with well-organized medullary rays of renal tubules. Similarly, renal sections from treatment groups receiving 10, 100, and 500 mg/kg (Figure) exhibited preserved morphology, with normal appearance of glomerular tufts, glomeruli, Bowman's capsule, Bowman's space, PCT, DCT, and CT, as well as intact medullary structures. Overall, both control and treated groups demonstrated healthy renal architecture, with no significant structural alterations observed across any treatment dose (**Figure 4.15** and **Figure 4.16**).

4.4.2.7.3 Histopathology of heart

Histopathological examination of cardiac tissue revealed normal myocardial architecture in all treatment groups (10, 100, and 500 mg/kg) as well as in the control group for both male (Figure) and female (Figure) rats. The myocardium exhibited well-preserved cellular organization with intact striations and no evidence of structural alterations. Importantly, no histopathological abnormalities such as myocardial fibre fragmentation, interstitial hemorrhage, inflammatory cell infiltration, widened intercellular spaces, or extensive tissue hemorrhage were observed in any of the examined sections (**Figure 4.17** and **Figure 4.18**).

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4.4.2.7.4 Histopathology of lung

Histopathological evaluation of pulmonary parenchyma in both male and female Wistar albino rats demonstrated preserved lung architecture across all treatment groups (10, 100, and 500 mg/kg b.w.) as well as in controls. The airway structures, including bronchus (B), bronchiole (BC), terminal bronchiole (TB), and respiratory bronchioles (RB), along with alveolar ducts (AD), alveolar sacs (AS), and alveoli passage structures as alveoli (A) with interalveolar septa (black arrow), exhibited normal histological organization. The bronchial walls showed the expected connective tissue composition with relatively low proportions of smooth muscle, elastic fibres, and cartilage, while the bronchioles were lined by columnar and low-columnar epithelium transitioning into the respiratory bronchioles. In control animals, female lung sections displayed normal bronchus, terminal bronchioles, alveolar ducts, blood vessels, interalveolar septa, and alveoli, whereas male controls similarly exhibited intact respiratory bronchioles, alveolar sacs, and interalveolar septa with no pathological evidence. Importantly, no histopathological alterations such as inflammation, congestion, fibrosis, or architectural distortion were detected in any of the treated groups (**Figure 4.17 and Figure 4.18**).

4.4.2.7.5 Histopathology of Brain

Histological evaluation of the cerebral cortex revealed normal tissue organization in both male and female Wistar albino rats across all treatment groups when compared with controls. The cerebral cortex displayed well-preserved structural integrity, characterized by the orderly arrangement of small pyramidal neurons (N), glial cells (GC), and blood capillaries (Cp), embedded within a clearly defined neuropil (Np) network. The cortical architecture of treated rats was comparable to that of control animals, with no evidence of neuronal degeneration, cytoplasmic vacuolation, gliosis, or vascular abnormalities. Overall, the cerebral cortex maintained its typical histological pattern, and no treatment-related alterations in cortical morphology were detected at any administered dose (**Figure 4.19 and Figure 4.20**).

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4.4.2.7.6 Histopathology of Colon

Histopathological examination of the colonic tissue from both male and female Wistar albino rats revealed normal structural organization of the mucosal and submucosal layers across all treatment groups as well as in controls. The mucosa exhibited well-preserved crypts (C) containing goblet cells, supported by the lamina propria (LP), crypts of Lieberkühn (CL), and the muscularis mucosae (MM), all of which appeared structurally intact. No histopathological abnormalities such as epithelial degeneration, crypt distortion, inflammatory changes, or mucosal injury were observed. The colonic sections of rats administered TPFME at doses of 10, 100, and 500 mg/kg were comparable to those of control animals, indicating no treatment-related alterations in colonic architecture in either sex (**Figure 4.19 and Figure 4.20**).

4.4.2.7.7 Histopathology of spleen

Histopathological analysis of spleen sections from both male and female Wistar albino rats demonstrated normal splenic architecture across all treated groups and controls. The red pulp (RP) exhibited normal morphology with hemosiderin pigment localized predominantly within macrophages, whereas the white pulp (WP) showed well-defined lymphoid follicles with no evidence of disruption or atrophy. In both control and treated animals, the white pulp appeared more prominent than the red pulp, consistent with normal splenic histology. No pathological alterations such as macrophage proliferation, congestion, necrosis, or inflammatory changes were observed in any group (**Figure 4.19 and Figure 4.20**).

4.4.2.7.8 Histopathology of testis

Histopathological examination of the testes in control rats revealed normal testicular architecture, characterized by well-organized seminiferous tubules (ST) separated by interstitial Leydig cells (ISC). The seminiferous epithelium was composed of stratified germinal cells and Sertoli cells, with the germinal layer displaying different stages of spermatogenesis, including spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and mature sperm (SP) located

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toward the lumen of the ST. In the treatment groups, the seminiferous tubules similarly exhibited intact germinal epithelium with normal spermatogenic cell (SG) progression, although a hyalinized luminal center (HC) containing a few sperm was observed. Importantly, no histo-architectural abnormalities, degenerative changes, or disruptions in spermatogenesis were evident across any treatment groups (**Figure 4.21**).

4.4.2.7.8 Histopathology of ovary

Histopathological evaluation of ovarian tissue from both control and TPFME-treated rats demonstrated comparable ovarian architecture with well-preserved follicular morphology. Different stages of follicular development, including germinal follicles (GF), secondary follicles (SF) with antra (&), and granulosa cell (GC) layers supported by the ovarian stroma (OS), were clearly observed. The presence of atretic follicles (AF) was noted as a normal physiological feature. In addition, all groups, including controls, exhibited a predominance of mature corpus luteum (CL) along with a few early corpus luteum (ECL), reflecting normal cyclic activity. No treatment-related alterations in ovarian architecture, follicular development, or luteal structures were detected across any of the dose groups (**Figure 4.21**).

4.5 DISCUSSION

All civilizations and cultures have used medications made from plants, therefore plants have always played a crucial role in our global health systems. These drugs are generally the only available choice, monetarily viable, and socially acceptable (**Villas-Boas et al., 2021**). Medicinal plants are utilized as natural therapeutic drugs due to their bioactive compounds. Generally, these plants are regarded as safe, posing no significant health risks, and are thus commonly employed for self-medication (**Vysakh et al., 2020**). However, the use of herbal medications that lack adequate scientific validation has raised concerns regarding their potential toxicity (**Neergheen-Bhujun, 2013**). Consequently, it is crucial to conduct toxicity evaluations to ensure the safe use of any medicinal plant. This involves characterizing the nature and significance of adverse effects and investigating the

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exposure levels at which these effects manifest (**Ibrahim et al., 2016**). The rat model is preferred by researchers for studying organ-specific toxicities, pharmacokinetics, and long-term safety measures, owing to similarities in physiological, metabolic, and genetic characteristics with humans, which allow for their responses to toxicants to serve as predictors for human health. Rats are relatively small compared to other rodents; they are easy to breed, have a shorter lifespan, and are cost-effective. This makes them a suitable model organism for conducting various experiments (**Gad, 2006**).

T. palmata is primarily distributed in the northeastern states of India, especially in Mizoram, and is classified among ethnomedicinal plants. Various parts of *T. palmata* are utilised as food and in traditional medicinal practices to address certain health issues (**Manoharan et al., 2023**). A literature survey revealed only one acute oral study assessing the toxicity of *T. palmata*, which involved the methanolic extract of its leaves in rats; this study found no mortality up to 2000 mg/kg body weight (b.w.) (**Victoria et al., 2019**). In our study, we investigated both acute and sub-acute toxicity for the first time, using the methanolic extract of flower buds in Wistar albino rats of both sexes. The findings from the acute toxicity assessment, conducted in accordance with OECD test guidelines (**OECD, 1996**), indicated that TPFME did not present any signs of toxicity or mortality at doses of 2000-3000 mg/kg in Wistar albino rats, corroborating the results reported by Victoria et al. (**2023**). Furthermore, administering a dose of 5000 mg/kg b.w. to Wistar albino rats resulted in clinical abnormalities, such as slowed movement and diarrhoea in female rats. The combination of slowed movement and diarrhoea typically suggests gastrointestinal toxicity (**Ayena et al., 2022**).

Further evaluation of the toxicity profile was conducted across three groups, including a normal control. The high-dose group of both sexes was included only in the histopathological examination due to a high mortality rate: 60% for males and 80% for females. At a dose of 5000 mg/kg in the female rat group, only one out of five rats survived, while in the male group, two out of five rats survived. The acute toxicity study indicates that higher doses are unsafe for rats. According to OECD 423

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TG, if all animals survive until the end of the experiment, the extract can be deemed safe and classified as category 5 under the Globally Harmonised Classification System decision tree, indicating very low toxicity (**Brondani et al., 2017; Duan & Liang, 2011**). Consequently, doses up to 3000 mg/kg were considered safe. Using the Spearman-Kärber method, LD₅₀ values for TPFME were calculated as 4,400 mg/kg for male rats and 4,200 mg/kg for female rats. According to the toxicity scale proposed by Hodge and Sterner (**Hodge and Sterner, 1980**), the LD₅₀ values of TPFME fall into the slightly toxic class, which ranges from approximately 500 to 5,000 mg/kg body weight.

Body weight alteration serves as a crucial indicator of toxicity (**Loha et al., 2019**). The acute toxicity test revealed no significant changes in mean body weight, food or water consumption, or rectal temperature. Furthermore, the differences in weight gain or loss between the control and treatment groups were statistically insignificant. The absolute and relative organ weights of various organs in both sexes remained unchanged in the 2000 and 3000 mg/kg groups when compared to the control. Haematological analysis indicated an elevated count of red blood cells (RBC), white blood cells (WBC), and neutrophils (NP) in the 3000 mg/kg treatment group; however, these values were still within the normal range, supporting the findings of normal clinical pathology (**Giknis and Clifford, 2008; Patel et al., 2024**). Conversely, biochemical parameters showed an increase in serum ALT levels, while lipid levels and blood glucose decreased in both sexes. Certain phytochemicals found in medicinal plants may influence liver toxicity, potentially leading to elevated levels of liver enzymes in the blood, such as AST, ALT, and ALP (**Vysakh et al., 2020**); however, the experimental results for serum biochemistry parameters remained within the normal reference range.

Additionally, neither the liver nor the kidney exhibited significant increases or decreases in oxidative stress and antioxidant levels, further affirming that there was no redox imbalance (**Sies, 2015**). Despite the absence of changes in organ weights, histopathological examination of hepatic and renal tissue sections showed no substantial damage to architecture or structural organization when compared to

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the control and treatment groups at the 2000-3000 mg/kg doses. No damage was observed in the 5000 mg/kg group, thereby confirming and validating that TPFME extract at doses up to 3000 mg/kg was not toxic and was safe for acute exposure in Wistar albino rats.

The clinical use of acute toxicity data is limited for longer-duration treatment. Thus, a repeated dose-related toxicity study was employed. Chronic illness conditions may necessitate repeated dose toxicological evaluation of substances due to potential buildup in the body, resulting in progressive effects on tissues and organs (**Bariweni et al., 2018; Ugwah-Oguejiofor et al., 2019**). Evaluation of the target organ and the haematological or biochemical effects of extracts is aided by subacute toxicity testing, as these effects are typically not detectable in acute toxicity testing. It is also crucial for ensuring human safety, particularly for developing medications, with the help of this study it is used to determination of the no observed effect level (NOEL) (**Loha et al., 2019**). By measuring feed and water intake, body weight, and haematological, biochemical, and histological parameters, the sub-acute toxicity profile of the flower buds of *T. palmata* extract was assessed in rats in this study. The repeated doses of TPFME were 10 mg/kg, 100 mg/kg and 500 mg/kg showed no mortality, no changes in clinical observation in both sexes. The total health status of experimental animals has been evaluated using body weight, feed, and water consumption as indicators (**El-Hilaly et al., 2004**). Our study showed

Body weight, food, water, body weight gain and rectal temperature decrease at 500 mg/kg in both sexes. This reduction is not considered as sign of toxicity since diets, thermoregulation can induce a decrease in these parameters until there are no significant or severe changes in biochemical, hematological and histological parameters in this study (**Andrade et al., 2002; Rivas et al., 2013; Ouchi et al., 2021; Villas-Boas et al., 2021**)

In toxicity studies, organ weight changes are very sensitive indicators of toxicity (**Michael et al., 2007**) They could represent damage to target organs, physiological disturbances, and effects on enzymes. Absolute and relative organ weight showed no significant changes except small changes in female colon weight

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at 10 and 100 mg/kg; uterus at 500 mg/kg of absolute weight. According to Teo et al. (2002), a rise in organ weight indicates hypertrophy, whereas a fall indicates necrosis in the target organ. Organ weight data must be evaluated in conjunction with gross pathology, clinical pathology, and histopathology results, even if organ weights offer helpful indicators of test article-related effects (Sellers et al., 2007). Our study, examination of histopathology of all organs in both sexes showed normal histology, structural organization and development which attribute that despite slight increase in absolute organ weight is not considered as toxic sign. Moreover, it could be reasoned that extract may possess phytochemicals that act on female organs more than the male rats.

The plant extract's blood-related properties can be verified using the haematological parameters. An essential indicator of physiology and disease in both humans and animals, the hemopoietic system constitutes one of the most vulnerable organs to harmful substances (Porwal et al., 2017). In sub-acute study our result showed there were no significant changes in blood parameters except the WBC, there was a slight increase at dose 500 mg/kg in both male and female rats as compared with control group; however, blood parameter values did not exceed the normal reference range for the animals tested in this experiment (Giknis and Clifford, 2008). Slight increase of WBC may be due to immune modulatory response to extract, as because WBC is the first line of cellular defense system.

For animals to survive, the liver and kidneys play a crucial role. In the toxicological assessment of xenobiotics, serum biochemical analysis is essential for measuring their functioning (Olorunnisola et al., 2012). Liver dysfunction was assessed through a look at the liver's enzymatic activity, including alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). The levels of liver enzymes are often elevated during acute hepatotoxicity but tend to fall after sustained intoxication as a result of liver damage (Imafidon and Okunrobo, 2012). Albumin, bilirubin, and serum total protein were used to measure the liver's function. Lower levels of albumin, bilirubin, and total proteins in the blood indicate decreased synthetic function, which is a sign of liver illness or injury.

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Usually, malignant circumstances or a high-protein diet cause these values to rise (Tietz et al., 1994). Our study results revealed that there were no significant changes in any of these parameters in both sexes as supporting the result of liver histopathology study.

Urea, blood urea nitrogen, creatinine, uric acid, glucose and specific dissolved salts (serum electrolyte) are measured in the standard blood test that evaluates kidney function. These are the markers is important to evaluate metabolism and detoxification of plant extract. In this study, there were no significant differ levels of Urea, Uric acid, and creatinine but electrolyte such as sodium, chloride was decreased in both sexes and calcium and potassium were decrease in females. The levels of kidney function parameters are with normal range.

Oxidative stress indicators like MDA (malondialdehyde) and antioxidants like SOD (superoxide dismutase), CAT (catalase), and GPx (glutathione peroxidase) in liver and kidney tissue are essential for determining both damage and protective effects in sub-acute toxicity studies analyzing plant extracts. These indicators demonstrate the plant extract's antioxidant capability in addition to the existence of oxidative damage (Samarghandian et al., 2016; Watafua et al., 2022). The results of our investigation showed that, in comparison to the control groups, there was a modest increase in MDA levels in the liver and kidney, but that SOD and CAT levels were greater, which is consistent with compensatory upregulation of the endogenous defense mechanism of redox signaling that produces damage. According to Sies (2015), the upregulation of enzymes in histopathology usually signifies adaptability as opposed to toxicity.

Based on above result and observation, the No Observed Adverse Effect Level (NOAEL) for methanolic extract of *T. palmata* considered as 100 mg/kg for both sexes and also the Lowest Observed Adverse Effect Level as 500 mg/kg due to depressed growth, reduce intake and supporting changes of thermoregulation, leukocytes and oxidative markers. Furthermore, a 90-day trial (OECD, 408) with TK and recovery cohorts would evaluate the reversibility of 500 mg/kg/day effects and

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refine NOAEL or target-organ findings. The absence of toxicokinetic (TK) data further restricts the interpretation of exposure margins and sex differences.

The safety profile of TPFME extract will be strengthened by further research on genotoxicity, carcinogenicity, and teratogenicity (**Sundaram et al., 2021**). However, it is proposed that this constraint might be the focus of our further studies.

4.6 KEY FINDINGS

➤ Acute

- Methanolic extract of *Trevesia palmata* flower buds (TPFME) was prepared by cold maceration (100% methanol; 1:4 w/v), yielding a stable crude extract stored at -20°C for in vivo studies.
- Acute oral toxicity was evaluated in Wistar albino rats (both sexes) following OECD TG 423, using single doses of 2000, 3000 and 5000 mg/kg b.w.
- Sub-acute (28-day) oral toxicity was evaluated according to OECD TG 407 at daily doses of 10, 100 and 500 mg/kg b.w. via oral gavage in both sexes.
- No clinical signs of toxicity or mortality were observed at 2000 and 3000 mg/kg in either sex.
- At 5000 mg/kg, dose-related toxic signs appeared (slow movement in both sexes; loose stool and diarrhoea in females) with 60% mortality in males and 80% in females.
- LD_{50} values were 4400 mg/kg (males) and 4200 mg/kg (females), placing TPFME in the “slightly toxic” category on the Hodge and Sterner scale.
- No significant changes were found in body weight, percentage weight gain/loss, food and water intake, or rectal temperature at 2000 and 3000 mg/kg in either sex versus controls.
- Absolute and relative weights of liver, kidney, brain, heart, lungs, spleen, stomach, pancreas, colon and reproductive organs showed no significant differences at 2000 and 3000 mg/kg in both sexes.

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- Histopathology of liver and kidney was normal at 2000 and 3000 mg/kg, whereas 5000 mg/kg produced dose-dependent lesions: hepatic vacuolar degeneration, endothelial disruption, congestion and inflammatory infiltration, and renal alterations including atrophied glomeruli, congested tubules and hemorrhage.
- At 3000 mg/kg, total RBC, WBC and neutrophil counts increased significantly but all hematological values remained within normal reference ranges.
- A significant rise in serum ALT was observed at 3000 mg/kg, with no significant changes in AST, ALP, total protein, albumin, globulin, A/G ratio, bilirubin, creatinine, urea, uric acid or BUN.
- In males, triglycerides decreased at 3000 mg/kg; in females, total cholesterol and triglycerides decreased and serum glucose was reduced in both sexes, while values remained within physiological limits.
- No significant changes in MDA, SOD, CAT or GPx levels were detected in liver or kidney at 2000 and 3000 mg/kg, indicating absence of acute redox imbalance.

➤ Sub-acute

- Daily oral administration of 10, 100 and 500 mg/kg for 28 days caused no observable clinical signs of toxicity (physical or behavioural) and no mortality in either sex.
- At 500 mg/kg, both sexes showed significant reductions in final body weight, percentage weight gain, food intake and water intake, and increased rectal temperature; the changes at 10 and 100 mg/kg were minimal or non-significant.
- Despite these changes, there were no parallel adverse alterations in serum biochemical or histological endpoints, suggesting non-severe, adaptative responses rather than overt systemic toxicity.
- Most absolute and relative organ weights remained unchanged across doses in both sexes.

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- In females, small but significant changes were noted: increased colon weight at 10 and 100 mg/kg and altered uterus weight at 500 mg/kg; however, corresponding histopathology of these organs was normal.
- Hemoglobin, RBC, PCV, MCV, MCH, differential leukocyte counts and platelet counts remained within normal ranges at all doses.
- Total WBC count increased significantly at 500 mg/kg in both sexes, suggesting a possible mild immunomodulatory/defence response rather than frank hematotoxicity.
- No significant changes were found in hepatic biomarkers (AST, ALT, ALP, total protein, albumin, globulin, A/G ratio, total and direct bilirubin) or renal biomarkers (creatinine, urea, uric acid, BUN) at any dose.
- Biochemical profiles remained within accepted reference ranges, corroborating the absence of hepatotoxicity or nephrotoxicity in sub-acute exposure.
- Treatment reduced triglycerides, HDL-C, sodium and chloride in both sexes; in males, total cholesterol decreased and LDL-C increased, while females showed reduced serum calcium and potassium.
- Despite statistical significance, all lipid and electrolyte values stayed within physiological limits, and blood glucose did not differ significantly from controls.
- Kidney MDA levels were modestly elevated, indicating mild lipid peroxidation, while liver MDA remained essentially unchanged.
- In males, kidney SOD and hepatic CAT activities increased significantly, indicating compensatory up-regulation of antioxidant defences; GPx activity was unchanged in both organs and sexes.
- Histological examination of liver, kidney, heart, lungs, brain, colon, spleen, testis and ovary showed preserved normal architecture across all sub-acute doses in both sexes, with no evidence of necrosis, inflammation, fibrosis or degenerative lesions.

➤ Overall toxicological interpretation

- TPFME is slightly toxic by LD₅₀ classification but shows a wide safety margin in rats, with safe acute exposure up to 3000 mg/kg.
- For repeated 28-day dosing, the NOAEL is proposed as 100 mg/kg, and the LOAEL as 500 mg/kg, based on reduced growth, decreased intake, altered thermoregulation, leukocyte changes and mild oxidative stress at the highest dose, in the absence of overt organ damage

Table 4.1 Acute toxicity studies of *Trevesia palmata* flower buds methanolic extract in Wistar albino rats relating to clinic observations and survival.

[illegible]

	diarrhoea	diarrhoea	diarrhoea	diarrhoea	diarrhoea	diarrhoea	diarrhoea	diarrhoea observed
Coma	No signs of coma	No signs of coma	No signs of coma	No signs of coma	No signs of coma	No signs of coma	No signs of coma	No signs of coma
Drowsiness	No signs of drowsiness observed	No signs of drowsiness observed	No signs of drowsiness observed	No signs of drowsiness observed	Yes, signs of drowsiness observed	No signs of drowsiness observed	No signs of drowsiness observed	No signs of drowsiness observed
Tremor	No signs of tremor observed	No signs of tremor observed	No signs of tremor observed	No signs of tremor observed	No signs of tremor observed	No signs of tremor observed	No signs of tremor observed	No signs of tremor observed
Salivation	No signs of salivation observed	No signs of salivation observed	No signs of salivation observed	No signs of salivation observed	No signs of salivation observed	No signs of salivation observed	No signs of salivation observed	No signs of salivation observed
Sedation	No signs of sedation observed	No signs of sedation observed	No signs of sedation observed	No signs of sedation observed	No signs of sedation observed	No signs of sedation observed	No signs of sedation observed	No signs of sedation observed
Swollen paws with haemorrhage	No signs of swollen paws with haemorrhage	No signs of swollen paws with haemorrhage	No signs of swollen paws with haemorrhage	No signs of swollen paws with haemorrhage	No signs of swollen paws with haemorrhage	No signs of swollen paws with haemorrhage	No signs of swollen paws with haemorrhage	No signs of swollen paws with haemorrhage
Paralysis	No signs of paralysis observed	No signs of paralysis observed	No signs of paralysis observed	No signs of paralysis observed	No signs of paralysis observed	No signs of paralysis observed	No signs of paralysis observed	No signs of paralysis observed
Death	No mortality	No mortality	No mortality	No mortality	No mortality	No mortality	3 out of 5 rats dead (60%)	4 out of 5 rats dead (80%)

Table 4.2 Parameters for calculation of 50% lethal dose (LD₅₀) using the arithmetic methods of Karber

Dose (mg/kg)	Number of Animals (n)	Dose difference (mg/kg) (a)	Number of dead rats	Mean Mortality (b)	Product (a * b)	LD ₅₀ (mg/kg)
Male Group						
0	5	-	-	-	-	4400
2000	5	0	0	0	0	
3000	5	1000	0	0	0	
5000	5	2000	3	0+3/2 = 1.5	3000	
Female Group						
0	5	-	-	-	-	4200
2000	5	0	0	0	0	
3000	5	1000	0	0	0	
5000	5	2000	4	0+4/2 = 2	4000	

Table 4.3 Hodge and Sterner toxicity scale

Toxicity class LD₅₀ (rat, mouse) mg/kg
Extremely toxic <1
Highly toxic 1 – 50
Moderately toxic 50 – 500
Slightly toxic 500 – 5000
Practically no toxicity 5000 – 15000

Table 4.4 14-days observation in male rat treated by single dose of TPFME were recorded.

Animals/Sex	Observation	Treatment Group (mg/kg b.w)		
		Control	2000	3000
Male	Initial day body weight (g)	230.8 ± 2.518a	232.2 ± 1.200a	233.4 ± 1.208a
	Final day body weight (g)	240.8 ± 0.860a	238.0 ± 1.304a	237.6 ± 0.812a
	Body weight gain/loss (%)	4.378 ± 1.089a	2.502 ± 0.483a	1.853 ± 0.606a
	Mean body weight (g/day)	235.4 ± 0.958a	233.5 ± 0.914a	232.7 ± 1.323a
	Mean food consumption (g/day)	20.5 ± 0.685a	19.0 ± 0.722a	18.0 ± 0.886a
	Mean water consumption (mL/day)	25.3 ± 0.487a	26.5 ± 0.291a	26.7 ± 0.504a
	Mean rectal temperature (°C/day)	36.3 ± 0.241a	36.0 ± 0.195a	35.5 ± 0.256a
Female	Initial day body weight (g)	158.6 ± 0.812a	160.2 ± 1.530a	163.0 ± 2.098a
	Final day body weight (g)	169.6 ± 1.030a	171.4 ± 0.979a	169.0 ± 2.168a
	Body weight gain/loss (%)	6.953 ± 1.024a	7.041 ± 1.408a	3.688 ± 0.592a
	Mean body weight (g/day)	160.7 ± 1.266a	163.3 ± 1.247a	163.8 ± 0.947a
	Mean food consumption (g/day)	21.5 ± 0.488a	20.0 ± 0.805a	19.5 ± 0.562a
	Mean water consumption (mL/day)	23.0 ± 0.583a	25.7 ± 0.630a	23.7 ± 0.424a
	Mean rectal temperature (°C /day)	35.5 ± 0.270a	35.2 ± 0.264a	35.3 ± 0.243a

Values are expressed in Mean ± SEM, n = 5 animals/group/sex. Statistical comparison was performed using one-way ANOVA followed by Tukey's post-hoc tests for all pair-wise multiple comparisons. Similar alphabets row wise indicate that treatment groups are significantly different at $p < 0.05$.

Table 4.5 The absolute organ weight (g) and relative organ ratio (%) of various organs in Wistar albino rats were assessed following a single oral dosage of a methanolic extract of *Trevesia palmata* flower buds for an acute toxicity study.

Animals/Sex	Organs/Unit		Acute toxicity		
			Treatment Group (mg/kg b. w)		
			Control	2000	3000
Male	Liver	g	6.850 ± 0.297a	6.100 ± 0.348a	6.634 ± 0.101a
		%	2.910 ± 0.128a	2.604 ± 0.154a	2.855 ± 0.049a
	Kidneys	g	1.760 ± 0.062a	1.596 ± 0.063a	1.546 ± 0.050a
		%	0.747 ± 0.025a	0.680 ± 0.026a	0.681 ± 0.034a
	Brain	g	1.768 ± 0.024a	1.704 ± 0.031a	1.690 ± 0.013a
		%	0.751 ± 0.012a	0.727 ± 0.015a	0.727 ± 0.006a
	Heart	g	0.882 ± 0.054a	0.840 ± 0.019a	0.864 ± 0.029a
		%	0.376 ± 0.022a	0.360 ± 0.010a	0.372 ± 0.013a
	Spleen	g	0.492 ± 0.029a	0.458 ± 0.016a	0.488 ± 0.018a
		%	0.209 ± 0.012a	0.195 ± 0.006a	0.210 ± 0.008a
	Colon	g	0.824 ± 0.031a	0.938 ± 0.089a	0.842 ± 0.064a
		%	0.350 ± 0.014a	0.400 ± 0.037a	0.362 ± 0.027a
	Stomach	g	1.290 ± 0.050a	1.238 ± 0.042a	1.186 ± 0.057a
		%	0.547 ± 0.021a	0.528 ± 0.020a	0.510 ± 0.024a
	Pancreas	g	0.570 ± 0.048a	0.480 ± 0.014a	0.542 ± 0.017a
		%	0.242 ± 0.020a	0.205 ± 0.005a	0.233 ± 0.008a
	Lungs	g	1.834 ± 0.057a	1.852 ± 0.035a	1.740 ± 0.056a
		%	0.779 ± 0.026a	0.790 ± 0.016a	0.748 ± 0.025a
	Testis	g	2.588 ± 0.076a	2.564 ± 0.086a	2.336 ± 0.072a

	Seminal vesicle	%	$1.099 \pm 0.032a$	$1.165 \pm 0.040a$	$1.060 \pm 0.032a$
		g	$0.882 \pm 0.020a$	$0.804 \pm 0.019a$	$0.820 \pm 0.048a$
Female	Liver	%	$0.374 \pm 0.008a$	$0.342 \pm 0.075a$	$0.352 \pm 0.021a$
		g	$4.492 \pm 0.080a$	$4.174 \pm 0.102a$	$4.190 \pm 0.216a$
	Kidneys	%	$2.789 \pm 0.032a$	$2.591 \pm 0.076a$	$2.649 \pm 0.122a$
		g	$1.256 \pm 0.037a$	$1.144 \pm 0.036a$	$1.156 \pm 0.039a$
	Brain	%	$0.778 \pm 0.016a$	$0.710 \pm 0.020a$	$0.734 \pm 0.026a$
		g	$1.628 \pm 0.025a$	$1.610 \pm 0.011a$	$1.646 \pm 0.011a$
	Heart	%	$1.011 \pm 0.015a$	$0.999 \pm 0.010a$	$1.042 \pm 0.012a$
		g	$0.628 \pm 0.015a$	$0.584 \pm 0.019a$	$0.586 \pm 0.020a$
	Spleen	%	$0.389 \pm 0.007a$	$0.362 \pm 0.012a$	$0.371 \pm 0.010a$
		g	$0.328 \pm 0.008a$	$0.342 \pm 0.011a$	$0.352 \pm 0.012a$
	Colon	%	$0.206 \pm 0.004a$	$0.212 \pm 0.007a$	$0.222 \pm 0.006a$
		g	$0.668 \pm 0.022a$	$0.664 \pm 0.019a$	$0.646 \pm 0.022a$
	Stomach	%	$0.414 \pm 0.013a$	$0.412 \pm 0.013a$	$0.409 \pm 0.014a$
		g	$0.908 \pm 0.026a$	$0.860 \pm 0.014a$	$0.864 \pm 0.025a$
	Pancreas	%	$0.564 \pm 0.019a$	$0.533 \pm 0.007a$	$0.547 \pm 0.015a$
		g	$0.606 \pm 0.031a$	$0.504 \pm 0.032a$	$0.518 \pm 0.018a$
	Lungs	%	$0.377 \pm 0.022a$	$0.313 \pm 0.021a$	$0.328 \pm 0.013a$
		g	$1.614 \pm 0.054a$	$1.636 \pm 0.115a$	$1.448 \pm 0.041a$
	Ovaries	%	$1.002 \pm 0.029a$	$1.014 \pm 0.069a$	$0.916 \pm 0.060a$
		g	$0.062 \pm 0.004a$	$0.070 \pm 0.005a$	$0.060 \pm 0.004a$
	Uterus	%	$0.038 \pm 0.002a$	$0.043 \pm 0.003a$	$0.038 \pm 0.003a$
		g	$0.388 \pm 0.017a$	$0.440 \pm 0.028a$	$0.430 \pm 0.030a$
		%	$0.241 \pm 0.012a$	$0.273 \pm 0.019a$	$0.271 \pm 0.017a$

Values are expressed in Mean $\pm$ SEM, n = 5 animals/group/sex. Statistical comparison was performed using one-way ANOVA followed by Tukey's post-hoc tests for all pair-wise multiple comparisons. Different alphabets row wise indicate that treated and non-treated groups. Similar alphabets indicate not statistically significant at $p > 0.05$.

Table 4.6 The impact of a single-dosage of a methanolic extract of *Trevesia palmata* flower buds given orally for 14 days on hematological parameters in Wister albino rats

Animals/Sex	Parameters	Unit	Normal range*	Treatment Group (mg/kg b. w)		
				Control	2000	3000
Male	Haemoglobin (Hb.)	g/dL	10.2-16.6	13.74 ± 0.52a	14.30 ± 0.31a	14.42 ± 0.50a
	Total red blood cells (RBC's)	10 ⁶ /μL	5-10	7.74 ± 0.15a	8.00 ± 0.16a	8.87 ± 0.14b
	Packed cell volume (PCV)	%	39-49	42.18 ± 1.56a	43.87 ± 0.94a	44.23 ± 1.50a
	Mean corpuscular volume (MCV)	fL	48-54	54.54 ± 2.14a	54.98 ± 2.22a	49.88 ± 1.62a
	Mean corpuscular haemoglobin (MCH)	Pg	17-21	17.77 ± 0.71a	17.92 ± 0.73a	16.26 ± 0.54a
	Mean corpuscular haemoglobin concentration (MCHC)	g/dL	32-38	32.57 ± 0.02a	32.59 ± 0.01a	32.60 ± 0.02a
	Total white blood cells (WBC's)	10 ³ /μL	6-15	6.13 ± 0.14a	6.80 ± 0.33a	8.6 ± 0.41b
	Neutrophils (NP)	%	10-40	12.36 ± 0.63a	15.12 ± 0.81a	18.12 ± 1.04b
	Lymphocytes (LC)	%	55-95	78.48 ± 2.20a	79.92 ± 2.04a	80.98 ± 2.53a
	Monocytes (MC)	%	1-4	1.22 ± 0.17a	1.3 ± 0.15a	1.8 ± 0.19a
	Eosinophils (EP)	%	0-4	0.84 ± 0.17a	1.14 ± 0.21a	1.36 ± 0.21a

	Platelets (PLT)	10 ³ /μL	782-985	853.6 ± 23.96a	874.6 ± 37.62a	910.7 ± 25.57a
Female	Haemoglobin (Hb.)	g/dL	10.2-16.6	13.84 ± 0.47a	14.08 ± 0.31a	14.20 ± 0.30a
	Total red blood cells (RBC's)	10 ⁶ /μL	5-10	7.93 ± 0.14a	8.10 ± 0.24a	8.83 ± 0.15b
	Packed cell volume (PCV)	%	39-49	42.49 ± 1.42a	43.21 ± 0.87a	44.23 ± 0.92a
	Mean corpuscular volume (MCV)	fL	48-54	53.72 ± 2.40a	53.48 ± 1.74a	49.45 ± 1.82a
	Mean corpuscular haemoglobin (MCH)	Pg	17-21	17.50 ± 0.80a	17.43 ± 0.61a	16.11 ± 0.60a
	Mean corpuscular haemoglobin concentration (MCHC)	g/dL	32-38	32.57 ± 0.02a	32.58 ± 0.01a	32.59 ± 0.01a
	Total white blood cells (WBC's)	10 ³ /μL	6-15	6.27 ± 0.21a	6.79 ± 0.24a	8.05 ± 0.35b
	Neutrophils (NP)	%	10-40	12.58 ± 0.70a	13.46 ± 1.21a	17.44 ± 1.14b
	Lymphocytes (LC)	%	55-95	76.44 ± 3.04a	78.30 ± 3.37a	80.80 ± 2.18a
	Monocytes (MC)	%	1-4	1.12 ± 0.12a	1.22 ± 0.18a	1.74 ± 0.19a
	Eosinophils (EP)	%	0-4	0.76 ± 0.19a	1.22 ± 0.17a	1.42 ± 0.35a
	Platelets (PLT)	10 ³ /μL	782-985	872.7 ± 29.86a	893.6 ± 34.21a	915.4 ± 20.58a

Values are expressed in Mean ± SEM, n = 5 animals/group/sex. Statistical comparison was performed using one-way ANOVA followed by Tukey's post-hoc tests for all pair-wise multiple comparisons. Different alphabets row wise indicate that treated and non-treated groups are significantly different at p < 0.05 and with similar alphabets indicate not statistically significant at p > 0.05.

*(Giknis and Clifford, 2008)

Table 4.7 Effect on hepatic and renal function biomarkers, lipid profile, blood serum glucose level, and major ion/electrolytes from blood serum of Wister albino rats in relating to acute toxicity study (14 days single dose treatment) of methanolic extract of *Trevesia palmata* flower buds.

Animals/Sex	Biochemical Parameters	Treatment Group (mg/kg b. w)			
		Control	2000	3000	Reference value (Normal range)
Liver and renal function test					
Male	Total protein (g/dL)	5.720 ± 0.17a	6.040 ± 0.12a	6.540 ± 0.33a	5.2-7.1 ^{##}
	Albumin (g/dL)	3.720 ± 0.11a	3.600 ± 0.15a	3.740 ± 0.18a	3.3-4.9 [#]
	Globulin (g/dL)	2.000 ± 0.16a	2.440 ± 0.21a	2.720 ± 0.17a	1.5-2.4 [#]
	Albumin/Globulin ratio	1.924 ± 0.20a	1.564 ± 0.25a	1.740 ± 0.55a	1.7-3.0 [#]
	Aspartate aminotransferase (U/L)	81.20 ± 2.57a	84.60 ± 2.54a	89.00 ± 1.94a	74-143 ^{##}
	Alanine aminotransferase (U/L)	19.80 ± 0.58a	21.80 ± 0.58a	24.80 ± 0.96b	18-45 ^{##}
	Alkaline phosphatase (U/L)	115.6 ± 1.28a	116.2 ± 1.46a	121.2 ± 2.41a	62-230 ^{##}
	Total bilirubin (mg/dL)	0.572 ± 0.03a	0.584 ± 0.02a	0.636 ± 0.03a	0.3-0.8*
	Direct bilirubin (mg/dL)	0.338 ± 0.01a	0.266 ± 0.03a	0.288 ± 0.02a	0.2-0.5**
	Creatinine (mg/dL)	0.412 ± 0.01a	0.416 ± 0.02a	0.404 ± 0.02a	0.3-0.5 ^{##}
	Uric acid (mg/dL)	2.888 ± 0.07a	2.992 ± 0.12a	2.566 ± 0.15a	3.5-48***
	Urea (mg/dL)	23.55 ± 0.63a	23.84 ± 1.17a	23.77 ± 0.71a	10.7-20 ^{##}
	Blood urea nitrogen (mg/dL)	11.00 ± 0.29a	11.12 ± 0.54a	11.10 ± 0.32a	8-33 ^{###}
Female	Total protein (g/dL)	6.040 ± 0.23a	6.440 ± 0.21a	7.040 ± 0.42a	5.5-7.7 ^{##}
	Albumin (g/dL)	3.780 ± 0.10a	4.440 ± 0.13a	4.540 ± 0.28a	3.3-4.9 [#]
	Globulin (g/dL)	2.260 ± 0.33a	2.000 ± 0.12a	2.360 ± 0.50a	1.5-2.4 [#]
	Albumin/Globulin ratio	1.846 ± 0.29a	2.246 ± 0.12a	2.710 ± 0.76a	1.7-3.0 [#]
	Aspartate aminotransferase (U/L)	68.00 ± 1.14a	70.20 ± 1.39a	71.60 ± 1.43a	65-203 ^{##}
	Alanine aminotransferase (U/L)	17.40 ± 0.06a	18.60 ± 0.51a	24.00 ± 0.70b	16-48 ^{##}
	Alkaline phosphatase (U/L)	74.80 ± 1.72a	76.60 ± 1.69a	79.40 ± 1.07a	26-147 ^{##}

	Total bilirubin (mg/dL)	0.536 ± 0.02a	0.546 ± 0.02a	0.570 ± 0.01a	0.3-0.8*
	Direct bilirubin (mg/dL)	0.254 ± 0.02a	0.222 ± 0.04a	0.224 ± 0.03a	0.2-05**
	Creatinine (mg/dL)	0.514 ± 0.06a	0.442 ± 0.03a	0.470 ± 0.03a	0.3-0.6 ^{##}
	Uric acid (mg/dL)	2.948 ± 0.13a	2.700 ± 0.21a	2.548 ± 0.17a	3.5-48d***
	Urea (mg/dL)	23.68 ± 1.01a	23.52 ± 1.10a	24.69 ± 0.84a	11.7-25 ^{##}
	Blood urea nitrogen (mg/dL)	11.08 ± 0.47a	10.98 ± 0.52a	11.54 ± 0.38a	8-33 ^{###}
Lipid profiles and Blood serum glucose level					
Male	Total Cholesterol (mg/dL)	67.2 ± 3.22a	61.4 ± 3.75a	59.4 ± 3.62a	37-95 ^{##}
	Triglycerides (mg/dL)	83.0 ± 4.24a	77.8 ± 4.16a	67.4 ± 3.69b	27-160 ^{##}
	HDL Cholesterol (mg/dL)	35.8 ± 2.13a	30.0 ± 1.23a	30.2 ± 1.16a	21-54 ^{\$}
	LDL Cholesterol (mg/dL)	14.8 ± 4.24a	15.8 ± 2.88a	15.8 ± 4.10a	nil
	Glucose (mg/dL)	181.4 ± 1.56a	122.6 ± 1.36b	162.4 ± 1.61c	106-184 ^{##}
Female	Total Cholesterol (mg/dL)	43.8 ± 1.35a	36.8 ± 1.15b	36.2 ± 1.40b	23-97 ^{##}
	Triglycerides (mg/dL)	45.8 ± 3.20a	35.8 ± 2.35a	28.8 ± 2.85b	16-175 ^{##}
	HDL Cholesterol (mg/dL)	28.2 ± 2.29a	23.0 ± 1.23a	23.8 ± 1.28a	21-54 ^{\$}
	LDL Cholesterol (mg/dL)	6.6 ± 2.11a	6.8 ± 1.32a	6.4 ± 1.47a	nil
	Glucose (mg/dL)	141.6 ± 1.29a	74.8 ± 2.03b	92.2 ± 1.16c	89-163 ^{##}
Blood serum ion/electrolytes concentration					
Male	Calcium (mg/dL)	7.72 ± 0.17a	9.36 ± 0.16b	10.24 ± 0.25c	9.1-11.9 ^{##}
	Sodium (mmol/L)	140 ± 2.84a	140.8 ± 1.82a	149.8 ± 4.68a	137-147 ^{##}
	Potassium (mmol/L)	5.78 ± 0.34a	5.52 ± 0.15a	7.25 ± 0.60b	3.88-6.11 ^{##}
	Chloride (mmol/L)	103 ± 2.51a	105.8 ± 1.9a	106.8 ± 2.71a	98-106 ^{##}
Female	Calcium (mg/dL)	9.12 ± 0.21a	6.72 ± 0.18b	6.92 ± 0.14b	9.5-12.1 ^{##}
	Sodium (mmol/L)	140.6 ± 2.02a	140.2 ± 2.33a	143.2 ± 1.80a	135-143 ^{##}

	Potassium (mmol/L)	5.68 ± 0.46a	5.21 ± 0.38a	4.92 ± 0.22a	3.37-5.11 ^{##}
	Chloride (mmol/L)	105.6 ± 3.07a	103 ± 2.30a	107.2 ± 2.47a	97-106 ^{##}

All values are expressed in Mean ± SEM, n = 5 animals/group/sex. Statistical comparison was performed using one-way ANOVA followed by Tukey's post-hoc tests for all pair-wise multiple comparisons. Different alphabets row wise indicate that treated and non-treated groups are significantly different at p < 0.05 and with similar alphabets indicate not statistically significant at p > 0.05. BUN: Blood urea nitrogen; HDL: High density lipoproteins; LDL: Low density lipoproteins

(Villas-Boas et al., 2021); ## (Giknis and Clifford, 2008); ### (Upadhyay et al., 2019); * (Mulyati et al., 2020); ** (Augustine et al., 2020)

*** (Verma et al., 2023); \$ (El Malik and Sabahelkhier, 2019)

Table 4.8 Effects of single-dosage administration of TPFME for acute toxicity study (14-days) on markers of oxidative stress and antioxidant in Wister albino rats.

Sex/Organ		Parameters	Treatment Group (mg/kg b. w)		
			Control	2000	3000
Male	Liver	MDA (nmol MDA/mg of protein)	13.67 ± 0.58a	15.83 ± 0.66a	15.65 ± 0.58a
		SOD (U SOD/mg of protein)	0.36 ± 0.03a	0.39 ± 0.03a	0.46 ± 0.05a
		GSH-Px (μmol GSH oxidize/min/mg of protein)	0.19 ± 0.01a	0.18 ± 0.01a	0.23 ± 0.02a
		CAT (KU/mg of protein)	0.34 ± 0.02a	0.44 ± 0.03a	0.46 ± 0.04a
	Kidney	MDA (nmol MDA/mg of protein)	17.18 ± 0.52a	20.08 ± 1.17a	19.81 ± 0.80a
		SOD (U SOD/mg of protein)	0.37 ± 0.02a	0.41 ± 0.03a	0.40 ± 0.03a
		GSH-Px (μmol GSH oxidize/min/mg of protein)	0.07 ± 0.00a	0.08 ± 0.01a	0.07 ± 0.00a
		CAT (KU/mg of protein)	0.46 ± 0.03a	0.45 ± 0.01a	0.43 ± 0.03a
Female	Liver	MDA (nmol MDA/mg of protein)	21.21 ± 0.49a	22.79 ± 0.96a	22.41 ± 0.65a
		SOD (U SOD/mg of protein)	0.25 ± 0.03a	0.27 ± 0.02a	0.32 ± 0.01a
		GSH-Px (μmol GSH oxidize/min/mg of protein)	0.11 ± 0.01a	0.09 ± 0.00a	0.10 ± 0.00a
		CAT (KU/mg of protein)	0.38 ± 0.01a	0.47 ± 0.04a	0.47 ± 0.01a
	Kidney	MDA (nmol MDA/mg of protein)	21.55 ± 0.52a	23.11 ± 0.78a	23.15 ± 0.51a
		SOD (U SOD/mg of protein)	0.27 ± 0.02a	0.28 ± 0.03a	0.36 ± 0.04a
		GSH-Px (μmol GSH oxidize/min/mg of protein)	0.08 ± 0.00a	0.07 ± 0.00a	0.07 ± 0.00a
		CAT (KU/mg of protein)	0.44 ± 0.02a	0.47 ± 0.01a	0.44 ± 0.01a

All values are expressed in Mean ± SEM, n = 5 animals/group/sex. Statistical comparison was performed using one-way ANOVA followed by Tukey's post hoc tests for all pair-wise multiple comparisons. Similar alphabets indicate not statistically significant at p > 0.05. MDA: malondialdehyde; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase; CAT: catalase.

Table 4.9. Sub-acute toxicity studies of *Trevesia palmata* flower buds methanolic extract in Wistar albino rats relating to clinic observations and survival

[illegible]

[illegible]

Table 4.10 28-days oral toxicity observation in male rat treated by repeated dose of TPFME were recorded.

Animals/Sex	Observation	Treatment Group (mg/kg b.w)			
		Control	10	100	500
Male	Initial day body weight (g)	131.2 ± 0.583a	133.8 ± 0.73a	134.6 ± 0.74b	132.8 ± 0.73a
	Final day body weight (g)	151.6 ± 0.92a	150.0 ± 0.70a, b	147.2 ± 0.86b	140.8 ± 0.86c
	Body weight gain/loss (%)	15.57 ± 1.18a	12.11 ± 0.40a, b	9.37 ± 0.83b	6.04 ± 0.94c
	Mean body weight (g/day)	139.0 ± 1.53a	137.0 ± 1.33a	135.3 ± 1.22a, b	131.5 ± 1.14b
	Mean food consumption (g/day)	21.57 ± 0.46a	21.14 ± 0.47a	20.07 ± 0.39a, b	18.57 ± 0.49b
	Mean water consumption (mL/day)	24.0 ± 0.29a	23.14 ± 0.28a	21.46 ± 0.35b	21.11 ± 0.34b
	Mean rectal temperature (°C/day)	34.21 ± 0.14a	34.73 ± 0.14a, b	35.11 ± 0.17b, c	35.43 ± 0.18c
Female	Initial day body weight (g)	136.2 ± 1.77a	138.4 ± 1.20a	136.2 ± 2.05a	134.4 ± 1.28a
	Final day body weight (g)	151.6 ± 2.06a	148.6 ± 1.50a	151.0 ± 0.54a	140.2 ± 0.37c
	Body weight gain/loss (%)	11.31 ± 0.48a	7.39 ± 1.18a	10.95 ± 1.41a	4.35 ± 1.20b
	Mean body weight (g/day)	139.9 ± 1.27a	138.5 ± 1.11a	138.1 ± 1.30a	132.2 ± 0.83c
	Mean food consumption (g/day)	20.57 ± 0.56a	19.89 ± 0.50a, b	19.86 ± 0.52a, b	18.21 ± 0.47b
	Mean water consumption (mL/day)	21.29 ± 0.38a	20.21 ± 0.40a, b	19.82 ± 0.30b	18.89 ± 0.39b
	Mean rectal temperature (°C /day)	34.61 ± 0.15a	34.91 ± 0.13a, b	34.96 ± 0.17a, b	35.45 ± 0.20b

Values are expressed in Mean ± SEM, n = 5 animals/group/sex. Statistical comparison was performed using one-way ANOVA followed by Tukey's post hoc tests for all pair-wise multiple comparisons. Different alphabets row wise indicate that treatment groups are significantly different at p < 0.05.

Table 4.11 Effect of methanolic extract of *Trevesia palmata* flower buds on absolute organ weight of different organs of Wister albino rat in sub-acute (28-days repeated dose) oral toxicity study.

Animals/Sex	Organs/Unit		Sub-acute toxicity			
			Treatment Group (mg/kg b. w)			
			Control	10	100	500
Male	Liver	g	4.726 ± 0.326a	5.144 ± 0.385a	4.856 ± 0.400a	4.674 ± 0.371a
		%	3.604 ± 0.254a	3.840 ± 0.270a	3.606 ± 0.290a	3.515 ± 0.263a
	Kidneys	g	0.984 ± 0.041a	0.882 ± 0.030a	0.860 ± 0.056a	0.854 ± 0.030a
		%	0.750 ± 0.032a	0.659 ± 0.024a	0.638 ± 0.041a	0.643 ± 0.022a
	Brain	g	1.462 ± 0.383a	1.532 ± 0.566a	1.514 ± 0.393a	1.484 ± 0.041a
		%	1.115 ± 0.328a	1.444 ± 0.374a	1.125 ± 0.030a	1.117 ± 0.024a
	Heart	g	0.889 ± 0.045a	0.914 ± 0.058a	0.982 ± 0.058a	0.878 ± 0.043a
		%	0.678 ± 0.037a	0.683 ± 0.036a	0.728 ± 0.040a	0.660 ± 0.029a
	Spleen	g	0.358 ± 0.013a	0.376 ± 0.031a	0.397 ± 0.019a	0.400 ± 0.011a
		%	0.273 ± 0.010a	0.281 ± 0.023a	0.295 ± 0.013a	0.301 ± 0.008a
	Colon	g	0.634 ± 0.043a	0.618 ± 0.038a	0.772 ± 0.043a	0.750 ± 0.023a
		%	0.483 ± 0.032a	0.461 ± 0.026a	0.573 ± 0.032a	0.564 ± 0.016a
	Stomach	g	0.731 ± 0.469a	0.744 ± 0.041a	0.728 ± 0.034a	0.712 ± 0.042a
		%	0.557 ± 0.036a	0.556 ± 0.032a	0.540 ± 0.025a	0.535 ± 0.029a
	Pancreas	g	0.510 ± 0.017a	0.488 ± 0.021a	0.521 ± 0.019a	0.518 ± 0.019a
		%	0.388 ± 0.014a	0.365 ± 0.017a	0.387 ± 0.014a	0.390 ± 0.015a
	Lungs	g	1.380 ± 0.052a	1.302 ± 0.053a	1.346 ± 0.034a	1.324 ± 0.055a
		%	1.052 ± 0.041a	0.973 ± 0.040a	1.001 ± 0.038a	0.996 ± 0.038a
	Testis	g	1.254 ± 0.061a	1.140 ± 0.070a	1.230 ± 0.033a	1.292 ± 0.056a
		%	0.956 ± 0.048a	0.853 ± 0.056a	0.914 ± 0.027a	0.973 ± 0.045a

	Seminal vesicle	g	$0.772 \pm 0.029a$	$0.762 \pm 0.039a$	$0.856 \pm 0.028a$	$0.872 \pm 0.031a$
		%	$0.588 \pm 0.020a$	$0.569 \pm 0.028a$	$0.636 \pm 0.022a$	$0.656 \pm 0.025a$
Female	Liver	g	$4.502 \pm 0.171a$	$4.620 \pm 0.253a$	$4.470 \pm 0.221a$	$4.160 \pm 0.213a$
		%	$3.092 \pm 0.101a$	$3.221 \pm 0.150a$	$3.135 \pm 0.133a$	$3.146 \pm 0.154a$
	Kidneys	g	$0.982 \pm 0.043a$	$1.144 \pm 0.051a$	$1.090 \pm 0.049a$	$0.978 \pm 0.034a$
		%	$0.676 \pm 0.036a$	$0.798 \pm 0.031a$	$0.764 \pm 0.028a$	$0.740 \pm 0.027a$
	Brain	g	$1.340 \pm 0.027a$	$1.374 \pm 0.015a$	$1.358 \pm 0.022a$	$1.288 \pm 0.019a$
		%	$0.922 \pm 0.030a$	$0.959 \pm 0.012a$	$0.953 \pm 0.010a$	$0.974 \pm 0.014a$
	Heart	g	$0.760 \pm 0.027a$	$0.782 \pm 0.033a$	$0.770 \pm 0.024a$	$0.676 \pm 0.016a$
		%	$0.522 \pm 0.019a$	$0.545 \pm 0.021a$	$0.540 \pm 0.013a$	$0.511 \pm 0.010a$
	Spleen	g	$0.380 \pm 0.019a$	$0.401 \pm 0.017a$	$0.358 \pm 0.016a$	$0.377 \pm 0.020a$
		%	$0.260 \pm 0.010a$	$0.280 \pm 0.013a$	$0.251 \pm 0.012a$	$0.285 \pm 0.014a$
	Colon	g	$0.762 \pm 0.039a$	$0.780 \pm 0.031a, b$	$0.788 \pm 0.023a, b$	$0.646 \pm 0.022a$
		%	$0.523 \pm 0.028a$	$0.550 \pm 0.012a$	$0.553 \pm 0.012a$	$0.488 \pm 0.015a$
	Stomach	g	$0.708 \pm 0.012a$	$0.714 \pm 0.032a$	$0.730 \pm 0.034a$	$0.680 \pm 0.018a$
		%	$0.486 \pm 0.005a$	$0.498 \pm 0.018a$	$0.512 \pm 0.020a$	$0.514 \pm 0.014a$
	Pancreas	g	$0.528 \pm 0.035a$	$0.580 \pm 0.048a$	$0.534 \pm 0.029a$	$0.582 \pm 0.034a$
		%	$0.362 \pm 0.022a$	$0.405 \pm 0.017a$	$0.374 \pm 0.018a$	$0.440 \pm 0.027a$
	Lungs	g	$0.860 \pm 0.026a$	$0.736 \pm 0.160a$	$0.868 \pm 0.044a$	$0.784 \pm 0.027a$
		%	$0.591 \pm 0.019a$	$0.515 \pm 0.011a$	$0.610 \pm 0.035a$	$0.592 \pm 0.018a$
	Ovaries	g	$0.068 \pm 0.004a$	$0.074 \pm 0.005a$	$0.064 \pm 0.005a$	$0.064 \pm 0.005a$
		%	$0.046 \pm 0.003a$	$0.052 \pm 0.004a$	$0.044 \pm 0.003a$	$0.048 \pm 0.004a$
	Uterus	g	$0.428 \pm 0.014a$	$0.402 \pm 0.008a, b$	$0.404 \pm 0.017a, b$	$0.368 \pm 0.010b$
		%	$0.294 \pm 0.011a$	$0.280 \pm 0.005a$	$0.284 \pm 0.014a$	$0.278 \pm 0.008a$

Values are expressed in Mean $\pm$ SEM, n = 5 animals/group/sex. Statistical comparison was performed using one-way ANOVA followed by Tukey's post hoc tests for all pair-wise multiple comparisons. Different alphabets row wise indicate that treated and non-treated groups are significantly different at $p < 0.05$ and with similar alphabets indicate not statistically significant at $p > 0.05$.

Table 4.12 The impact of a single dosage of a methanolic extract of *Trevesia palmata* flower buds given orally for 28 days repeated dose on certain hematological parameters in male rats.

Animals/Sex	Parameters	Unit	Normal range*	Treatment Group (mg/kg b. w)			
				Control	10	100	500
Male	Haemoglobin (Hb.)	g/dL	10.2-16.6	13.42 ± 0.69a	13.42 ± 0.43a	13.94 ± 0.38a	14.18 ± 0.49a
	Total red blood cells (RBC's)	10 ⁶ /μL	5-10	7.74 ± 0.40a	7.82 ± 0.31a	8.04 ± 0.32a	8.20 ± 0.36a
	Packed cell volume (PCV)	%	39-49	41.34 ± 2.12a	41.22 ± 1.30a	42.79 ± 1.14a	43.51 ± 1.49a
	Mean corpuscular volume (MCV)	fL	48-54	52.18 ± 0.74a	52.39 ± 0.67a	53.99 ± 0.49a	53.69 ± 0.50a
	Mean corpuscular haemoglobin (MCH)	Pg	17-21	17.01 ± 0.53a	17.26 ± 0.85a	17.38 ± 0.38a	17.35 ± 0.54a
	Mean corpuscular haemoglobin concentration (MCHC)	g/dL	32-38	32.46 ± 0.08a	32.55 ± 0.02a	32.58 ± 0.01a	32.59 ± 0.05a
	Total white blood cells (WBC's)	10 ³ /μL	6-15	6.80 ± 0.16a	7.23 ± 0.20a	7.39 ± 0.24a	8.55 ± 0.37b
	Neutrophils (NP)	%	10-40	20.11 ± 1.23a	16.95 ± 1.64a	17.35 ± 1.92a	15.56 ± 1.06a
	Lymphocytes (LC)	%	55-95	76.14 ± 2.65a	78.19 ± 3.30a	77.83 ± 3.57a	79.47 ± 3.81a
	Monocytes (MC)	%	1-4	1.32 ± 0.15a	1.62 ± 0.10a	1.66 ± 0.17a	1.93 ± 0.28a
	Eosinophils (EP)	%	0-4	1.10 ± 0.26a	1.15 ± 0.19a	1.80 ± 0.25a	1.91 ± 0.24a

	Platelets (PLT)	10 ³ /μL	782-985	1002 ± 41.11a	909 ± 45.81a	1035 ± 75.44a	975 ± 63.34a
Female	Haemoglobin (Hb.)	g/dL	10.2-16.6	12.62 ± 0.41a	12.16 ± 0.42	13.50 ± 0.32a	13.38 ± 0.30a
	Total red blood cells (RBC's)	10 ⁶ /μL	5-10	7.44 ± 0.23a	7.14 ± 0.18a	7.67 ± 0.14a	7.65 ± 0.17a
	Packed cell volume (PCV)	%	39-49	38.81 ± 1.25a	37.43 ± 1.26a	41.46 ± 0.96a	41.07 ± 0.92a
	Mean corpuscular volume (MCV)	fL	48-54	52.18 ± 0.74a	52.39 ± 0.67a	53.99 ± 0.49a	53.69 ± 0.50a
	Mean corpuscular haemoglobin (MCH)	Pg	17-21	16.97 ± 0.24a	17.02 ± 0.23a	17.58 ± 0.17a	17.48 ± 0.16a
	Mean corpuscular haemoglobin concentration (MCHC)	g/dL	32-38	32.51 ± 0.02a	32.49 ± 0.02a	32.56 ± 0.01	32.55 ± 0.01a
	Total white blood cells (WBC's)	10 ³ /μL	6-15	6.30 ± 0.34a	6.21 ± 0.28a	6.47 ± 0.33a	8.36 ± 0.29b
	Neutrophils (NP)	%	10-40	19.41 ± 1.73a	19.15 ± 1.48a	20.90 ± 1.88a	16.12 ± 1.54a
	Lymphocytes (LC)	%	55-95	77.12 ± 4.20a	77.56 ± 2.67a	75.56 ± 2.96a	81.80 ± 2.85a
	Monocytes (MC)	%	1-4	1.87 ± 0.28a	1.82 ± 0.19a	1.57 ± 0.21a	2.1 ± 0.23a
	Eosinophils (EP)	%	0-4	1.01 ± 0.10a	0.88 ± 0.13a	1.29 ± 0.19a	1.64 ± 0.27a
	Platelets (PLT)	10 ³ /μL	782-985	808 ± 51.12a	886 ± 35.08a	863 ± 30.58a	977 ± 57.78

Values are expressed in Mean ± SEM, n = 5 animals/group/sex. Statistical comparison was performed using one-way ANOVA followed by Tukey's post-hoc tests for all pair-wise multiple comparisons. Different alphabets row wise indicate that treated and non-treated groups are significantly different at $p < 0.05$ and with similar alphabets indicate not statistically significant at $p > 0.05$.

*(Giknis and Clifford, 2008

Table 4.13 Effect on hepatic and renal function biomarkers, lipid profile, blood serum glucose level, and major ion/electrolytes from blood serum of male rats in relating to sub-acute toxicity study (28 days repeated dose) of methanolic extract of *Trevesia palmata* flower buds.

Animals/Sex	Biochemical Parameters	Treatment Group (mg/kg b. w)				
		Control	10	100	500	Reference value (Normal range)
Liver and renal function test						
Male	Total protein (g/dL)	5.860 ± 0.39a	5.060 ± 0.23a	5.444 ± 0.27a	5.380 ± 0.41a	5.2-7.1 ^{##}
	Albumin (g/dL)	3.480 ± 0.17a	3.140 ± 0.20a	3.400 ± 0.66a	3.300 ± 0.15a	3.3-4.9 [#]
	Globulin (g/dL)	2.400 ± 0.34a	1.940 ± 0.25a	2.040 ± 0.50a	2.080 ± 0.33a	1.5-2.4 [#]
	Albumin/Globulin ratio	1.580 ± 0.21a	1.780 ± 0.32a	2.340 ± 0.72a	1.840 ± 0.37a	1.7-3.0 [#]
	Aspartate aminotransferase (U/L)	79.80 ± 4.90a	84.20 ± 5.80a	89.00 ± 5.30a	92.40 ± 6.45a	74-143 ^{##}
	Alanine aminotransferase (U/L)	19.20 ± 1.98a	21.40 ± 2.22a	25.40 ± 2.13a	27.40 ± 2.20a	18-45 ^{##}
	Alkaline phosphatase (U/L)	174.4 ± 5.69a	176.2 ± 3.54a	188.4 ± 4.99a	190.4 ± 4.00a	62-230 ^{##}
	Total bilirubin (mg/dL)	0.420 ± 0.08a	0.434 ± 0.04a	0.454 ± 0.06a	0.480 ± 0.06a	0.3-0.8*
	Direct bilirubin (mg/dL)	0.210 ± 0.02a	0.288 ± 0.45a	0.304 ± 0.02a	0.240 ± 0.46a	0.2-0.5**
	Creatinine (mg/dL)	0.332 ± 0.04a	0.373 ± 0.04a	0.356 ± 0.03a	0.396 ± 0.02a	0.3-0.5 ^{##}
	Uric acid (mg/dL)	1.909 ± 0.28a	1.388 ± 0.36a	1.180 ± 0.21a	0.981 ± 0.22a	3.5-48***
	Urea (mg/dL)	20.39 ± 0.66a	22.11 ± 1.16a	23.05 ± 0.63a	23.71 ± 0.89a	10.7-20 ^{##}
	Blood urea nitrogen (mg/dL)	9.521 ± 0.30a	10.32 ± 0.54a	10.77 ± 0.29a	11.07 ± 0.41a	8-33 ^{###}
Female	Total protein (g/dL)	6.100 ± 0.27a	5.640 ± 0.34a	6.140 ± 0.15a	6.200 ± 0.18a	5.5-7.7 ^{##}
	Albumin (g/dL)	3.580 ± 0.17a	3.540 ± 0.18a	3.620 ± 0.31a	3.580 ± 0.34a	3.3-4.9 [#]
	Globulin (g/dL)	2.500 ± 0.32a	2.100 ± 0.23a	2.500 ± 0.39a	2.620 ± 0.45a	1.5-2.4 [#]
	Albumin/Globulin ratio	1.560 ± 0.26a	1.760 ± 0.17a	1.740 ± 0.44a	1.940 ± 0.82a	1.7-3.0 [#]
	Aspartate aminotransferase (U/L)	75.00 ± 3.92a	78.20 ± 6.31a	83.60 ± 6.31a	87.40 ± 5.58a	65-203 ^{##}
	Alanine aminotransferase (U/L)	21.20 ± 2.35a	22.20 ± 1.77a	26.20 ± 2.35a	29.40 ± 2.80a	16-48 ^{##}
	Alkaline phosphatase (U/L)	147.0 ± 5.52a	148.8 ± 1.96a	159.4 ± 5.13a	166.2 ± 6.93a	26-147 ^{##}
	Total bilirubin (mg/dL)	0.474 ± 0.01a	0.432 ± 0.03a	0.412 ± 0.04a	0.402 ± 0.03a	0.3-0.8*
	Direct bilirubin (mg/dL)	0.280 ± 0.05a	0.226 ± 0.05a	0.262 ± 0.04a	0.274 ± 0.02a	0.2-05**

	Creatinine (mg/dL)	0.392 ± 0.03a	0.386 ± 0.02a	0.394 ± 0.03a	0.316 ± 0.01a	0.3-0.6 ^{##}
	Uric acid (mg/dL)	2.107 ± 0.26a	1.758 ± 0.14a	1.461 ± 0.25a	1.597 ± 0.12a	3.5-48d ^{***}
	Urea (mg/dL)	22.55 ± 0.71a	25.11 ± 0.68a	23.53 ± 0.75a	24.19 ± 0.94a	11.7-25 ^{##}
	Blood urea nitrogen (mg/dL)	10.53 ± 0.33a	11.72 ± 0.31a	10.99 ± 0.35a	11.30 ± 0.44a	8-33 ^{###}
	Lipid profiles and Blood serum glucose level					
Male	Total Cholesterol (mg/dL)	58.8 ± 1.15a	60.6 ± 1.25a, b	47.18 ± 2.05c	53.04 ± 1.41a, c	37-95 ^{##}
	Triglycerides (mg/dL)	73.4 ± 1.66a	62.8 ± 2.03b,	50.8 ± 3.87c,	55.80 ± 2.2b, c	27-160 ^{##}
	HDL Cholesterol (mg/dL)	37.2 ± 0.97a	39.1 ± 1.79a	29.32 ± 1.13b	26.3 ± 1.37b	21-54 ^{\$}
	LDL Cholesterol (mg/dL)	6.96 ± 0.46a	8.98 ± 0.58a	7.70 ± 2.38a	15.5 ± 1.89b	nil
	Glucose (mg/dL)	90.86 ± 2.28a	98.26 ± 3.67a	101.8 ± 2.43a	99.90 ± 2.96a	106-184 ^{##}
Female	Total Cholesterol (mg/dL)	66.6 ± 1.60a	50.3 ± 1.45a	53.8 ± 1.52a	49.7 ± 1.63a	23-97 ^{##}
	Triglycerides (mg/dL)	69.2 ± 2.15a	49.80 ± 5.51b	50.8 ± 1.98b	50.0 ± 1.44b	16-175 ^{##}
	HDL Cholesterol (mg/dL)	44.62 ± 1.67a	33.36 ± 1.17b	33.76 ± 1.34b	25.98 ± 1.68d	21-54 ^{\$}
	LDL Cholesterol (mg/dL)	8.22 ± 2.59a	7.50 ± 1.27a	9.96 ± 2.30a	13.78 ± 2.38a	nil
	Glucose (mg/dL)	93.10 ± 3.20a	93.98 ± 2.25a	84.50 ± 2.14a	83.22 ± 3.89a	89-163 ^{##}
	Blood serum ion/electrolytes concentration					
Male	Calcium (mg/dL)	10.7 ± 0.35a	10.1 ± 0.53a	9.73 ± 0.30a	9.99 ± 0.32a	9.1-11.9 ^{##}
	Sodium (mmol/L)	147.4 ± 1.48a	147.2 ± 0.73a	147.0 ± 0.98a	141.0 ± 1.32b	137-147 ^{##}
	Potassium (mmol/L)	5.80 ± 0.19a	5.87 ± 0.24a	5.26 ± 0.18a	5.21 ± 0.20a	3.88-6.11 ^{##}
	Chloride (mmol/L)	95.3 ± 2.18a	99.0 ± 2.83a	92.0 ± 1.90a	73.8 ± 2.52b	98-106 ^{##}
Female	Calcium (mg/dL)	10.2 ± 0.22a	9.6 ± 0.15a	9.7 ± 0.08a	11.1 ± 0.20b	9.5-12.1 ^{##}
	Sodium (mmol/L)	145.3 ± 0.54a	147.9 ± 0.43a	140.7 ± 1.02b	146.7 ± 0.64a	135-143 ^{##}
	Potassium (mmol/L)	5.86 ± 0.29a	4.87 ± 0.15b	4.74 ± 0.20b	4.94 ± 0.18b	3.37-5.11 ^{##}
	Chloride (mmol/L)	92.1 ± 2.33a	83.8 ± 2.32a	71.6 ± 2.17b	85.1 ± 2.59a	97-106 ^{##}

All values are expressed in Mean $\pm$ SEM, n = 5 animals/group/sex. Statistical comparison was performed using one-way ANOVA followed by Tukey's post-hoc tests for all pair-wise multiple comparisons. Different alphabets row wise indicate that treated and non-treated groups are significantly different at $p < 0.05$ and with similar alphabets indicate not statistically significant at $p > 0.05$. BUN: Blood urea nitrogen; HDL: High density lipoproteins; LDL: Low density lipoproteins

(Villas-Boas et al., 2021); ## (Giknis and Clifford, 2008); ### (Upadhyay et al., 2019); * (Mulyati et al., 2020); ** (Augustine et al., 2020)

*** (Verma et al., 2023); \$ (El Malik and Sabahelkhier, 2019)

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Table 4.14 Effects of repeated-dosage administration of TPFME for sub-acute toxicity study (28-days) on markers of oxidative stress and antioxidant in Wister albino rats.

Sex/Organ		Parameters	Treatment Group (mg/kg b. w)			
			Control	10	100	500
Male	Liver	MDA (nmol MDA/mg of protein)	7.74 ± 0.37a	9.48 ± 0.53a	9.30 ± 0.64a	8.01 ± 0.38a
		SOD (U SOD/mg of protein)	0.28 ± 0.05a	0.21 ± 0.03a	0.32 ± 0.04a	0.30 ± 0.02a
		GSH-Px (μmol GSH oxidize/min/mg of protein)	0.22 ± 0.04a	0.26 ± 0.03a	0.27 ± 0.03a	0.31 ± 0.02a
		CAT (KU/mg of protein)	0.31 ± 0.02a	0.40 ± 0.03a	0.53 ± 0.02b	0.57 ± 0.03b
	Kidney	MDA (nmol MDA/mg of protein)	12.94 ± 0.84a	19.94 ± 0.79b	15.57 ± 0.61a	14.75 ± 0.71a
		SOD (U SOD/mg of protein)	0.24 ± 0.04a	0.51 ± 0.03b	0.45 ± 0.04b	0.35 ± 0.03a, b
		GSH-Px (μmol GSH oxidize/min/mg of protein)	0.17 ± 0.02a	0.27 ± 0.02a	0.18 ± 0.03a	0.19 ± 0.02a
		CAT (KU/mg of protein)	0.29 ± 0.01a	0.23 ± 0.02a	0.26 ± 0.03a	0.31 ± 0.02a
Female	Liver	MDA (nmol MDA/mg of protein)	10.70 ± 0.52a	11.18 ± 0.26a	19.59 ± 0.57b	11.86 ± 0.43a
		SOD (U SOD/mg of protein)	0.37 ± 0.02a	0.44 ± 0.02a	0.41 ± 0.03a	0.38 ± 0.03a
		GSH-Px (μmol GSH oxidize/min/mg of protein)	0.25 ± 0.02	0.19 ± 0.02a	0.26 ± 0.03a	0.20 ± 0.01a
		CAT (KU/mg of protein)	0.33 ± 0.02a	0.40 ± 0.02a	0.42 ± 0.03a	0.44 ± 0.03a
	Kidney	MDA (nmol MDA/mg of protein)	13.56 ± 0.46a	17.74 ± 0.69b	17.27 ± 0.36b	16.61 ± 0.56b
		SOD (U SOD/mg of protein)	0.27 ± 0.04a	0.25 ± 0.04a	0.23 ± 0.05a	0.26 ± 0.02a
		GSH-Px (μmol GSH oxidize/min/mg of protein)	0.16 ± 0.03a	0.20 ± 0.03a	0.24 ± 0.20a	0.18 ± 0.02a
		CAT (KU/mg of protein)	0.23 ± 0.03a	0.28 ± 0.04a	0.29 ± 0.01a	0.25 ± 0.02a

All values are expressed in Mean $\pm$ SEM, n = 5 animals/group/sex. Statistical comparison was performed using one-way ANOVA followed by Tukey's post hoc tests for all pair-wise multiple comparisons. Different alphabets row wise indicate that treated and non-treated groups are significantly different at $p < 0.05$ and with similar alphabets indicate not statistically significant at $p > 0.05$. MDA: malondialdehyde; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase; CAT: catalase

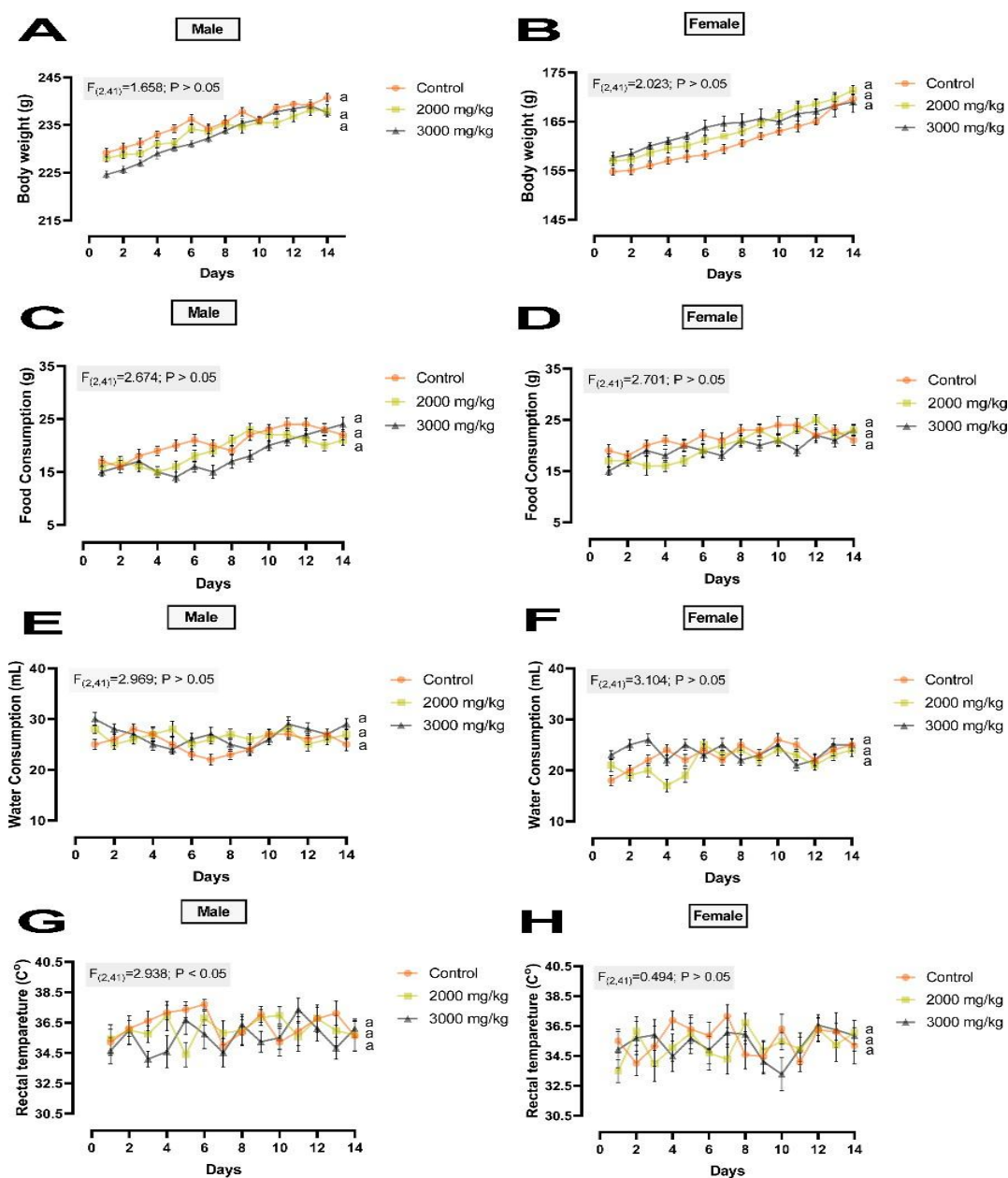


Figure 4.1 Assessment of body weight, food consumption, water consumption and rectal temperature from rats after single dose of treatment of 14-days (acute oral toxicity) in different treatment groups. Body weight (g) (**Figure A and B**), food consumption (g) (**Figure C and D**), water consumption (mL) (**Figure E and F**) and rectal temperature (C°) (**Figure G and H**) in male and female group respectively; Data values are represented in mean $\pm$ SEM, n=5 animals/group/sex, (ANOVA/Tukey test). Connected line with different alphabets indicate statistically significant at $p < 0.05$.

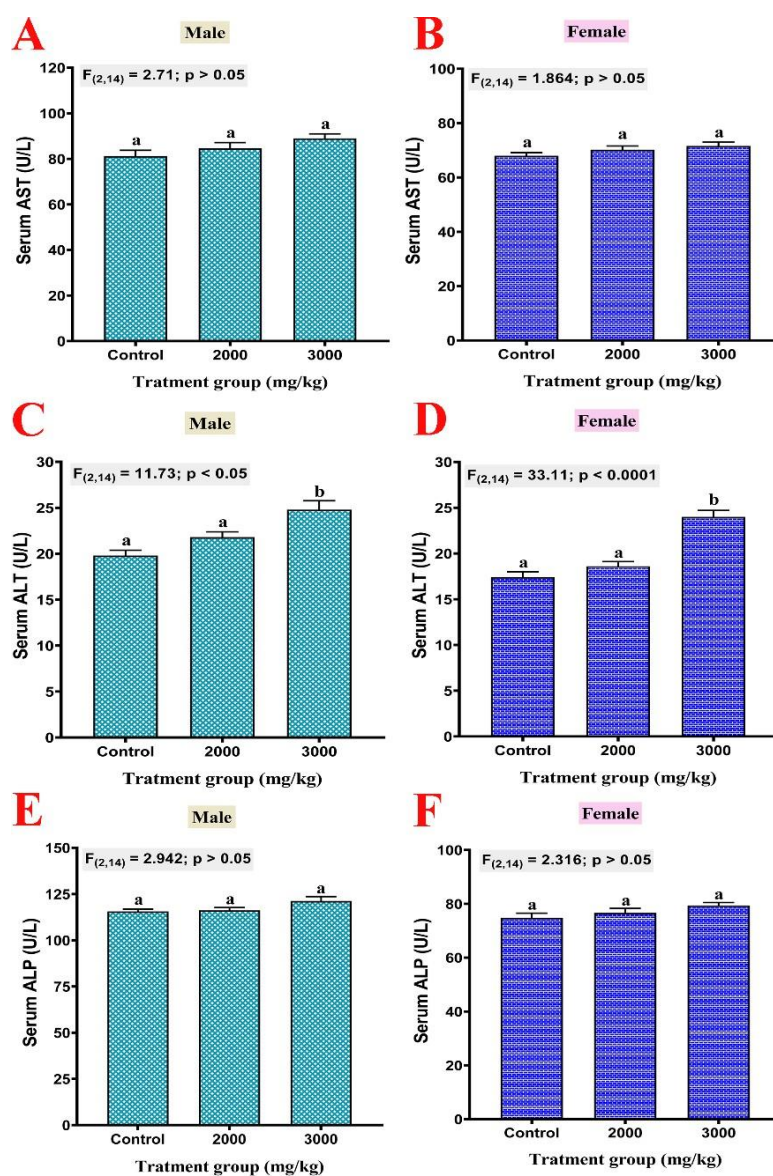


Figure 4.2 Effects of *Trevesia palmata* methanolic extract on serum level concentration of aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in male and female rats after single dose of treatment of 14 days (acute oral toxicity) as compared to the control group. The figure has representation of **A** and **D** – AST; **B** and **E**- ALT; **C** and **F**- ALP in male and female respectively. Data values are represented in mean $\pm$ SEM, n=5 animals/group/sex, (ANOVA/Tukey test). Bar with different alphabets indicate statistically significant at $p < 0.05$.

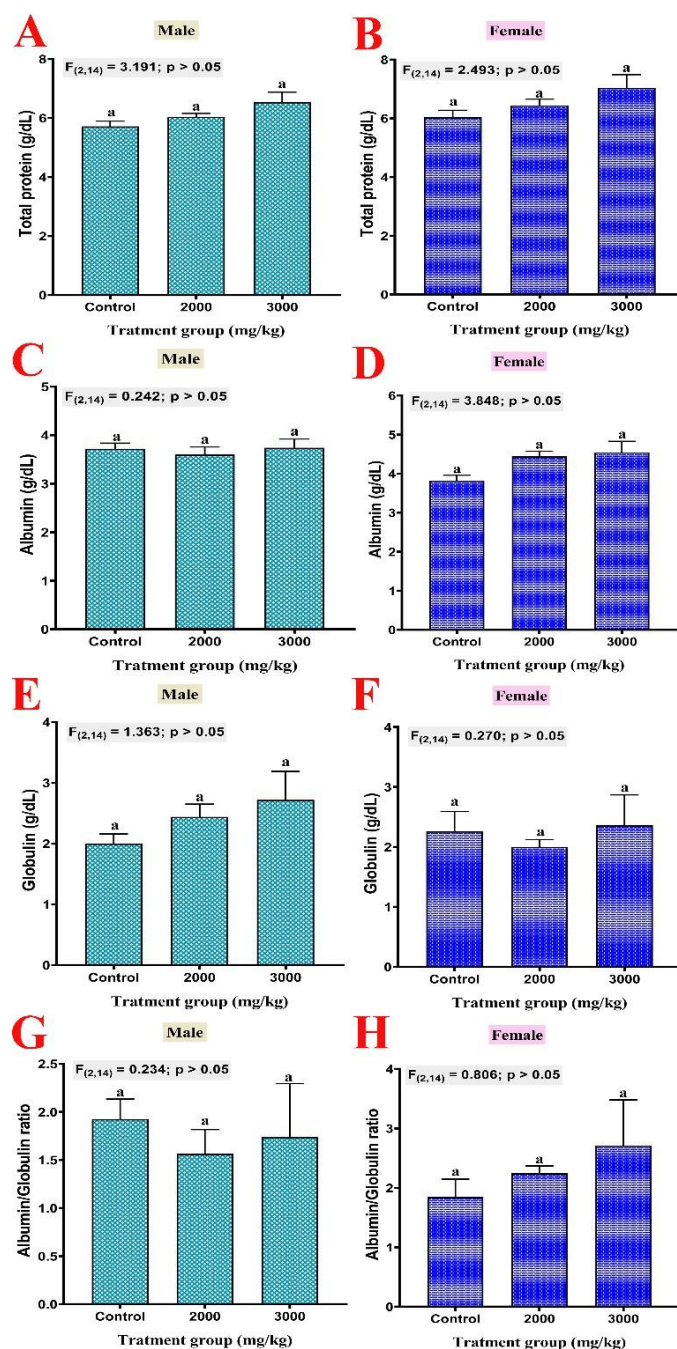


Figure 4.3 Effects of *Trevesia palmata* methanolic extract on serum level concentration of total protein, albumin, globulin, A/G ratio in male and female rats after single dose of treatment of 14 days (acute oral toxicity) as compared to the control group. The figure has representation of **A** and **B** – total protein; **C** and **D**–albumin; **E** and **F**–Globulin; **G** and **H** in male and female respectively. Data values are represented in mean $\pm$ SEM, n=5 animals/group/sex, (ANOVA/Tukey test). Bar with similar alphabets indicate statistically significant at $p > 0.05$.

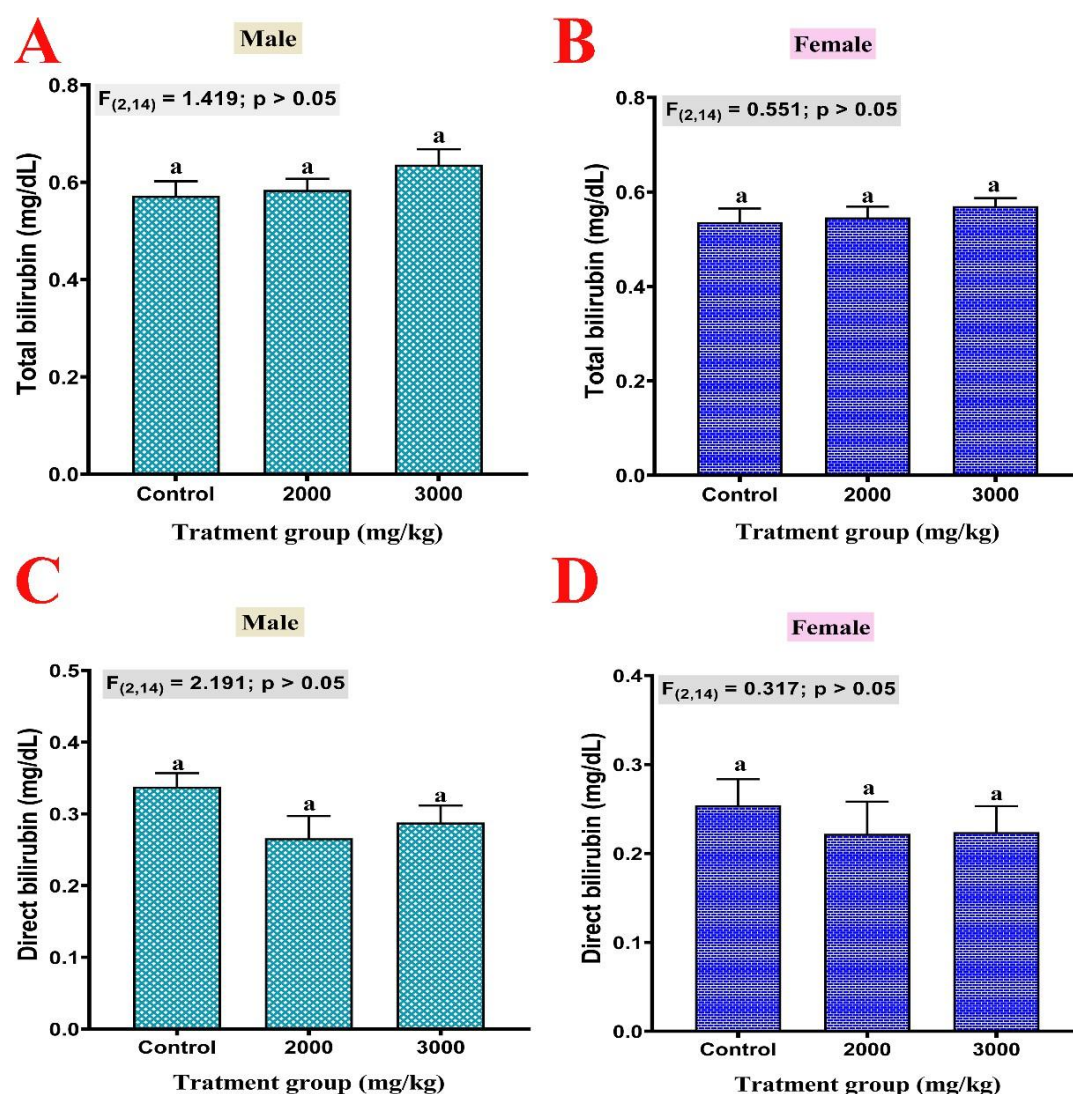


Figure 4.4 Effects of *Trevesia palmata* methanolic extract on serum level concentration of total bilirubin and direct bilirubin in male and female rats after single dose of treatment of 14 days (acute oral toxicity) as compared to the control group. The figure has representation of **A** and **B** – total bilirubin; **C** and **D**- direct bilirubin in male and female respectively. Data values are represented in mean $\pm$ SEM, n=5 animals/group/sex, (ANOVA/Tukey test). Bar with similar alphabets indicate statistically significant at $p > 0.05$.

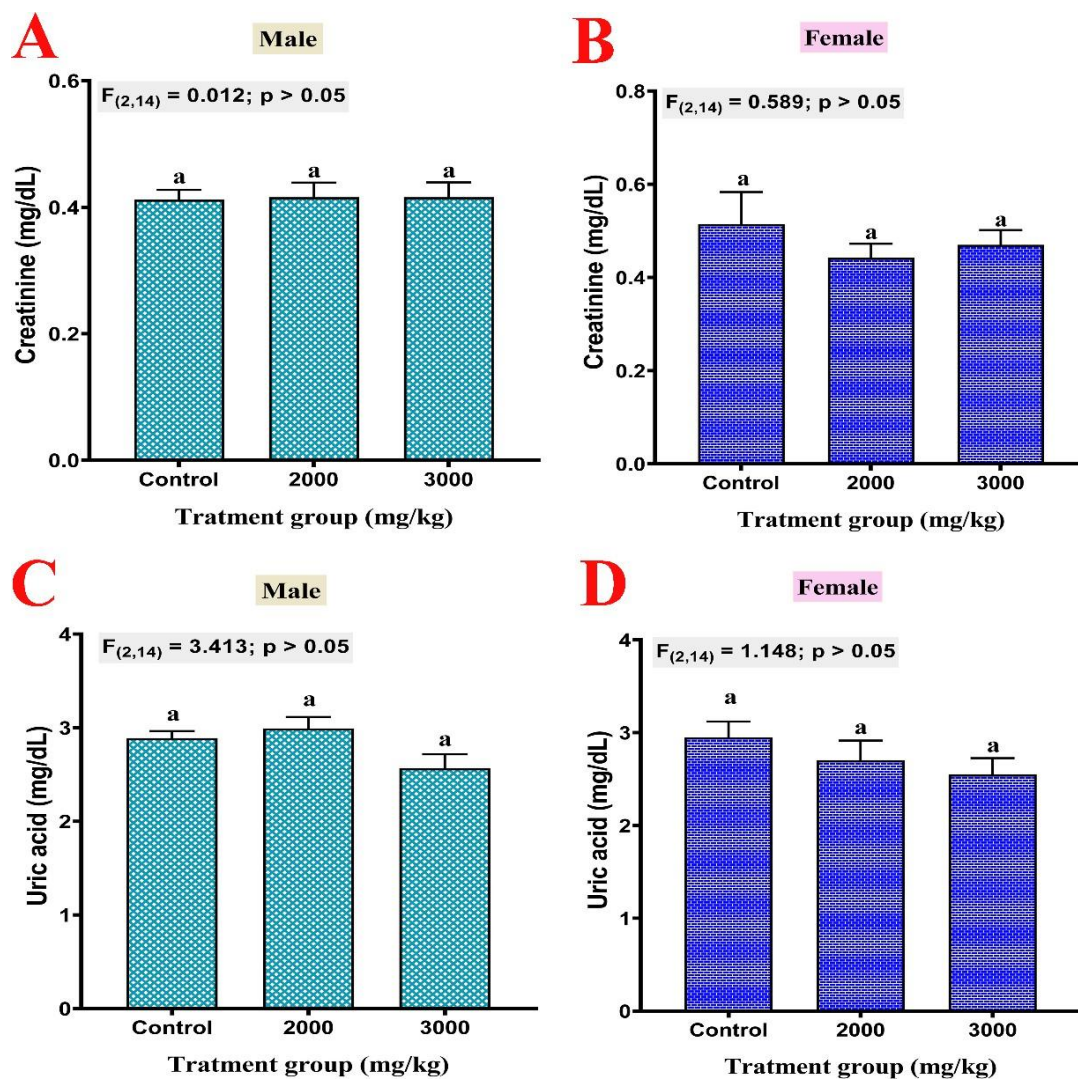


Figure 4.5 Effects of *Trevesia palmata* methanolic extract on serum level concentration of creatinine and uric acid in male and female rats after single dose of treatment of 14 days (acute oral toxicity) as compared to the control group. The figure has representation of **A** and **B** – Creatinine; **C** and **D**- Uric acid in male and female respectively. Data values are represented in mean $\pm$ SEM, n=5 animals/group/sex, (ANOVA/Tukey test). Bar with similar alphabets indicate statistically significant at $p > 0.05$.

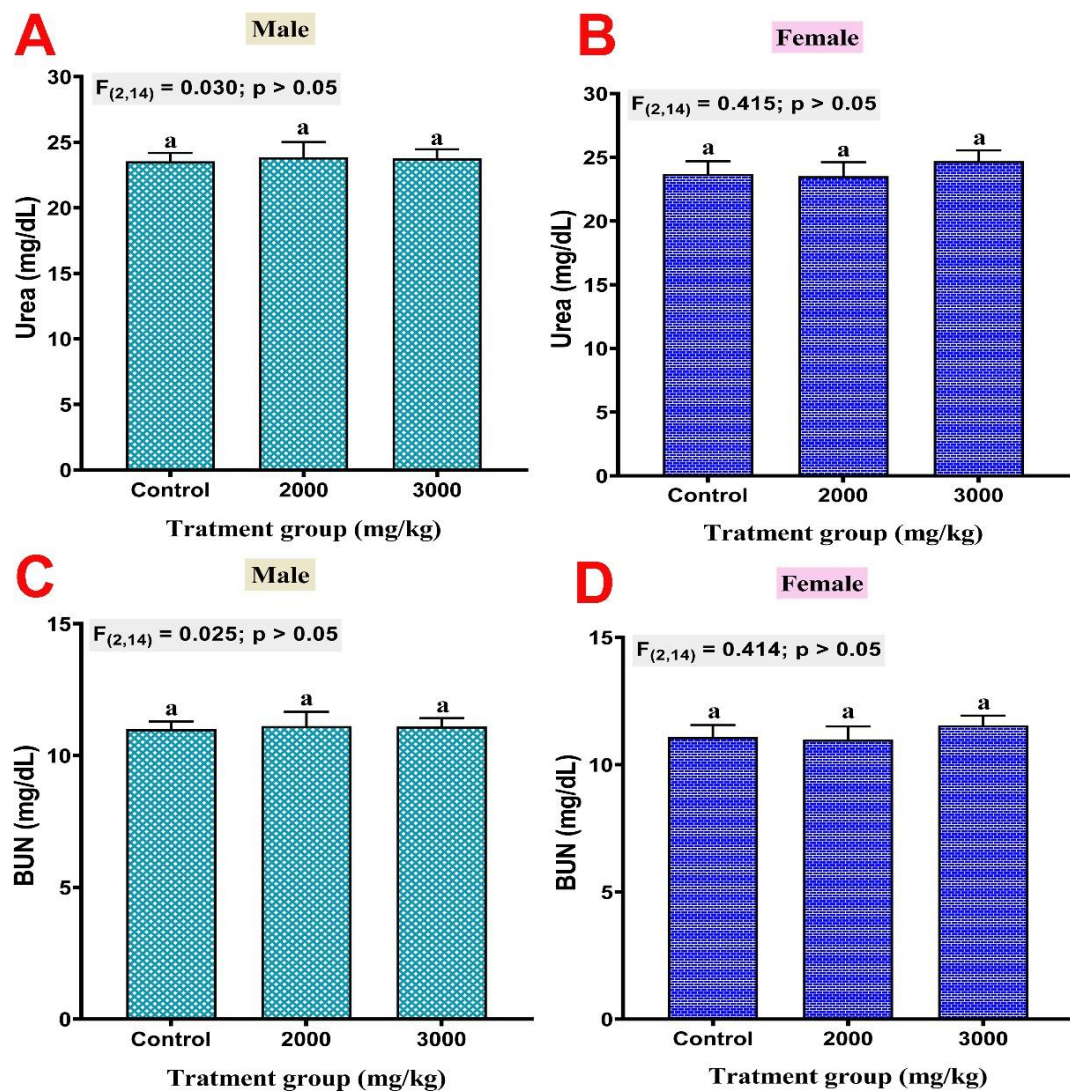


Figure 4.6 Effects of *Trevesia palmata* methanolic extract on serum level concentration of Urea and BUN in male and female rats after single dose of treatment of 14 days (acute oral toxicity) as compared to the control group. The figure has representation of **A** and **B** – Urea; **C** and **D**- Blood urea nitrogen (BUN) in male and female respectively. Data values are represented in mean $\pm$ SEM, n=5 animals/group/sex, (ANOVA/Tukey test). Bar with similar alphabets indicate statistically significant at $p > 0.05$.

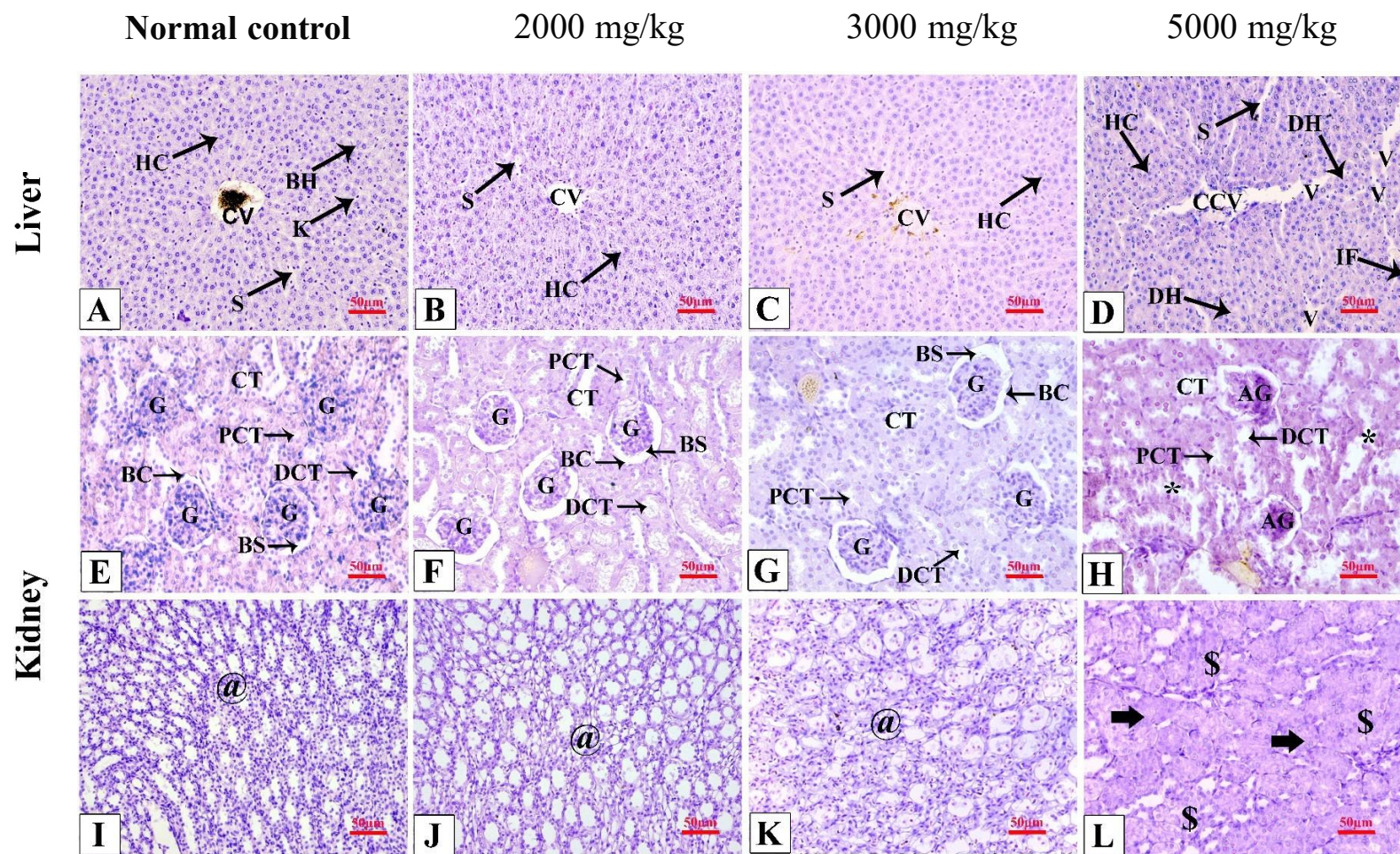


Figure 4.7 Histopathological analysis of the organ liver and kidney of Wister albino male rats exposed to methanolic extract from *T. palmata* flower buds in the acute oral toxicity test. **(A-D)** light photomicrograph of liver tissue sections (H&E). **(E-H)** photomicrograph of cortex section of kidney tissue (H&E). **(I-L)** photomicrograph of medulla section of kidney tissue (H&E). H&E: Hematoxylin and eosin staining, **scale bar = 50 µm for 20X magnification**; AG: Atrophied glomerulus; BC: Bowman's capsule; BH: Binucleated hepatocytes; BS: Bowman's Space; CT: Collecting tubules; CV: Central vein; CCV: Congested central vein; DCT: Distal convoluted tubules; DH: Degenerative hepatocytes; G: Glomerulus; HC: Hepatocytes; IF: Inflammatory cell infiltration; K: Kupffer cell; PCT: Proximal convoluted tubules; S: Sinusoids; V: Vacuolar degeneration; @: Medullary renal tubules; \$(Dolar): Hemorrhage; *(Single star): Congested collecting tubules; (Black arrow): Congestion of medullary rays.

5000 mg/kg

Kidney

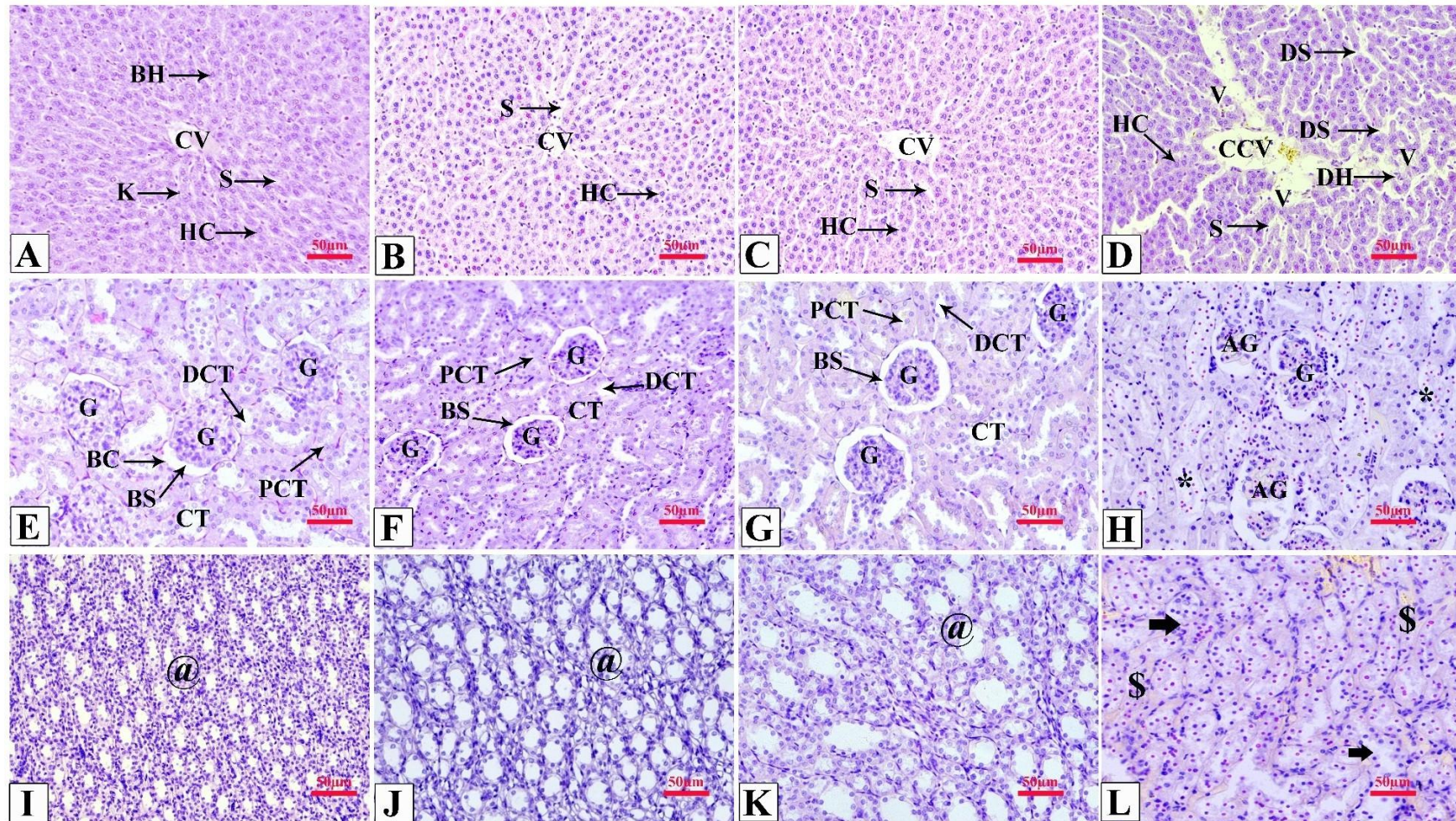


Figure 4.8. Histopathological examination of liver and kidney of Wister albino female rats exposed to methanolic extract from *T. palmata* flower buds in the acute oral toxicity study. **(A-D)** light photomicrograph of liver tissue sections (H&E). **(E-H)** photomicrograph of cortex section of kidney tissue (H&E). **(I-L)** photomicrograph of medulla section of kidney tissue (H&E). H&E: Hematoxylin and eosin staining, **scale bar = 50 μ m for 20X magnification**; AG: Atrophied glomerulus; BC: Bowman's capsule; BH: Binucleated hepatocytes; BS: Bowman's Space; CT: Collecting tubules; CV: Central vein; CCV: Congested central vein; DCT: Distal convoluted tubules; DH: Degenerative hepatocytes; DS: Dilated hepatic sinusoids; G: Glomerulus; HC: Hepatocytes; K: Kupffer cell; PCT: Proximal convoluted tubules; S: Sinusoids; V: Vacuolar degeneration; @: Medullary renal tubules; \$(Dolar): Hemorrhage; *(Single star): Congested collecting tubules; (Black arrow): Congestion of medullary rays.

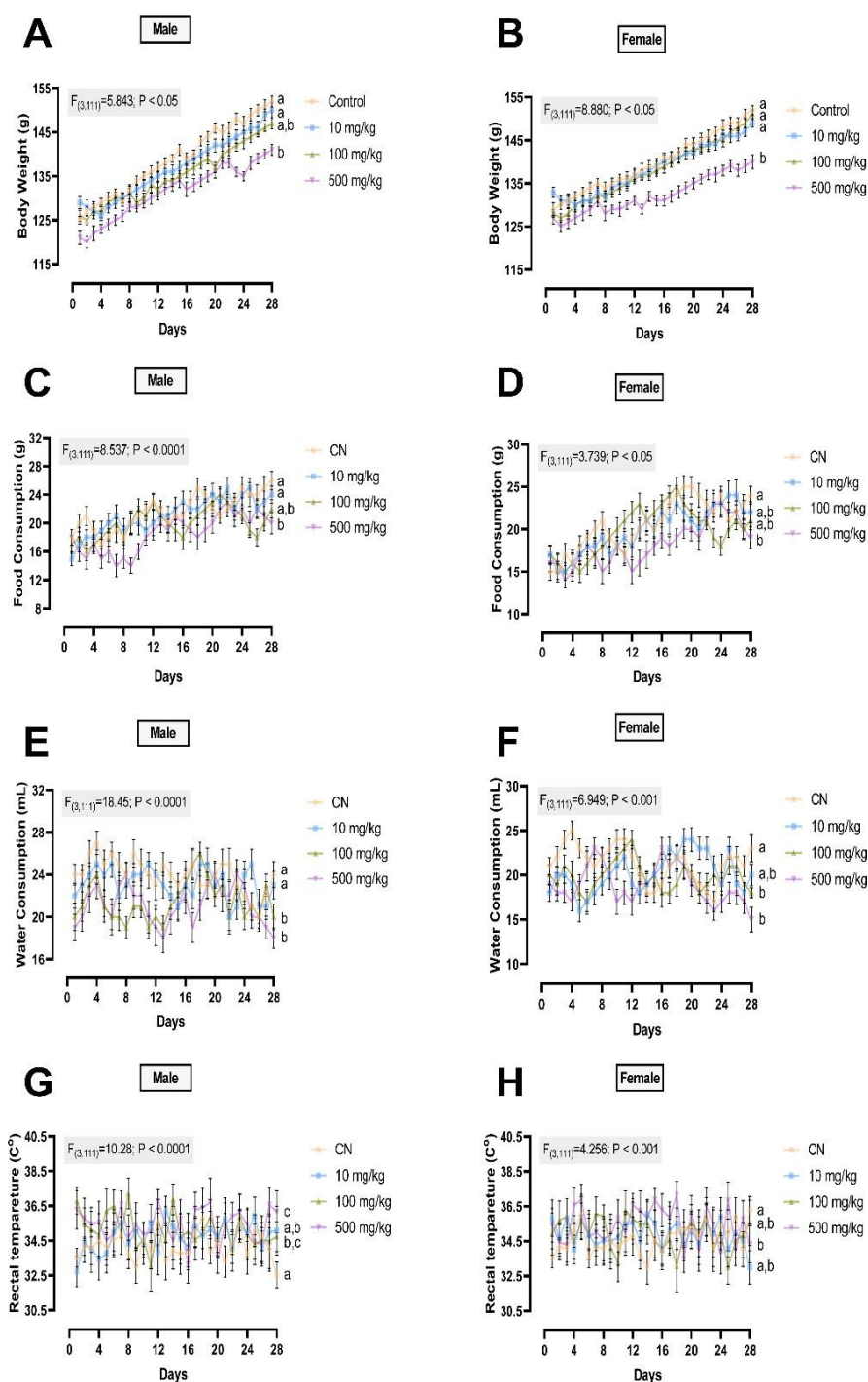


Figure 4.9 Assessment of body weight, food consumption, water consumption and rectal temperature from rats after repeated dose of treatment of 28 days (Sub-acute oral toxicity) in different treatment groups. body weight (g) (**Figure A and B**), food consumption (g) (**Figure C and D**), water consumption (mL) (**Figure E and F**) and rectal temperature (°C) (**Figure G and H**) in male and female group respectively; Data values are represented in mean $\pm$ SEM, n=5 animals/group/sex, (ANOVA/Tukey test). Point with connected line with different alphabets indicate statistically significant at $p < 0.05$.

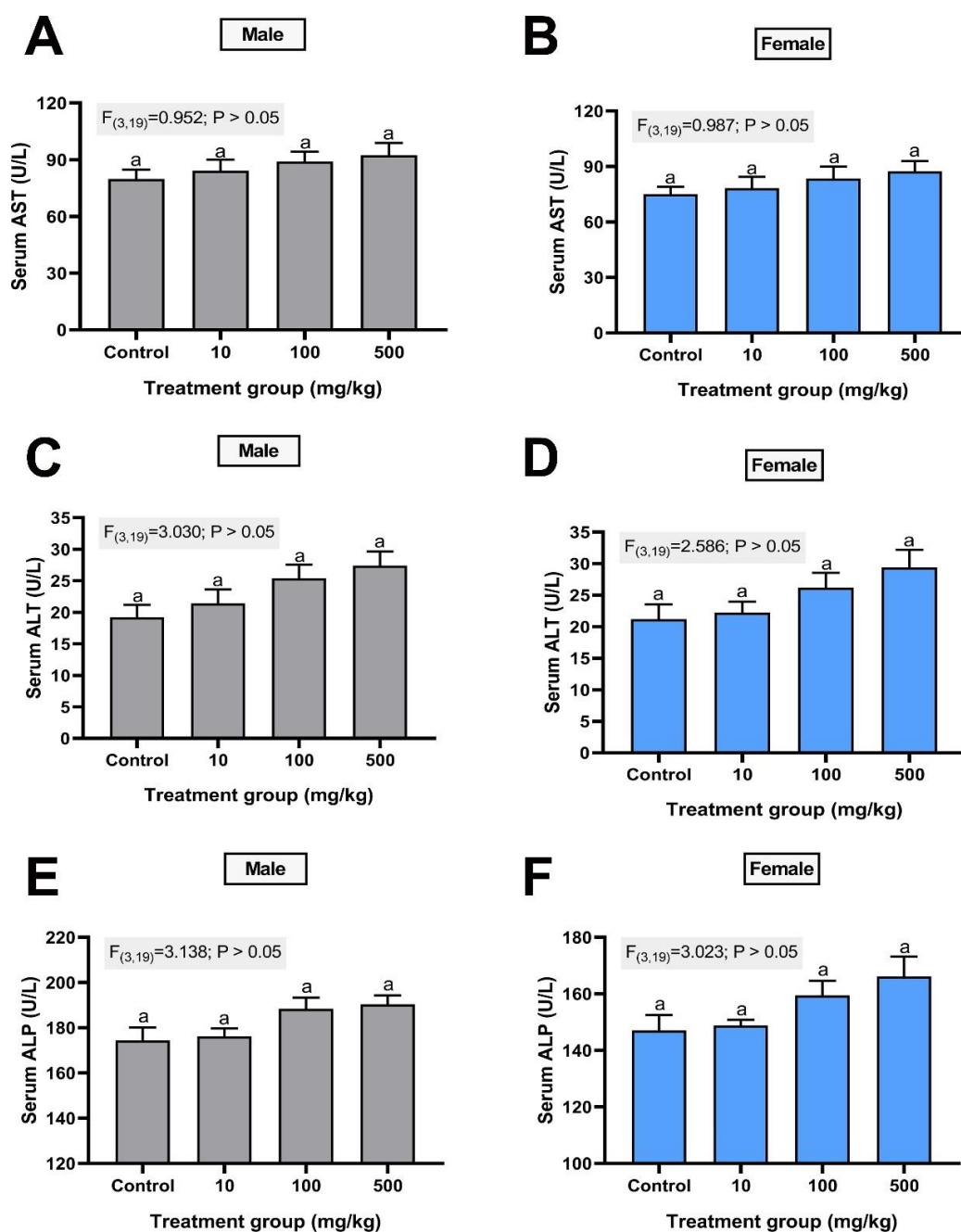


Figure 4.10 Effects of *Trevesia palmata* methanolic extract on serum level concentration of aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in male and female rats after repeated dose of treatment of 28 days (Sub-acute oral toxicity) as compared to the control group. The figure has representation of **A** and **D** – AST; **B** and **E**- ALT; **C** and **F**- ALP in male and female respectively. Data values are represented in mean $\pm$ SEM, n=5 animals/group/sex, (ANOVA/Tukey test). Bar with similar alphabets indicate statistically significant at $p > 0.05$.

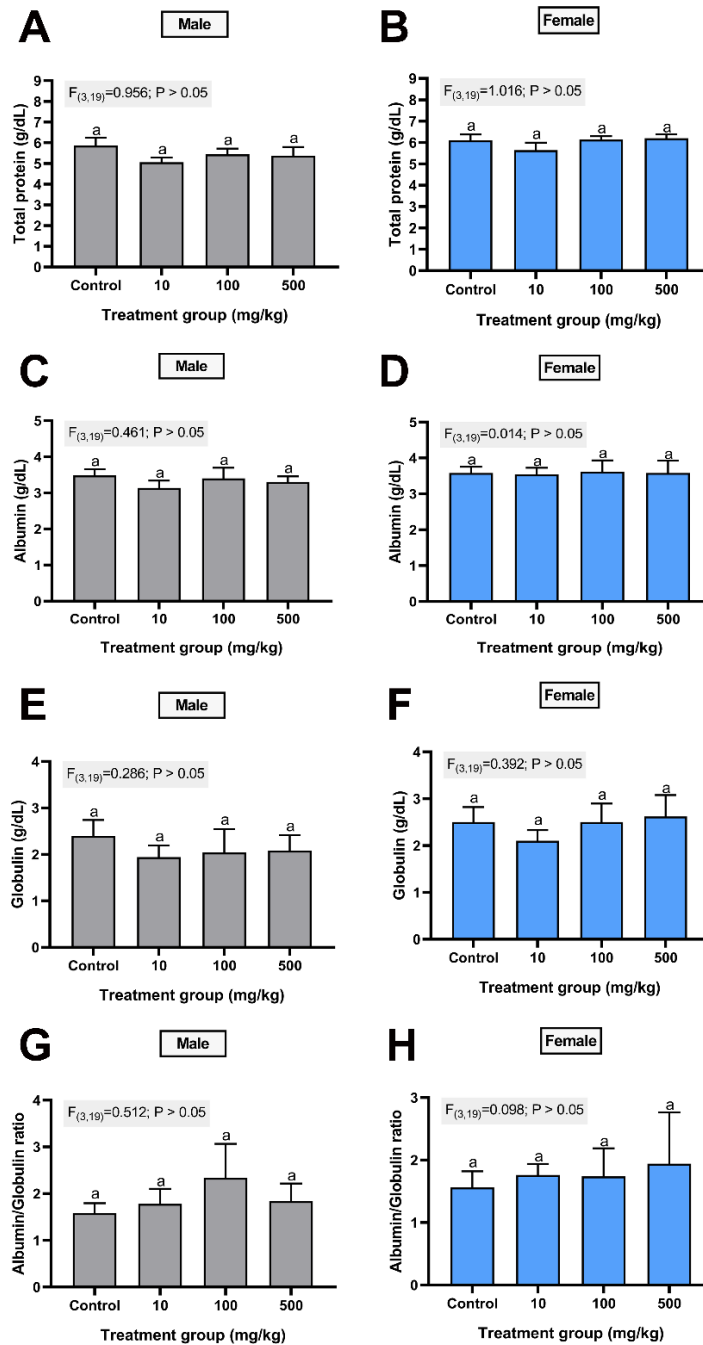


Figure 4.11 Effects of *Trevesia palmata* methanolic extract on serum level concentration of total protein, albumin, globulin, A/G ratio in male and female rats after repeated dose of treatment of 28 days (Sub-acute oral toxicity) as compared to the control group. The figure has representation of **A** and **B** – total protein; **C** and **D**– albumin; **E** and **F**–Globulin; **G** and **H** in male and female respectively. Data values are represented in mean $\pm$ SEM, $n=5$ animals/group/sex, (ANOVA/Tukey test). Bar with similar alphabets indicate statistically significant at $p > 0.05$.

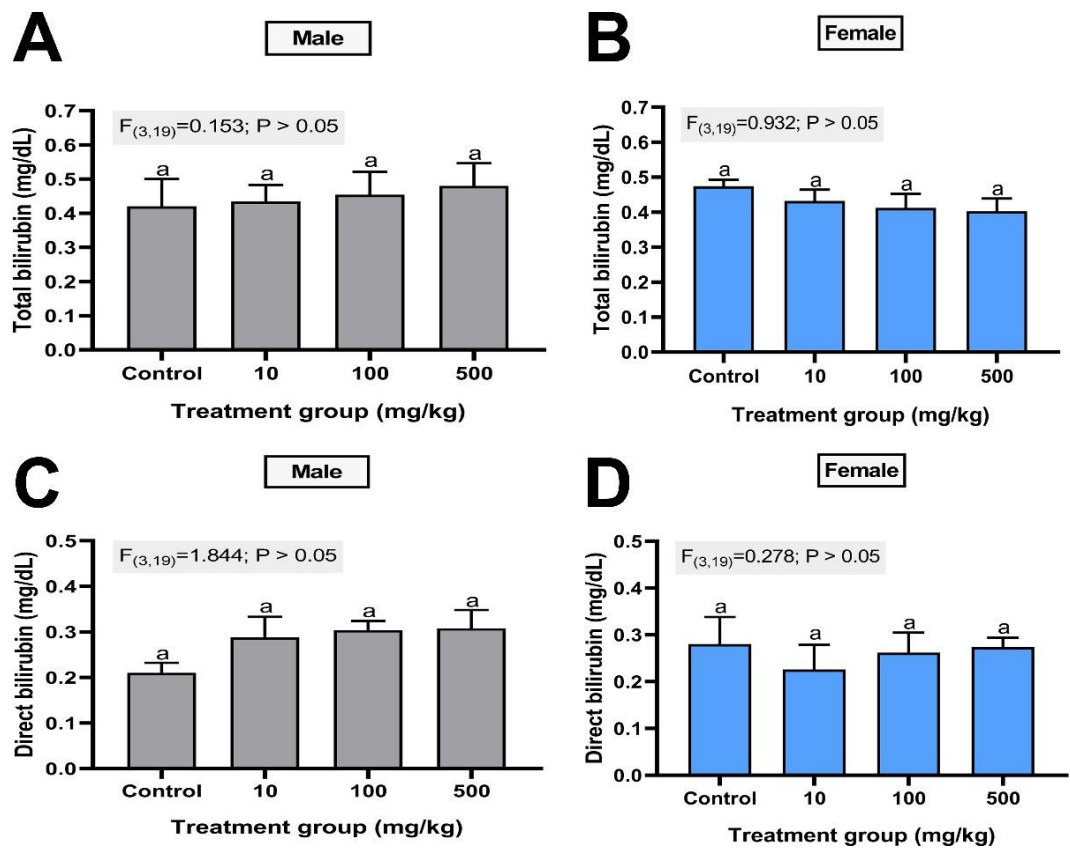


Figure 4.12 Effects of *Trevesia palmata* methanolic extract on serum level concentration of total bilirubin and direct bilirubin in male and female rats after repeated dose of treatment of 28 days (Sub-acute oral toxicity) as compared to the control group. The figure has representation of **A** and **B** – total bilirubin; **C** and **D**- direct bilirubin in male and female respectively. Data values are represented in mean $\pm$ SEM, n=5 animals/group/sex, (ANOVA/Tukey test). Bar with similar alphabets indicate statistically significant at $p > 0.05$.

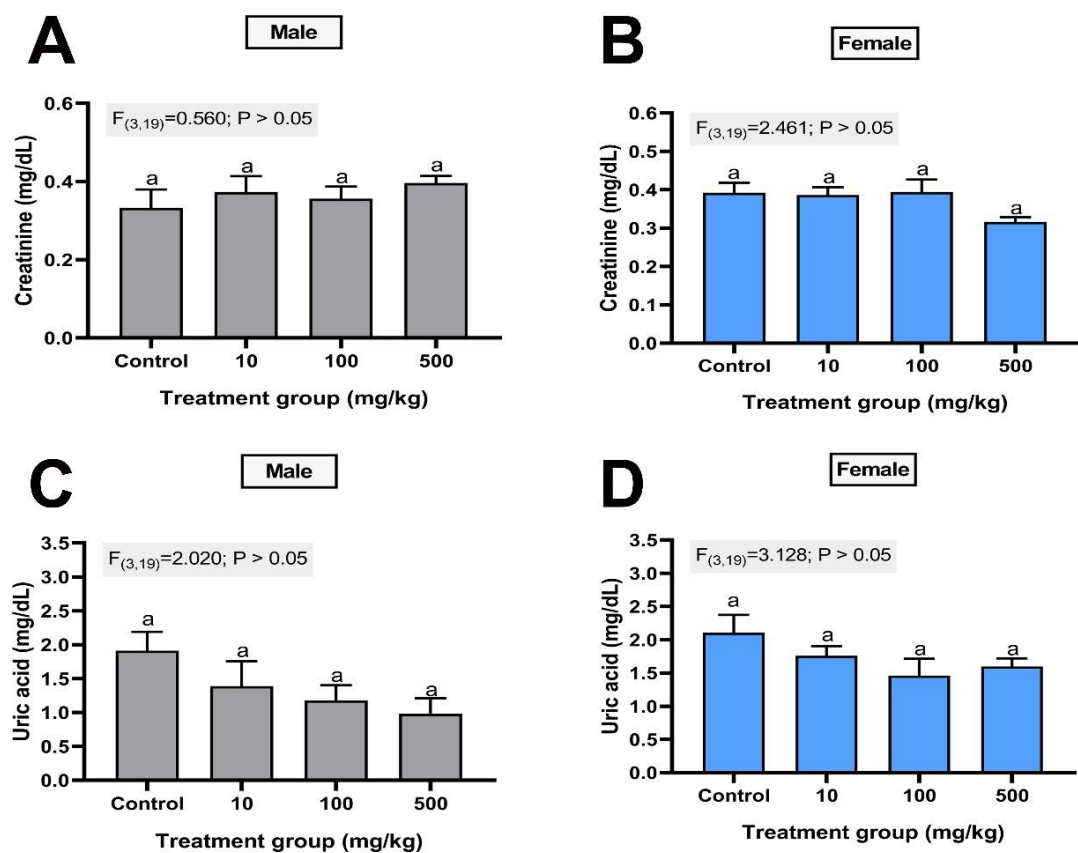


Figure 4.13 Effects of *Trevesia palmata* methanolic extract on serum level concentration of creatinine and uric acid in male and female rats after repeated dose of treatment of 28 days (Sub-acute oral toxicity) as compared to the control group. The figure has representation of **A** and **B** – Creatinine; **C** and **D**- Uric acid in male and female respectively. Data values are represented in mean $\pm$ SEM, $n=5$ animals/group/sex, (ANOVA/Tukey test). Bar with similar alphabets indicate statistically significant at $p > 0.05$.

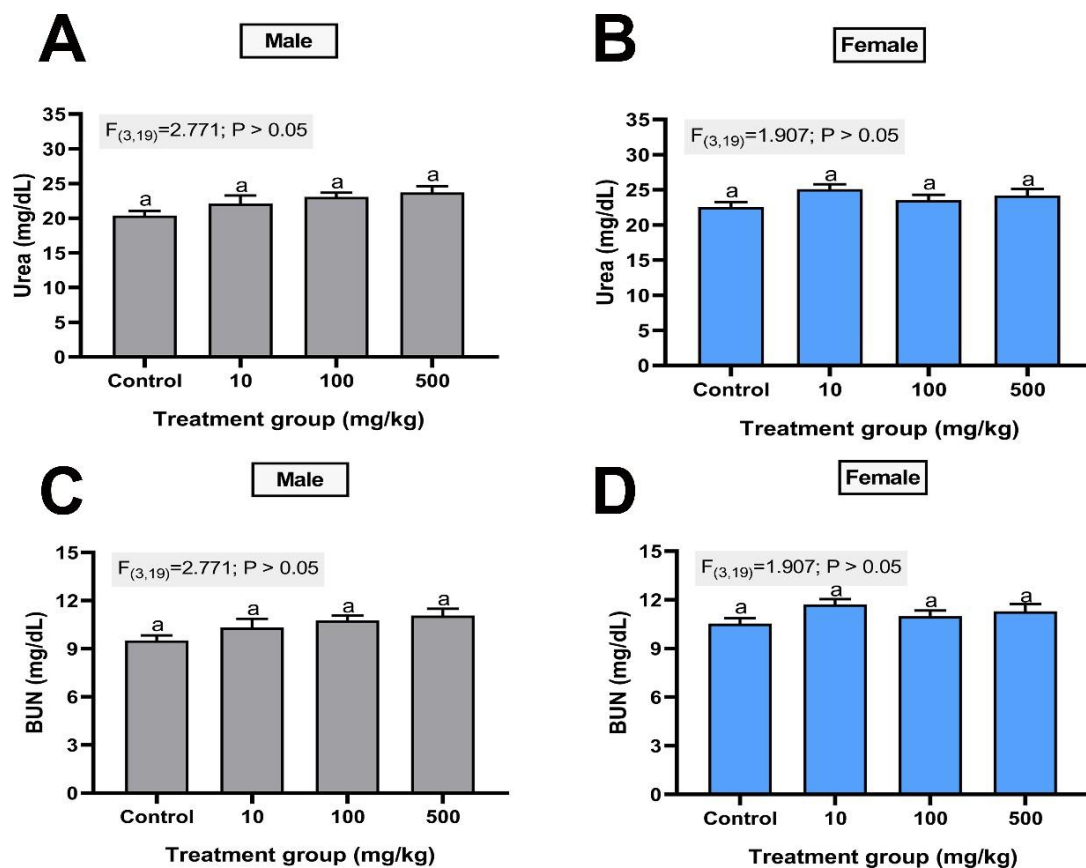


Figure 4.14 Effects of *Trevesia palmata* methanolic extract on serum level concentration of Urea and BUN in male and female rats after repeated dose of treatment of 28 days (Sub-acute oral toxicity) as compared to the control group. The figure has representation of **A** and **B** – Urea; **C** and **D**- Blood urea nitrogen (BUN) in male and female respectively. Data values are represented in mean $\pm$ SEM, n=5 animals/group/sex, (ANOVA/Tukey test). Bar with similar alphabets indicate statistically significant at $p > 0.05$.

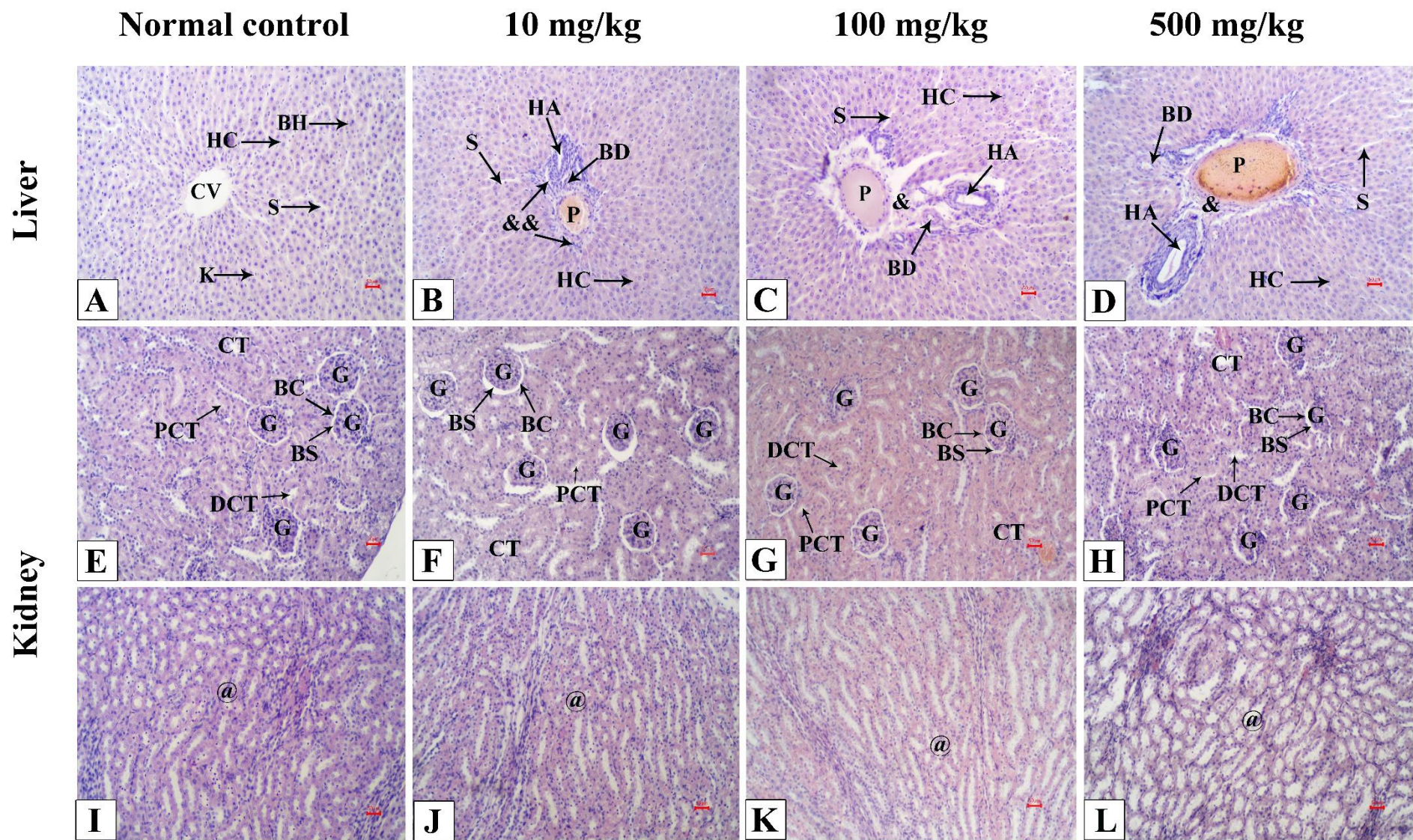


Figure 4.15 Histopathological results of liver and kidney of Wister albino male rats after administration of TPFME for 28 days in sub-acute toxicity study. **(A-D)** photomicrograph of liver tissue (H&E). **(E-H)** photomicrograph of kidney cortex section (H&E) and **(I-L)** Photomicrograph of kidney medulla section (H&E). H&E: Hematoxylin and eosin staining, **scale bar = 50 μ m for 20X magnification**; BC: Bowman's capsule; BD: Bile duct; BH: Binucleated hepatocytes; BS: Bowman's Space; CT: Collecting tubules; CV: Central vein; DCT: Distal convoluted tubules; G: Glomerulus; HA: Hepatic artery; HC: Hepatocytes; K: Kupffer cell; P: Portal vein; PCT: Proximal convoluted tubules; S: Sinusoids; @: Medullary renal tubules; &- Interlobular connective tissue; &&- Connective tissue of portal area.

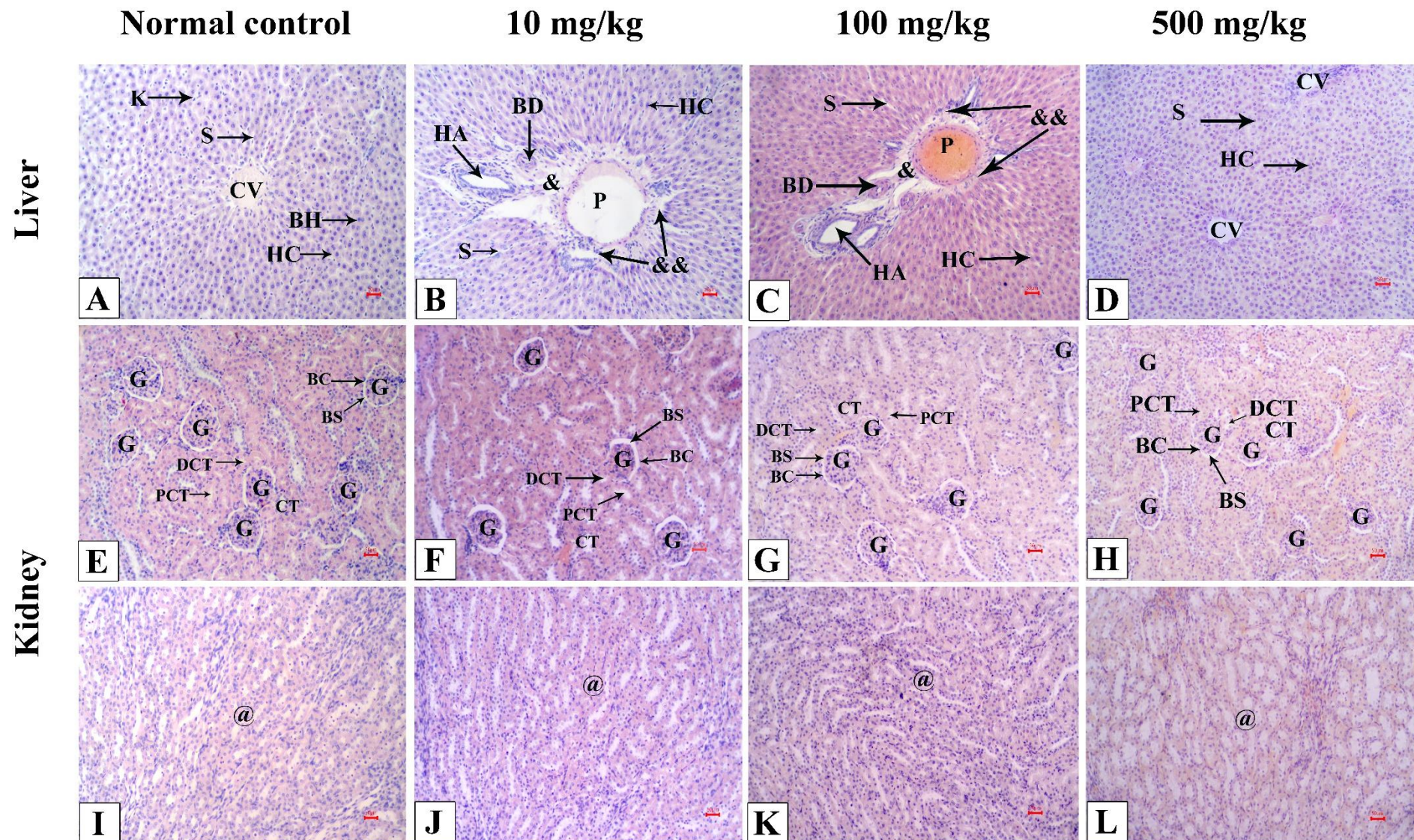


Figure 4.16 Histopathological examination of liver and kidney of Wister albino female rats after administration of TPFME for 28 days in sub-acute toxicity study. **(A-D)** photomicrograph of liver tissue (H&E). **(E-H)** photomicrograph of kidney cortex section (H&E) and **(I-L)** Photomicrograph of kidney medulla section (H&E). H&E: Hematoxylin and eosin staining, **scale bar = 50 μ m for 20X magnification**; BC: Bowman's capsule; BD: Bile duct; BH: Binucleated hepatocytes; BS: Bowman's Space; CT: Collecting tubules; CV: Central vein; DCT: Distal convoluted tubules; G: Glomerulus; HA: Hepatic artery; HC: Hepatocytes; K: Kupffer cell; P: Portal vein; PCT: Proximal convoluted tubules; S: Sinusoids; @: Medullary renal tubules; &- Interlobular connective tissue; &&- Connective tissue of portal area.

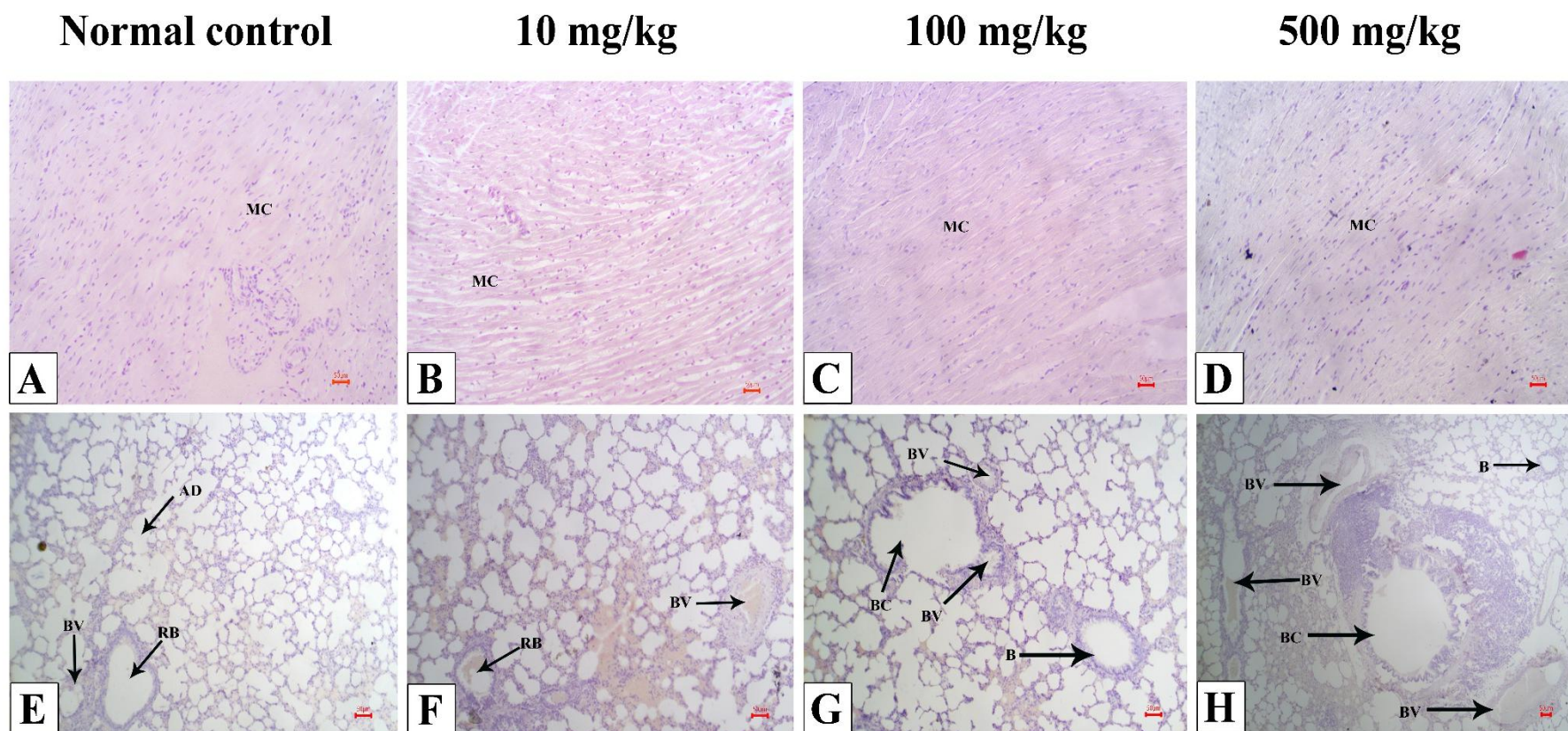


Figure 4.17 Histopathological analysis of heart and lung of Wister albino male rats exposed to methanolic extract from *T. palmata* flower buds in the sub-acute oral toxicity test. (A-D) photomicrograph of heart tissue slice (H&E). (E-H) photomicrograph of lung(airway) tissue section (H&E). Hematoxylin and eosin staining, scale bar = 50 µm for 20X (A-D) and 10X (E-L) magnification; A: Alveoli; AD: Alveolar duct; AC: Alveolar sac; B: Bronchus; BC: Bronchiole; BV: Blood vessel; MC: Myocardium.

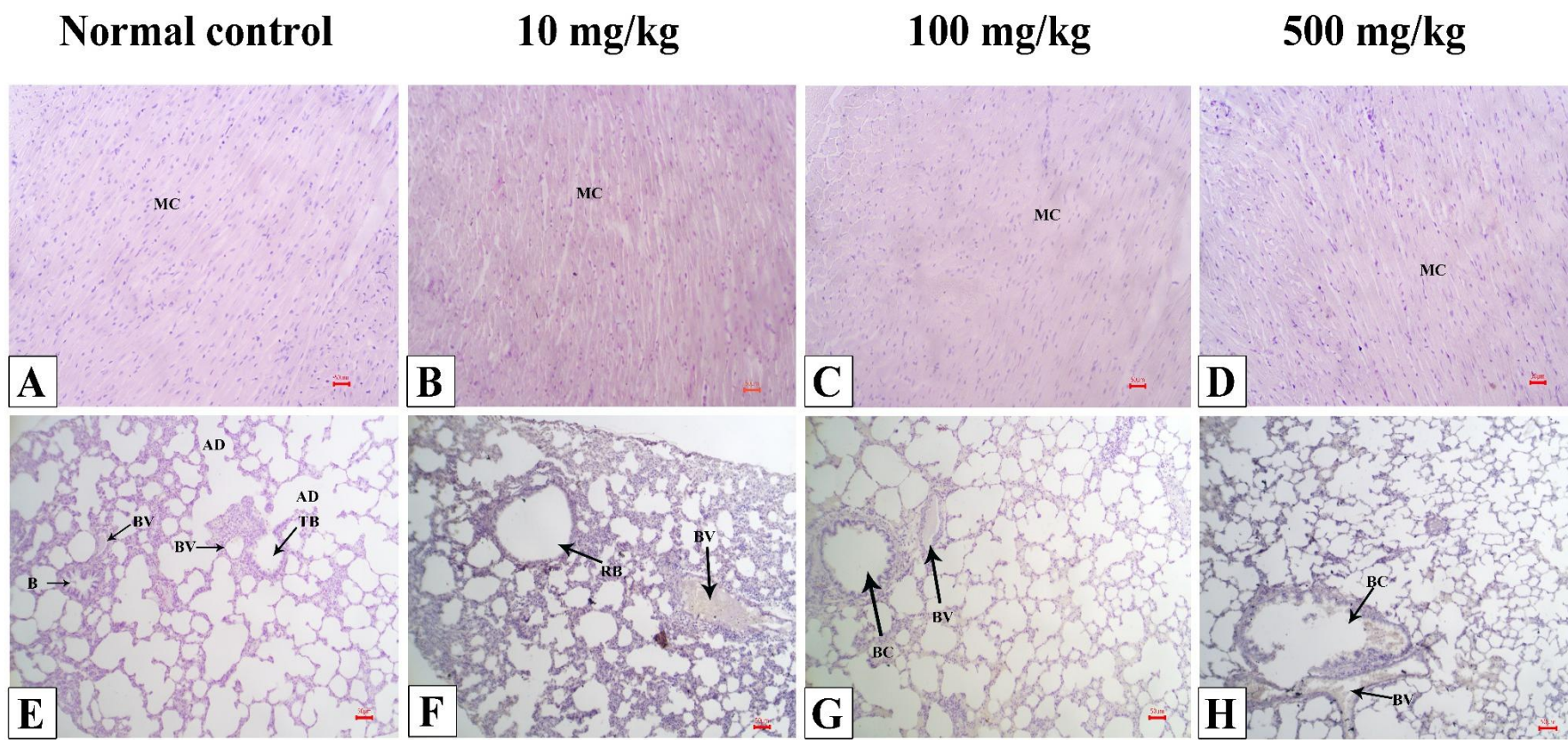


Figure 4.18 Histopathological examination of heart and lung of Wister albino female rats exposed to methanolic extract from *T. palmata* flower buds in the sub-acute oral toxicity test. **(A-D)** photomicrograph of heart tissue slice (H&E). **(E-H)** photomicrograph of lung(airway) tissue section (H&E) and **(I-L)** photomicrograph of lung(alveoli) tissue section (H&E). H&E: Hematoxylin and eosin staining, **scale bar = 50 μm for 20X (A-D) and 10X (E-L)** magnification; AD: Alveolar duct; B: Bronchus; BC: Bronchiole; BV: Blood vessel; MC: Myocardium; TB: Terminal bronchiole; RB: Respiratory bronchiole; (Black arrow): Intervalveolar septa.

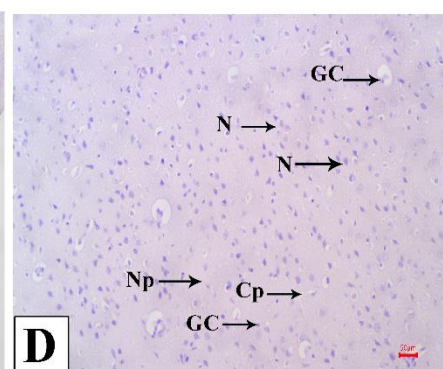
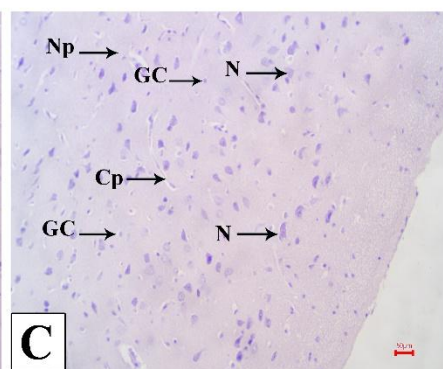
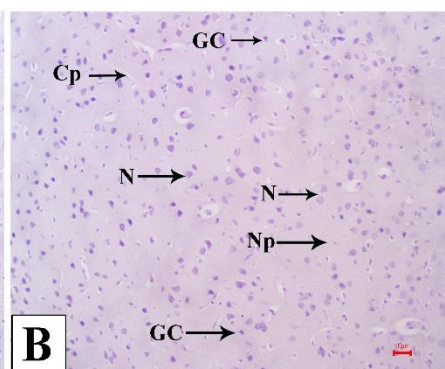
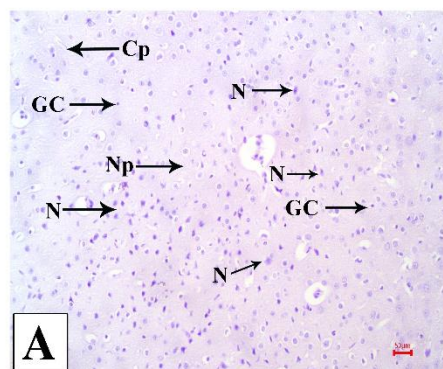
Normal control

10 mg/kg

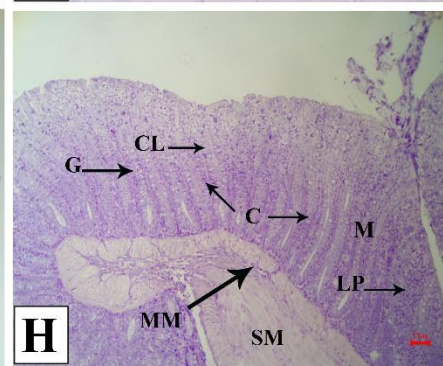
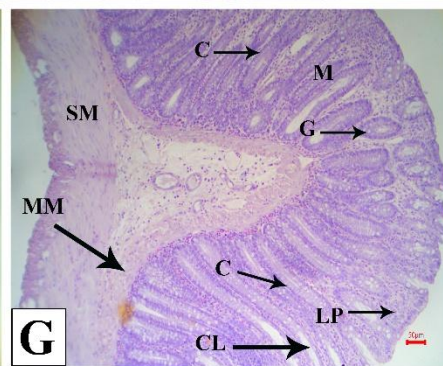
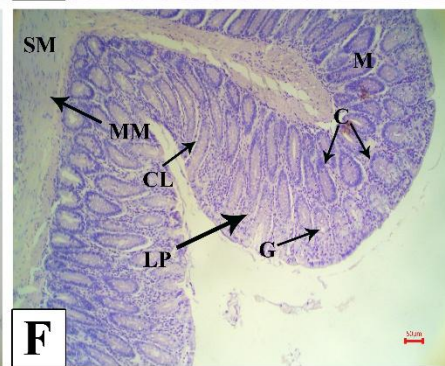
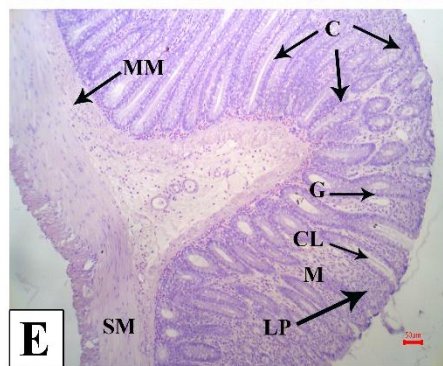
100 mg/kg

500 mg/kg

Brain



Colon



Spleen

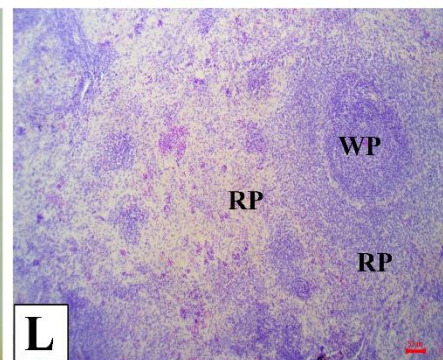
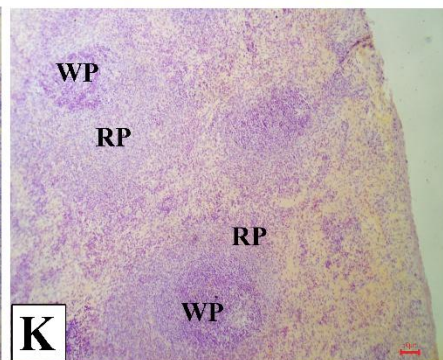
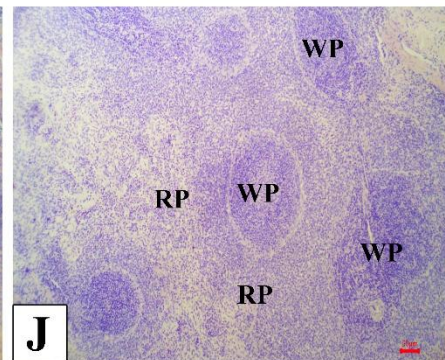
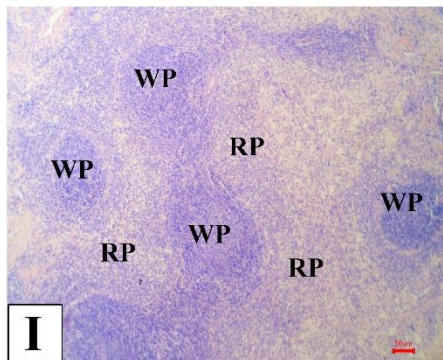


Figure 4.19 Histopathological evaluation of organ brain, colon and spleen of Wister albino male rats exposed to methanolic extract from *T. palmata* flower buds in the sub-acute oral toxicity test. **(A-D)** photomicrograph of section of cerebral cortex of brain region (H&E). **(E-H)** photomicrograph of colon section represented mucosa and sub-mucosa layer (H&E) and **(I-L)** photomicrograph of section of spleen. H&E: Hematoxylin and eosin staining, **scale bar = 50 µm for 20X (A-D) and 10X (E-L)** magnification. C: Crypt; Cp: Capillary; CL: Crypt of Lieberkühn; G: Goblet cell; GC: Glial cell; LP: Lamina propria; M: Mucosa; N: Neurons; Np: Neurophil; SM: Sub mucosa; RP: Red Pulp; WP: White pulp;

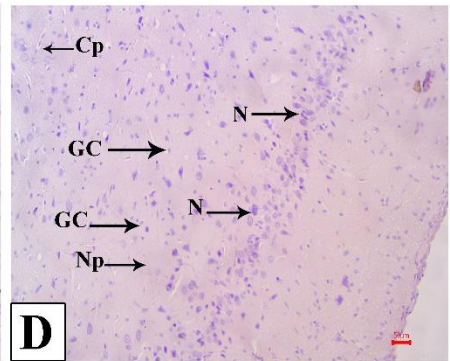
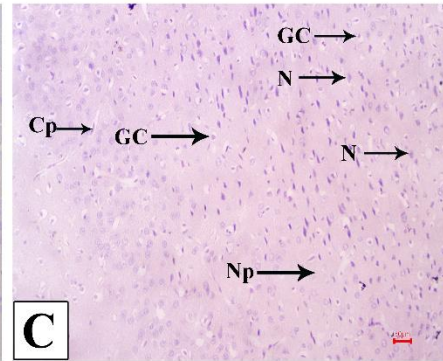
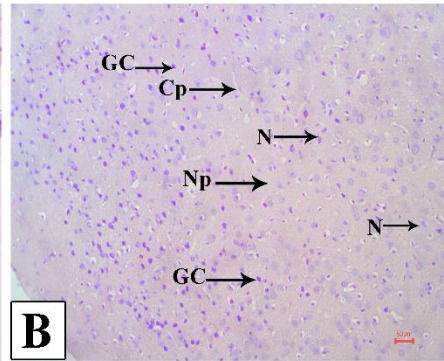
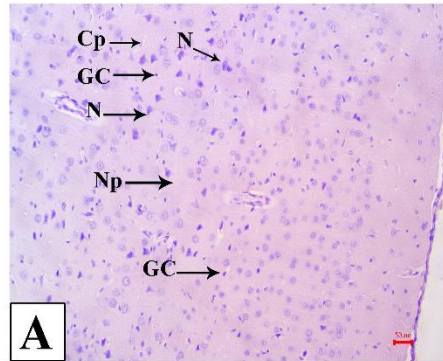
Normal control

10 mg/kg

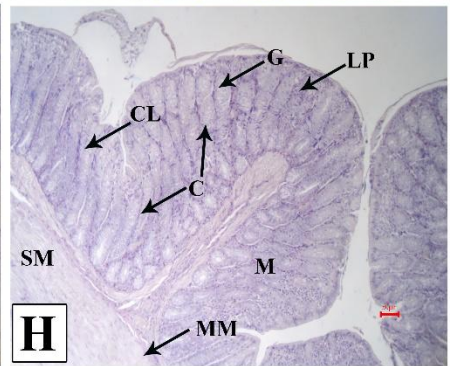
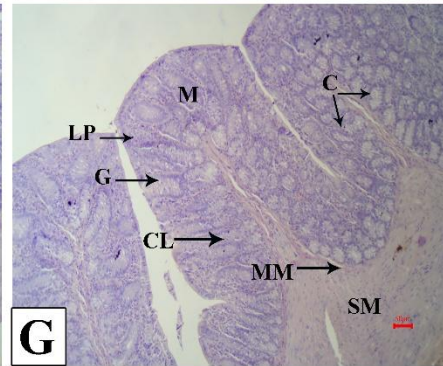
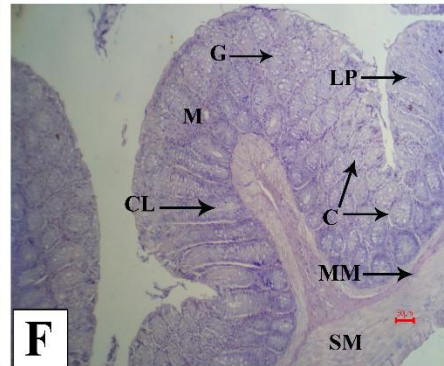
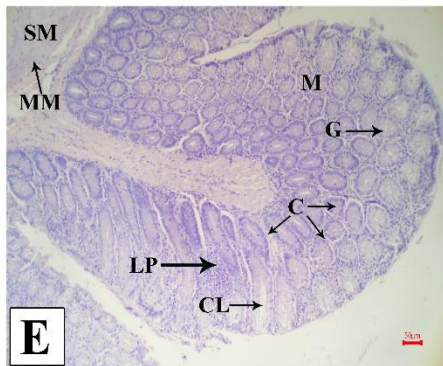
100 mg/kg

500 mg/kg

Brain



Colon



Spleen

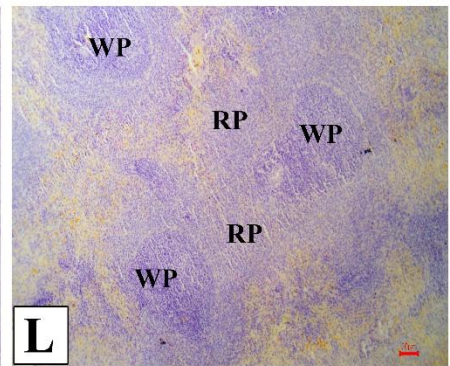
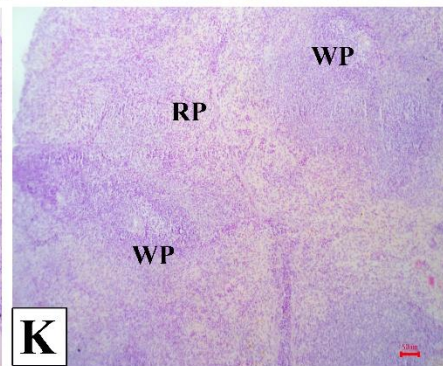
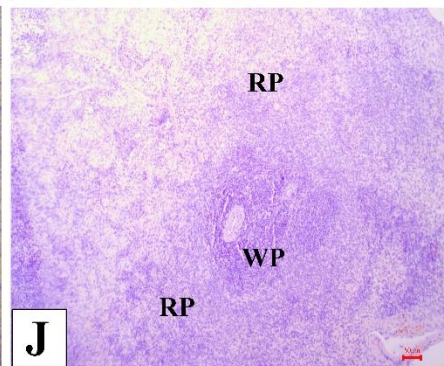
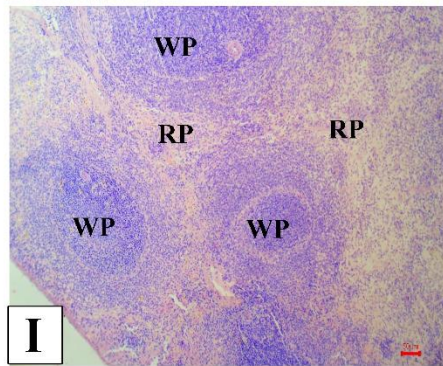


Figure 4.20 Histopathological evaluation of organ brain, colon and spleen of Wister albino female rats exposed to methanolic extract from *T. palmata* flower buds in the sub-acute oral toxicity test. **(A-D)** photomicrograph of section of cerebral cortex of brain region (H&E). **(E-H)** photomicrograph of colon section represented mucosa and sub-mucosa layer (H&E) and **(I-L)** photomicrograph of section of spleen. H&E: Hematoxylin and eosin staining, **scale bar = 50 µm for 20X (A-D) and 10X (E-L)** magnification. C: Crypt; Cp: Capillary; CL: Crypt of Lieberkühn; G: Goblet cell; GC: Glial cell; LP: Lamina propria; M: Mucosa; N: Neurons; Np: Neurophil; SM: Sub mucosa; RP: Red Pulp; WP: White pulp;

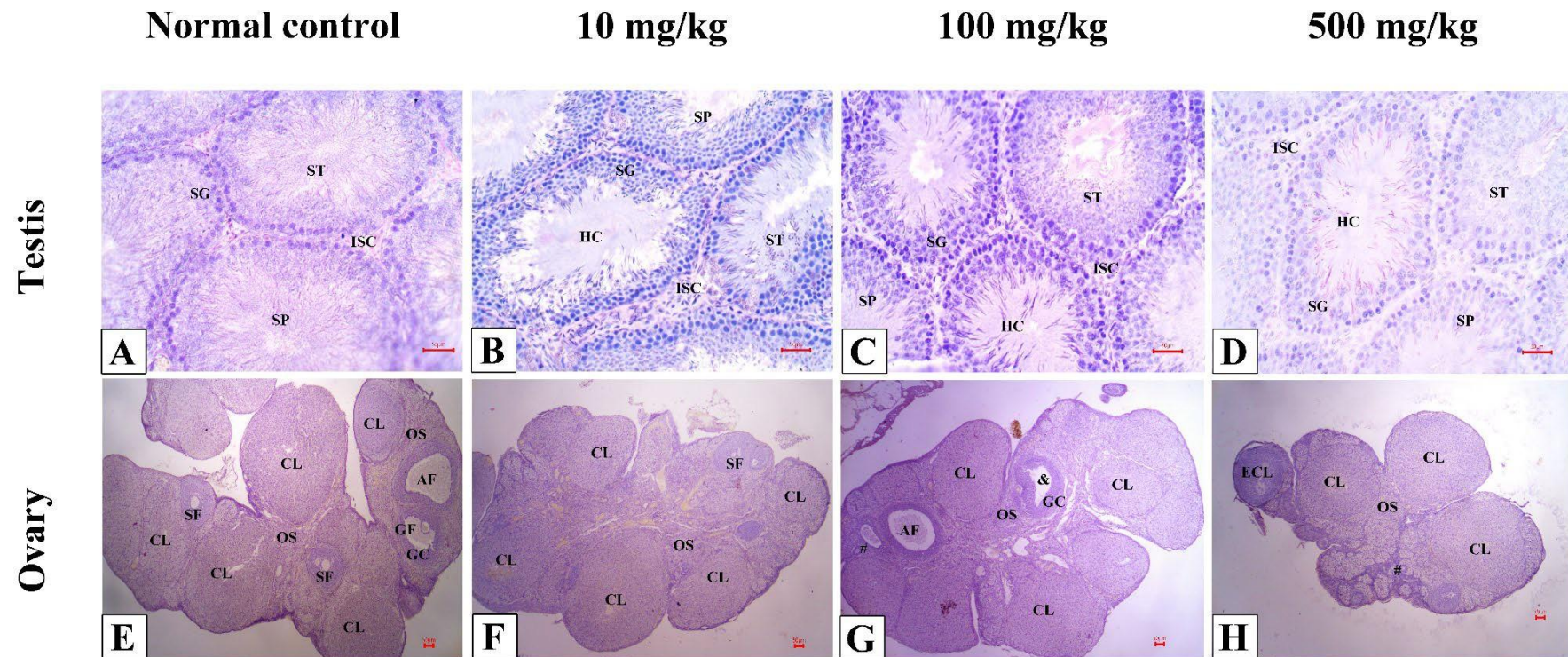


Figure 4.21 Histopathological examination of organ testes and ovary of Wister albino rats exposed to methanolic extract from *T. palmata* flower buds in the sub-acute oral toxicity test. (A-D) Photomicrograph of section of testes (H&E) and (E-H) Photomicrograph of section of ovary (H&E). H&E: Hematoxylin and eosin staining, scale bar = 50 μ m for 4X (A-D) and 40X (E-L) magnification. AF: Atretic follicles; CL: Corpus luteum; (ECL) Early corpus luteum; GC: Granulosa cell; GF: Germinal follicles; ISC: Interstitial Leydig cells; HC: Hyalinized luminal center; OS: Ovarian stroma; SG: Spermatogenic cell; SF: Secondary follicles; SP: Sperm; ST: Seminiferous tubules; &- Antra.

5.1 INTRODUCTION

5.1.1 Brief overview

Inflammatory bowel disease (IBD) comprises chronic inflammatory disorders affecting the gastrointestinal tract, with ulcerative colitis (UC) being a major form characterised by continuous inflammation and ulceration of the colonic mucosa. Patients commonly experience abdominal pain, diarrhoea, rectal bleeding, and weight loss. Despite therapeutic advances, many current UC treatments still present challenges, including inadequate long-term efficacy, adverse effects, and difficulty achieving sustained remission (**McDowell et al., 2023**).

In recent years, considerable interest has emerged in medicinal plants as sources of natural compounds with potential therapeutic value in inflammatory diseases (**Duan et al., 2021**). *Trevesia palmata* (TP) is one such plant noted for its diverse pharmacological properties, including antioxidant, anti-inflammatory, and immunomodulatory activities. *T. palmata* contains numerous bioactive phytochemicals such as phenolics, flavonoids, terpenoids, and alkaloids. Extracts from the plant have demonstrated pronounced antioxidant and anti-inflammatory effects in various experimental models (**Islam et al., 2022; Hussain et al., 2021; Thao et al., 2018; Rahman et al., 2014; De Tommasi et al., 2000**). However, the specific therapeutic actions of isolated phytocompounds from *T. palmata* in the context of ulcerative colitis remain insufficiently explored.

The trinitrobenzene sulfonic acid (TNBS) rat model is widely used to replicate the pathophysiological features of human UC, including mucosal erosion, inflammatory infiltration, and oxidative stress (**Antoniou et al., 2016**). This model is therefore valuable for examining novel therapeutic interventions. The present study investigates the antioxidant, anti-inflammatory, and modulatory effects of phytocompounds isolated from *Trevesia palmata* leaf extract (TPLE) in TNBS-induced ulcerative colitis in rats. Through biochemical, molecular, and histopathological analyses, this research aims to clarify the mechanisms underlying their protective effects. These findings may support the development of plant-derived therapies with improved efficacy and safety profiles for UC management.

5.1.2 Reactive Oxygen Species in Inflammatory Bowel Disease

Oxidative stress and inflammation are closely interconnected processes involved in the development of numerous chronic conditions (Biswas, 2016). Reactive oxygen species (ROS), generated during normal metabolism, play essential roles in cell signalling and immune defence. However, excessive ROS can cause oxidative damage to lipids, proteins, and DNA, contributing to degenerative and inflammatory diseases (Jomova et al., 2023; Biswas, 2016). In ulcerative colitis, dysregulated immune responses lead to overproduction of ROS by activated neutrophils and macrophages within the intestinal mucosa (Hitoshi et al., 2018). Although ROS support microbial killing, uncontrolled release results in mucosal damage and amplification of inflammatory pathways. Reactive species such as superoxide, hydrogen peroxide, hydroxyl radicals, and hypochlorous acid contribute directly to tissue injury. Interactions between neutrophils, macrophages, and platelets further exacerbate oxidative stress (Ashique et al., 2023).

Physiological antioxidant systems such as superoxide dismutase (SOD), catalase, glutathione peroxidase, and non-enzymatic antioxidants normally mitigate ROS-mediated injury. However, UC patients often exhibit impaired antioxidant defence, contributing to chronic oxidative stress and tissue destruction (Jarmakiewicz-Czaja; Rajput et al., 2021). Animal studies using the TNBS model have consistently demonstrated elevated ROS levels and enhanced inflammatory activity, highlighting oxidative stress as a central driving force in UC. Thus, targeting redox pathways has emerged as a promising therapeutic strategy (Hambardikar & Mandlik, 2022). Medicinal plants rich in phenolic and flavonoid compounds, such as *Trevesia palmata*, have shown considerable antioxidant and anti-inflammatory potential in models of UC, supporting their possible therapeutic role (Islam et al., 2022; Hussain et al., 2021; Thao et al., 2018; Rahman et al., 2014; De Tommasi et al., 2000).

5.1.3 Antioxidant and Anti-inflammatory Effects of Plants Containing Phenolic and Flavonoid Compounds

Plant-derived antioxidants play a crucial role in mitigating oxidative stress and limiting the formation of free radicals. Phenolic and flavonoid compounds possess potent antioxidant and anti-inflammatory properties that help reduce cellular injury and inflammatory responses (Al-Khayri et al., 2022; Tungmunnithum et al., 2018). Pro-inflammatory mediators such as TNF- α and nitric oxide (NO) can react with free radicals, causing irreversible cell membrane damage and promoting inflammatory tissue destruction. Antioxidants can counteract these mechanisms by scavenging reactive intermediates and preventing the propagation of oxidative damage (Garcia-Sanchez et al., 2020). Studies also suggest that antioxidants may enhance immune responses and promote overall physiological resilience (Chaudhary et al., 2023). Polyphenols have demonstrated significant benefits in experimental colitis, acting as antioxidants, immune regulators, and modulators of the intestinal microbiota (Asakura & Kitahora, 2018). Medicinal plants containing abundant flavonoids, alkaloids, phenolics, and terpenoids therefore exhibit broad therapeutic potential (Roy et al., 2022).

T. palmata exhibits strong antioxidant, anti-inflammatory, antibacterial, cytotoxic, and membrane-stabilising effects. Its methanolic leaf extract possesses considerable medicinal promise and has been explored for diagnostic and therapeutic applications (Manoharan et al., 2023). Flavonoids present in high concentrations in *T. palmata* are especially recognised for their protective effects on gastrointestinal tissues and their ability to modulate inflammatory processes (Panche et al., 2016). Accordingly, this study compares the anti-inflammatory activity of flavonoid compounds isolated from TPFME with sulfasalazine, a standard therapeutic agent, in TNBS-induced ulcerative colitis in rats.

5.2 REVIEW OF LITERATURE

Ulcerative colitis (UC) is a chronic inflammatory disease involving oxido-nitrosative stress, dysregulated cytokine production, and aberrant apoptosis. Although modern pharmacotherapies have improved patient outcomes, remission

rates and long-term treatment durability remain limited, highlighting the need for exploration of alternative therapeutic strategies (**Guo & Wang, 2023; Lasa et al., 2022; Ungaro et al., 2019**).

The aetiology of UC is multifactorial, encompassing genetic susceptibility, immune system dysfunction, microbial imbalance, and environmental influences. Standard treatments including corticosteroids, immunosuppressants, and amino salicylic drugs often produce undesirable side effects, stimulating interest in natural compounds and dietary interventions as complementary therapeutic approaches (**Ungaro et al., 2019**). The “IBD integrome” concept integrates genetic, environmental, microbial, and immunological components to enhance understanding of IBD pathogenesis (**Fiocchi, 2015**). Animal models, particularly TNBS-induced colitis, are invaluable for studying cytokine patterns, immunological disturbances, and potential therapeutic interventions (**Au et al., 2023; Goyal et al., 2014**). Oxidative stress (OS), driven by an imbalance between ROS and antioxidant defences, is a central factor in UC. Excess ROS impairs epithelial barrier integrity, activates inflammatory signalling pathways, and accelerates mucosal injury (**Tian et al., 2017**). Strengthening antioxidant defences or inhibiting ROS-producing enzymes may therefore alleviate disease progression (**Sahoo et al., 2023**).

Genetically engineered animal models have expanded understanding of IBD mechanisms and supported the development of targeted therapies (**Mizoguchi et al., 2020**). Histopathological analysis remains critical for differentiating UC from Crohn’s disease and enhancing diagnostic accuracy (**Fabian & Bajer, 2022**). Collectively, these research insights emphasise the significance of animal models in understanding gastrointestinal pathology and guiding the development of novel therapeutic interventions for UC.

5.3 MATERIALS AND METHODS

5.3.1 Chemicals and materials

2,4,6-Trinitrobenzenesulfonic acid solution (TNBS) was obtained from SIGMA-ALDRICH Co. Ltd. (USA, CAS No. 2508-19-2), Kits for Rat MPO, IL-6, IL-1, IL-10, T-AOC, PG-E2, CALP, FAD-linked sulfhydryl oxidase ALR, and LT-B4 ELISA assay of Bioassay Technology Laboratory (Zhejiang, China) was obtained from Hind Biotech, Kolkata, West Bengal. All anti-bodies (primary and secondary) were purchased from Hind Biotech, Kolkata, West Bengal. Dimethyl sulphoxide (DMSO) obtained from HiMedia Laboratories Pvt., Ltd. (Mumbai, India). Sulfasalazine medicine (Tablet, 500 mg), polyurethane tube for enteral feeding (FG 10) and molecular grade ethanol from local store.

5.3.2 Animal ethics and maintenance

This study complied with the ARRIVE guidelines for the care and use of laboratory animals (**Du Sert et al., 2020**). All procedures were reviewed and approved by the Institutional Animal Ethical Committee, Mizoram University, Aizawl, Mizoram, India (Approval No. MZU/IAEC/2021-22/08; dated 03 September 2021). Measures were implemented before and throughout the experiments to minimize pain, distress, and discomfort.

A total of 40 male Wistar albino rats (*Rattus norvegicus*) (N = 40), aged 10–12 weeks and weighing 150–170 g, were sourced from the Animal Care and Housing Facility at Mizoram University, Aizawl. Animals were single-housed in clean polypropylene cages (421 × 290 × 190 mm) under controlled conditions (20 ± 2 °C; relative humidity 50–70%) with a 12:12 h light:dark photoperiod. Rats received a standard commercial pellet diet at regular intervals and had ad libitum access to ultrapure water. Cages and housing areas were sanitized daily, and animal health was monitored routinely.

5.3.3 Induction of ulcerative colitis

All animals were fasted for 24 hours prior to ulcerative colitis induction. Rats were lightly anesthetized using ketamine (90 mg/kg) and xylazine (10 mg/kg) (i.p) body weight. Based on the protocol of Motavallian-Naeini et al. (2012) and Li et al, (2011) colitis was induced by intrarectal administration of TNBS (50 mg/kg) dissolved in 50% (v/v) ethanol through a 2 mm diameter polyurethane feeding tube inserted approximately 8 cm proximal to the anus. To minimize leakage of the instilled solution, the rats were maintained in a head-down position for one minute. Control animals received an enema of 0.25 ml of 0.9% physiological saline following the same procedure (Motavallian-Naeini et al., 2012).

5.3.4 Experimental group, drug intervention and sample collection

Fourty animals were randomly divided into five experimental groups (N = 8/group) (Table 5.1). The groups were classified as follows:

Group I (Control): Received physiological saline in a volume of 0.25 mL (once, intra-rectally) and 5ml/kg of 1 % aqueous solution of DMSO p. o for 7 days.

Group II TNBS induced control animal: Received TNBS (50 mg/kg) in a volume of 0.25 mL (once, intra-rectally) and 5ml/kg of 1% aqueous solution of DMSO p.o for 7 days.

Group III TNBS induced and sulfasalazine (50 mg/kg): Received TNBS (50 mg/kg) in a volume of 0.25 mL (once, intra-rectally) and treatment with sulfasalazine (50 mg/kg) in 1 % aqueous solution of DMSO, p.o for 7 days

Group IV TNBS induced and methanolic extract of *T. palmata* flower buds (10 mg/kg) treated animals: Received TNBS (50 mg/kg) in a volume of 0.25 mL (once, intra-rectally) and treatment with methanolic extract of *T. palmata* flower buds (10 mg/kg in 5ml/kg of 1% aqueous solution of DMSO, p.o.) for 7 days.

Group V TNBS induced and methanolic extract of *T. palmata* flower buds (20 mg/kg) treated animals: Received TNBS (50 mg/kg) in a volume of 0.25 mL (once,

intra-rectally) and treatment with methanolic extract of *T. palmata* flower buds (20 mg/kg in 5ml/kg of 1% aqueous solution of DMSO, p.o.) for 7 days.

The dose of sulfasalazine was taken from published literature (Li et al., 2011) and the dose of TPF used in this study was selected based on acute and subacute study of methanolic extract of *T. palmata* flower buds

Animals were then allowed to recover and observed daily throughout the duration of the study. Clinical symptoms, including the amount of food consumed, water consumed, stool appearance, visible stool, and occult bleeding were tested daily. Consistency and frequency of stools and the change of body weight and rectal temperature monitored until issue harvest. On the 8th day, rats will be sequentially euthanized by anesthesia ketamine (90 mg/kg) and xylazine (10 mg/kg) (i.p) s. After that animals were scarified by decapitation blood was withdrawn. Each blood sample were collected into separate vials for determination of blood and serum parameters. After blood collection, the colon will be excised then assessment of colonic damage was performed and frozen immediately and stored at -80° C for further biochemical and molecular examination. A part of the freshly excised liver, kidney and colon of five animals from each group were washed with saline and preserved in Bouin's fixative solution for histopathological and immunohistochemistry studies.

5.3.5 Assessment of body weight, food and water intake and rectal temperature

After induction of UC, the animals were thoroughly observed 20-30 minutes until get full conscious. Throughout the treatment animals monitors was conducting two times in a day. Food and water intake were recorded in g and ml, respectively. Body weight, food and water consumption and rectal temperature were assessed during the experiment (prior to the administration of TNBS or normal saline and subsequent to the procedure daily for 7 days).

5.3.6 Measurement of organ weights and colon length

After the sacrifice of animals and before sample stored the organ weight of colon, was noted and in colon organ, measure of length was done.

5.3.7 Anal bleeding and stool consistency

Anal bleeding and stool consistency were monitored daily to assess the severity of ulcerative colitis in the TNBS-induced rat model. Each animal was individually observed, and freshly passed fecal pellets were examined. Stool consistency was scored on a 0–3 scale as follows: 0 = normal, well-formed pellets; 1 = slightly soft but formed stools; 2 = loose, unformed stools; 3 = watery diarrhea. Anal/rectal bleeding was scored on a 0–3 scale based on visual inspection and the presence of blood on the perianal region and feces: 0 = no visible blood; 1 = minimal blood staining around the anus or on the stool; 2 = obvious blood in stool and moderate perianal staining; 3 = gross rectal bleeding with severe perianal staining and/or blood drops in the cage. Scores were recorded at the same time each day, and mean values were calculated for each group as an index of disease activity associated with TNBS-induced ulcerative colitis.

5.3.8 Colon length and weight ratio

At the end of the experimental period, animals were euthanized, and the entire colon was carefully excised from the cecum to the rectum. The colon was gently flushed with ice-cold saline to remove luminal contents and blotted dry with filter paper. Colon length (cm) was measured under slight tension, avoiding overstretching of the tissue. The cleaned colon was then weighed on a digital balance, and the colon weight-to-length ratio (mg/cm) was calculated as an index of tissue edema and inflammation in the TNBS-induced ulcerative colitis model. An increased colon weight-to-length ratio was considered indicative of more severe colonic damage and inflammatory response.

5.3.9 Assessment of disease activity index

Disease activity index (DAI) was evaluated based on body weight loss, stool consistency, and blood in stools, which were recorded daily throughout the experimental period. Body weight loss was scored as follows: 0 = no weight loss, 1 = 1–5% loss, 2 = 6–10% loss, 3 = 11–20% loss, and 4 = >20% loss. Stool consistency was scored as 0 for normally formed pellets, 2 for pasty, semi formed pellets, and 4 for liquid stools. Occult or gross bleeding was scored as 0 for negative hemoccult, 2 for positive hemoccult, and 4 for visible blood or gross rectal bleeding. For each animal, the scores of the three parameters were summed to obtain a total score ranging from 0 to 12, which was then divided by 3 to yield the final DAI value. Body weight was measured daily from the start of the experiment until termination, and all animals were monitored throughout the study to assess changes in DAI in response to treatment, following the method described by **Yang et al. (2014)**.

5.3.10 Macroscopic assessment

Macroscopic assessment of colonic damage was performed at the end of the experiment to evaluate the severity of TNBS-induced ulcerative colitis. After euthanasia, the colon was excised from the cecum to the rectum, gently flushed with ice-cold saline to remove luminal contents, opened longitudinally along the antimesenteric border, and laid flat on a black background. The mucosal surface was examined for hyperemia, edema, wall thickening, ulceration, necrosis, and adhesions, and macroscopic damage was scored on a 0–10 scale according to the method described by Morris et al. (1989), where 0 represents normal mucosa and higher scores indicate increasing extent and severity of ulceration and inflammation. Colons were scored by an observer blinded to the treatment groups, and the mean macroscopic score for each group was used as an index of colonic injury in the TNBS-induced ulcerative colitis model (**Morris et al., 1989**).

5.3.11 Oxidative stress and antioxidant activity

Colon tissues were collected immediately after sacrifice, rinsed with ice-cold saline to remove blood and luminal contents, blotted dry, and homogenized in appropriate buffers (10% w/v) under cold conditions. The homogenates were centrifuged, and the resulting supernatants were used for the estimation of lipid peroxidation (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT). Protein content was determined separately, and all results were expressed per mg protein.

5.3.11.1 Lipid peroxidation (MDA)

Lipid peroxidation in colon tissue was assessed by measuring malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances (TBARS) method described by **Ohkawa et al. (1979)**. Briefly, an aliquot of tissue homogenate was mixed with sodium dodecyl sulfate, acetic acid, and thiobarbituric acid (TBA) reagent and heated in a boiling water bath ($\approx 95\text{--}100\text{ }^{\circ}\text{C}$) for approximately 60 min. After cooling, the mixture was centrifuged, and the absorbance of the pink chromogen in the supernatant was measured spectrophotometrically at 532 nm. MDA levels were calculated using an appropriate molar extinction coefficient and expressed as nmol MDA/mg protein.

5.3.11.2 Reduced glutathione (GSH)

GSH levels in colon tissue were determined according to the method of **Rahman et al. (2006)**, based on the reaction with Ellman's reagent. In brief, tissue homogenate was deproteinized (e.g., with sulfosalicylic acid), and the clear supernatant was incubated with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) in phosphate buffer. The formation of the yellow-colored 5-thio-2-nitrobenzoic acid (TNB) was measured at 412 nm. GSH concentration was calculated from a standard curve and expressed as μmol GSH/mg protein.

5.3.11.3 Superoxide dismutase (SOD)

SOD activity in colon tissue was measured using the method of Kakkar et al. (1984), which is based on the inhibition of NADH–phenazine methosulfate–nitroblue tetrazolium (NBT) reduction. The reaction mixture contained tissue supernatant, sodium pyrophosphate buffer, phenazine methosulfate, NBT, and NADH. After incubation at room temperature for a fixed time, the reaction was stopped by adding glacial acetic acid, and the color intensity of the resulting chromogen was measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit the rate of NBT reduction by 50%, and the activity was expressed as U/mg protein.

5.3.11.4 Catalase (CAT).

CAT activity was estimated according to the method of **Sinha (1972)**, which is based on the decomposition of hydrogen peroxide (H_2O_2) and subsequent formation of a chromogen with dichromate/acetic acid. Briefly, tissue homogenate was incubated with H_2O_2 in phosphate buffer for a defined period, and the reaction was terminated by the addition of dichromate–acetic acid reagent. The tubes were then heated in a boiling water bath to develop a stable-colored complex. After cooling, absorbance was measured at 570 nm. CAT activity was calculated from a standard curve and expressed as U/mg protein.

5.3.12 Histopathology study of colon tissue

Rats were euthanized, and the colon was carefully excised from the cecum to the rectum. Representative distal colonic segments were gently flushed with ice-cold saline to remove luminal contents and immediately fixed in Bouin's fluid fixative for 24h. Fixed tissues were processed by routine histological techniques, including dehydration through graded alcohols, clearing in xylene, and embedding in paraffin wax. Paraffin blocks were sectioned at 5 μm thickness using a rotary microtome, and sections were mounted on glass slides and stained with hematoxylin and eosin (H&E) following standard procedures described by **Bancroft and Gamble (2002)**. Stained sections were examined under a light microscope for characteristic features

of ulcerative colitis, such as epithelial erosion or ulceration, crypt architectural distortion or loss, goblet cell depletion, mucosal and submucosal edema, and inflammatory cell infiltration in the mucosa and submucosa. Histological damage was evaluated semi-quantitatively on a graded scale, considering both the extent and severity of inflammation and structural injury. All slides were assessed by an observer blinded to the treatment groups, and mean histological scores were calculated for each group as an index of colonic damage in the TNBS-induced ulcerative colitis model.

5.3.14 ELISA assay

Determination of nitric oxide (NO), myeloperoxidase (MPO), calprotectin (CALP), interleukin-6 (IL-6), interleukin-1 (IL-1), leukotriene-B4 (LT-B4), FAD-linked sulphhydryl oxidase ALR, prostaglandin E2 (PG-E2), total antioxidant capacity (TAOC) and interleukin-10 (IL-10) was carried out using commercially available rat ELISA kits (Bioassay Technology Laboratory, Rat ELISA kit). All assays were performed strictly in accordance with the manufacturer's instructions to ensure reliability and reproducibility of the measurements.

Briefly, appropriate standards and samples were added to the pre-coated ELISA plates, followed by incubation with the specific detection antibodies and enzyme conjugates supplied with each kit. After completion of the incubation steps and subsequent washing to remove unbound components, substrate solution was added to develop color. The reaction was terminated using the recommended stop solution, and the absorbance was measured at the specified wavelength using a microplate reader. Concentrations of each analyte were calculated from the corresponding standard

5.3.14 Immunohistochemistry

Immunohistochemistry was performed to evaluate the tissue distribution of heat shock protein 70 (HSP70) using a protocol previously standardized in our laboratory (Jeremy et al., 2017). Briefly, paraffin-embedded sections were deparaffinised in xylene and rehydrated through a graded ethanol series. Non-

specific binding was blocked by incubating the sections with goat serum (Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:100, Lot# H2114) for 30 min.

The slides were then incubated overnight at 4 °C with the following primary antibodies: NF κ B p65 (1:1000, mouse monoclonal IgG, Cat# E-AB-22066, Elabscience), iNOS (1:800, rabbit polyclonal IgG, Lot# XC3539385, Bioss), TNF- α (1:800, rabbit polyclonal IgG, Cat# BS-2081R, Bioss), anti-active caspase-3 (1:800, mouse monoclonal antibody, Cat# E-AB-22115, Elabscience), NRF2 (1:800, rabbit polyclonal antibody, E-AB-93081), HO-1 (1:800, rabbit polyclonal antibody, E-AB-93081) and COX-2 (1:800, rabbit polyclonal IgG, EB-AB-31012, Elabscience). After rinsing with PBS, the sections were incubated for 3 h at room temperature with a goat anti-mouse secondary antibody conjugate. Immunoreactivity was visualized using 0.05% 3,3'-diaminobenzidine tetrahydrochloride (DAB) in Tris-HCl buffer (pH 7.6) containing hydrogen peroxide, followed by dehydration and mounting with DPX. Stained slides were examined and imaged using a Euromax microscope.

5.3.15 Western blotting:

Western blot analysis was performed on colon protein lysates, as previously described (Jeremy et al., 2017). The total protein concentration of each lysate was determined using the Bradford technique (Bradford, 1976). Equal amounts of protein (50 μ g) were separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) at 150 V, and the resolved proteins were transferred onto polyvinylidene difluoride (PVDF) membranes using a Bio-Rad Mini-PROTEAN Blotting (1703930) device for 20–30 minutes. Membranes were then blocked for at least 1 hour at room temperature in a blocking solution containing 0.05% Tween 20 in phosphate-buffered saline (PBS) and 10% non-fat skimmed milk (Cat# GRM1254-500G, HiMedia Laboratory Private Limited, Mumbai, India). Subsequently, membranes were incubated overnight at 4 °C in a humidified chamber with the following primary antibodies: BCL-2 (1:2000, mouse polyclonal antibody, Cat# sc-7382, Santa Cruz Biotechnology, Inc., Dallas, USA), BAX (1:1000, mouse polyclonal IgG, Cat# sc-7480, Santa Cruz Biotechnology, Inc., Dallas, USA), tumour necrosis factor alpha (TNF- α) (1:1000, rabbit polyclonal IgG, Cat# BS-2081R,

BIOSS), interleukin-6 (IL-6) (1:1000, rabbit monoclonal IgG, BIOS), nuclear factor kappa B p65 (NFκB p65) (1:2000, mouse monoclonal IgG, Cat# E-AB-22066, Elabscience), NOX2 (NADPH oxidase 2, also called CYBB/cytochrome b-245) (1:1000, rabbit polyclonal antibody, Cat# E-AB-60387, Elabscience), and peroxisome proliferator-activated receptor gamma (PPARG) (1:1000, rabbit polyclonal IgG, Elabscience). Membranes probed with rabbit primary antibodies were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary IgG antibody (1:4000, Cat# E-AB-1003, Elabscience), and membranes probed with mouse primary antibodies were incubated with HRP-conjugated goat anti-mouse secondary IgG antibody (1:4000, Cat# E-AB-1001, Elabscience) for 3 hours at room temperature with constant agitation. Following incubation, membranes were washed four times with PBST and then immersed in enhanced chemiluminescence (ECL) solution (Cat# 1705061, Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions and developed on X-ray film in a dark room. Band intensities were quantified using ImageJ software. β-Tubulin (1:1500, rabbit monoclonal IgG, Cat# E-AB-20070, Elabscience) was used as a loading control for all antibodies employed in this study.

5.3.14 Statistical analysis

All quantitative data are presented as the mean $\pm$ standard error of the mean (SEM). Statistical comparisons among experimental groups were carried out using one-way analysis of variance (one-way ANOVA), which was employed to determine whether there were overall significant differences between group means. A probability value (P) of less than 0.05 was considered to indicate statistical significance for all analyses, and only differences meeting this threshold were interpreted as meaningful in the context of the study.

5.4 RESULTS

5.4.1 Alterations in body weight, food consumption, water consumption and rectal temperature in TNBS-induced colitis rats treated with various therapeutic regimens

TNBS treatment resulted in a significant decrease in body weight in rats compared to the control group. However, treatment with *Trevesia palmata* flower bud methanolic extract (TPF) at doses of 10 mg/kg and 20 mg/kg significantly prevented this weight loss, showing a protective effect compared to TNBS alone. Sulfasalazine (SPS50) also prevented weight loss, but its effect was not as strong as that of TPF at both doses. Statistical analysis revealed significant differences between the treatment groups, with different letters marking statistically significant differences ($p < 0.05$) (**Figure 5.1A**). Food intake was significantly reduced in TNBS-treated rats compared to the control group. However, treatment with TPF (10 mg/kg and 20 mg/kg) significantly increased food intake, similar to the effect observed with SPS50. Both the control and TPF-treated groups showed significantly higher food intake compared to the TNBS-only group (**Figure 5.1B**). TNBS treatment led to a significant reduction in water intake compared to the control group. Treatment with TPF (10 mg/kg and 20 mg/kg) reversed this decrease in water intake, while the SPS50 group also showed some recovery, though not to the same extent as TPF. Statistical differences were marked with different letters, indicating significant effects ($p < 0.05$) (**Figure 5.1C**). TNBS treatment significantly lowered rectal temperature compared to control animals. The TPF (10 mg/kg and 20 mg/kg) treatments resulted in higher rectal temperatures, indicating a protective effect against TNBS-induced hypothermia. While the SPS50 group showed some improvement in rectal temperature, the effect was less pronounced than with the TPF treatments (**Figure 5.1D**).

5.4.2 Survival rate in TNBS-induced colitis rats treated with various therapeutic regimens

The Kaplan-Meier survival curves shown in **Figure 5.2** illustrate the survival rate of control and TNBS-induced ulcerative colitis rats treated with standard drug (SPS50) or *Trevesia palmata* flower-bud extract (TPF10 and TPF20) over 7 days following TNBS administration. Rats in the control group, treated with Dimethyl sulfoxide (1%) and saline (0.9%), showed 100% survival throughout the 7 days, indicating normal health without induced colitis. In contrast, the TNBS group, treated with Trinitrobenzene sulfonic acid (TNBS) alone, exhibited a marked decline in survival, with survival rates dropping to about 40% by Day 5, demonstrating the severe effects of TNBS-induced colitis. The SPS50-treated rats showed better survival than the TNBS-only group, with survival rates around 60% by Day 5, though survival did not improve to the levels observed in the control group. Rats treated with 10 mg/kg of TPF10 exhibited a notable improvement in survival, with about 80% survival by Day 7, indicating that TPF10 provided protective effects against TNBS-induced ulcerative colitis. The highest survival rate was observed in the TPF20 group, with approximately 100% survival by Day 7, suggesting a strong protective effect and recovery from the TNBS-induced colitis compared to the other groups. These results indicate that *T. palmata* flower-bud extract, especially at the higher dose (TPF20), demonstrates significant protective effects in TNBS-induced ulcerative colitis rats, highlighting its potential as a therapeutic candidate for managing ulcerative colitis and related inflammatory conditions (**Figure 5.2**).

5.4.3 Rectal bleeding and inflammation alterations in colon architecture in TNBS-induced colitis rats treated with various therapeutic regimens

In the control group, no significant rectal bleeding or visible damage was observed. The anal region appeared normal, with no signs of inflammation or injury. In the TNBS-treated rats, a significant increase in rectal bleeding was evident. The anus appeared inflamed and bleeding, clearly indicating the colitis induced by TNBS. Rats treated with sulfasalazine (SPS50) showed moderate improvement in rectal bleeding compared to the TNBS-only group. While there was some reduction in

bleeding, signs of inflammation and irritation were still noticeable, indicating that the therapeutic effect of SPS50 was partial. Treatment with 10 mg/kg of TPF10 significantly reduced rectal bleeding and inflammation. The anal region showed less bleeding and fewer signs of injury, suggesting that TPF offers a protective effect against TNBS-induced colitis. Rats treated with the higher dose of TPF20 (20 mg/kg) exhibited the most significant improvement, with the least amount of rectal bleeding and minimal signs of inflammation. This result suggests that the higher dose of TPF provides a stronger protective effect compared to the 10 mg/kg dose. These observations suggest that TPF offers significant protection against TNBS-induced rectal bleeding and inflammation. The 20 mg/kg dose provided the most pronounced therapeutic effect, supporting the potential of TPF as a candidate for treating inflammatory conditions such as TNBS-induced colitis (**Figure 5.3A-E**).

5.4.4 Stool Consistency in TNBS-induced colitis rats treated with various therapeutic regimens

In the control group (**A**), the stool appeared well-formed and consistent, exhibiting the typical characteristics of healthy rats, with no visible signs of distress or abnormalities. In contrast, the TNBS group (**B**) showed abnormal stool with a liquid consistency and visible signs of diarrhoea, which indicates the induction of colitis, leading to gastrointestinal disturbances typical of TNBS treatment. Rats treated with Sulfasalazine (SPS50) (**C**) showed some improvement in stool consistency compared to the TNBS-only group, but the stool remained slightly loose and not fully formed. This suggests a partial therapeutic recovery from the TNBS-induced condition, though the effect was not as significant as other treatments. Treatment with 10 mg/kg of TPF (**D**) resulted in stool with relatively better consistency compared to the TNBS-only group. The stool was somewhat firmer, indicating partial improvement in gastrointestinal function and a reduction in the symptoms of colitis. Rats treated with 20 mg/kg of TPF (**E**) showed the most significant improvement in stool consistency, with near-normal, well-formed stools. This indicates a stronger protective effect of TPF at the higher dose in alleviating TNBS-induced gastrointestinal disturbances. These findings suggest that TPF helps

improve stool consistency in TNBS-induced colitis. The 20 mg/kg dose of TPF exhibited the most pronounced effect, restoring stool consistency to nearly normal levels and highlighting its potential as a therapeutic option for inflammatory bowel diseases such as TNBS-induced colitis (**Figure 5.4A-E**).

5.4.5 Alterations in colon architecture in TNBS-induced colitis rats treated with various therapeutic regimens

Figure 5.5 shows the alterations in colon architecture in TNBS-induced colitis rats treated with various therapeutic regimens. In the Control group, the colon architecture appeared normal, with no signs of inflammation or damage. The TNBS group showed significant changes, including severe damage to the colon, marked by thickening, bleeding, and ulcerations, indicating substantial inflammation and tissue injury. The rats treated with SPS50 (Sulfasalazine) exhibited some improvement compared to the TNBS group, with a reduction in inflammation, although mild ulcerations and swelling were still evident in the colon tissue. The TPF10 (*Trevesia palmata* flower-bud extract at 10 mg/kg) group showed moderate improvement, with less swelling, fewer ulcerations, and a more organised colon structure than the TNBS group, suggesting a protective effect of TPF against TNBS-induced damage (**Figure 5.5**). The TPF20 (*Trevesia palmata* flower-bud extract at 20 mg/kg) treatment demonstrated the most significant protective effect, with the colon architecture appearing almost normal, showing minimal signs of inflammation and no visible ulcerations or swelling. These findings suggest that *Trevesia palmata* flower-bud extract (TPF), especially at the higher dose (TPF20), has a strong potential for protecting against TNBS-induced ulcerative colitis, improving colon health and reducing tissue damage (**Figure 5.5**).

5.4.6 Colon length and colon weight/length ratio in TNBS-induced colitis rats treated with various therapeutic regimens.

The results presented in **Figure 5.6** show significant alterations in colon length and colon weight/length ratio in TNBS-induced colitis rats, as well as the therapeutic effects of *Trevesia palmata* flower bud methanolic extract (TPF) at

different doses. In the control group, the colon length remained the highest, reflecting healthy colon morphology. TNBS administration caused a pronounced reduction in colon length, indicating severe inflammation and tissue contraction typical of ulcerative colitis. Treatment with SPS50, TPF10, and TPF20 significantly attenuated this reduction, with all treatment groups showing longer colon lengths than the TNBS group. Among them, TPF20 demonstrated the greatest improvement, nearly restoring colon length toward normal values (**Figure 5.6**). The colon weight/length ratio, an indicator of colon oedema and inflammation, was lowest in the control group, consistent with healthy tissue. TNBS administration led to a substantial increase in this ratio, reflecting marked inflammation, oedema, and thickening of the colon wall. Treatment with SPS50, TPF10, and TPF20 significantly reduced the elevated colon weight/length ratio compared to the TNBS group. Although all treatments showed improvement, TPF20 again exhibited the strongest effect, demonstrating a lower colon weight/length ratio closer to the control group, which suggests a pronounced reduction in inflammation. Overall, these results indicate that *T. palmata* flower bud methanolic extract provides dose-dependent protection against TNBS-induced colonic damage. Both colon length restoration and reduction in colon weight/length ratio confirm the anti-inflammatory and tissue-protective properties of TPF, with the 20 mg/kg dose showing the highest therapeutic potential (**Figure 5.6**).

5.4.7 Disease Activity Index (DAI) score of TNBS-induced ulcerative colitis rats treated with different therapeutic regimens

The Disease Activity Index (DAI) scores for TNBS-induced ulcerative colitis rats treated with different therapeutic regimens are shown in **Figure 5.7**. The control group, treated with Dimethyl sulfoxide (1%) and saline (0.9%), had the lowest DAI score, indicating the absence of disease activity. In contrast, the TNBS group, which received Trinitrobenzene sulfonic acid alone, showed the highest DAI score, reflecting severe inflammation and marked disease activity. Rats treated with SPS50 (Sulfasalazine) exhibited a significant reduction in DAI score compared to the TNBS group, although their scores remained higher than those of the control group, indicating partial protection against TNBS-induced colitis. Treatment with TPF10

(*Trevesia palmata* flower bud methanolic extract at 10 mg/kg) also led to a significant decrease in DAI score compared to the TNBS group, demonstrating a beneficial effect in lowering disease severity. The TPF20 group showed the lowest DAI score among the treated groups, approaching values similar to the control group, suggesting that the higher dose provided the strongest protection against TNBS-induced colitis. Overall, the TNBS group exhibited the highest disease activity, while SPS50, TPF10, and TPF20 significantly reduced the DAI scores, with TPF20 showing the most pronounced improvement (**Figure 5.7**).

5.4.8 Alleviative effects of *Trevesia palmata* flower bud methanolic extract on the gross morphology of the rat colon in TNBS-induced ulcerative colitis

The gross morphology of the colon in TNBS-induced ulcerative colitis rats showed clear differences among the treatment groups. In the control group, the colons appeared smooth, intact, and free from any visible signs of inflammation or ulceration. In contrast, the TNBS group displayed severe pathological changes, including extensive ulceration, dark necrotic patches, thickening of the colon wall, and marked tissue damage, indicating successful induction of ulcerative colitis (**Figure 5.8**). Rats treated with SPS50 exhibited noticeable improvement compared to the TNBS group. The colons showed reduced ulceration and inflammation, although mild lesions and irregularities were still present. Treatment with TPF10 resulted in further improvements, with fewer ulcerated areas, less tissue thickening, and a more uniform surface appearance, suggesting a moderate protective effect of the extract at this dose. The most significant improvement was observed in the TPF20 group, where the colons showed minimal signs of ulceration and inflammation, and the tissue surface appeared closer to normal morphology. This indicates that the higher dose of *T. palmata* flower bud methanolic extract provided substantial protection against TNBS-induced colonic injury, reducing both damage and visible pathological alterations. Overall, the visual assessment of colon morphology demonstrates a dose-dependent protective effect of *T. palmata* flower bud methanolic extract, with TPF20 showing the strongest ability to alleviate TNBS-induced colonic damage (**Figure 5.8**).

5.4.9 Alleviative effects of *Trevesia palmata* flower bud methanolic extract (TPF) on the colon macroscopic score of rats in TNBS-induced ulcerative colitis

The results shown in **Figure 5.9** highlight the macroscopic score of the colon in TNBS-induced ulcerative colitis rats treated with different therapeutic regimens. The control group, treated with Dimethyl sulfoxide (1%) and saline (0.9%), showed the lowest macroscopic score, indicating no signs of inflammation or ulceration. In contrast, the TNBS group, which received Trinitrobenzene sulfonic acid (TNBS) alone, exhibited a high macroscopic score of approximately 4, reflecting severe inflammation and ulceration in the colon (**Figure 5.9**). Rats treated with SPS50 (Sulfasalazine) showed a moderate reduction in macroscopic score compared to the TNBS group, with a score of around 2.5, indicating some improvement in inflammation but still significant tissue damage. Treatment with TPF10 led to a further reduction in the macroscopic score, approximately 2.2, showing some beneficial effects, though some inflammation and minor ulcerations were still present. The most significant improvement was observed in the TPF20 group, which showed the lowest macroscopic score of approximately 1, indicating a marked reduction in ulcerations and inflammation, approaching normal colon morphology (**Figure 5.9**). Statistical analysis showed significant differences between the groups, with the TNBS group showing the highest macroscopic score and the TPF20 group exhibiting the most significant alleviation of colon damage. These findings suggest that *T. palmata* flower bud methanolic extract, especially at the 20 mg/kg dose, provides substantial protective effects against TNBS-induced ulcerative colitis (**Figure 5.9**).

5.4.10 Histopathological alterations in colon sections of TNBS-induced ulcerative colitis rats treated with different therapeutic regimens.

The histological analysis of colon sections in TNBS-induced ulcerative colitis rats treated with different therapeutic regimens revealed distinct findings across the groups. In the control group (A), the colon exhibited normal histological architecture with an orderly arrangement of the mucosa, submucosa, muscularis, and abundant goblet cells. The lamina propria was intact, and no inflammatory cell infiltration was

observed (**Figure 5.10**). In the TNBS group (B), there were significant structural changes, including the disruption of crypts (indicated by arrows), extensive inflammatory cell infiltration, and severe damage to tissue architecture, as evidenced by the loss of goblet cells (marked by asterisks). These changes indicate extensive damage due to TNBS-induced colitis. In the TNBS + SPS50 group (C), treatment with Sulfasalazine (50 mg/kg) resulted in some restoration of the crypt architecture (arrows), with preserved goblet cells and partial reduction in inflammatory infiltration. However, some damage remained, suggesting partial recovery from the inflammation and tissue damage induced by TNBS (**Figure 5.10**). The TNBS + TPF10 (10 mg/kg) and TNBS + TPF20 (20 mg/kg) groups (D and E) showed significant improvements in histological structure. Both TPF10 and TPF20 treatments led to the preservation of crypt architecture (arrows) and a reduction in inflammatory infiltration. Goblet cells were also preserved, and the lamina propria appeared more intact compared to the TNBS group, suggesting that *T. palmata* flower bud methanolic extract at both doses provided significant protection against TNBS-induced colonic damage. In conclusion, *T. palmata* flower bud methanolic extract, particularly at the 20 mg/kg dose, showed a pronounced effect in protecting the colon from the structural damage associated with TNBS-induced ulcerative colitis (**Figure 5.10**).

5.4.11 Biochemical alterations in oxidative stress markers in colon tissue of TNBS-induced ulcerative colitis rats treated with sulfasalazine and *Trevesia palmata* flower bud extract.

The results from the data shown in panels A, B, C, and D reflect the effects of *T. palmata* flower bud methanolic extract (TPF10 and TPF20) on various biochemical markers related to oxidative stress and antioxidant activity in rats with TNBS-induced colitis (**Figure 5.11A-D**). Malondialdehyde (MDA) levels, a marker of lipid peroxidation, were significantly elevated in the TNBS group compared to the control, indicating increased oxidative damage. Treatment with SPS50 (Sulfasalazine) and both TPF10 and TPF20 significantly reduced MDA levels, with TPF20 showing the most substantial decrease, suggesting its potent antioxidative

properties in reducing lipid peroxidation (**Figure 5.11A**). The TNBS group exhibited the lowest SOD activity, reflecting impaired antioxidant defence. SPS50 and both TPF10 and TPF20 treatments significantly improved SOD activity, with TPF20 resulting in the highest SOD levels, indicating enhanced antioxidative defence (**Figure 5.11B**). Reduced glutathione (GSH) levels were significantly reduced in the TNBS group, indicating depletion of this crucial antioxidant in response to inflammation. Treatment with SPS50 and TPF formulations led to a significant increase in GSH levels, with TPF20 showing the most pronounced recovery, indicating effective restoration of the antioxidant pool (**Figure 5.11C**). The TNBS group had significantly reduced catalase activity compared to controls. Both SPS50 and TPF treatments improved catalase activity, with TPF20 showing the most substantial increase, further supporting the protective antioxidant effects of *T. palmata* flower bud methanolic extract (**Figure 5.11D**). Overall, the findings suggest that *T. palmata* flower bud methanolic extract, particularly at the 20 mg/kg dose, effectively modulates oxidative stress by restoring key antioxidant activities and reducing lipid peroxidation in TNBS-induced colitis rats.

5.4.12 Inflammatory cytokines and oxidative stress markers in colon tissue of TNBS-induced ulcerative colitis rats treated with different therapeutic regimens.

Myeloperoxidase (MPO) activity, a marker of neutrophil infiltration, was significantly elevated in the TNBS + saline group, indicating inflammation. Treatment with sulfasalazine (SPS) and both doses of *T. palmata* flower bud methanolic extract (TPF10 and TPF20) significantly reduced MPO levels, with TPF20 showing the most significant reduction (**Figure 5.12A**). Regarding IL-6 levels, the TNBS group had elevated IL-6, a pro-inflammatory cytokine. Sulfasalazine and TPF treatments significantly lowered IL-6 levels, with TPF20 showing the greatest reduction, indicating its potential to modulate inflammation (**Figure 5.12B**). IL-1 β levels were also increased in the TNBS group, indicating inflammation. Similar to IL-6, treatment with SPS and TPF formulations decreased IL-1 β , with TPF20 achieving the lowest levels, suggesting its anti-inflammatory

potential (**Figure 5.12C**). The TNBS + saline group had elevated LTB4 levels, which were significantly reduced by treatment with SPS and both TPF doses, with TPF20 being the most effective (**Figure 5.12D**). Calprotectin (CALP) levels were higher in the TNBS group, indicating increased cellular stress. Both TPF doses and SPS treatment reduced CALP levels, suggesting the restoration of cellular homeostasis by the treatments (**Figure 5.12E**). FAD-linked sulphydryl oxidase (flavin adenine dinucleotide-linked sulphydryl oxidase) activity levels were significantly elevated in the TNBS group. Treatment with SPS and TPF formulations reduced FAD-linked sulphydryl oxidase levels, particularly TPF20, indicating improved liver function (**Figure 5.12F**). PGE2 levels, a key mediator of inflammation, were elevated in the TNBS group. SPS and TPF treatments reduced PGE2, with TPF20 showing the most significant reduction, indicating its anti-inflammatory effects (**Figure 5.12G**). Total antioxidant capacity (TAOC) levels were significantly lower in the TNBS group. Treatment with TPF formulations significantly improved TAOC, with the highest effect seen in the TPF20 group, suggesting enhanced antioxidant capacity (**Figure 5.12H**). Although the TNBS group showed low IL-10 levels, treatment with SPS and TPF formulations, particularly TPF20, significantly increased IL-10, highlighting its potential to promote an anti-inflammatory environment (**Figure 5.12I**). Overall, the results demonstrate that both sulfasalazine and *T. palmata* flower bud methanolic extract, especially at the 20 mg/kg dose, significantly reduce inflammation, enhance antioxidant capacity, and improve colitis symptoms in TNBS-induced rats (**Figure 5.12**).

5.4.13 Modulation of TNBS-induced alterations in colonic NF- κ B p65 expression by *T. palmata* flower bud extract (TPF)

The immunohistochemical analysis of NF- κ B p65 expression in the colon tissues of TNBS-induced ulcerative colitis rats revealed significant differences between the treatment groups (**Figure 5.13**). In the control group (**Figure 5.13A**), the NF- κ B p65 expression was minimal, indicating normal tissue function. The TNBS-treated group (**Figure 5.13B**) exhibited strong NF- κ B p65 expression, particularly in the epithelial and subepithelial regions, suggesting increased inflammatory activity.

The group treated with sulfasalazine (SPS50) (**Figure 5.13C**) showed moderate NF- κ B p65 expression, reflecting partial suppression of inflammation. In the groups treated with *T. palmata* flower bud methanolic extract (TPF10 and TPF20) (**Figure 5.13D and E**), a dose-dependent reduction in NF- κ B p65 expression was observed, with the 20 mg/kg dose (E) showing the most pronounced reduction. This suggests that *T. palmata* flower bud extract treatment effectively modulates NF- κ B p65 activity, thereby reducing inflammation associated with TNBS-induced ulcerative colitis. The statistical analysis (**Figure 5.13F**) confirmed significant differences in NF- κ B p65 expression across the groups. The TNBS group exhibited the highest levels of NF- κ B p65 expression, while the *T. palmata* -treated groups, particularly at the higher dose (TPF20), showed significantly lower NF- κ B p65 levels, indicating a protective effect against the inflammation associated with ulcerative colitis (**Figure 5.13**).

5.4.14 Modulation of colonic tumour necrosis factor alpha (TNF- α) protein expression by *T. palmata* flower bud extract (TPF)

Immunohistochemical analysis of TNF- α expression in the colon tissues of TNBS-induced ulcerative colitis rats demonstrated significant differences across the treatment groups (**Figure 5.14**). In the control group (**Figure 5.14A**), TNF- α expression was minimal, indicating normal tissue function with little inflammation. The TNBS-treated group (**Figure 5.14B**) exhibited intense TNF- α staining, particularly in the epithelial and subepithelial regions, indicating a heightened inflammatory response. The group treated with sulfasalazine (SPS50) (**Figure 5.14C**) showed moderate TNF- α expression, reflecting some degree of inflammation suppression. In the groups treated with *T. palmata* flower bud methanolic extract (TPF10 and TPF20) (**Figure 5.14D and E**), a dose-dependent reduction in TNF- α expression was observed. The 20 mg/kg dose (E) showed the most significant reduction, suggesting that *T. palmata* extract effectively attenuates TNF- α -mediated inflammation in the colon. The statistical analysis (**Figure 5.14F**) confirmed that the TNBS group had the highest TNF- α expression, while the *T. palmata* -treated

groups, particularly the higher dose (TPF20), showed significantly lower TNF- α levels, demonstrating the anti-inflammatory effect of the extract (**Figure 5.14**).

5.4.15 Modulation of colonic COX-2 (Cyclooxygenase-2) protein expression by *T. palmata* flower bud extract (TPF)

The immune-histochemical expression of COX-2 in the colons of different treatment groups are displayed in **Figure 5.15A-F**. In the control group (**Figure 5.15A**), there is minimal COX-2 expression, with slight labelling in the epithelial cells. In the TNBS group (**Figure 5.15B**), significant COX-2 expression is observed, especially in the epithelial layer, indicating inflammation. The TNBS + Sulfasalazine (**Figure 5.15C**) group shows a moderate reduction in COX-2 expression, with less intense staining compared to the TNBS group. The TNBS + TPF10 (**Figure 5.15D**) and TNBS + TPF20 (**Figure 5.15E**) groups show decreased COX-2 expression, with TPF20 treatment resulting in the lowest COX-2 activity among the treatment groups, suggesting a potential anti-inflammatory effect. The bar graph (**Figure 5.15F**) quantifies the COX-2 expression area, demonstrating statistically significant differences between the groups. The TNBS group exhibits the highest COX-2 expression, while the TPF10 and TPF20 treatments significantly reduce this expression, with TPF20 showing the most notable effect (**Figure 5.15**).

5.4.16 Modulation of colonic inducible nitric oxide synthase (iNOS) protein expression by *T. palmata* flower bud extract (TPF)

The immune-histochemical staining of iNOS expression in the colon tissues showed significant differences between the treatment groups (**Figure 5.16A-F**). In the control group (**Figure 5.16A**), a minimal expression of iNOS was observed. In contrast, the TNBS group (**Figure 5.16B**) exhibited a pronounced increase in iNOS expression, with high activity seen in the inflamed tissues. Treatment with Sulfasalazine (**Figure 5.16C**) significantly reduced iNOS expression compared to the TNBS group, although some activity remained. The treatment with *T. palmata* flower bud extract at both 10 mg/kg (**Figure 5.16D**) and 20 mg/kg (**Figure 5.16E**) doses showed a reduction in iNOS expression compared to the TNBS group, but the

expression levels were still higher than the control group. Quantitative analysis of iNOS expression in the different treatment groups (**Figure 5.16F**) revealed that the TNBS group had the highest iNOS area percentage, while treatment with Sulfasalazine and *T. palmata* flower bud extract (both doses) significantly reduced iNOS expression. However, the reduction was less pronounced in the 10 mg/kg TPF group. Statistical analysis indicated significant differences ($p < 0.0001$) among all groups, with the control group showing the least iNOS activity (**Figure 5.16**).

5.4.17 Alterations in colonic Nrf-2 protein expression induced by TNBS were attenuated by treatment with TPF.

The immunohistochemical analysis showed a significant change in the expression of Nrf2 in the colon tissues across the different treatment groups (**Figure 5.17A-F**). The control group (**Figure 5.17A**) displayed minimal expression of Nrf2, with marked presence in the crypts (indicated by arrows). The TNBS group (**Figure 5.17B**) showed a notable decrease in Nrf2 expression, with limited staining. In contrast, treatment with Sulfasalazine (**Figure 5.17C**) and *T. palmata* flower bud methanolic extract at 10 mg/kg (**Figure 5.17D**) and 20 mg/kg (**Figure 5.17E**) resulted in increased expression of Nrf2 in the colon tissues, with the highest area of Nrf2 expression observed in the TNBS + TPF20 group. The bar graph (**Figure 5.17F**) quantifying the Nrf2 expression area revealed significant differences between groups, with the TNBS group having the lowest expression and the TPF20 group having the highest. Statistical analysis confirmed a significant difference between the groups, with different letters indicating significant variation at $p < 0.0001$ (**Figure 5.17**).

5.4.18 Alterations in colonic protein expression of heme oxygenase-1 (HO-1) induced by TNBS were attenuated by treatment with TPF.

The immunohistochemical staining of HO-1 in colon tissues from different treatment groups showed significant alterations (**Figure 5.18A-F**). The control group (**Figure 5.18A**) displayed prominent HO-1 expression in the colonic cells, as indicated by the arrows. The TNBS group (**Figure 5.18B**) exhibited weak HO-1

expression, with reduced staining in the colonic tissues. Treatment with Sulfasalazine (**Figure 5.18C**) resulted in a moderate increase in HO-1 expression compared to the TNBS group. The groups treated with *T. palmata* flower bud methanolic extract (TPF10 and TPF20) (**Figure 5.18D and E**) showed enhanced HO-1 expression, with TPF20 demonstrating the most substantial increase in HO-1 area. The bar graph (**Figure 5.18F**) quantifying HO-1 expression revealed significant differences between groups, with the TNBS group showing the lowest expression and TPF20 showing the highest. Statistical analysis confirmed that the differences in HO-1 expression were significant at $p < 0.0001$, with different letters indicating statistical differences between groups (**Figure 5.18**).

5.4.19 Alterations in colonic protein expression of active caspase-3 induced by TNBS were attenuated by treatment with TPF.

The expression of active caspase-3 was evaluated in the colon tissue of rats following different treatment regimens (**Figure 5.19A-F**). Immunohistochemical analysis of active caspase-3 in the colon tissues showed varying levels of expression across different groups. The TNBS group exhibited a significant increase in active caspase-3 staining (**Figure 5.19B**) compared to the control (**Figure 5.19A**), indicating a higher level of apoptosis. In contrast, treatment with Sulfasalazine (**Figure 5.19C**) and the methanolic extract of *T. palmata* flower buds (**Figure 5.19D and E**) significantly reduced the active caspase-3 expression, showing a protective effect on apoptosis in the colon tissue. The quantification of active caspase-3 area (**Figure 5.19F**) revealed that the TNBS group had the highest expression, while treatment with Sulfasalazine and TPF10 and TPF20 reduced this expression significantly, with the TPF20 group showing the most significant decrease in active caspase-3 staining (**Figure 5.19**).

5.4.20 Effects of *T. palmata* flower bud methanolic extract on the protein expression of inflammation, oxidative stress, cell signalling and apoptotic markers in colon tissue of TNBS-induced ulcerative colitis

5.4.20.1 NF- κ B p65, TNF- α , and COX-2

Western blot analysis of NF- κ B p65, TNF- α , and COX-2 protein expressions showed notable differences across the treatment groups (**Figure 5.20 A-C**). The NF- κ B p65 protein expression in the TNBS group was significantly higher compared to the control group, indicating increased inflammation, but treatment with Sulfasalazine (SPS50), TPF10, and TPF20 reduced its expression. TPF20 treatment exhibited the most effective reduction in NF- κ B p65 levels, as evidenced by the quantification of the NF- κ B p65/ β -tubulin ratio (**Figure 5.20A**). Similarly, TNF- α expression was highest in the TNBS group and was significantly reduced with all treatments, with the TPF20 group showing the lowest expression (**Figure 5.20B**). For COX-2 expression, the TNBS group showed a marked increase compared to the control, and again, all treatments significantly lowered COX-2 levels, with TPF20 being the most effective (**Figure 5.20C**). The statistical analysis revealed significant differences between treatment groups, with the highest expression in the TNBS group and the lowest in the TPF20 group, as indicated by the different letters ($p < 0.0001$) (**Figure 5.20**).

5.4.20.2 IL-6, NOX-2, and p53

The results of the expression of inflammation, oxidative stress, and cell signalling markers in TNBS-induced ulcerative colitis rats treated with various regimens showed that: Interleukin-6 (IL-6) (**Figure 5.21A**) expression was significantly elevated in the TNBS group compared to the control. Treatment with Sulfasalazine and TPF (10 mg/kg and 20 mg/kg) reduced IL-6 expression significantly compared to the TNBS group. NADPH oxidase (NOX2) (**Figure 5.21B**) expression was significantly higher in the TNBS group compared to the control. Both Sulfasalazine and TPF treatment reduced NOX2 expression, with TPF showing a more prominent effect, especially at the 20 mg/kg dose. p53 (**Figure**

5.21C) expression was significantly increased in the TNBS group. Treatment with Sulfasalazine and TPF (10 mg/kg and 20 mg/kg) led to a significant reduction in p53 expression compared to TNBS, with TPF (20 mg/kg) showing the most considerable decrease (**Figure 5.21**).

5.4.20.3 PPAR- γ , Bax, and Bcl-2

The results for the expression of apoptosis-related proteins in TNBS-induced ulcerative colitis rats treated with various regimens in **Figure 5.22** are as follows: PPAR- γ expression was significantly lower in the TNBS group compared to the control. Treatment with Sulfasalazine and TPF (10 mg/kg and 20 mg/kg) significantly increased PPAR- γ expression, with the highest expression observed in the TPF (20 mg/kg) group (**Figure 5.22A**). Bax expression was significantly elevated in the TNBS group, indicating increased apoptosis. Treatment with Sulfasalazine and TPF (10 mg/kg and 20 mg/kg) reduced Bax expression significantly, with TPF (20 mg/kg) showing the most prominent effect (**Figure 5.22B**). Bcl-2 expression was significantly reduced in the TNBS group. Sulfasalazine and TPF treatments increased Bcl-2 expression, with TPF (10 mg/kg and 20 mg/kg) showing the most substantial improvement (**Figure 5.22C**). The Bax/Bcl-2 ratio was significantly higher in the TNBS group, reflecting the elevated apoptotic activity (**Figure 5.22D**). Both Sulfasalazine and TPF treatment reduced this ratio, with TPF (20 mg/kg) having the most significant effect, indicating a protective effect against apoptosis (**Figure 5.22**).

5.5 DISCUSSION

5.5.1 Alterations in body weight, food consumption, water consumption and rectal temperature in TNBS-induced colitis rats treated with various therapeutic regimens

The results from the clinical parameters demonstrated that *T. palmata* flower bud methanolic extract (TPF) has protective effects against TNBS (trinitrobenzene sulfonic acid)-induced damage in rats, as evidenced by significant improvements in body weight, food intake, water intake, and rectal temperature. Specifically, TNBS treatment resulted in a notable decrease in body weight, food and water intake, and

rectal temperature, compared to the control group. However, treatment with TPF (10 mg/kg and 20 mg/kg) significantly mitigated these effects, with the 20 mg/kg dose showing the most pronounced protective impact. In contrast, the standard treatment, Sulfasalazine (SPS50), also prevented some of the TNBS-induced damage, though its effects were less significant than those of TPF. These findings are broadly consistent with and support earlier reports of protective effects of natural products in TNBS-induced colitis and related inflammatory conditions. Previous studies have shown that TNBS administration causes significant body-weight loss and reduced food intake in colitis models, with a progressively lower food intake apparent shortly after induction (Ferreira-Duarte et al., 2021). Treatment with anti-inflammatory or plant-derived agents has been shown to reverse these changes: for instance, Geniposide restored TNBS-decreased body weight and ameliorated experimental colitis in rats (Xu et al., 2017). Similarly, herbal and natural-product-based therapies, such as Chang-An-Shuan, have been reported to significantly reduce weight loss and improve disease-related symptoms in TNBS-induced colitis (Mi et al., 2017). More recently, a study on (-)-Fenchone demonstrated that administration of this natural compound reduced the severity of colitis, improved weight loss, and ameliorated histopathological and inflammatory damage in TNBS-treated animals (Araruna et al., 2024). Taken together, our observation — that *T. palmata* flower bud methanolic extract (TPF) mitigates TNBS-induced reductions in body weight, food and water intake, and rectal temperature — aligns well with the established literature on natural-product interventions in TNBS-induced colitis. These converging results reinforce the potential of plant-derived extracts as complementary or alternative therapeutic agents in inflammatory bowel disease models and support further mechanistic and translational investigations into TPF's efficacy and mode of action.

5.5.1 Survival rate in TNBS-induced colitis rats treated with various therapeutic regimens

The present study investigated the survival rate of TNBS-induced colitis rats treated with various therapeutic regimens, including the standard drug Sulfasalazine (SPS50) and *T. palmata* flower-bud extract (TPF), at doses of 10 mg/kg (TPF10) and

20 mg/kg (TPF20). The Kaplan-Meier survival curves illustrated significant variations in survival rates among the different treatment groups, demonstrating the potential protective effects of TPF, particularly at the higher dose. In the control group, which received only Dimethyl sulfoxide (1%) and saline (0.9%), the survival rate remained at 100% throughout the 7 days, indicating no adverse effects and normal health. This aligns with previous findings that show standard control groups under non-inflammatory conditions exhibit stable survival rates (**Xu et al., 2017**). In contrast, the TNBS group, which was treated with TNBS alone to induce ulcerative colitis, exhibited a substantial decline in survival. The survival rate dropped to approximately 40% by Day 5, reflecting the severe inflammatory effects of TNBS-induced colitis, which has been well-documented in animal models (**Ferreira-Duarte et al., 2021**). TNBS-induced colitis leads to extensive damage to the colonic mucosa, intestinal barrier dysfunction, and increased systemic inflammation, contributing to the observed mortality (**Araruna et al., 2024**). The rats treated with Sulfasalazine (SPS50), a standard therapeutic agent for inflammatory bowel disease, exhibited a modest improvement in survival, with about 60% survival by Day 5. Although this represents a better outcome than the TNBS-only group, survival rates did not reach the levels observed in the control group, highlighting the limited efficacy of Sulfasalazine in preventing severe colitis-related mortality in this model. Sulfasalazine, while widely used in clinical practice, is known to have limited effectiveness in some cases, particularly in severe or chronic colitis (**Licznarska et al., 2015**). Moreover, Sulfasalazine's mode of action primarily targets inflammatory pathways but may not fully address tissue damage and immune dysfunction induced by TNBS.

Interestingly, the rats treated with 10 mg/kg of *T. palmata* flower-bud methanolic extract (TPF10) exhibited a notable improvement in survival, with approximately 80% survival by Day 7. This suggests that TPF10 provided significant protection against the detrimental effects of TNBS-induced ulcerative colitis. Previous studies have reported the therapeutic potential of various plant extracts in ameliorating the severity of TNBS-induced colitis by modulating inflammatory responses and oxidative stress (**Mi et al., 2017; Xu et al., 2017**). The beneficial

effects of TPF10 may be attributed to its ability to reduce pro-inflammatory cytokines, oxidative stress, and tissue damage, which are key contributors to disease progression in colitis models (Altaf et al., 2023).

The highest survival rate was observed in the TPF20 group, with approximately 100% survival by Day 7, indicating that the higher dose of TPF provided strong protective effects against TNBS-induced colitis. These results are consistent with previous reports demonstrating dose-dependent effects of plant-based extracts in colitis models, where higher doses often lead to more significant therapeutic outcomes (Balmus et al. 2016). TPF20's ability to completely prevent mortality in the TNBS model suggests that *T. palmata* flower-bud extract, especially at higher doses, could be a promising therapeutic candidate for the management of ulcerative colitis and other related inflammatory conditions. The observed protective effects of TPF20 could be related to its potential antioxidant and anti-inflammatory properties, as well as its ability to restore mucosal integrity (Balmus et al. 2016; Altaf et al., 2023). The protective effects of *T. palmata* flower-bud extract observed in this study highlight the therapeutic potential of natural products in treating inflammatory diseases such as ulcerative colitis. While the exact mechanisms of action remain to be fully elucidated, TPF likely exerts its effects through the modulation of immune responses, reduction of oxidative stress, and protection of colonic tissue integrity. Further mechanistic studies are necessary to determine the specific bioactive compounds responsible for these effects and to explore their potential for clinical application. In conclusion, the results of this study suggest that *T. palmata* flower-bud extract, particularly at higher doses, provides significant protection in TNBS-induced ulcerative colitis, with survival rates comparable to those of control animals. These findings support the potential of TPF as a natural therapeutic candidate for the management of ulcerative colitis and highlight the need for further research to explore its full therapeutic potential.

5.5.3 Rectal bleeding and inflammation alterations in colon architecture in TNBS-induced colitis rats treated with various therapeutic regimens

The results of this study demonstrate that *T. palmata* flower bud methanolic extract (TPF) offers significant protective effects against TNBS-induced ulcerative colitis in rats, as evidenced by improvements in rectal bleeding, inflammation, and colon architecture. The therapeutic potential of TPF, particularly at the higher dose (TPF20, 20 mg/kg), was shown to effectively mitigate the inflammatory damage induced by TNBS, offering strong protection against rectal bleeding and colon injury. In the control group, which received Dimethyl sulfoxide (1%) and saline (0.9%), no rectal bleeding or signs of inflammation were observed, reflecting normal health. This is consistent with previous studies that indicate healthy control groups do not exhibit any pathological changes in colonic architecture or rectal function (**Xu et al., 2017**). In contrast, the TNBS-treated rats demonstrated a significant increase in rectal bleeding and visible inflammation, which is a hallmark of TNBS-induced colitis models. The severity of these symptoms is consistent with reports that TNBS treatment leads to the induction of acute colitis with characteristic mucosal damage and ulceration (**Araruna et al., 2024; Ferreira-Duarte et al., 2021**).

The rats treated with Sulfasalazine (SPS50) showed moderate improvements in rectal bleeding compared to the TNBS-only group, but inflammation and irritation were still noticeable. Sulfasalazine, a commonly used anti-inflammatory drug for ulcerative colitis, has been shown to reduce inflammation and bleeding in some cases, but its effects are often limited, particularly in severe colitis models (**Licznarska et al., 2015**). In the current study, while Sulfasalazine provided partial protection, it did not fully restore normal colon architecture, highlighting the need for more effective treatments, particularly those derived from natural products. In contrast, treatment with TPF (10 mg/kg, TPF10) led to significant reductions in rectal bleeding and inflammation. The anal region of the treated rats showed reduced signs of injury and less bleeding compared to the TNBS-only group, suggesting that TPF confers a protective effect against TNBS-induced colitis. Previous research has reported that plant-derived extracts, such as those from *T. palmata*, possess anti-

inflammatory and antioxidant properties, which can alleviate the symptoms of colitis and promote mucosal healing (Mi et al., 2017; Balmus et al. 2016). The protective effects observed in this study support the hypothesis that TPF modulates inflammatory pathways and reduces oxidative stress, both of which are critical contributors to TNBS-induced colitis.

The most significant therapeutic effect was observed in the TPF20 group (20 mg/kg), which exhibited the least amount of rectal bleeding and minimal signs of inflammation. The higher dose of TPF (20 mg/kg) provided a stronger protective effect compared to the lower dose (10 mg/kg), suggesting a dose-dependent response. These findings align with previous studies showing that higher doses of plant extracts often result in more pronounced therapeutic effects, likely due to enhanced bioavailability and more robust modulation of inflammatory pathways (Altaf et al., 2023; Licznarska et al., 2015). The ability of TPF to reduce both the severity of inflammation and the extent of tissue damage at the higher dose underscores its potential as a candidate for the treatment of inflammatory bowel diseases (IBD) like ulcerative colitis.

In terms of molecular mechanisms, TPF likely exerts its protective effects through the modulation of key inflammatory pathways, including the inhibition of NF- κ B signalling, reduction of pro-inflammatory cytokines (e.g., TNF- α), and the restoration of mucosal integrity (Mi et al., 2017). Additionally, the antioxidant properties of TPF, which have been documented in previous studies, may contribute to the reduction of oxidative stress and the promotion of tissue repair in the colon (Balmus et al. 2016; Altaf et al., 2023).

In conclusion, the findings from this study indicate that *T. palmata* flower-bud extract (TPF), particularly at the higher dose (20 mg/kg), provides significant protection against TNBS-induced rectal bleeding and inflammation. The results suggest that TPF has the potential to serve as an effective therapeutic agent for the management of ulcerative colitis and other related inflammatory conditions. These observations also emphasise the importance of exploring plant-based extracts as

complementary or alternative treatments for IBD, highlighting the need for further mechanistic studies to understand the full range of biological effects of TPF.

5.5.4 Stool Consistency in TNBS-induced colitis rats treated with various therapeutic regimens

The findings from this study demonstrate that *T. palmata* flower bud methanolic extract (TPF) significantly improves stool consistency in rats with TNBS-induced colitis, particularly at the higher dose of 20 mg/kg (TPF20). The TNBS-induced colitis model, which is widely used to study ulcerative colitis and other gastrointestinal disorders, results in characteristic symptoms such as diarrhoea, abdominal discomfort, and abnormal stool consistency, as seen in the TNBS group in this study. The therapeutic effects of TPF, especially at higher doses, were evident in the significant improvement in stool consistency, highlighting its potential as a treatment for inflammatory bowel diseases (IBD) like ulcerative colitis. In the control group, the stool appeared well-formed and consistent, as expected, with no signs of distress or abnormalities, which is consistent with findings in other studies using healthy rodent models (Xu et al., 2017). In contrast, the TNBS-treated rats exhibited abnormal stool with a liquid consistency, which is indicative of the gastrointestinal disturbances caused by TNBS-induced colitis. TNBS treatment leads to severe inflammation of the colon, which disrupts normal gastrointestinal function and results in loose stools or diarrhoea, a hallmark of colitis in animal models (Araruna et al., 2024). The rats treated with Sulfasalazine (SPS50) showed some improvement in stool consistency, though it remained slightly loose and not fully formed, suggesting partial therapeutic recovery. This observation is consistent with previous research indicating that while Sulfasalazine can reduce inflammation and improve colitis symptoms, its efficacy may be limited, particularly in more severe cases of colitis (Licznarska et al., 2015). Although Sulfasalazine is a standard treatment for inflammatory bowel disease, it does not always restore normal gastrointestinal function or completely address the underlying causes of colitis. In contrast, treatment with TPF (10 mg/kg, TPF10) resulted in relatively firmer stool compared to the TNBS-only group. The partial improvement in stool consistency

observed in the TPF10 group suggests that TPF has some beneficial effects in managing gastrointestinal disturbances caused by TNBS-induced colitis. This is in line with other studies where plant-derived extracts have been shown to exert therapeutic effects in colitis models by modulating inflammatory pathways and promoting gut health (Mi et al., 2017; Altaf et al., 2023). However, the most significant improvement was observed in the TPF20 group (20 mg/kg), where stool consistency was restored to near-normal levels, reflecting the strongest therapeutic effect of TPF. These results are consistent with the dose-dependent effects observed with other natural products, where higher doses often lead to more pronounced therapeutic outcomes (Balmus et al. 2016). The improvement in stool consistency in the TPF-treated groups, particularly at the higher dose, likely reflects the extract's ability to reduce inflammation, restore mucosal integrity, and modulate gut microbiota. Previous research has demonstrated that plant-based therapies, including those derived from *T. palmata*, have anti-inflammatory, antioxidant, and gut-healing properties, all of which are crucial for restoring normal gastrointestinal function in colitis models (Altaf et al., 2023; Mi et al., 2017). Inflammatory cytokines such as TNF- α and interleukins, as well as oxidative stress, are key contributors to the pathogenesis of colitis, and TPF's modulation of these factors may explain the observed improvements in stool consistency.

The findings of this study suggest that TPF, particularly at a dose of 20 mg/kg, significantly improves gastrointestinal function in TNBS-induced colitis, making it a promising candidate for further investigation as a therapeutic agent for inflammatory bowel diseases. The protective effects of TPF against TNBS-induced gastrointestinal disturbances highlight its potential as a natural alternative or complementary treatment to conventional therapies. Future studies should explore the underlying mechanisms through which TPF exerts its effects, including its impact on the gut microbiota, inflammatory pathways, and oxidative stress markers, to better understand its full therapeutic potential.

5.5.5 Colon Architecture

The findings from this study demonstrate that *T. palmata* flower bud methanolic extract (TPF) provides significant protection against TNBS-induced ulcerative colitis in rats, as evidenced by improvements in colon architecture, colon length, colon weight/length ratio, disease activity index (DAI), and gross morphology. These results underscore the therapeutic potential of TPF, particularly at higher doses, as a candidate for treating inflammatory bowel diseases such as ulcerative colitis. In the control group, the colon appeared normal with no visible signs of inflammation or damage, which is consistent with the expected outcome in healthy animals (Xu et al., 2017). In contrast, TNBS treatment caused significant damage to the colon architecture, including thickening, ulcerations, and bleeding. These pathological changes are characteristic of TNBS-induced colitis, a well-established model for studying ulcerative colitis (Araruna et al., 2024). The rats treated with Sulfasalazine (SPS50) exhibited some improvement in colon architecture, as shown by reduced inflammation and fewer ulcerations, but they still exhibited mild swelling and irregularities, indicating that the effects of Sulfasalazine were partial. This observation aligns with prior reports suggesting that Sulfasalazine can reduce inflammation in colitis but may not fully restore normal colon architecture (Licznarska et al., 2015). In contrast, treatment with TPF10 (10 mg/kg) led to moderate improvements in colon structure, with reduced swelling, fewer ulcerations, and a more organised tissue structure, suggesting a protective effect of TPF. The most significant protection was observed in the TPF20 (20 mg/kg) group, where the colon architecture appeared almost normal, with minimal signs of inflammation and no visible ulcerations. These findings are consistent with studies that show plant-derived extracts, such as those from *T. palmata*, can effectively mitigate colonic damage and inflammation in colitis models (Mi et al., 2017; Altaf et al., 2023). The dose-dependent protection observed in this study further supports the therapeutic potential of TPF, especially at higher doses.

5.5.6 Colon Length and Colon Weight/Length Ratio

The results for colon length and colon weight/length ratio (**Figure 5.6**) further demonstrate the protective effects of TPF in TNBS-induced colitis. In the control group, the colon length remained normal, reflecting healthy tissue (**Xu et al., 2017**). However, TNBS administration led to a pronounced reduction in colon length, which is commonly associated with severe inflammation and tissue contraction in colitis models (**Araruna et al., 2024**). Treatment with SPS50, TPF10, and TPF20 significantly attenuated this reduction, with TPF20 showing the greatest improvement, nearly restoring colon length toward normal values. The colon weight/length ratio, which serves as an indicator of oedema and inflammation, was highest in the TNBS group, reflecting marked inflammation and thickening of the colon wall. All treatment groups showed a significant reduction in the colon weight/length ratio, with TPF20 demonstrating the most pronounced effect, indicating a substantial reduction in inflammation. These results are consistent with previous studies that highlight the role of herbal treatments in reducing colon oedema and inflammation in colitis models (**Mi et al., 2017; Balmus et al. 2016**).

5.5.7 Disease Activity Index (DAI)

The DAI scores, which reflect disease severity, were lowest in the control group, as expected, and highest in the TNBS group, indicating severe inflammation and disease activity (**Figure 5.7**). The SPS50 group showed a significant reduction in DAI score, suggesting partial protection against TNBS-induced colitis. However, the scores remained higher than those of the control group, indicating that Sulfasalazine alone may not be sufficient to fully control disease activity in severe colitis models (**Licznarska et al., 2015**). In contrast, both TPF10 and TPF20 significantly reduced the DAI score, with TPF20 showing the most significant reduction, approaching values similar to the control group. This finding suggests that TPF, particularly at higher doses, has the potential to provide robust protection against disease progression in TNBS-induced colitis, likely through modulation of inflammatory pathways and tissue repair (**Altaf et al., 2023**).

5.5.8 Gross Morphology and Colon Macroscopic Score

The gross morphology of the colon was assessed to further evaluate the extent of tissue damage and inflammation. In the TNBS group, the colon exhibited severe ulceration, necrotic patches, and thickening of the wall, consistent with the typical pathology of TNBS-induced colitis (**Figure 8**). Rats treated with Sulfasalazine exhibited some improvement, with reduced ulceration and inflammation, though mild lesions remained. This partial protection is consistent with previous reports on the limited efficacy of Sulfasalazine in certain colitis models (**Licznarska et al., 2015**). In the TPF10 group, the colon showed fewer ulcerated areas, less tissue thickening, and a more uniform surface, indicating moderate protection. However, the most significant improvement was observed in the TPF20 group, where the colon showed minimal ulceration, no visible inflammation, and near-normal tissue morphology. These findings are consistent with the literature on the protective effects of natural products in colitis models, where higher doses often result in more significant tissue protection and healing (**Mi et al., 2017; Balmus et al. 2016**).

The colon macroscopic score, which quantitatively assesses the severity of inflammation and ulceration, was highest in the TNBS group, with scores indicating extensive damage (**Figure 9**). The SPS50 group showed a moderate reduction in the macroscopic score, suggesting partial therapeutic effects. However, the TPF10 and TPF20 groups exhibited a significant reduction in macroscopic scores, with TPF20 showing the most pronounced improvement. These results indicate that TPF, especially at the higher dose, provides substantial protection against TNBS-induced colonic injury and reduces both visible tissue damage and inflammation, consistent with previous findings on the efficacy of plant-based extracts in colitis (**Balmus et al. 2016; Altaf et al., 2023**).

In conclusion, the results of this study suggest that *Trevesia palmata* flower-bud methanolic extract (TPF) provides dose-dependent protection against TNBS-induced ulcerative colitis, with the 20 mg/kg dose (TPF20) showing the most pronounced therapeutic effects. The significant improvements in colon architecture, colon length, colon weight/length ratio, disease activity index, and gross morphology

highlight the potential of TPF as a natural therapeutic candidate for the treatment of inflammatory bowel diseases such as ulcerative colitis. These findings warrant further studies to explore the underlying mechanisms of action and to optimise the therapeutic use of TPF in clinical settings.

5.5.10 Histopathological Alterations in Colon Sections

This study aimed to evaluate the therapeutic effects of *Trevesia palmata* flower-bud methanolic extract (TPF) in TNBS-induced ulcerative colitis, focusing on histopathological alterations, biochemical markers of oxidative stress, and inflammatory cytokines. The results suggest that TPF, particularly at higher doses (20 mg/kg), provides significant protection against TNBS-induced colitis, restoring colon architecture, reducing oxidative stress, and modulating inflammatory cytokines. Below is a detailed discussion on each aspect of the study. Histological analysis of colon sections revealed significant differences between the treatment groups. The control group exhibited normal histological architecture with intact mucosa, submucosa, muscularis, and abundant goblet cells, with no signs of inflammation. This aligns with the expected findings in healthy animals (**Xu et al., 2017**). In contrast, the TNBS group showed severe alterations in colon structure, including crypt disruption, inflammatory cell infiltration, and loss of goblet cells, which is characteristic of TNBS-induced colitis (**Araruna et al., 2024**). The presence of extensive tissue damage underscores the severity of inflammation and mucosal injury associated with this model of ulcerative colitis.

Sulfasalazine (SPS50), a standard treatment for colitis, showed partial improvement in colon architecture, with some restoration of crypts and preservation of goblet cells. However, mild ulcerations and inflammation persisted, indicating that Sulfasalazine may not fully address the underlying tissue damage in severe colitis (**Licznarska et al., 2015**). The TPF10 and TPF20 groups demonstrated significant improvements in colon structure, with preserved crypts, reduced inflammatory infiltration, and intact goblet cells. These results suggest that *T. palmata* flower-bud methanolic extract offers notable protection against TNBS-induced colonic damage, with TPF20 showing the most pronounced effects, consistent with the findings of

other natural products that modulate inflammation and promote tissue healing (**Mi et al., 2017; Altaf et al., 2023**).

5.5.11 Biochemical Alterations in Oxidative Stress Markers

The biochemical markers of oxidative stress, including Malondialdehyde (MDA), Superoxide Dismutase (SOD), Reduced Glutathione (GSH), and Catalase (CAT), were assessed to evaluate the antioxidant properties of TPF. The results showed that TNBS administration significantly increased MDA levels, a marker of lipid peroxidation, indicating increased oxidative damage. These findings align with previous studies that have shown elevated oxidative stress in TNBS-induced colitis, contributing to tissue injury and inflammation (**Ferreira-Duarte et al., 2021**). Treatment with Sulfasalazine and TPF (both doses) reduced MDA levels, with TPF20 showing the most substantial decrease, suggesting potent antioxidative properties of *T. palmata* flower-bud extract in reducing lipid peroxidation (**Altaf et al., 2023**). SOD activity, a key enzyme in the defence against oxidative stress, was significantly reduced in the TNBS group, confirming impaired antioxidant defence. Both Sulfasalazine and TPF treatments significantly improved SOD activity, with TPF20 achieving the highest SOD levels. This improvement in SOD activity supports the hypothesis that TPF restores the antioxidant defence mechanisms in TNBS-induced colitis, which has been demonstrated in other plant-based therapies (**Balmus et al. 2016**). GSH levels, a crucial antioxidant in maintaining cellular redox balance, were also depleted in the TNBS group. Treatment with Sulfasalazine and TPF formulations led to a significant increase in GSH levels, with TPF20 showing the most pronounced recovery. This finding suggests that TPF effectively restores the antioxidant pool, which is critical for managing inflammation and preventing oxidative damage in colitis (**Mi et al., 2017**). CAT activity, another important antioxidant enzyme, was significantly reduced in the TNBS group. Both Sulfasalazine and TPF treatments improved catalase activity, with TPF20 showing the most substantial increase, further supporting the antioxidative effects of TPF in reducing oxidative stress and promoting tissue repair (**Altaf et al., 2023**).

5.12 Inflammatory Cytokines and Oxidative Stress Markers

The effects of TPF on inflammatory cytokines and oxidative stress markers were further assessed. Myeloperoxidase (MPO) activity, which serves as a marker of neutrophil infiltration, was significantly elevated in the TNBS group, indicating the presence of inflammation. Treatment with Sulfasalazine and both doses of TPF reduced MPO activity, with TPF20 showing the most significant reduction. This is consistent with previous studies showing that the modulation of MPO activity correlates with the resolution of inflammation in colitis models (**Licznarska et al., 2015; Altaf et al., 2023**). IL-6 and IL-1 β , pro-inflammatory cytokines, were significantly elevated in the TNBS group, further supporting the inflammatory nature of the colitis induced by TNBS. Treatment with Sulfasalazine and TPF formulations reduced the levels of these cytokines, with TPF20 showing the greatest reduction. This suggests that TPF has significant anti-inflammatory effects, potentially by modulating immune responses and inhibiting the activation of inflammatory pathways such as NF- κ B and MAPK (**Mi et al., 2017; Altaf et al., 2023**). Elevated levels of leukotriene B4 (LTB4), another marker of inflammation, were also reduced by Sulfasalazine and TPF treatments, particularly TPF20, indicating a broader anti-inflammatory effect of TPF. Calprotectin (CALP), a marker of cellular stress and inflammation, was significantly reduced by both TPF doses and Sulfasalazine treatment, suggesting that TPF helps restore cellular homeostasis and protect against inflammatory damage (**Balmus et al. 2016**). FAD-linked sulphydryl oxidase (FAD), an enzyme involved in oxidative stress and tissue damage, was also reduced by TPF, particularly TPF20, highlighting its role in improving liver function and oxidative balance (**Altaf et al., 2023**).

Prostaglandin E2 (PGE2), a key mediator of inflammation, was elevated in the TNBS group, as expected in models of ulcerative colitis. Treatment with Sulfasalazine and TPF formulations reduced PGE2 levels, with TPF20 showing the most significant reduction, further confirming the anti-inflammatory effects of TPF (**Altaf et al., 2023**). Additionally, total antioxidant capacity (TAOC) levels, which reflect the overall antioxidative status, were significantly lower in the TNBS group.

TPF formulations, particularly TPF20, significantly increased TAOC levels, supporting the role of TPF in enhancing the antioxidant defence system. Finally, IL-10, an anti-inflammatory cytokine, was elevated in the TPF-treated groups, particularly TPF20, suggesting that TPF promotes an anti-inflammatory environment, which is crucial for resolving colitis (Mi et al., 2017; Balmus et al. 2016).

In conclusion, the findings from this study suggest that *Trevesia palmata* flower-bud methanolic extract (TPF) provides significant therapeutic benefits in TNBS-induced ulcerative colitis, particularly at the higher dose (TPF20). TPF was shown to protect against histopathological damage, modulate oxidative stress markers, and reduce inflammation by lowering pro-inflammatory cytokines and enhancing antioxidant defence. These results support the potential of TPF as a natural therapeutic candidate for the treatment of ulcerative colitis and other related inflammatory conditions.

5.5.13 Modulation of NF- κ B p65 Expression

This study aimed to evaluate the therapeutic effects of *Trevesia palmata* flower bud methanolic extract (TPF) in TNBS-induced ulcerative colitis by analysing histopathological alterations, biochemical markers of oxidative stress, and inflammation. The results show that TPF, especially at higher doses (TPF20, 20 mg/kg), effectively alleviated colonic damage, reduced inflammation, and improved the oxidative stress status in TNBS-induced ulcerative colitis rats. Below, we discuss the findings in the context of relevant literature. The immunohistochemical analysis of NF- κ B p65 expression revealed significant differences between the treatment groups. In the TNBS group, there was a marked increase in NF- κ B p65 expression, particularly in the epithelial and subepithelial regions, suggesting heightened inflammatory activity. The NF- κ B pathway plays a critical role in the regulation of inflammation and is often activated in response to inflammatory stimuli such as TNBS (Yang et al., 2021). Treatment with Sulfasalazine and TPF led to a dose-dependent reduction in NF- κ B p65 expression, with TPF20 showing the most significant effect. This finding supports previous reports that plant-based extracts,

such as *Trevesia palmata*, possess anti-inflammatory properties by modulating the NF- κ B pathway, which is crucial for the resolution of colitis (Mi et al., 2017; Balmus et al. 2016).

5.5.14 Modulation of TNF- α Expression

TNF- α is a key pro-inflammatory cytokine involved in the pathogenesis of ulcerative colitis, and its expression was significantly elevated in the TNBS group. The results of this study show that TPF treatment significantly reduced TNF- α expression, particularly at the higher dose (TPF20). TNF- α is known to promote inflammation through the activation of NF- κ B, and its inhibition has been shown to improve colitis symptoms (Altaf et al., 2023). The reduction in TNF- α expression by TPF further confirms its anti-inflammatory potential and aligns with findings from other studies on herbal extracts, which have been reported to attenuate TNF- α -induced inflammation in colitis models (Balmus et al. 2016).

5.5.15 Modulation of COX-2 Expression

Cyclooxygenase-2 (COX-2) is an enzyme involved in the production of pro-inflammatory prostaglandins, and its expression is significantly elevated in colitis. In this study, the TNBS group exhibited high COX-2 expression, which was significantly reduced by treatment with Sulfasalazine and TPF. The 20 mg/kg dose (TPF20) showed the most substantial reduction in COX-2 levels, suggesting a potent anti-inflammatory effect of TPF. This is consistent with other studies showing that COX-2 inhibition can reduce inflammation and improve symptoms in inflammatory bowel disease models (Licznarska et al., 2015; Altaf et al., 2023).

5.5.16 Modulation of iNOS Expression

Inducible nitric oxide synthase (iNOS) is another key player in inflammation, and its elevated expression is associated with increased production of nitric oxide (NO), which can contribute to tissue damage in colitis. The results of this study showed that the TNBS group had significantly higher iNOS expression compared to the control group, indicating elevated inflammatory activity. Treatment with

Sulfasalazine and TPF (both doses) significantly reduced iNOS expression, with the greatest effect seen in the TPF20 group. This reduction in iNOS expression is consistent with previous studies demonstrating that plant-based treatments can reduce iNOS activity and attenuate inflammation in colitis models (Altaf et al., 2023; Mi et al., 2017).

5.5.17 Modulation of Nrf-2 Expression

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that regulates the expression of antioxidant genes and plays a key role in protecting against oxidative stress. The TNBS group showed reduced Nrf2 expression, indicating a compromised antioxidant defence in the colon. Treatment with Sulfasalazine and TPF increased Nrf2 expression, with TPF20 showing the most significant increase. This result suggests that TPF may help restore the antioxidant defence in colitis, which is crucial for mitigating the oxidative stress that contributes to colonic damage (Mi et al., 2017; Balmus et al. 2016).

5.5.18 Modulation of HO-1 Expression

Heme oxygenase-1 (HO-1) is another important antioxidant enzyme that protects tissues from oxidative stress. In this study, the TNBS group exhibited reduced HO-1 expression, while treatment with Sulfasalazine and TPF restored HO-1 levels, with the highest expression seen in the TPF20 group. The upregulation of HO-1 by TPF suggests its potential to protect against oxidative damage and promote tissue repair in colitis (Altaf et al., 2023).

5.5.19 Modulation of Active Caspase-3 Expression

Caspase-3 is a key marker of apoptosis, and its increased expression in the TNBS group reflects the elevated apoptosis in colonic tissues induced by inflammation. Treatment with Sulfasalazine and TPF significantly reduced active caspase-3 expression, with TPF20 showing the most significant decrease. The reduction in caspase-3 expression suggests that TPF may protect against apoptosis

and tissue damage in colitis, which has been reported in other studies on herbal treatments (Mi et al., 2017).

5.5.20 Western Blot Analysis of Inflammatory Markers

The results of the western blot analysis of NF- κ B p65, TNF- α , and COX-2 protein expression (**Figure 20**) showed that all treatments significantly reduced the expression of these inflammatory markers compared to the TNBS group. The TPF20 group exhibited the most significant reductions in all three markers, indicating the powerful anti-inflammatory effects of *Trevesia palmata* flower bud methanolic extract. This aligns with findings from previous studies showing that herbal extracts can modulate inflammatory pathways to reduce colonic damage in ulcerative colitis (Balmus et al. 2016; Altaf et al., 2023).

5.5.21 Expression of IL-6, NOX-2, and p53

The expression of IL-6, NOX-2, and p53 was significantly elevated in the TNBS group, indicating heightened inflammation and oxidative stress. Treatment with Sulfasalazine and TPF (both doses) significantly reduced the expression of these markers, with TPF20 showing the most pronounced effects. The reduction in IL-6 and NOX-2 expression suggests that TPF modulates inflammatory and oxidative stress pathways to protect the colon (Licznarska et al., 2015; Mi et al., 2017). Furthermore, the decrease in p53 expression indicates reduced apoptosis, supporting the potential of TPF in protecting against cell death in colitis (Balmus et al. 2016).

5.5.22 Expression of PPAR- γ , Bax, and Bcl-2

PPAR- γ , Bax, and Bcl-2 are key markers involved in apoptosis regulation. In this study, PPAR- γ expression was significantly reduced in the TNBS group, and treatment with Sulfasalazine and TPF significantly increased PPAR- γ levels, with TPF20 showing the highest increase. Bax expression, which reflects increased apoptosis, was also reduced by TPF, particularly at the 20 mg/kg dose, while Bcl-2 expression, which promotes cell survival, was significantly increased by TPF

treatment. The Bax/Bcl-2 ratio, an indicator of apoptosis, was significantly reduced by TPF, indicating its protective effect against apoptosis in colitis (Mi et al., 2017).

The results of this study demonstrate that *Trevesia palmata* flower bud methanolic extract (TPF), especially at the higher dose (TPF20), provides significant therapeutic effects in TNBS-induced ulcerative colitis by modulating key inflammatory and oxidative stress markers. TPF reduced the expression of NF- κ B p65, TNF- α , COX-2, iNOS, IL-6, and NOX-2, while enhancing antioxidant markers like Nrf2, HO-1, and PPAR- γ . Additionally, TPF reduced apoptotic markers, including Bax and caspase-3, suggesting a protective effect on cell survival. These findings highlight the potential of TPF as a natural therapeutic agent for inflammatory bowel diseases and underscore the importance of further research to understand its full therapeutic mechanisms.

5.6 KEY FINDINGS

Overall effect of TPF

- TNBS induced marked clinical, macroscopic, histological, biochemical, and molecular changes consistent with severe ulcerative colitis.
- *Trevesia palmata* flower-bud methanolic extract (TPF) produced dose-dependent protection, with **TPF20** consistently showing the strongest therapeutic effect, often superior to sulfasalazine (SPS50).
- **Clinical signs and general condition**
 - **Body weight, food & water intake, rectal temperature:** TNBS caused weight loss, hypophagia, reduced water intake, and decreased rectal temperature. Both TPF10 and TPF20 prevented these changes, with TPF20 giving near-normal values; SPS50 showed only partial correction.

- **Stool consistency & rectal bleeding:** TNBS produced diarrhoeic, blood-stained stools. TPF treatment, especially TPF20, markedly improved stool consistency and reduced rectal bleeding; SPS50 showed moderate benefit.
- **Survival rate:** Survival was lowest in the TNBS group (~40%). TPF20 achieved **100% survival**, TPF10 ~80%, and SPS50 ~60%, indicating strong protection by TPF.
- **Disease Activity Index (DAI):** TNBS produced the highest DAI scores. TPF10 and particularly TPF20 significantly lowered DAI, with TPF20 approaching control levels.
- **Colon length and weight/length ratio:** TNBS shortened colon length and increased weight/length ratio (edema, inflammation). Both TPF doses and SPS50 improved colon length and reduced the ratio, with TPF20 showing the greatest normalization.
- **Macroscopic colon changes**
 - TNBS caused thickening, ulcerations, hemorrhage, and high macroscopic scores.
 - TPF10 reduced ulceration and inflammation moderately, whereas **TPF20 almost restored normal gross architecture**, with minimal thickening and ulceration.
 - Macroscopic score was highest in the TNBS group (score ~4) and lowest in TPF20 (score ~1), with SPS50 showing intermediate improvement.

- **Histopathology**

- TNBS resulted in severe mucosal damage: distorted crypts, epithelial disruption, marked inflammatory cell infiltration, and goblet cell depletion.
- TPF10 and TPF20 markedly improved histology, showing preserved crypt architecture, reduced cellular infiltration, and restored goblet cells; **TPF20 closely resembled control tissue**. SPS50 produced partial repair.

- **Oxidative stress and antioxidant status (MDA, SOD, GSH)**

- TNBS significantly increased MDA (lipid peroxidation) and decreased SOD and GSH, indicating intense oxidative stress.
- TPF, especially TPF20, **reduced MDA and restored SOD and GSH**, demonstrating strong antioxidative capacity. SPS50 showed modest correction.

- **Inflammatory cytokines and inflammatory signaling**

- TNBS markedly elevated MPO, IL-6, IL-1 β , TNF- α , NF- κ B p65, COX-2, NOX-2 and other inflammatory/oxidative markers.
- SPS50, TPF10, and TPF20 all reduced these markers, with **TPF20 consistently producing the largest decreases** in IL-6, IL-1 β , TNF- α , NF- κ B p65, COX-2, MPO, and NOX-2.
- These changes confirm that TPF effectively suppresses both inflammatory cytokine production and downstream NF- κ B–COX-2–TNF- α signaling, as well as NOX-2–mediated oxidative stress.

- **Key inflammatory and oxidative enzymes (NF- κ B, TNF- α , COX-2, iNOS)**
 - TNBS upregulated NF- κ B p65, TNF- α , COX-2, and iNOS in colonic tissue.
 - TPF treatment, particularly TPF20, significantly downregulated all these proteins, indicating potent anti-inflammatory and anti-nitrosative effects, often superior to SPS50.
- **Nrf2/HO-1 cytoprotective axis**
 - TNBS suppressed Nrf2 and HO-1 expression, weakening endogenous antioxidant defense.
 - TPF, especially TPF20, **restored and enhanced Nrf2 and HO-1 levels**, supporting activation of cytoprotective, antioxidant pathways.
- **Apoptosis and cell survival (caspase-3, PPAR- γ , Bax, Bcl-2, Bax/Bcl-2, p53)**
 - TNBS increased active caspase-3, Bax, and p53 and decreased PPAR- γ and Bcl-2, leading to a high Bax/Bcl-2 ratio and enhanced apoptosis.
 - TPF (more so TPF20) reduced caspase-3, Bax, and p53, increased PPAR- γ and Bcl-2, and lowered the Bax/Bcl-2 ratio, indicating **attenuation of apoptosis and improved cell survival**. SPS50 showed beneficial but generally less pronounced effects.
- **Integrated conclusion from key findings**
 - Across clinical indices, macroscopic and microscopic pathology, oxidative stress markers, inflammatory mediators, and apoptotic regulators, **TPF20 consistently provided the greatest protection** against TNBS-induced ulcerative colitis.

- These data collectively support *Trevesia palmata* flower-bud methanolic extract—particularly at 20 mg/kg—as a promising phytotherapeutic candidate that mitigates inflammation, oxidative stress, and apoptosis in experimental colitis.

Table 5.1. Tabulated form of treatment group details and drug schedule of induction of ulcerative colitis of TNBS model in rat

Group no.	Animals group	Treatment	Dose	Route of administration	No. of Animals	Days of Treatment		
						0 day	1-7 th days	8 th day
I	Control	Dimethyl sulfoxide (DMSO) (1 %)	5 ml/kg	p. o	8	-	√	Blood sample were collected and animals were sacrificed for biochemical, molecular and histopathological examination.
		Physiological saline (0.9 %)	0.25 mL	Intra-rectally	8	-	√	
II	TNBS	Dimethyl sulfoxide (DMSO) (1 %)	5 ml/kg	p. o	8	-	√	
		Trinitrobenzene sulfonic acid (TNBS)	50 mg/kg 0.25 mL	Intra-rectally	8	√	-	
III	TNBS + SPS50	Sulfasalazine in Dimethyl sulfoxide (DMSO) (1 %)	50 mg/kg 5 ml/kg	p. o	8	-	√	
		Trinitrobenzene sulfonic acid (TNBS)	50 mg/kg 0.25 mL	Intra-rectally	8	√	-	
IV	TNBS + TPF10	methanolic extract of <i>T. palmata</i> flower buds in in Dimethyl sulfoxide (DMSO) (1 %)	10 mg/kg 5 ml/kg	p. o	8	-	√	
		Trinitrobenzene sulfonic acid (TNBS)	50 mg/kg 0.25 mL	Intra-rectally	8	√	-	
V	TNBS + TPF20	methanolic extract of <i>T. palmata</i> flower buds in in Dimethyl sulfoxide (DMSO) (1 %)	20 mg/kg 5 ml/kg	p. o	8	-	√	Anesthesia Dose ketamine (90 mg/kg) and xylazine (10 mg/kg) (i.p) body weight
		Trinitrobenzene sulfonic acid (TNBS)	50 mg/kg 0.25 mL	Intra-rectally	8	√	-	

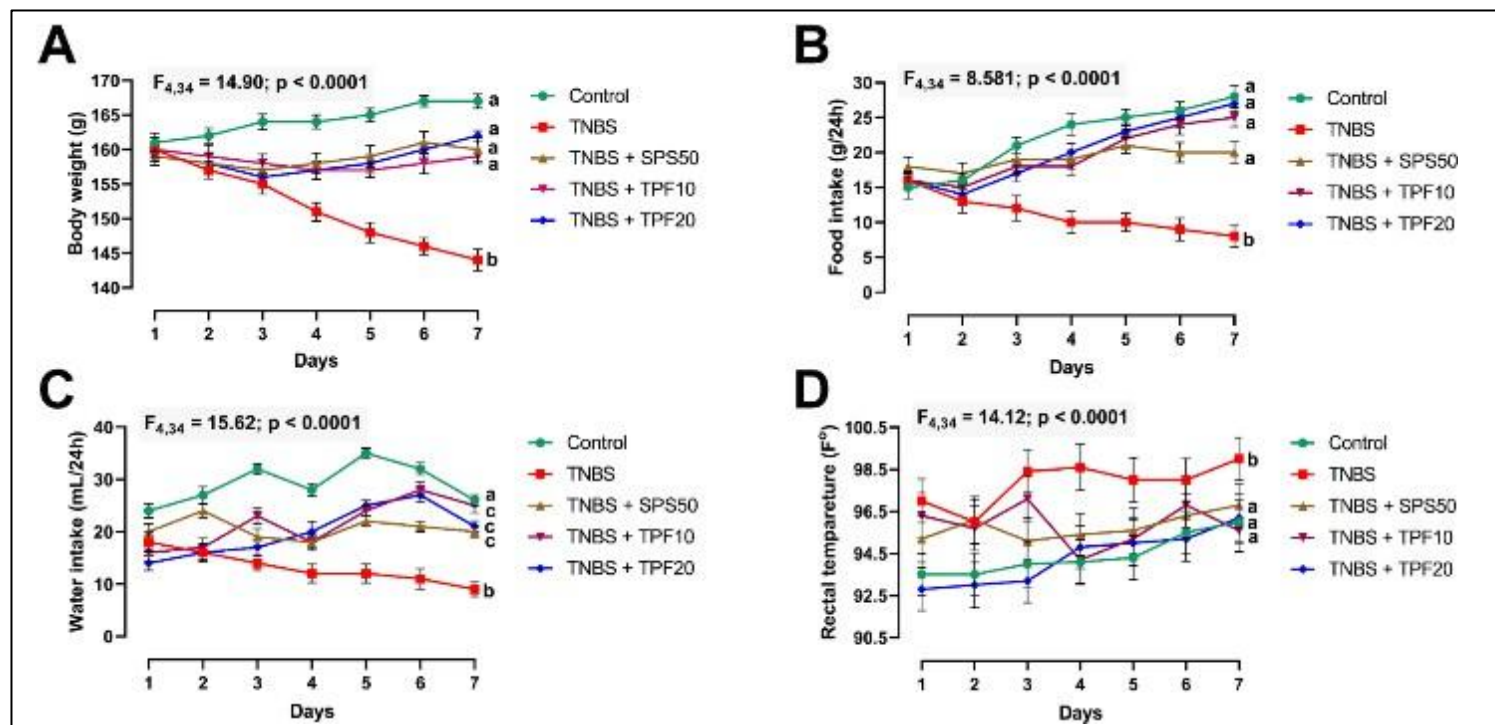


Figure 5.1 Body weight, food consumption, water consumption and rectal temperature in TNBS-induced ulcerative colitis rats across different treatment groups. Body weight (g) (A), Food intake (g) (B), Water intake (mL/24) (C) and rectal temperature (°C) (D). Data values are represented in mean $\pm$ SEM, n=5 animals/group, (ANOVA/Tukey test). Connected line with different alphabets indicate statistically significant at $p < 0.05$. **Control:** Dimethyl sulfoxide (1 %) and saline (0.9%) **TNBS:** Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); **SPS50:** Sulfasalazine in DMSO (50 mg/kg, p.o.); **TPF10:** *Trevesia palmata* flower bud methanolic extract (10 mg/kg, p.o.); **TPF20:** *Trevesia palmata* flower bud methanolic extract (20 mg/kg, p.o.).

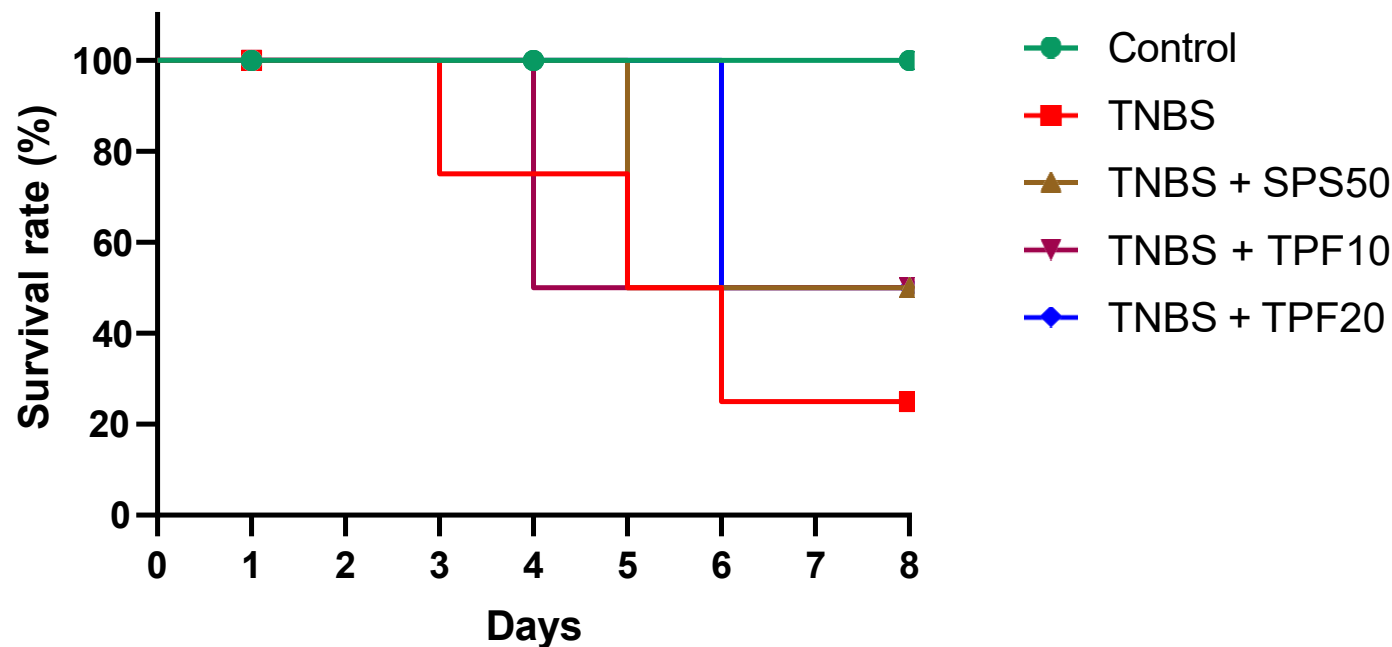


Figure 5.2 Survival Rate in TNBS-induced ulcerative colitis rats across different treatment groups. Kaplan–Meier survival curves showing the percentage survival (n=8) of control rats and TNBS-induced colitis rats treated with standard drug (SPS 50) or *Trevesia palmata* flower-bud extract (TPF10 and TPF20) over 7 days following TNBS administration. **Control:** Dimethyl sulfoxide (1 %) and saline (0.9%) **TNBS:** Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); **SPS50:** Sulfasalazine in DMSO) (50 mg/kg, p.o.); **TPF10:** *Trevesia palmata* flower bud methanolic extract (10 mg/kg, p.o.); **TPF20:** *Trevesia palmata* flower bud methanolic extract (20 mg/kg, p.o.).

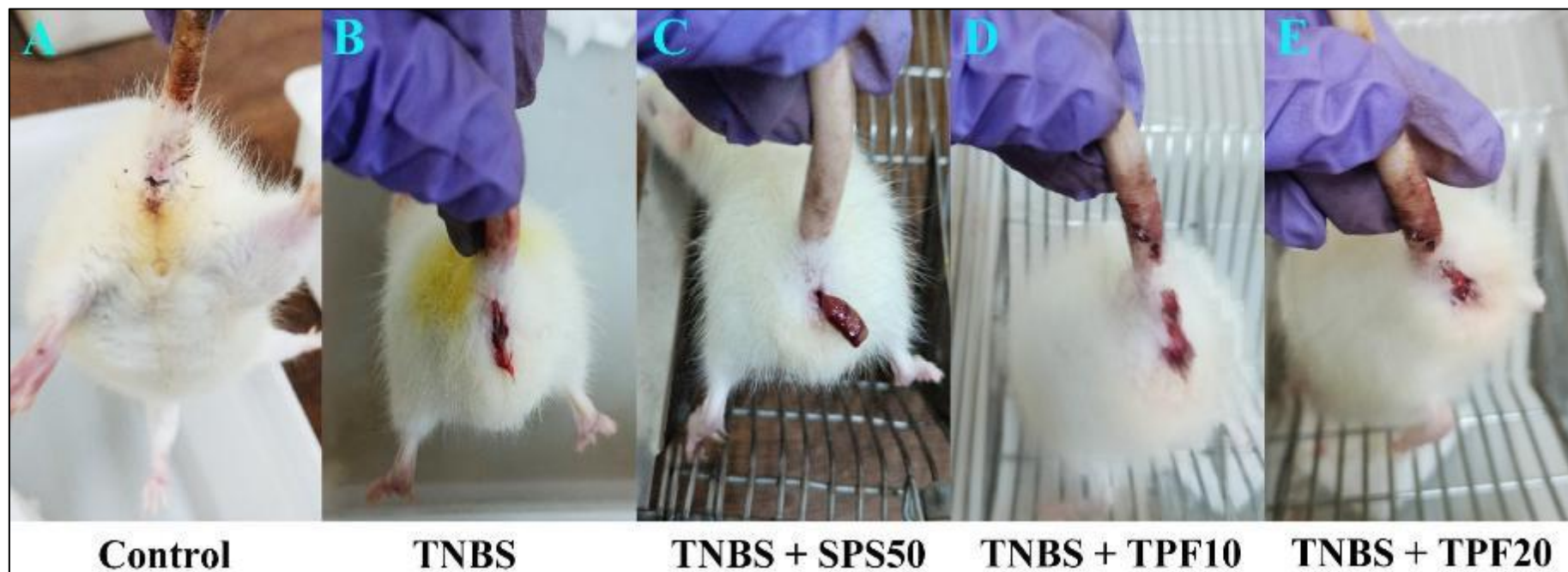


Figure 5.3 Gross appearance of anus with rectal bleeding in *Trevesia palmata* flower bud methanolic extract (TPF) treatment on body weight, food consumption, water consumption and rectal temperature in TNBS- induced rats after 7 days in different treatment groups. **Control:** Dimethyl sulfoxide (1 %) and saline (0.9%) **TNBS:** Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); **SPS50:** Sulfasalazine in DMSO) (50 mg/kg, p.o.); **TPF10:** *Trevesia palmata* flower bud methanolic extract (10 mg/kg, p.o.); **TPF20:** *Trevesia palmata* flower bud methanolic extract (20 mg/kg, p.o.).

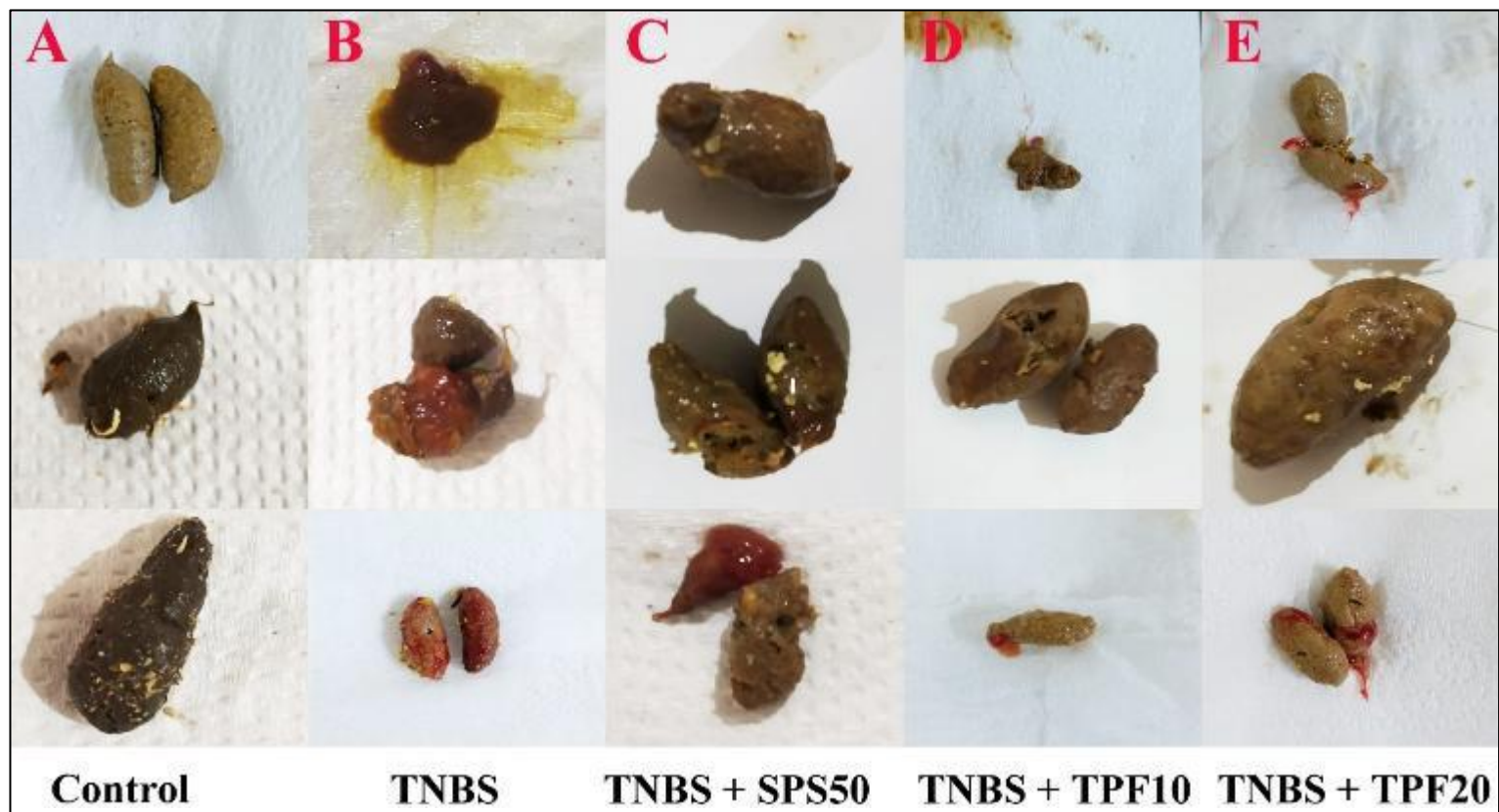


Figure 5.4 Stool consistency in different treatment groups of TNBS-induced ulcerative colitis in rats. **Control:** Dimethyl sulfoxide (1 %) and saline (0.9%) **TNBS:** Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); **SPS50:** Sulfasalazine in DMSO) (50 mg/kg, p.o.); **TPF10:** *Trevesia palmata* flower bud methanolic extract (10 mg/kg, p.o.); **TPF20:** *Trevesia palmata* flower bud methanolic extract (20 mg/kg, p.o.).

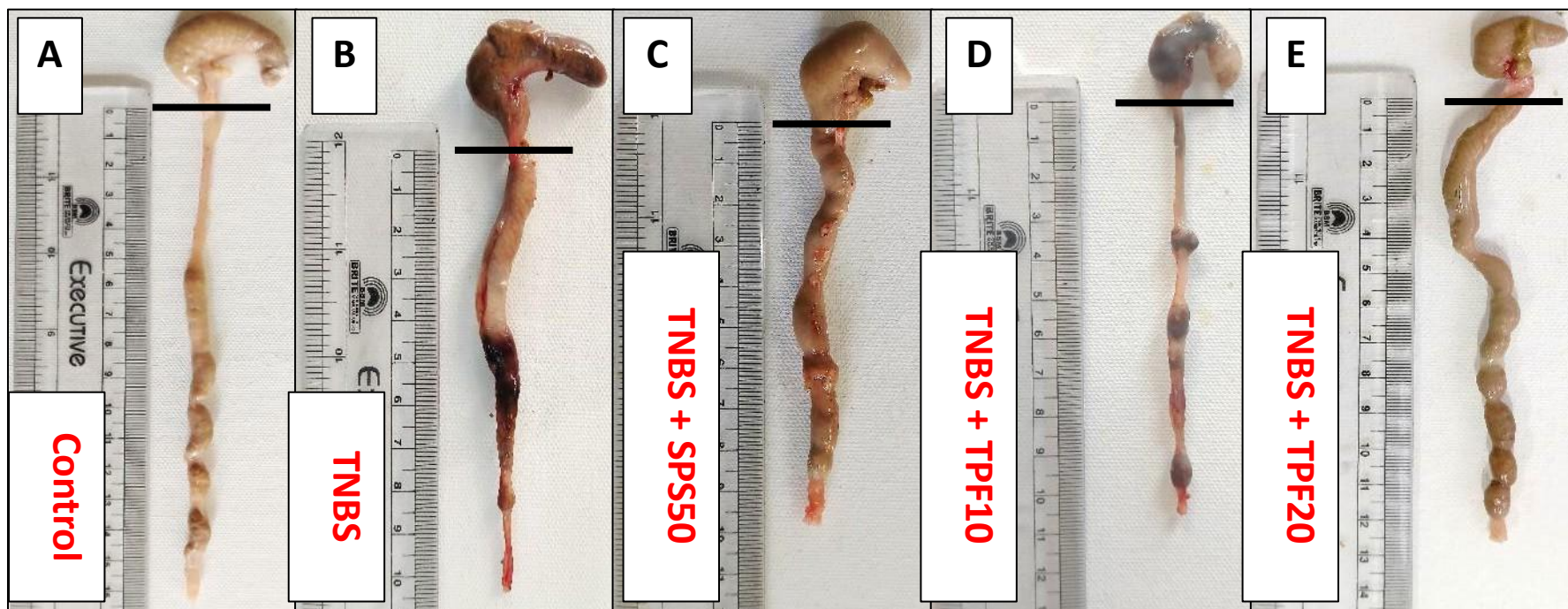


Figure 5.5 Alterations in colon architecture in TNBS-induced rats across different treatment groups. **Control:** Dimethyl sulfoxide (1 %) and saline (0.9%) **TNBS:** Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); **SPS50:** Sulfasalazine in DMSO) (50 mg/kg, p.o.); **TPF10:** *Trevesia palmata* flower bud methanolic extract (10 mg/kg, p.o.); **TPF20:** *Trevesia palmata* flower bud methanolic extract (20 mg/kg, p.o.).

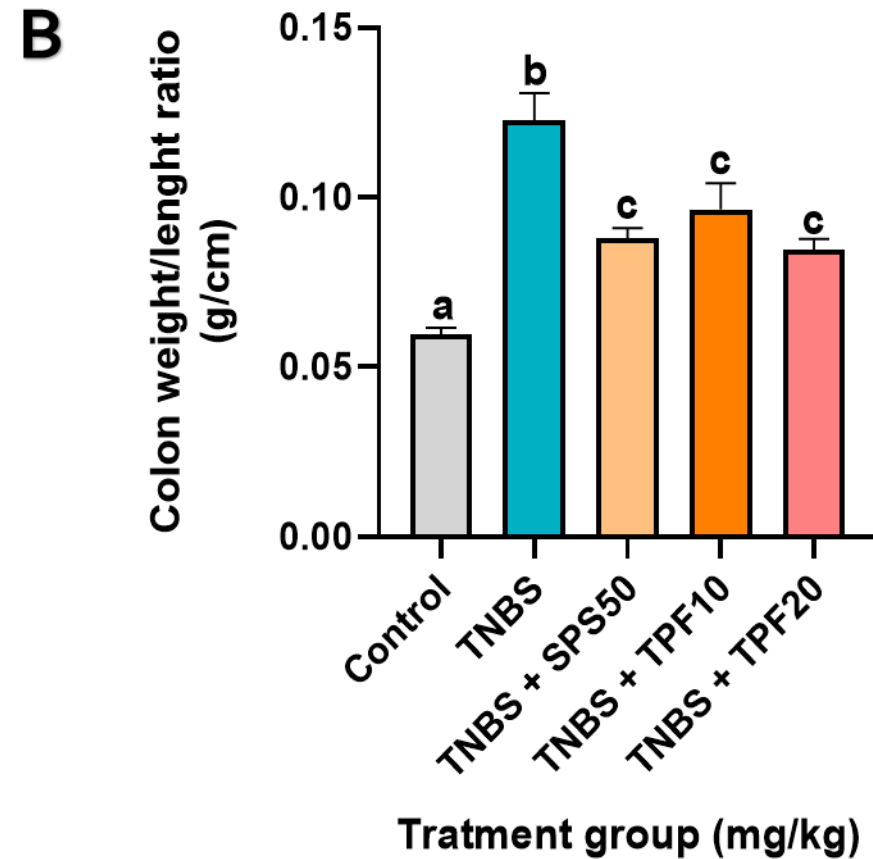
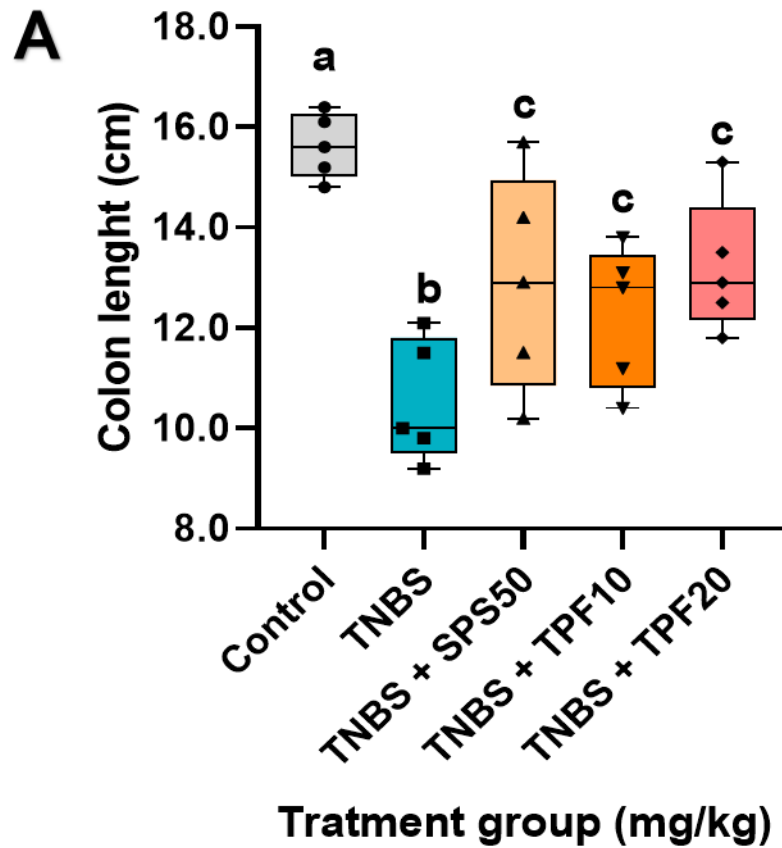


Figure 22. Effects of *Trevesia palmata* flower bud methanolic extract (TPF) on colon length (A) and colon weight/length ratio (B) in TNBS-induced colitis rats treated with various therapeutic regimens. Values are mean of five observations (n=5). Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison tests. Bar graph with different letter means statistically significant difference at $p < 0.05$ and similar letters are not significant ($p > 0.05$). Control: Dimethyl sulfoxide (1 %) and saline (0.9%) TNBS: Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); SPS50: Sulfasalazine in DMSO (50 mg/kg, p.o.); TPF10: *Trevesia palmata* flower bud methanolic extract (10 mg/kg, p.o.); TPF20: *Trevesia palmata* flower bud methanolic extract (20 mg/kg, p.o.).

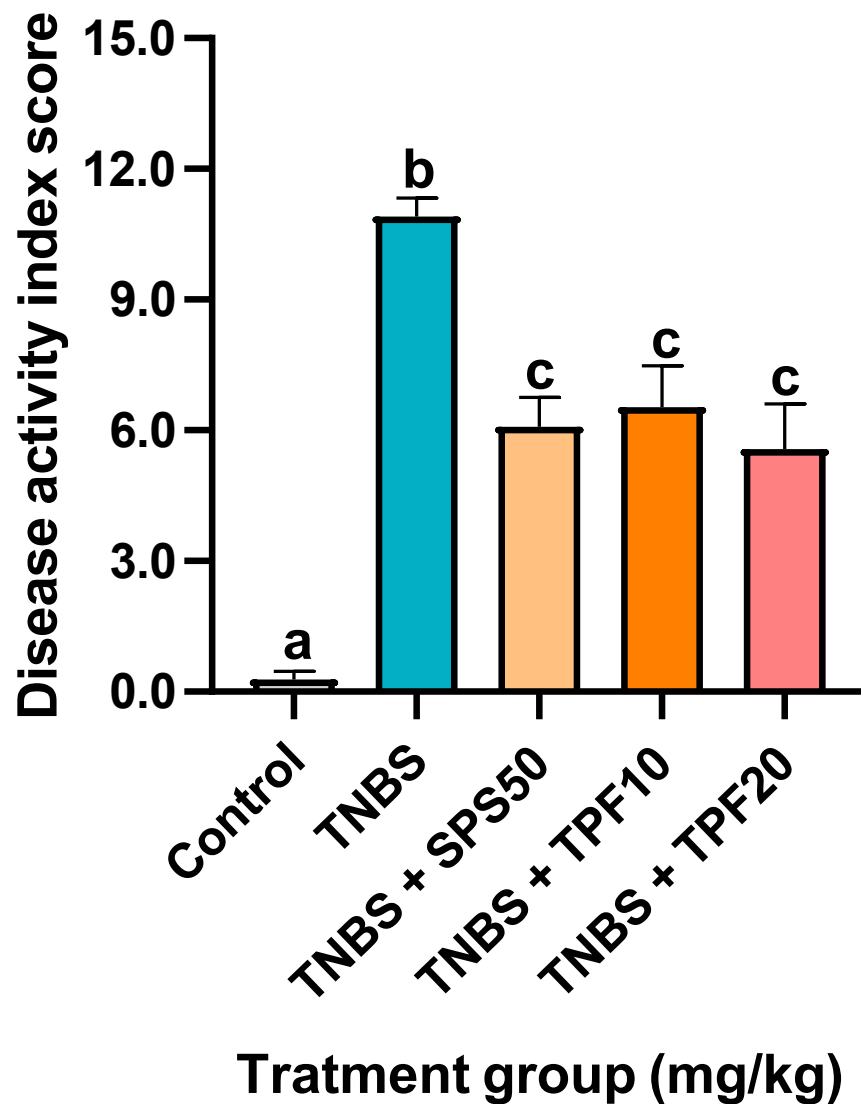


Figure 5.7 Disease Activity Index (DAI) score of TNBS-induced ulcerative colitis rats treated with different therapeutic regimens. Values are mean of five observations (n=5). Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison tests. Bar graph with different letter means statistically significant difference at $p < 0.05$ and similar letters are not significant ($p > 0.05$). **Control:** Dimethyl sulfoxide (1 %) and saline (0.9%) **TNBS:** Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); **SPS50:** Sulfasalazine in DMSO (50 mg/kg, p.o.); **TPF10:** *Trevesia palmata* flower bud methanolic extract (10 mg/kg, p.o.); **TPF20:** *Trevesia palmata* flower bud methanolic extract (20 mg/kg, p.o.)

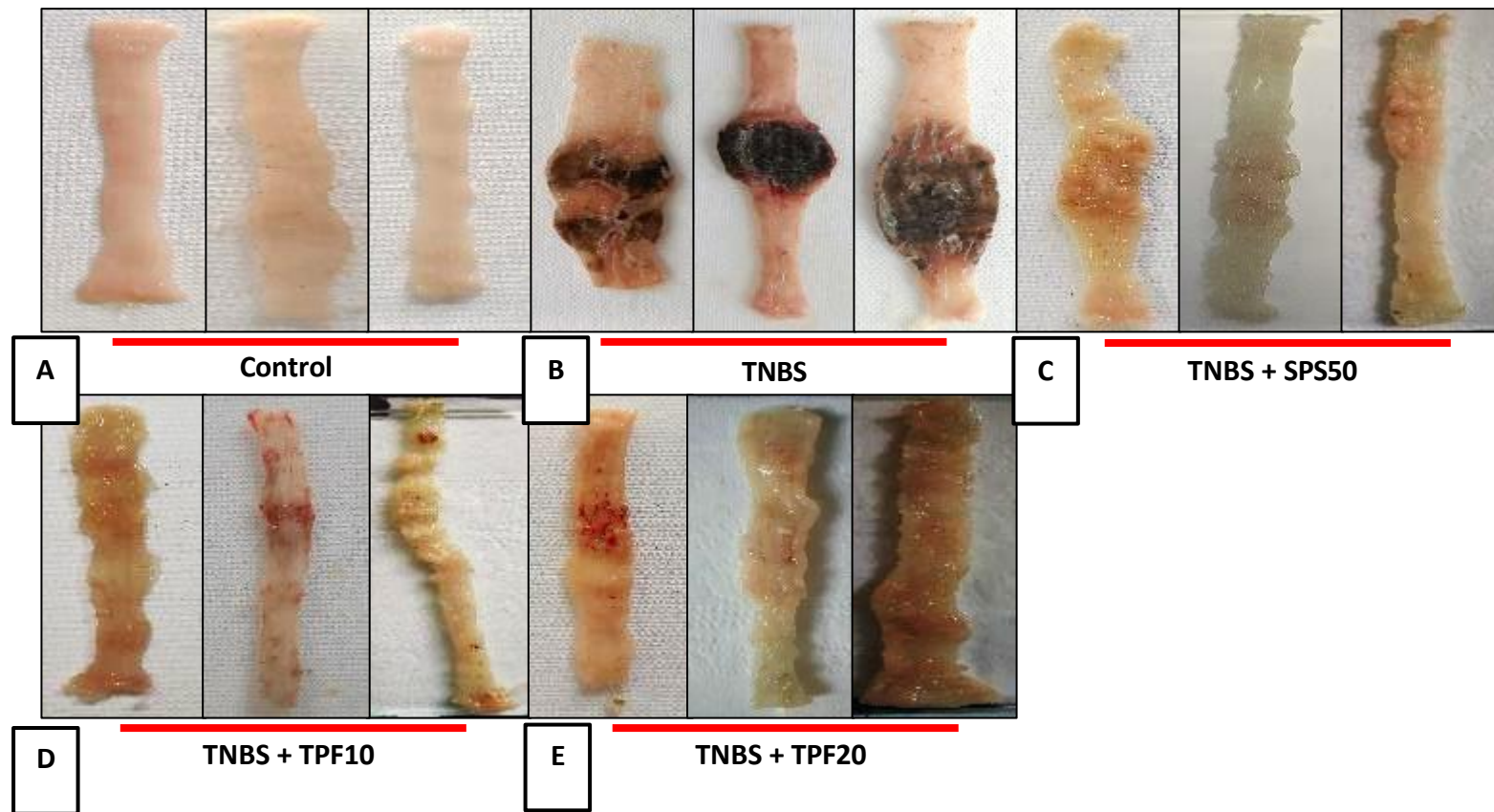


Figure 5.8 Alleviative effects of *Trevesia palmata* flower bud methanolic extract on the gross morphology of the rat colon in TNBS-induced ulcerative colitis. **Control:** Dimethyl sulfoxide (1 %) and saline (0.9%) **TNBS:** Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); **SPS50:** Sulfasalazine in DMSO) (50 mg/kg, p.o.); **TPF10:** *Trevesia palmata* flower bud methanolic extract (10 mg/kg, p.o.); **TPF20:** *Trevesia palmata* flower bud methanolic extract (20 mg/kg p. o).

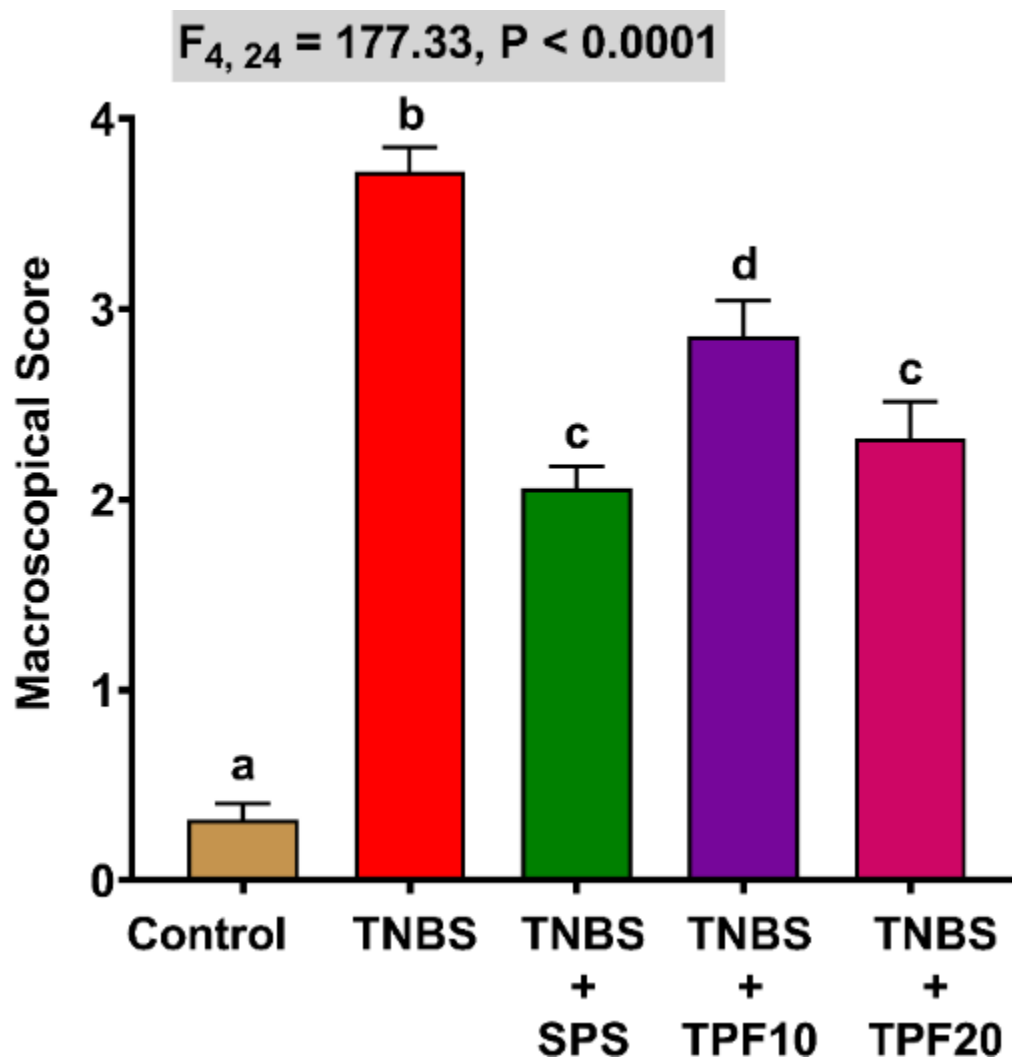


Figure 5.9 Alleviative effects of *Trevesia palmata* flower bud methanolic extract on the colon macroscopic score of rats in TNBS-induced ulcerative colitis. Values are mean of five observations (n=5). Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison tests. Bar graph with different letter means statistically significant difference at $p < 0.05$ and similar letters are not significant ($p > 0.05$). **Control:** Dimethyl sulfoxide (1 %) and saline (0.9%) **TNBS:** Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); **SPS50:** Sulfasalazine in DMSO) (50 mg/kg, p.o.); **TPF10:** *Trevesia palmata* flower bud methanolic extract (10 mg/kg, p.o.); **TPF20:** *Trevesia palmata* flower bud methanolic extract (20 mg/kg p. o).

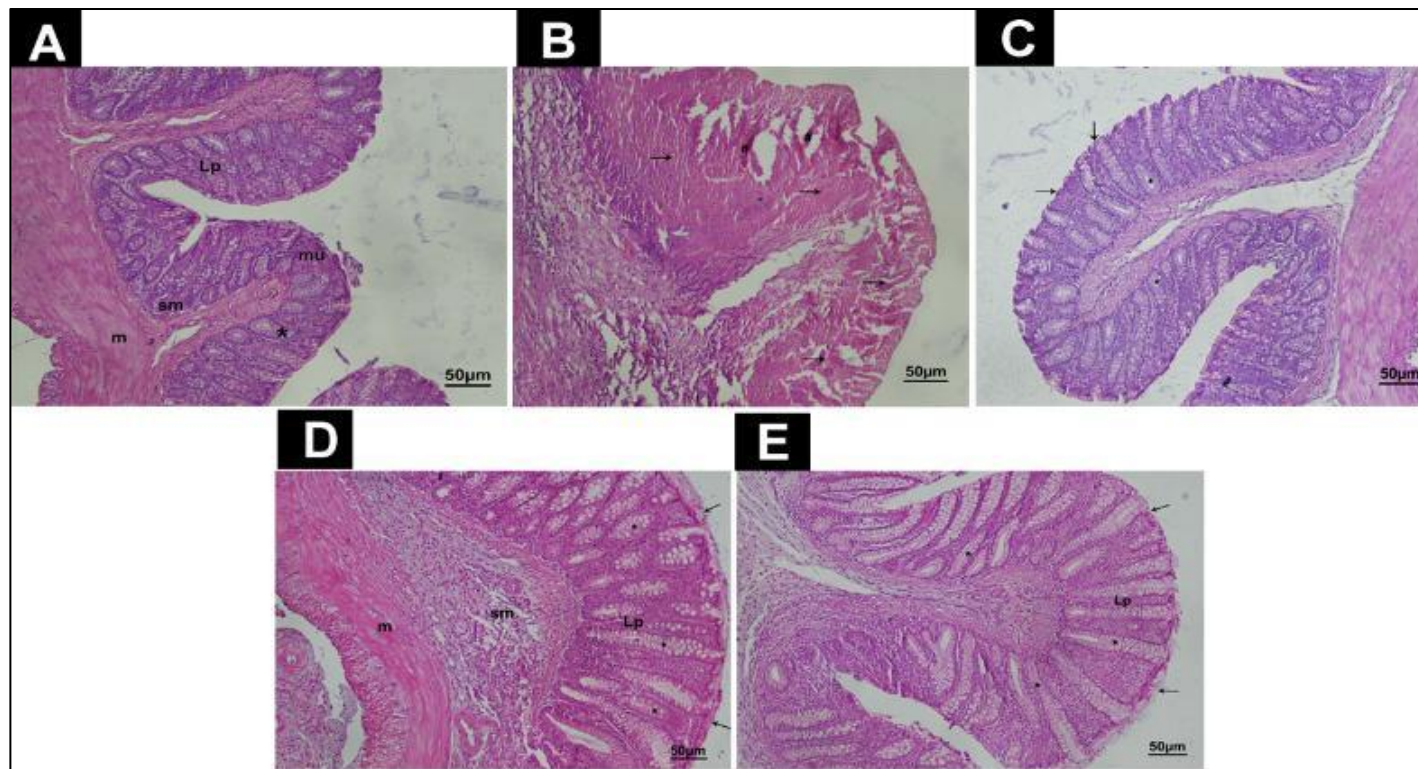


Figure 5.10 Histopathological alterations in colon sections of TNBS-induced ulcerative colitis rats treated with different therapeutic regimens. (A) **Control group**: Normal arrangement of mucosa (mu), submucosa (sm), muscularis (m), numerous goblet cells (*) and thin lamina propri (Lp). (B) **TNBS group**: Abnormal crypts (arrows), diffusion inflammatory cell infiltration (#) and severe damage of tissue architecture. (C) **TNBS with sulfasalazine (50 mg/kg) group**: preserved crypts (arrows), and goblet cells (*). (D, E) **TNBS + TPF 10 and TPF 20 group**: Protection of the colon from structural damage by *Trevesia palmata* flower bud methanolic extract (TPF10 and TPF20, 10 and 20 mg/kg). H&E: Hematoxylin and eosin staining, scale bar = 50 μm for 10X magnification

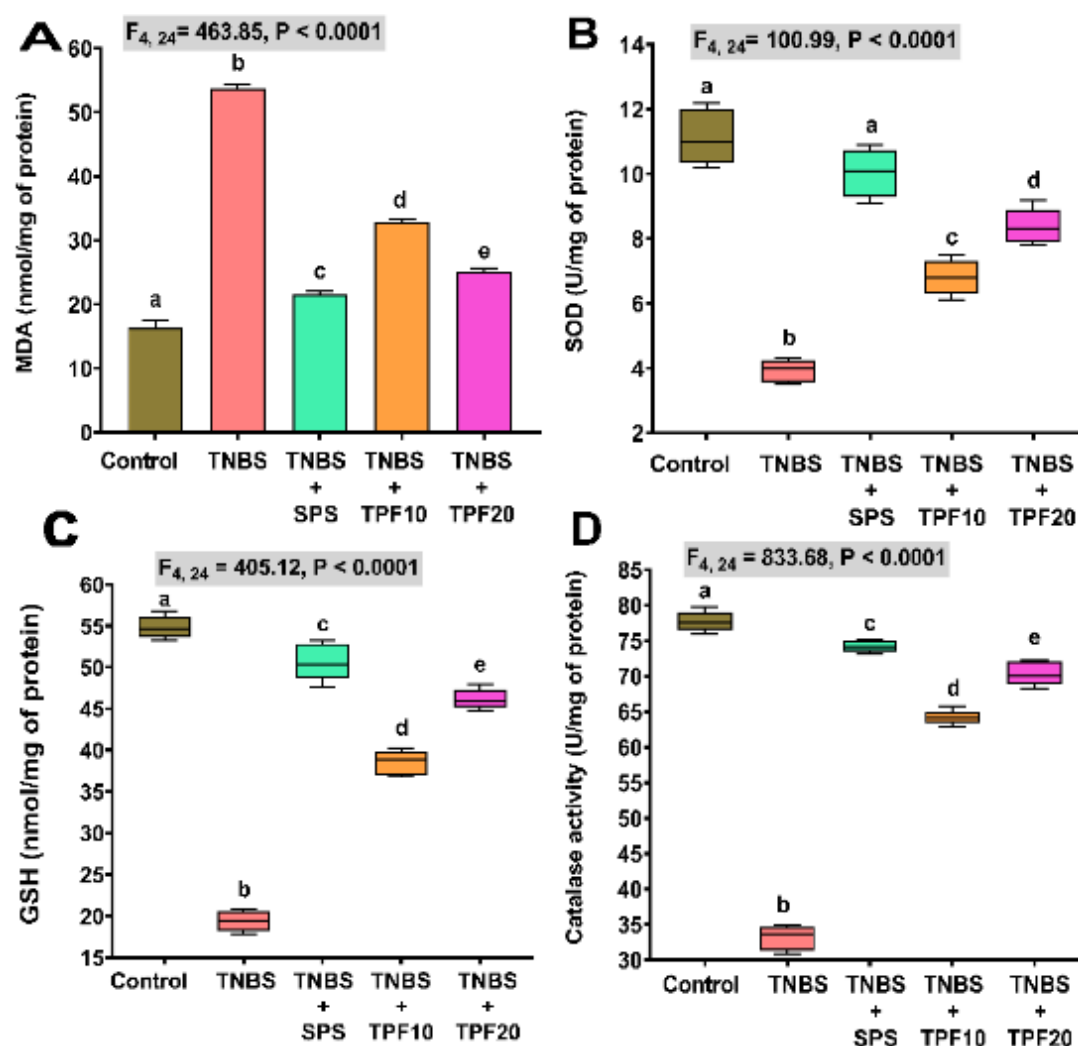


Figure 5.11 Biochemical alterations in oxidative stress markers in colon tissue of TNBS-induced ulcerative colitis rats treated with sulfasalazine and *Trevesia palmata* flower bud extract. Values are mean of five observations (n=5). Statistical comparison was performed using one-way ANOVA followed by Tukey's multiplecomparison tests. Bar graph with different letter means statistically significant difference at $p < 0.05$ and similar letters are not significant ($p > 0.05$). **Control:** Dimethyl sulfoxide (1 %) and saline (0.9%) **TNBS:** Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); **SPS50:** Sulfasalazine in DMSO) (50 mg/kg, p.o.); **TPF10:** *Trevesia palmata* flower bud methanolic extract (10 mg/kg, p.o.); **TPF20:** *Trevesia palmata* flower bud methanolic extract (20 mg/kg, p.o.).

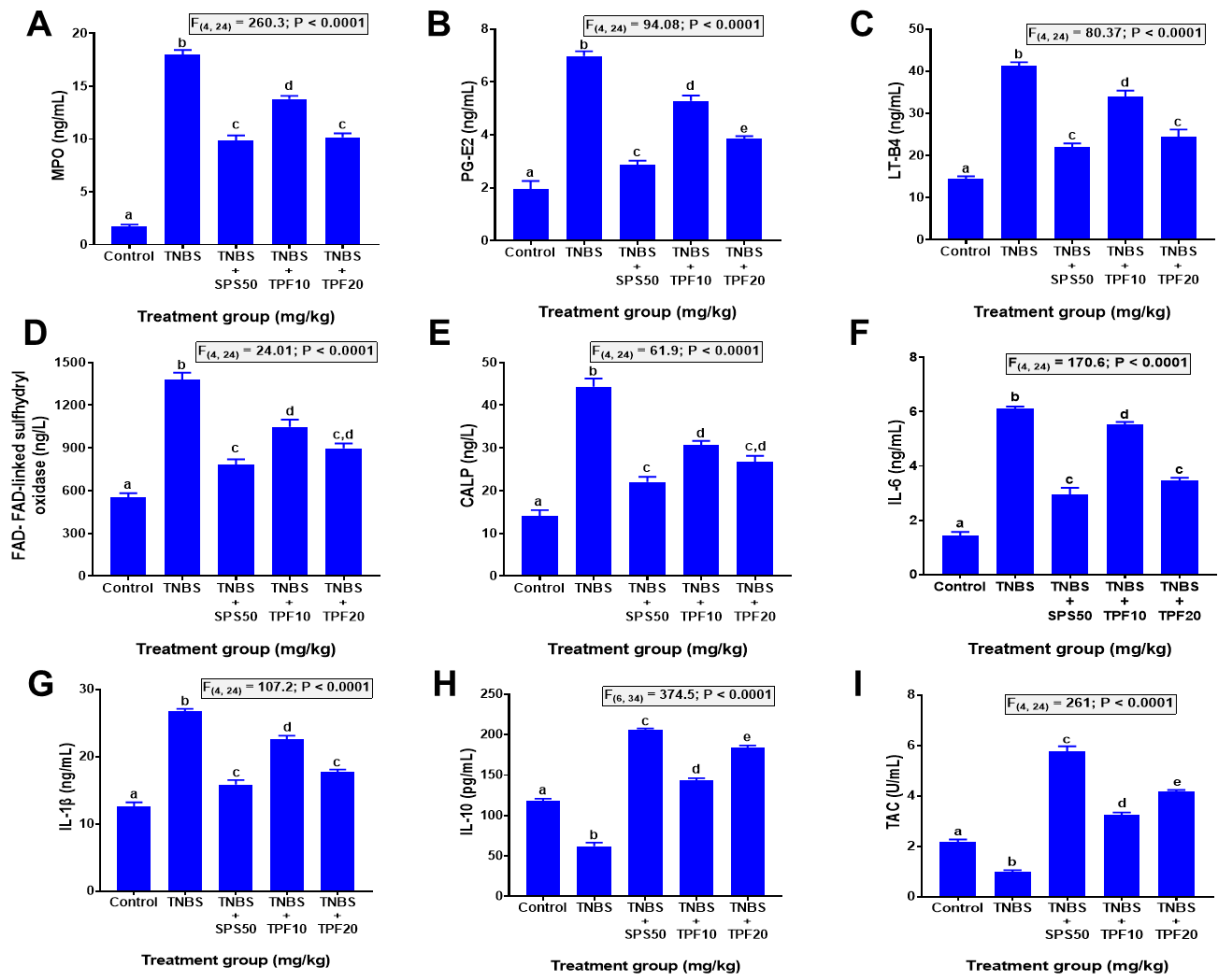


Figure 5.12: The inflammatory cytokine and oxidative stress markers in different treatment groups were assessed by measuring-(**A**) Myeloperoxidase (MOP) activity, (**B**) Prostaglandin E2 (PGE2), (**C**) (**D**) Flavin adenine dinucleotide-linked sulphhydryl oxidase (FAD), (**E**) Calprotectin (CALP), (**F**) Interleukin-6 (IL-6), (**G**) Interleukin-1 Beta (IL-1 β), (**H**) Interleukin-10 (IL-10)(**I**) total antioxidant capacity (TAC) levels. Data are presented as mean $\pm$ SEM (n=5), with statistical significance determined by ANOVA and post hoc tests ($p < 0.05$), where differences between groups are indicated by different letters

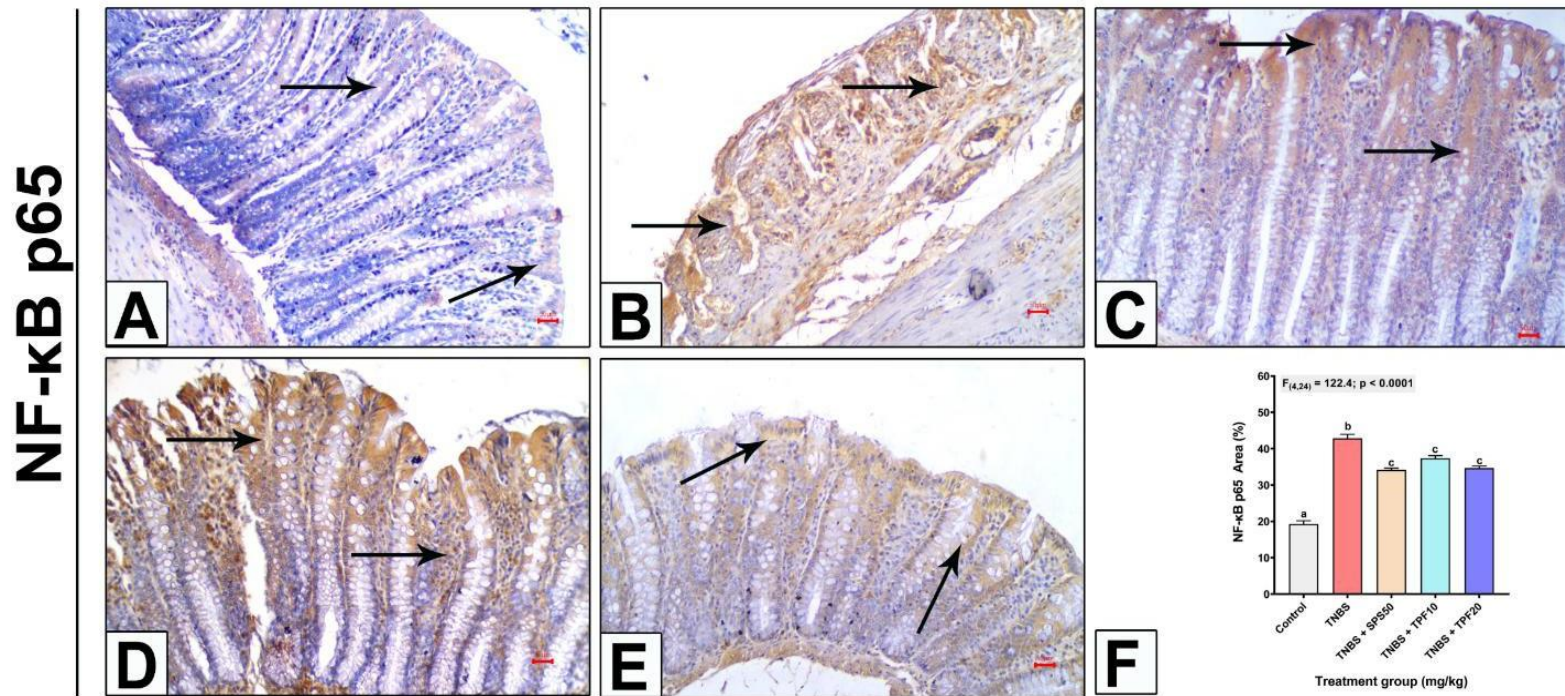


Figure 5.13. Modulation of TNBS-induced alterations in colonic **NF-κB p65** (Nuclear Factor kappa-light-chain-enhancer of activated B cells) expression by *T. palmata* flower bud extract (TPF). Photomicrograph of the immunohistochemical studied protein expression of **NF-κB p65** in the colons of the (A) control, (B) Trinitrobenzene sulfonic acid (TNBS), (C) TNBS + Sulfasalazine 50 mg/kg (TNBS +SPS50), TNBS + methanolic extract of (D and E) *T. palmata* flower buds (10, 20) mg/kg (TNBS +TPF10; TNBS + TPF20). The bar graph (F) represents the expression area of each group. Values are means of five observations (n=5). Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison tests. Bar graph with different letter means statistically significant difference at $p < 0.05$ and similar letters are not significant ($p > 0.05$). **Control:** Dimethyl sulfoxide (1 %) and saline (0.9%) **TNBS:** Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); **SPS50:** Sulfasalazine in DMSO (50 mg/kg, p.o.); **TPF10:** *Trevesia palmata* flower bud methanolic extract (10 mg/kg, p.o.); **TPF20:** *Trevesia palmata* flower bud methanolic extract (20 mg/kg, p.o.). Scale bar = 50 μ m for 20X magnification

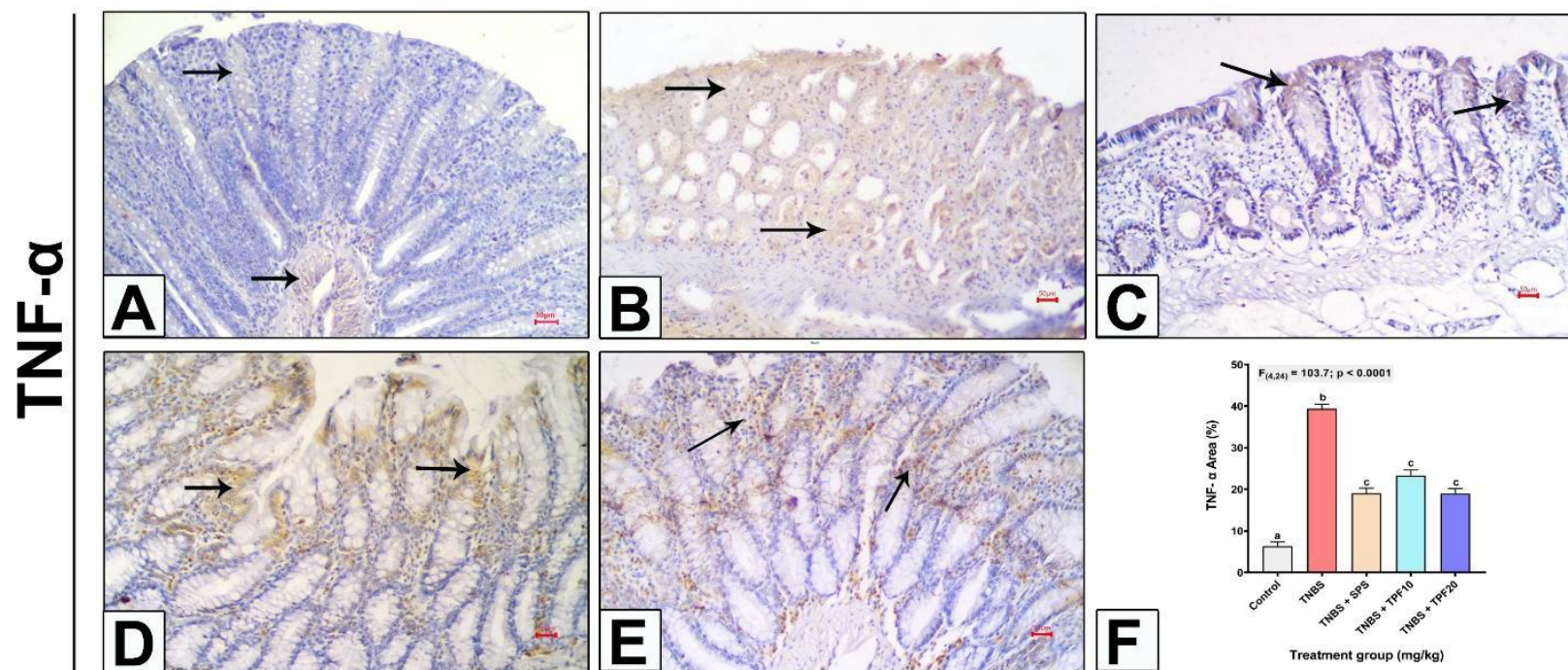


Figure 5.14 Modulation of colonic tumor necrosis factor alpha (TNF- α) protein expression by *Trevesia palmata* flower bud extract (TPF). Photomicrographs showing the immunohistochemical protein expression of TNF- α in the colons of (A) control, (B) Trinitrobenzene sulfonic acid (TNBS), (C) TNBS + Sulfasalazine 50 mg/kg (TNBS + SPS50), and (D, E) TNBS + methanolic extract of *T. palmata* flower buds (10, 20 mg/kg) (TNBS + TPF10; TNBS + TPF20). The bar graph (F) represents the expression area of each group. Values are means of five observations (n=5). Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison tests. Bar graph with different letter means statistically significant difference at $p < 0.05$, and similar letters are not significant ($p > 0.05$). Scale bar = 50 μ m for 20X magnification

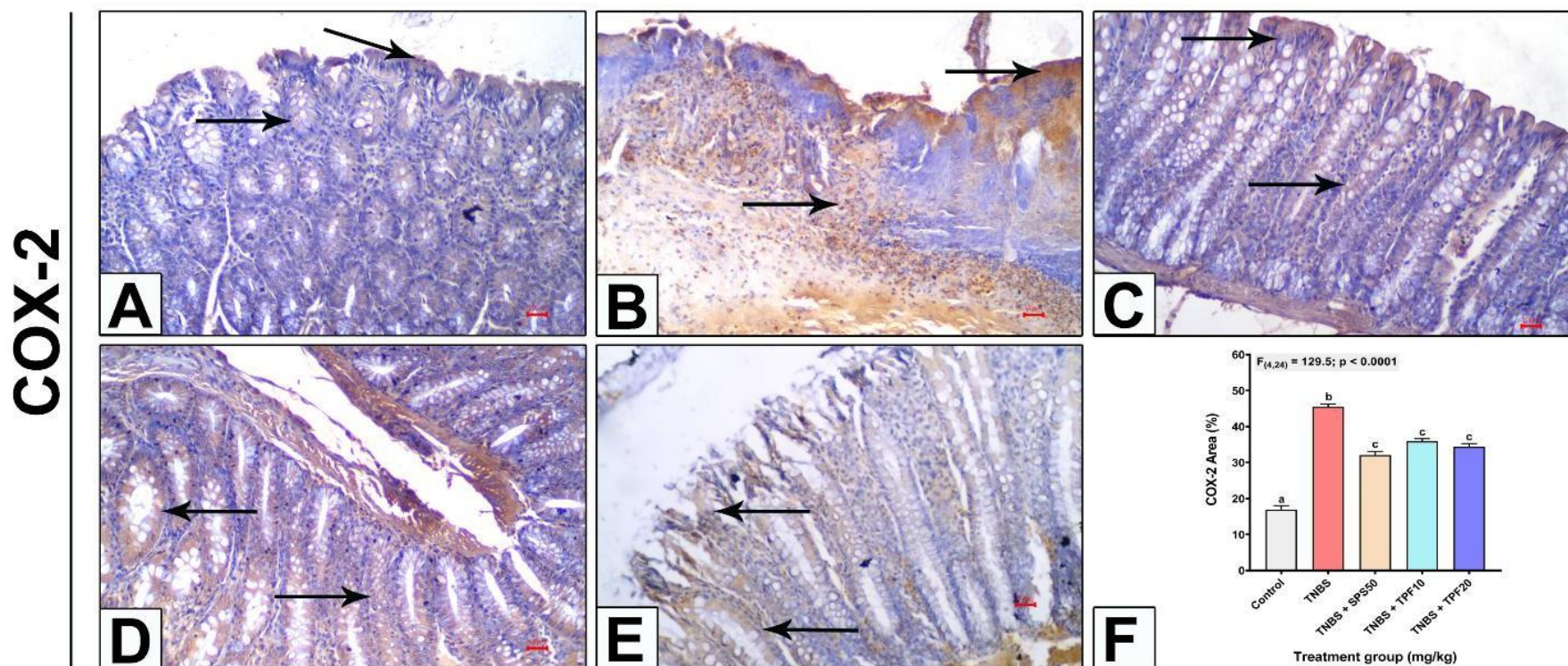


Figure 5.15. Effect of *Trevesia palmata* flower bud methanolic extract (TPF) on TNBS-induced alterations in colonic protein expression of Cyclooxygenase-2 (**COX-2**). Photomicrographs depicting the immunohistochemical expression of **COX-2** in the colons of different groups: (A) Control, (B) Trinitrobenzene sulfonic acid (TNBS), (C) TNBS + Sulfasalazine (50 mg/kg), (D) TNBS + TPF 10 mg/kg, and (E) TNBS + TPF 20 mg/kg. The bar graph (F) represents the expression area of each group. Values are means of five observations (n=5). Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison tests. Bar graph with different letter means statistically significant difference at $p < 0.05$, and similar letters are not significant ($p > 0.05$). **Control:** Dimethyl sulfoxide (1 %) and saline (0.9%) **TNBS:** Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); **SPS50:** Sulfasalazine in DMSO) (50 mg/kg, p.o.); **TP10:** *Trevesia palmata* flower bud methanolic extract (10 mg/kg, p.o.); **TP20:** *Trevesia palmata* flower bud methanolic extract (20 mg/kg, p.o.). Scale bar = 50 μ m for 20X magnification

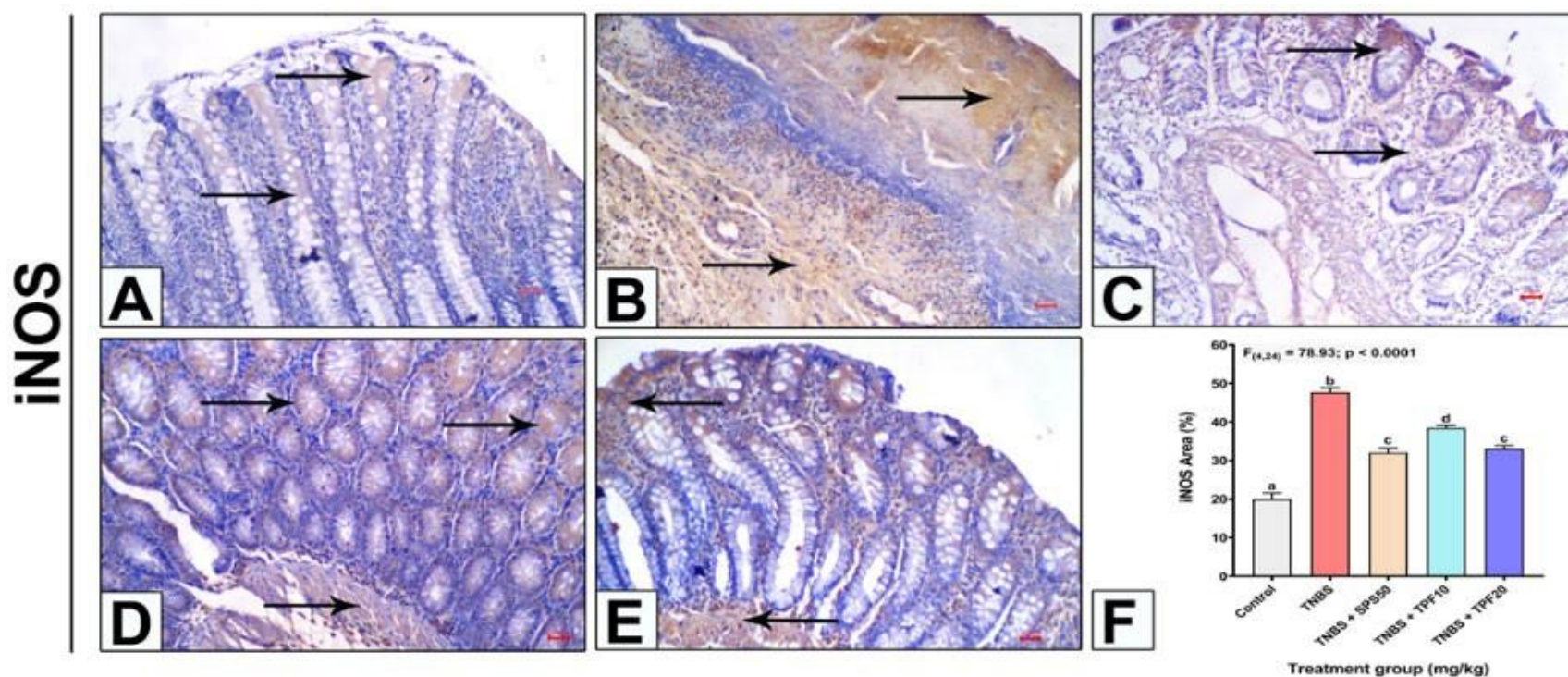


Figure 5.16. Treatment with *Trevesia palmata* flower bud methanolic extract (TPF) attenuated the alterations in colonic iNOS protein expression induced by TNBS. Photomicrograph of the immunohistochemical studied protein expression of iNOS in the colons of the (A) control, (B) Trinitrobenzene sulfonic acid (TNBS), (C) TNBS + Sulfasalazine 50 mg/kg (TNBS +SPS50), TNBS + methanolic extract of (D and E) *T. palmata* flower buds (10, 20) mg/kg (TNBS +TPF10; TNBS + TPF20). The bar graph (F) represents the expression area of each group. Values are means of five observations (n=5). Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison tests. Bar graph with different letter means statistically significant difference at $p < 0.05$ and similar letters are not significant ($p > 0.05$). **Control:** Dimethyl sulfoxide (1 %) and saline (0.9%) **TNBS:** Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); **SPS50:** Sulfasalazine in DMSO) (50 mg/kg, p.o.); **TP10:** *Trevesia palmata* flower bud methanolic extract (10 mg/kg, p.o.); **TP20:** *Trevesia palmata* flower bud methanolic extract (20 mg/kg, p.o.). Scale bar = 50 μ m for 20X magnification

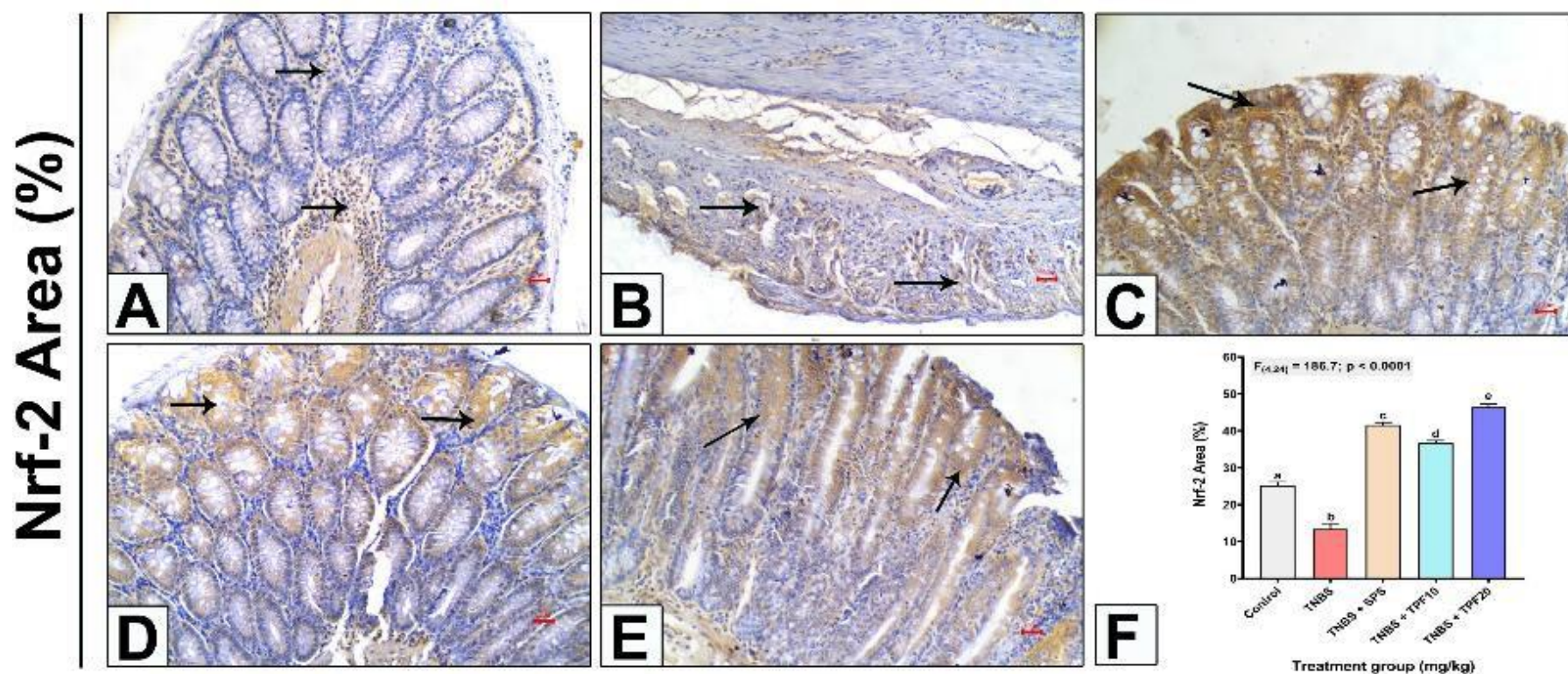


Figure 5.17 Alterations in colonic protein expression of nuclear factor erythroid 2-related factor 2 (**Nrf-2**) induced by TNBS were attenuated by treatment with TPF. Photomicrograph of the immunohistochemical studied protein expression of **Nrf-2** in the colons of the (A) control, (B) Trinitrobenzene sulfonic acid (TNBS), (C) TNBS + Sulfasalazine 50 mg/kg (TNBS +SPS50), TNBS + methanolic extract of (D and E) *T. palmata* flower buds (10, 20) mg/kg (TNBS +TPF10; TNBS + TPF20). The bar graph (F) represents the expression area of each group. Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison tests. Bar graph with different letter means statistically significant difference at $p < 0.05$ and similar letters are not significant ($p > 0.05$). **Control**: Dimethyl sulfoxide (1 %) and saline (0.9%) **TNBS**: Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); **SPS50**: Sulfasalazine in DMSO (50 mg/kg, p.o.); **TP10**: *Trevesia palmata* flower bud methanolic extract (10 mg/kg, p.o.); **TP20**: *Trevesia palmata* flower bud methanolic extract (20 mg/kg, p.o.). Scale bar = 50 μ m for 20X magnification

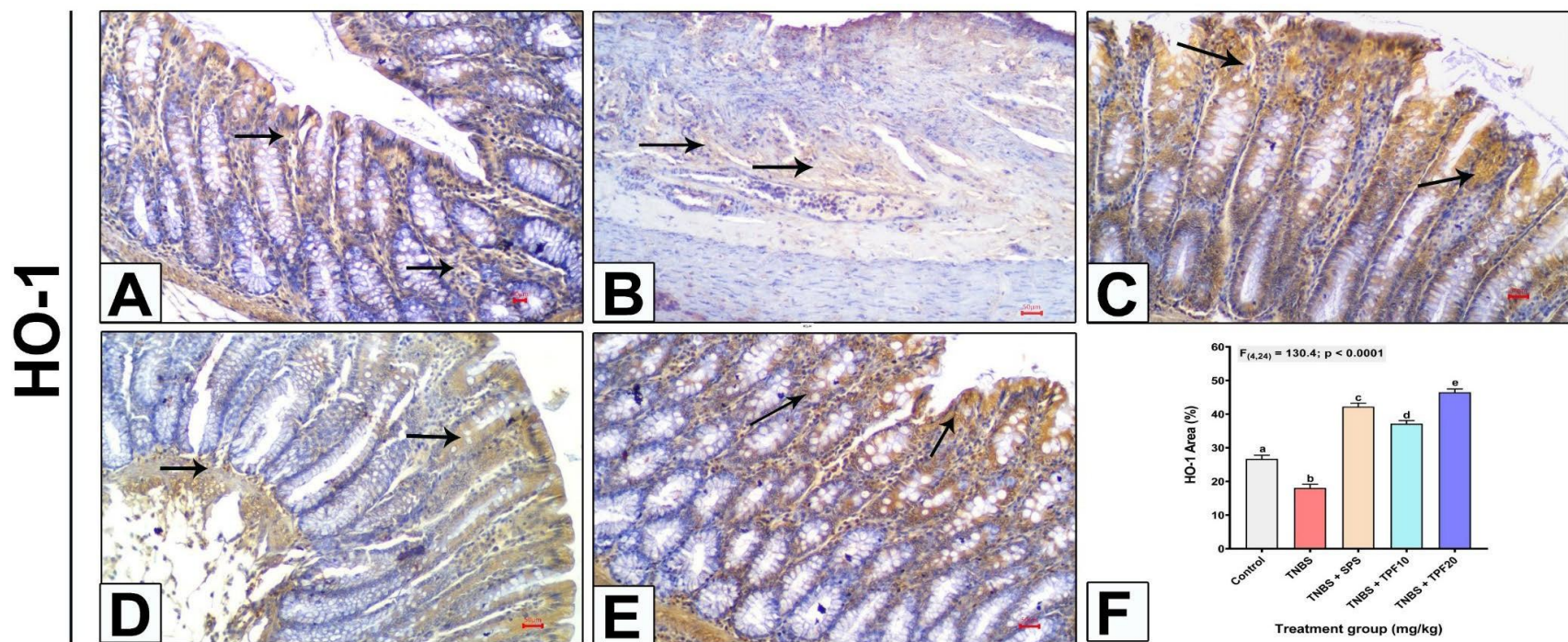


Figure 5.18 Alterations in colonic protein expression of heme oxygenase-1 (**HO-1**) induced by TNBS were attenuated by treatment with TPF. Photomicrograph of the immunohistochemical studied protein expression of **HO-1** in the colons of the (A) control, (B) Trinitrobenzene sulfonic acid (TNBS), (C) TNBS + Sulfasalazine 50 mg/kg (TNBS +SPS50), TNBS + methanolic extract of (D and E) *T. palmata* flower buds (10, 20) mg/kg (TNBS +TPF10; TNBS + TPF20). The bar graph (F) representing the expression area of each group. Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison tests. Bar graph with different letter means statistically significant difference at $p < 0.05$ and similar letters are not significant ($p > 0.05$). **Control**: Dimethyl sulfoxide (1 %) and saline (0.9%) **TNBS**: Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); **SPS50**: Sulfasalazine in DMSO) (50 mg/kg, p.o.); **TP10**: *Trevesia palmata* flower bud methanolic extract (10 mg/kg, p.o.); **TP20**: *Trevesia palmata* flower bud methanolic extract (20 mg/kg, p.o.). Scale bar = 50 μ m for 20X magnification

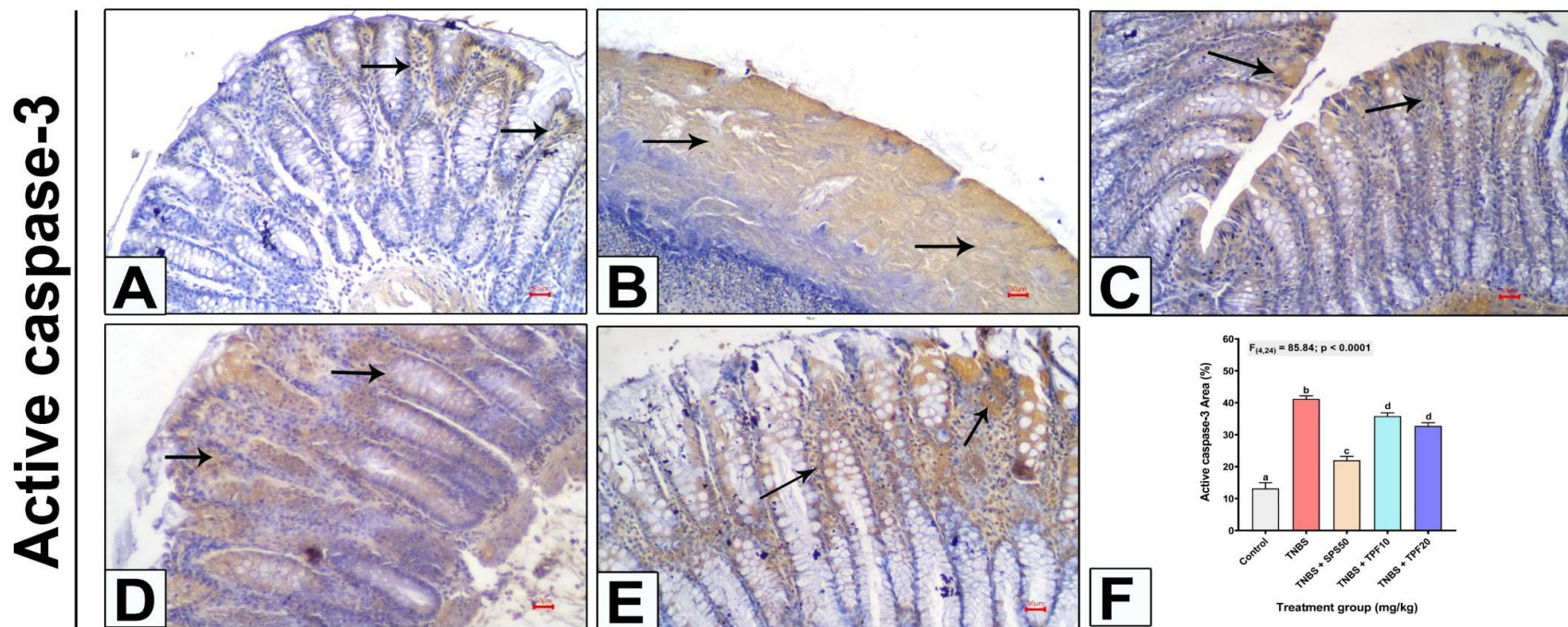


Figure 5.19 Alterations in colonic protein expression of **active caspase-3** induced by TNBS were attenuated by treatment with TPF. Photomicrograph of the immunohistochemical studied protein expression of **active caspase-3** in the colons of the (A) control, (B) Trinitrobenzene sulfonic acid (TNBS), (C) TNBS + Sulfasalazine 50 mg/kg (TNBS +SPS50), TNBS + methanolic extract of (D and E) *T. palmata* flower buds (10, 20) mg/kg (TNBS +TPF10; TNBS + TPF20). The bar graph (F) representing the expression area of each group. Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison tests. Bar graph with different letter means statistically significant difference at $p < 0.05$ and similar letters are not significant ($p > 0.05$). **Control:** Dimethyl sulfoxide (1 %) and saline (0.9%) **TNBS:** Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); **SPS50:** Sulfasalazine in DMSO) (50 mg/kg, p.o.); **TP10:** *Trevesia palmata* flower bud methanolic extract (10 mg/kg, p.o.); **TP20:** *Trevesia palmata* flower bud methanolic extract (20 mg/kg, p.o.). Scale bar = 50 μ m for 20X magnification.

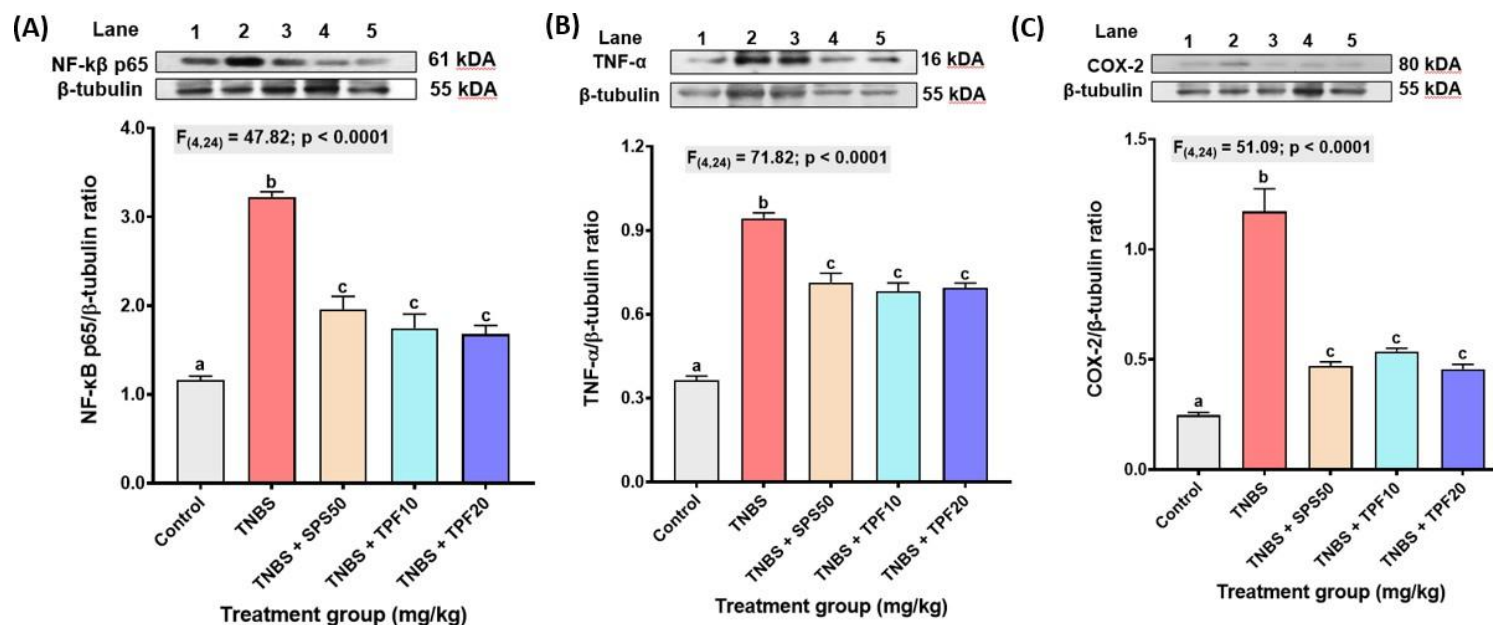


Figure 5.20 Effects of *Trevesia palmata* flower bud methanolic extract on the protein expression of inflammatory signaling markers, including NF-κB p65 (Nuclear Factor kappa-light-chain-enhancer of activated B cells, A), TNF-α (Tumor Necrosis Factor-alpha, B), and COX-2 (Cyclooxygenase-2, C) in colon tissue of TNBS-induced ulcerative colitis. The markers evaluated were all normalised to β-tubulin. Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison tests. Bar graph with different letter means statistically significant difference at $p < 0.05$, and similar letters are not significant ($p > 0.05$). **Control:** Dimethyl sulfoxide (1 %) and saline (0.9%) **TNBS:** Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); **SPS50:** Sulfasalazine in DMSO (50 mg/kg, p.o.); **TP10:** *Trevesia palmata* flower bud methanolic extract (10 mg/kg, p.o.); **TP20:** *Trevesia palmata* flower bud methanolic extract (20 mg/kg, p.o.).

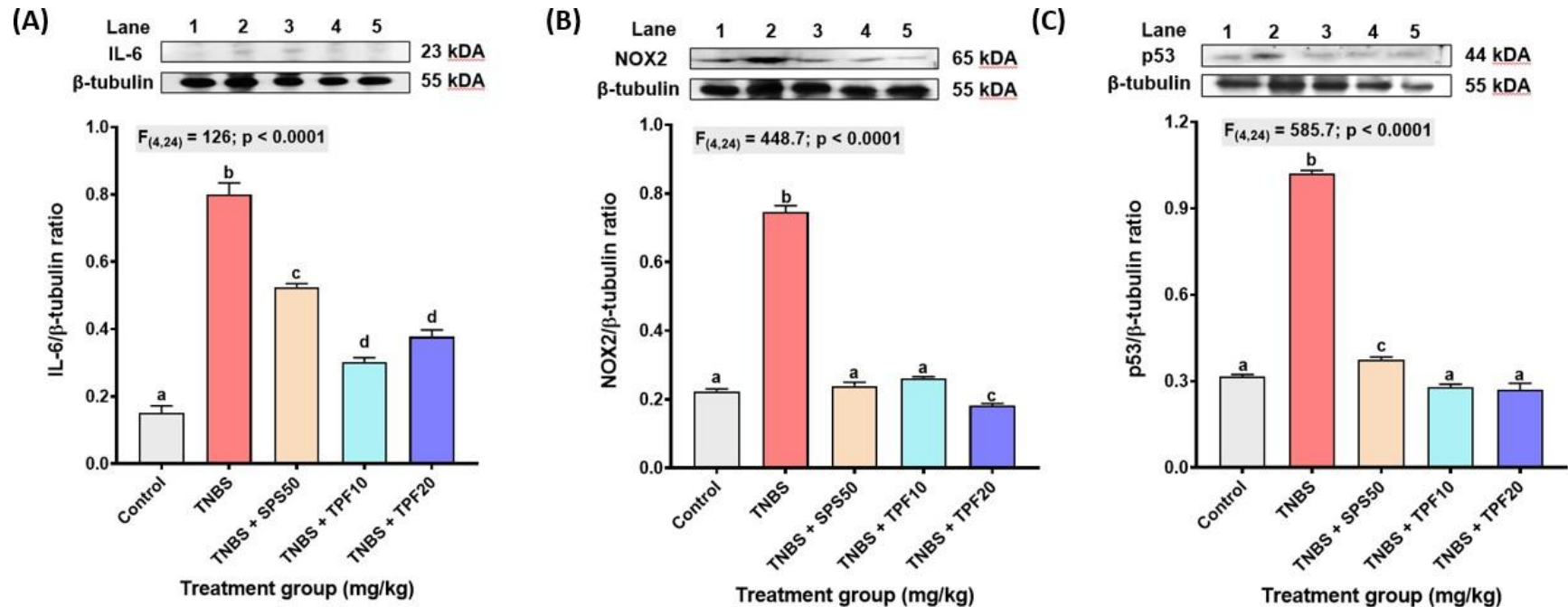


Figure 5.21 Effects of *Trevesia palmata* flower bud methanolic extract on the expression pattern of inflammation, oxidative stress, and cell signalling markers in colon tissue of TNBS-induced ulcerative colitis. The markers evaluated included (A) **Interlukin-6**, (B) **NOX-2** (NADPH Oxidase 2), and (C) **p53** (Tumor Protein 53) all normalised to β-tubulin. Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison tests. Bar graph with different letter means statistically significant difference at $p < 0.05$, and similar letters are not significant ($p > 0.05$). **Control**: Dimethyl sulfoxide (1 %) and saline (0.9%) **TNBS**: Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); **SPS50**: Sulfasalazine in DMSO (50 mg/kg, p.o.); **TP10**: *Trevesia palmata* flower bud methanolic extract (10 mg/kg, p.o.); **TP20**: *Trevesia palmata* flower bud methanolic extract (20 mg/kg, p.o.).

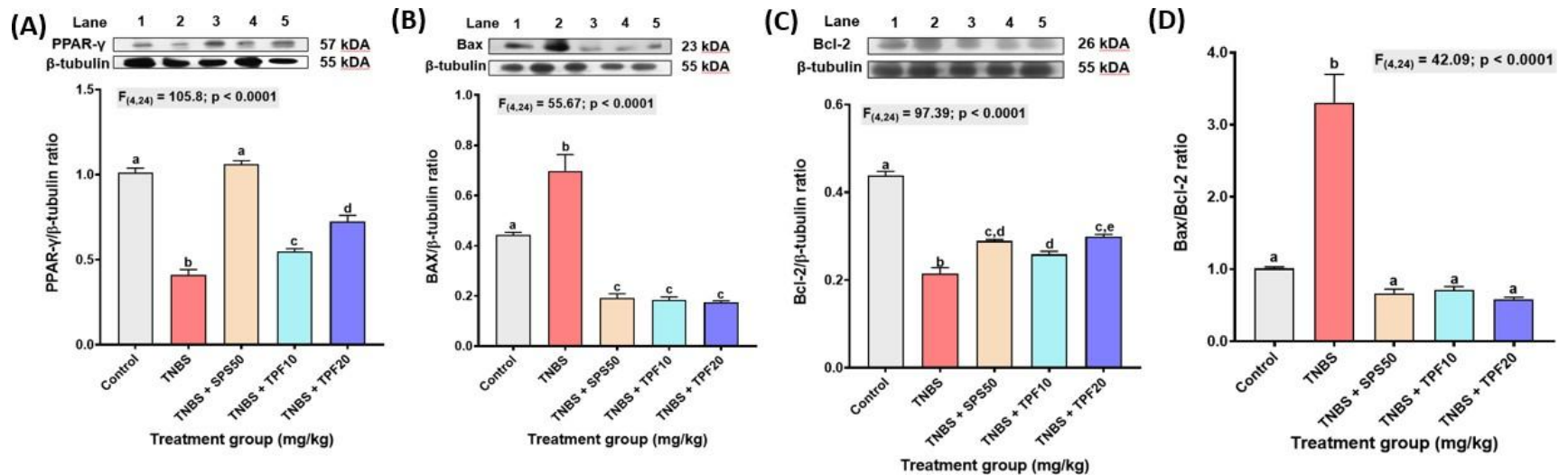


Figure 5.22 Effects of *Trevesia palmata* flower bud methanolic extract on the expression pattern of inflammation, and apoptotic markers in colon tissue of TNBS-induced ulcerative colitis. . The markers evaluated included (A) **PPAR-γ** (Peroxisome Proliferator-Activated Receptor Gamma), (B) **BAX** (Bcl-2-associated X protein), and (C) **Bcl-2** (B-cell lymphoma 2) all normalised to β-tubulin. Statistical comparison was performed using one-way ANOVA followed by Tukey's multiple comparison tests. Bar graph with different letter means statistically significant difference at $p < 0.05$, and similar letters are not significant ($p > 0.05$). **Control**: Dimethyl sulfoxide (1 %) and saline (0.9%) **TNBS**: Trinitrobenzene sulfonic acid (50 mg/kg, intra-rectally); **SPS50**: Sulfasalazine in DMSO) (50 mg/kg, p.o.); **TP10**: *Trevesia palmata* flower bud methanolic extract (10 mg/kg, p.o.); **TP20**: *Trevesia palmata* flower bud methanolic extract (20 mg/kg, p.o.).

6.1 INTRODUCTION

6.1.1 The Essence and Narrative of Ulcerative Colitis

Ulcerative colitis (UC) is a chronic, relapsing inflammatory bowel disease (IBD) that primarily affects the rectum and the proximal colon. It is characterized by inflammation and ulceration of the intestinal mucosa, leading to symptoms such as bloody stools, diarrhoea, abdominal pain, and mucus discharge. The pathophysiology of UC is complex and multifactorial, with a combination of genetic, environmental, and immunological factors playing key roles. While host genetics contribute to susceptibility, the inflammatory response and microbial imbalances in the gut also significantly influence disease progression (**De Souza et al., 2022**).

UC has been classified as a modern refractory disease by the World Health Organization due to its chronic nature and the challenges involved in effective management (Wang et al., 2022). The disease can range in severity, with some patients experiencing only mild symptoms and others facing extensive involvement of the colon, often resulting in the need for surgical intervention. Approximately 20-30% of UC patients may require surgery during their lifetime, and the disease places a significant financial burden on patients and families alike (**Shao et al., 2024**). Current pharmacological treatments, including corticosteroids, aminosalicylates, immunosuppressive agents, and biologics, are often accompanied by significant side effects, such as infections, fever, and high recurrence rates, leading to a pressing need for novel and safe therapeutic strategies (**Zou et al., 2020**). The Montreal classification system divides UC patients into categories based on disease extent, including E1 (proctitis), E2 (left-sided colitis), and E3 (severe colitis) (Lamb et al., 2023). Disease severity is linked to an increased risk of complications such as colorectal cancer, which is more common in patients with long-standing, active inflammation. Moreover, patients with UC are susceptible to extra-intestinal manifestations such as anemia, arthritis, and ocular issues, which further complicate disease management (**Lamb et al., 2023**). These multifaceted challenges highlight the need for improved therapies to manage UC and its associated complications effectively.

6.1.2 *Trevesia Palmata* and Its Potential in Treating Ulcerative Colitis

Ulcerative colitis (UC) is a chronic, inflammatory bowel disease (IBD) characterized by ongoing inflammation and ulceration of the colon, leading to symptoms such as abdominal pain, diarrhea, and rectal bleeding. The pathophysiology of UC is complex, involving both immune dysregulation and microbial imbalances in the gut. Current treatments primarily focus on suppressing inflammation through pharmaceuticals like corticosteroids, immunosuppressive agents, and biologics, but these therapies often come with side effects, and many patients experience recurrent flare-ups or limited efficacy (Lamb et al., 2023). Recent research has highlighted the pivotal role of the gut microbiota in UC development and progression. Dysbiosis, or the imbalance of microbial communities, is a well-documented factor in UC, with a reduction in beneficial microbes and an overgrowth of pathogenic species contributing to intestinal inflammation. This has prompted the exploration of alternative therapies aimed at restoring microbial balance as a potential therapeutic strategy for UC.

One such promising natural remedy is *Trevesia palmata*, a plant known for its rich bioactive compounds, including flavonoids, alkaloids, and terpenoids. Studies have suggested that *T. palmata* possesses anti-inflammatory, antimicrobial, and antioxidant properties, which may be beneficial in managing UC. By targeting the gut microbiota and potentially promoting the growth of beneficial bacterial species while suppressing harmful pathogens, *T. palmata* presents an innovative approach to modulating gut health and alleviating UC symptoms (Chen et., 2025).

6.1.3 Role of Gut Microbiota in Ulcerative Colitis

The development and progression of UC are not only influenced by genetic and immune factors but are also closely linked to the composition and diversity of the gut microbiota. The gut microbiota refers to the complex community of microorganisms, including bacteria, archaea, fungi, and viruses, that reside in the human gastrointestinal tract. This microbial ecosystem plays a crucial role in maintaining intestinal homeostasis by participating in digestion, modulating immune

responses, and influencing metabolic and neurological functions (Tilg et al., 2018). Disruptions in the delicate balance of the gut microbiota, known as dysbiosis, have been shown to contribute to the pathogenesis of a variety of diseases, including IBD.

In the case of UC, alterations in gut microbiota composition are commonly observed, with reduced microbial diversity and shifts towards pathogenic bacteria. Studies have shown that UC patients often exhibit a depletion of beneficial microorganisms, such as *Faecalibacterium prausnitzii* and *Roseburia hominis*, which are known for their anti-inflammatory properties. At the same time, there is an overgrowth of pathogenic species, such as *Escherichia coli* and *Bacteroides fragilis*, which can exacerbate inflammation and mucosal injury (Singh et al., 2021). For example, Andoh et al. (2011) reported an increase in *Bacteroides* abundance in the fecal microbiota of UC patients, while Machiels et al. (2014) found that UC patients had lower levels of *Faecalibacterium prausnitzii* compared to healthy controls. This microbial imbalance is thought to trigger an abnormal immune response that leads to chronic inflammation and tissue damage in the colon.

Restoring a balanced gut microbiota has therefore become a promising therapeutic strategy for UC. Probiotic therapies, which involve the administration of beneficial microorganisms such as *Lactobacillus* and *Bifidobacterium*, have shown some efficacy in reducing inflammation and improving symptoms in UC patients (Distrutti et al., 2016). However, the development of more targeted treatments, such as natural plant-based therapies, may offer an alternative approach to modulate the gut microbiota and mitigate UC symptoms.

6.1.4 Metagenomic Insights into Gut Microbiota

Metagenomics, the study of genetic material recovered directly from environmental samples, has revolutionized our understanding of the gut microbiota. Traditional methods of culturing bacteria in the laboratory often fail to capture the full diversity of microorganisms present in the gut, particularly those that are difficult to culture. In contrast, metagenomic sequencing allows for a more comprehensive analysis by directly sequencing DNA from fecal or intestinal samples, providing a

more accurate picture of microbial community structure and function (**Hegazy et al., 2023**). Through metagenomic approaches, researchers can assess the microbial composition in patients with UC and compare it to healthy controls, identifying specific microbial species or communities associated with disease onset and progression. For instance, studies have shown that UC patients exhibit a decrease in bacterial diversity, particularly in the abundance of beneficial microbes, which is often accompanied by an increase in pro-inflammatory species (**Singh et al., 2021**). Moreover, metagenomics can help identify microbial biomarkers that may serve as diagnostic tools or therapeutic targets for UC.

In the context of UC treatment, metagenomic profiling also provides valuable insights into how therapeutic interventions, such as the administration of plant extracts, can influence the gut microbiota. The methanolic extract of *Trevesia palmata* flower buds, for example, has been shown to have anti-inflammatory properties in animal models of UC. The use of metagenomic techniques to evaluate the effects of *T. palmata* on the gut microbiota can shed light on the specific microbial changes associated with its protective effects, offering a deeper understanding of the mechanisms through which this plant extract may help restore microbial balance and alleviate UC symptoms. By utilizing metagenomic sequencing technologies, researchers can not only gain a more detailed understanding of the gut microbiota's role in UC but also uncover novel therapeutic strategies that target the microbiome. In particular, plant-based therapies, which are rich in bioactive compounds, may offer a safer and more sustainable alternative to conventional pharmaceutical treatments for UC. The present study aims to investigate the potential of *Trevesia palmata* in modulating gut microbiota and providing therapeutic benefits in a TNBS-induced ulcerative colitis model in Wistar albino rats, using metagenomic profiling to uncover the mechanistic pathways of action.

6.2 REVIEW OF LITERATURE

6.2.1 Ulcerative colitis and intestinal microbiota

The pathogenesis of UC and the gut flora are closely related. The intestinal microbiota's stable state is crucial for preventing the out-of-control proliferation of specific microorganisms. Some diseases and conditions, including obesity, metabolic syndrome, autoimmune disorders, necrotizing enterocolitis, skin disease, UC, Crohn's disease (CD), and irritable bowel syndrome, may be influenced by intestinal microbiota dysbiosis (Xu et al., 2018).

The intestinal defense and immunoregulatory functions are diminished, the body's immunity is weakened, and the relative pathogenic factors are raised, which can lead to the invasion of the intestinal mucosa or worsen existing conditions, when the intestinal microbiota balance is disturbed. Interactions between the host and intestinal microbiota may play a significant role in the etiology of UC, which is a complex disease. Under typical conditions, the host's innate and adaptive immunity blocks the invasion of dangerous bacteria while tolerating the normal microbiota. Immunity is, however, jeopardized by an unbalanced microbiome. Inflammation can result from an overactive immune response in the gut mucosa (Zhang et al., 2021).

Intestinal inflammation can result from disturbed microbiota. Intestinal pathogenic bacteria quickly multiply when the microbiota is disrupted. Additionally, the production of immunosuppressive proteins leads to immune dysfunction and the release of enterotoxins increases intestinal mucosal permeability. Intestinal epithelial cells are directly invaded and harmed by expanding populations of pathogenic bacteria, harming the intestinal mucosal barrier. The excessive growth of some bacteria impacts energy and metabolic metabolism, resulting in intestinal inflammation and mucosal damage. The function of the intestinal mucosal barrier deteriorates, the intestinal wall's ability to act as a shield is reduced, and intestinal microbiota is moved around the body, further damaging the mucosal barrier and escalating the intestinal inflammatory response. Animal studies have shown that

altering gut bacterial balance can cause colitis in animal models. Colitis did not occur in IL-10 or HLA-B27 knockout sterile animals (**Bamias et al., 2012**).

Numerous studies have shown that UC patients have compromised intestinal microbiota in terms of both composition and function. There is a decrease in several bacteria, including *Akkermansia* (A.) *mutiniphila*. *A. mutiniphila*, which makes up 1% to 5% of the human intestinal microbiota, is one of the most prevalent species. There is a connection between UC and *A. mutiniphila*, according to numerous research. *A. mutiniphila* and *Roseburia* bacteria were reduced in UC patients (**Bajer et al., 2017**).

As a result, *A. mutiniphila* may become a new focus for UC. Patients with UC and CD commonly had abnormal microbiota, which lowered the complexity of the gut microbial environment (**Manichanh et al., 2012**). Since intestinal inflammation may be caused by a number of microorganisms, UC patients may also carry *Mycobacterium avium* paratuberculosis (**Secchi et al., 2023**), *Escherichia coli* that is adherent-invasive (**Mei et al., 2016**), *Clostridium* (C.) *difficile* (**Reddy et al., 2013; Deshpande et al., 2019**), *Helicobacter* species (**Paulson et al., 2011**), *Salmonella* species, *Yersinia* species, *Fusobacterium* species (**Ohkusa et al., 2024**), norovirus and *Listeria* species. The precise pathophysiology, nevertheless, is unknown.

6.2.2 Impact of Gut Microbiota Dysbiosis on Ulcerative Colitis Pathogenesis

The concept that gut microbiota plays a critical and active role in the pathogenesis of Ulcerative Colitis (UC) has gained substantial scientific support in recent years. Dysbiosis—defined as an imbalance in the composition, diversity, spatial organization, and functional capacity of the gut microbial community—is frequently observed in UC patients. Rather than being a passive consequence of inflammation, growing evidence indicates that dysbiosis actively contributes to both the onset and progression of UC (**Guo et al., 2020**). This imbalance disrupts the delicate host–microbe interactions necessary for intestinal homeostasis, ultimately promoting pathological responses that drive mucosal injury and chronic inflammation.

A key hallmark of dysbiosis in UC is the alteration of gut microbial composition and diversity. Numerous studies consistently report reduced microbial richness and species diversity in individuals with UC compared to healthy individuals. Beneficial bacterial groups—particularly those known for producing short-chain fatty acids (SCFAs), such as *Faecalibacterium* and *Roseburia*—are often diminished, while facultative anaerobes and opportunistic or pathogenic taxa may increase (Lopez-Siles et al., 2017; Nishida et al., 2023). These shifts not only represent a loss of protective microbial functions but also promote the expansion of organisms capable of thriving in inflammatory environments, thereby amplifying disease activity.

Beyond compositional changes, dysbiosis in UC profoundly affects the metabolic and functional output of the gut microbiome. Altered metagenomic and metabolomic signatures have been documented in UC patients, including disruptions in the production of key microbial metabolites such as SCFAs, bile acids, and tryptophan-derived compounds (Wang et al., 2022). These metabolites are essential for maintaining epithelial health, nourishing colonocytes, and modulating host immune responses. Their reduction compromises mucosal homeostasis, weakens epithelial metabolism, and diminishes the availability of immunoregulatory molecules, thereby contributing to disease progression.

Dysbiosis also plays a pivotal role in impairing intestinal barrier integrity and disrupting mucosal immune regulation. A healthy gut microbiota supports the structural and functional integrity of the intestinal barrier by promoting mucin production, regulating tight junction proteins, and stimulating antimicrobial peptide expression. In UC, however, dysbiosis is associated with increased epithelial permeability, diminished goblet cell numbers, and altered secretion of protective epithelial molecules (Carmody et al., 2019; Schultsz et al., 2022). These defects facilitate translocation of bacterial antigens and pathogen-associated molecular patterns (PAMPs), which in turn provoke exaggerated innate and adaptive immune responses. The resulting overproduction of inflammatory cytokines and recruitment

of immune cells create a self-perpetuating inflammatory environment characteristic of UC (Chin et al., 2024).

Importantly, the relationship between dysbiosis and inflammation forms a vicious cycle in UC pathogenesis. While dysbiosis can initiate or exacerbate inflammation, active inflammation further reshapes the microbial community by altering nutrient availability, oxygen gradients, pH, and mucus composition. This hostile inflammatory environment favors the growth of pro-inflammatory bacterial species, which in turn intensify immune activation and mucosal damage (Zhao et al., 2021). Thus, dysbiosis and inflammation mutually reinforce each other, supporting chronic disease progression.

Collectively, the accumulating body of evidence underscores that gut microbiota dysbiosis is not merely an epiphenomenon of UC but a central driver of its pathophysiology. By disrupting mucosal barrier function, metabolic homeostasis, and immune regulation, dysbiosis contributes directly to the persistent intestinal inflammation that defines UC (Lopez-Siles et al., 2017; Liu et al., 2021). Understanding these complex microbial interactions provides crucial insight into potential microbiota-targeted therapeutic strategies for managing UC.

6.2.3 Impact of Gut Microbiota on Ulcerative Colitis Pathogenesis and the Therapeutic Efficacy of *Trevesia palmata* in its Management

Given the growing evidence of the critical role that gut microbiota plays in the pathogenesis of Ulcerative Colitis (UC), targeting the microbiome to restore a balanced microbial environment, or eubiosis, has become an attractive therapeutic strategy. This approach aims to correct the microbial imbalances that contribute to the development and progression of UC, with the goal of improving symptoms, inducing remission, and potentially preventing disease relapse. Several interventions have been explored, ranging from the use of probiotics, prebiotics, and synbiotics to more complex treatments like faecal microbiota transplantation (FMT) and microbiota-derived metabolites. Among these, *Trevesia palmata* has emerged as a

promising alternative due to its potential to modulate gut microbiota, reduce inflammation, and restore microbial balance in UC.

Probiotics, which are live beneficial bacteria, have been widely studied for their ability to restore the diversity and balance of the gut microbiota. Strains like *Lactobacillus* and *Bifidobacterium* are commonly used for their ability to enhance gut barrier function, reduce pathogen colonization, and modulate immune responses. In UC, probiotics have shown the ability to reduce inflammation, improve remission rates, and help maintain long-term disease control, though the effectiveness often depends on the specific strain used, dosage, and individual patient microbiome characteristics. Alongside probiotics, prebiotics, which are non-digestible food components like fiber that promote the growth and activity of beneficial bacteria, are also used to enhance microbial balance. When combined, synbiotics, which include both prebiotics and probiotics, have synergistic effects, further promoting the growth of beneficial microbes and improving gut health. While studies on synbiotics in UC have shown promising results, further research is needed to establish their widespread effectiveness (**Leung et al., 2020**).

Fecal microbiota transplantation (FMT) is another promising intervention, involving the transfer of fecal material from a healthy donor to a UC patient to restore a healthy and diverse microbiome. FMT aims to reintroduce a balanced microbial community, potentially alleviating symptoms and inducing remission. Several clinical trials have reported positive results, including improved microbial diversity, reduced inflammation, and symptom relief. However, FMT remains controversial, as treatment outcomes can be variable, with some patients experiencing relapses or no improvement. The lack of standardized protocols for donor selection, dosing, and administration, as well as concerns about long-term safety, are challenges that need to be addressed before FMT can be widely adopted as a treatment option. Another emerging approach focuses on the use of microbiota-derived metabolites, such as short-chain fatty acids (SCFAs), bile acids, and tryptophan derivatives, which are essential for maintaining intestinal homeostasis and modulating immune responses. In UC, dysbiosis often results in the reduced

production of these metabolites, contributing to inflammation and loss of gut barrier integrity (Sokol et al., 2022). Therapeutic strategies that restore or mimic the beneficial effects of these metabolites are being developed, with some promising results in restoring gut health and immune function. Additionally, live-biotherapeutic products (LBPs), which use carefully selected strains of live bacteria, and postbiotics, which involve microbial metabolites, are being explored as more controlled, standardized therapies for modulating the microbiome (Zhang et al., 2021; Liao et al., 2023).

In this context, *Trevesia palmata* emerges as a potential therapeutic alternative. Recent studies have shown that *T. palmata*, has rich bioactive compounds such as flavonoids, alkaloids, and terpenoids, has anti-inflammatory, antimicrobial, and antioxidant properties. These properties make *T. palmata* a promising candidate for modulating the gut microbiota in UC. Its ability to promote the growth of beneficial gut bacteria, restore microbial balance, and reduce intestinal inflammation could make it an effective complementary or alternative treatment to traditional pharmacological interventions. By restoring gut microbiota composition and enhancing gut barrier function, *T. palmata* may help alleviate UC symptoms, providing a novel approach to managing the disease (Wang et al., 2022 & Rajagopalan et al., 2023).

6.3 MATERIAL AND METHODS

6.3.1 Sample collection:

Each rat fecal sample from every group was meticulously homogenized into smaller fragments within sterile microcentrifuge tubes. To maintain sterility, the scissors used in the process were thoroughly disinfected with 99% lab-grade ethanol prior to sample handling. After homogenization, all fecal samples were promptly stored at -80°C to preserve their integrity for further analysis.

6.3.2 DNA extraction

DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen 69504) according to the manufacturer's procedure, with some changes. To ensure bacterial cell wall lysis, the sample digestion time was increased to 60 minutes in a 57°C water bath, followed by mechanical lysis using 0.5 mm glass beads in a Fisherbrand Head Mill 4 Homogenizer (Fisher Scientific 15-340-1521) for 5 rounds of 60 seconds at 5 ms. To separate the lysate from the debris, the bead tubes were centrifuged for one minute at 8,000 rpm. The lysate was transferred to a NucleoSpin collection column for greater purification according to the manufacturer's instructions. Following extraction, each sample's total DNA concentration was determined using a nanophotometer (Implen, Desville Scientific Inc.). Extracted DNA was kept at -20°C until sequencing.

6.3.3 16S rRNA sequencing

The 16S rRNA in 15 Rat fecal samples was analyzed using high-throughput DNA sequencing. DNA was measured with NanoDrop and 2100 Bioanalyzer equipment, and the sequencing library was created with real-time PCR procedures. Quick-16S Primer Set V3-V4 amplified the V3-V4 region of the 16S rRNA gene. The final PCR products were then quantified and grouped together. Finally, the sequencing was done using more than 10% PhiX spike-in and DADA2 software to assign bacterial taxa.

6.3.4 V3-V4 Amplicon generation

DNA samples were diluted to 10 ng/μl and amplified for 16S rDNA (~1.5 KB) using 16S forward and 16S reverse primers (Refer. Primer sequence). A positive control (internal metagenomic DNA sample) and no template control were used in a step-up approach of 35 PCR cycles. These PCR results were analyzed on an agarose gel. All 16S PCR products were used to amplify the V3-V4 region (465bp) using particular primers, with positive 1:100 and no template controls, utilizing a step-up technique and 35 PCR cycles. Prior to library preparation, all V1-V4 amplified PCR

products were cleaned up (IX) with AMPure XP beads (Beckman Coulter, Cat# A63882) to remove non-specific fragments.

6.3.5 Library Preparation Protocol

The cleaned V3-Va product was then used to prepare a library using the NEBNext Ultra DNA Library Prep Kit for Illumina (NEIL, Cat# E7370L). First, the amplicon was end-repaired and mono-adenylated at the 3' end using a single enzymatic process. The NEB hairpin-loop adapters were then ligated to the DNA fragments using a 14-DNA ligase reaction. Following ligation, the loop containing Uracil was linearized using USER Enzyme (a mixture of UDG and Endo VIII), allowing it to serve as a substrate for PCR-based indexing in the following step. During PCR, barcodes were added in each sample using distinct primers, allowing for multiplexing. The Fragment Analyzer was used to examine the fragment distribution of all prepared libraries using the HS-NOS Fragment Kit (1-6000bp) (Agilent, Cat# DNF-474-1000).

6.3.6 Sequencing Protocol

Libraries were evaluated using the Qubit dsDNA HS Assay kit (Invitrogen, Cat# 032854). The resultant libraries were grouped and diluted to get the final optimum loading concentration. The pooled libraries were subsequently loaded onto the Illumina MiSeq V2 instrument, which generated 0.5M, 300bp Paired end reads per sample.

6.3.7 Bioinformatics analysis

This study's bioinformatics analysis used Illumina reads to examine the Quantitative Insights into Microbial Ecology 2 software. To extract ASV sequences, the sequences were demultiplexed with a native plugin and DADA2. Representative sequences were taxonomically assigned using the feature-classifier plugin in QIIME2, with the classify-consensus-vsearch technique applied to the Greengenes non-redundant database. Taxa bar plots are generated using the QIIME2 taxa plugin.

Alpha diversity was estimated using presence absence and Shannon index. Beta diversity was determined using qualitative variables (Ling et al, 2022).

6.3.8 Statistical analysis

All quantitative data are presented as the mean $\pm$ standard error of the mean (SEM). Statistical comparisons among experimental groups were carried out using one-way analysis of variance (one-way ANOVA), which was employed to determine whether there were overall significant differences between group means. A probability value (P) of less than 0.05 was considered to indicate statistical significance for all analyses, and only differences meeting this threshold were interpreted as meaningful in the context of the study.

6.4 RESULTS

6.4.1 Multiqc report on fecal composition of Wister albino male rats

The MultiQC report provides a comprehensive assessment of the sequencing data obtained from fecal samples of Wister albino female rats, focusing on the evaluation of gut microbiota composition. The report includes several key quality control metrics that ensure the reliability of the sequencing results, which are essential for downstream microbiome analysis. The sequence count (**Figure 6.1A**) is one of the first metrics evaluated, representing the total number of sequences obtained for each sample. A sufficient sequencing depth is crucial for accurate microbiome profiling, and the report reveals that all experimental groups had comparable sequence counts, ensuring that the sequencing depth was consistent across the samples. This consistency supports the reliability of the data, as variations in sequencing depth could otherwise skew the results. The mean quality score (**Figure 6.1B**) plot offers a high-level view of the sequencing quality across all samples, with most sequences showing high-quality scores indicated by the green regions. This suggests that the sequencing process was efficient and that there were minimal quality issues. However, the gradual decline in quality scores toward the later cycle positions, where the plot transitions from green to yellow and red, is a typical characteristic of sequencing data and does not significantly affect the overall

data quality. This decline is often seen in sequencing experiments and is not detrimental to the microbiome analysis. A more granular look at the sequencing quality is provided by the per sequence quality score (**Figure 6.1C**) graph, which examines the quality scores for individual sequences. This metric is crucial for identifying poorly generated sequences that may compromise the data. The report shows that the majority of sequences had high-quality scores, with only a small proportion falling below the expected threshold. Such occurrences are common in sequencing experiments and do not significantly impact the overall data quality or reliability.

The per sequence GC content (**Figure 6.1D**) graph reveals the distribution of guanine-cytosine (GC) content across all sequences. In a typical sequencing experiment, a normal distribution of GC content is expected, which indicates unbiased sequencing and minimal contamination. The results show that the GC content was normally distributed, suggesting that the sequencing process was unbiased and that no significant contamination occurred. This is an important finding, as deviations from normal GC content could indicate sequencing biases or contamination, which were not observed in this dataset. The per base N content (**Figure 6.1E**) metric measures the percentage of uncalled bases ("N"s) across different positions in the sequences. A low percentage of N bases indicates high sequencing accuracy. The report shows that most sequences had minimal N bases, further supporting the high accuracy and reliability of the sequencing process. This suggests that the sequencing was robust and that the results can be trusted for microbiome analysis. The sequence duplication level (**Figure 6.1F**) graph assesses the level of redundancy in the sequencing data, with high duplication levels possibly indicating over-sampling of the same microbial species. The report reveals that the level of sequence duplication was within acceptable limits, with only a small proportion of sequences being highly duplicated. This suggests that the sequencing captured a broad and diverse range of microbial species, which is critical for accurate microbiome profiling. High sequence duplication could skew the microbial composition, but in this case, it did not significantly affect the diversity captured. The overrepresented sequences (**Figure 6.1G**) chart identifies any sequences that are

present in disproportionately high numbers, which could indicate contamination or sequencing biases. The results show that only a small fraction of sequences were overrepresented, and these did not significantly alter the overall microbiota composition. This suggests that there were no major contamination issues, further confirming the reliability of the sequencing process and the integrity of the microbiome data. The adapter content (**Figure 6.1H**) graph monitors the presence of adapter sequences that may have been inadvertently included during the sequencing process. While adapter contamination is common in high-throughput sequencing, the report shows that adapter content was minimal, with only slight contamination detected in a few samples. This is a typical finding and does not significantly affect the overall data quality, which remains suitable for microbiome analysis.

Finally, the status check (**Figure 6.1I**) provides a summary of the overall sequencing quality for each sample. Most samples passed all quality control checks, with only a few exhibiting minor issues. These minor issues are typical in high-throughput sequencing experiments and do not compromise the overall quality or reliability of the data. The status check confirms that the majority of the samples were of high quality and suitable for downstream microbiome analysis. Thus, the MultiQC report confirms that the sequencing data used to assess the gut microbiota composition of Swiss albino female rats is of high quality.

6.4.2 Taxonomic distribution

The gut microbiota plays a crucial role in the development and progression of Ulcerative Colitis (UC), with dysbiosis an imbalance in the microbial community—contributing significantly to the inflammatory processes seen in UC. The data presented here demonstrate that the administration of *Trevesia palmata* (*T. palmata*) flower buds, particularly at higher doses (TPF20), significantly influences the gut microbiota composition, restoring microbial balance and potentially alleviating the symptoms of UC. The findings are described at multiple taxonomic levels, including phylum, class, family, and species, providing a comprehensive understanding of how *T. palmata* modulates gut microbiota in UC rats.

At the phylum level (**Figure 6.2**), the analysis shows clear differences between the experimental groups. In the healthy control rats, the gut microbiota is dominated by beneficial phyla such as Firmicutes, Bacteroidetes, and Proteobacteria, which play essential roles in digestion, immune modulation, and maintaining gut health. In contrast, the TNBS-induced UC group exhibited a significant decrease in Firmicutes and Bacteroidetes—key phyla associated with anti-inflammatory effects and the production of short-chain fatty acids (SCFAs). At the same time, the Proteobacteria phylum, often linked to gut inflammation and pathogenic overgrowth, was significantly increased. The treatment with *T. palmata*, especially at the TPF20 dose, resulted in a significant restoration of Firmicutes and Bacteroidetes abundance, suggesting that *T. palmata* helps rebalance the microbial profile towards a more beneficial and healthy composition. Notably, the increase in Firmicutes is crucial because these microbes are involved in producing SCFAs, which contribute to gut health by maintaining epithelial integrity and reducing inflammation. The reduction in Proteobacteria with TPF20 treatment further underscores the potential anti-inflammatory effects of *T. palmata*, as this phylum is often associated with disease exacerbation in UC.

At the class level (**Figure 6.2**), the changes in microbial communities are more specific and offer insights into functional shifts. In the control group, classes such as Clostridia, Bacteroidia, and Betaproteobacteria are prevalent. These classes are crucial for SCFA production, immune system modulation, and maintaining gut barrier integrity. The TNBS-induced UC group, however, exhibited a decrease in the abundance of Clostridia and Bacteroidia, while there was an increase in Enterobacteria and Lactobacillia, classes associated with inflammation and dysbiosis. Treatment with *T. palmata* (especially TPF20) significantly restored the abundance of Clostridia and Bacteroidia, both of which are associated with anti-inflammatory effects and the production of SCFAs. The increase in Clostridia, in particular, is important because this class is involved in producing beneficial metabolites that help reduce inflammation and strengthen the intestinal barrier. This suggests that *T. palmata* not only restores microbial diversity but also helps modulate the functional capacity of the gut microbiota to combat UC-related inflammation.

At the order level (**Figure 6.3**), the analysis provides further resolution. In healthy rats, the orders Clostridiales (within Firmicutes) and Bacteroidales (within Bacteroidetes) were abundant, both of which are essential for maintaining intestinal health through their roles in nutrient fermentation and SCFA production. In the TNBS-induced UC rats, there was a significant reduction in Clostridiales and Bacteroidales, which are beneficial for intestinal function, while Enterobacteriales (within Proteobacteria) increased, correlating with pathogenic overgrowth and inflammation. Treatment with *T. palmata* (especially at TPF20) resulted in a marked restoration of Clostridiales and Bacteroidales, indicating that *T. palmata* helps restore the gut microbiota to a more health-promoting state. This suggests that *T. palmata* not only promotes microbial diversity at the class level but also supports the growth of key microbial orders involved in gut homeostasis. At the family level (**Figure 6.4**), the composition of microbial families further reflects the effects of *T. palmata* treatment. The control group primarily hosts families such as Lachnospiraceae and Ruminococcaceae, which are involved in the production of SCFAs and the maintenance of intestinal health. In the TNBS-induced UC rats, however, there is a clear reduction in these beneficial families, accompanied by an increase in pathogenic families like Enterobacteriaceae, which are often associated with intestinal inflammation and dysbiosis. Treatment with *T. palmata* (TPF10 and TPF20) significantly increased the abundance of Lachnospiraceae and Ruminococcaceae, suggesting that *T. palmata* supports the growth of beneficial families that are crucial for gut health. This indicates that *T. palmata* can help restore microbial communities that promote a healthy gut environment, enhancing the production of SCFAs and supporting immune function. In genus level (**Figure 6.5**), a detailed analysis revealed the influence of *T. palmata* on specific microbial genera. In healthy control rats, genera like *Faecalibacterium*, *Bacteroides*, and *Lachnospira* dominated the microbiota, and these genera are known for their anti-inflammatory effects and their role in SCFA production. In the TNBS-induced UC rats, there was a significant reduction in *Faecalibacterium* and *Bacteroides*, which are essential for maintaining gut health, while genera like *Escherichia* and *Enterococcus* became more prevalent, which are associated with pathogenicity in UC. Treatment with *T. palmata* (especially at TPF20) led to the restoration of *Faecalibacterium prausnitzii*

and *Bacteroides fragilis*, both of which are beneficial for immune regulation and maintaining gut epithelial integrity. This suggests that *T. palmata* plays a crucial role in promoting the growth of health-promoting genera, which are essential for counteracting the inflammation and dysbiosis associated with UC.

Finally, at the species level (**Figure 6.6**), the specific microbial populations present in the gut were analyzed. In healthy control rats, species like *Faecalibacterium prausnitzii* and *Bacteroides fragilis* dominate, both of which are known for their anti-inflammatory properties and ability to maintain gut barrier integrity. In the TNBS-induced UC group, these beneficial species were significantly reduced, while harmful species like *Escherichia coli* and *Enterococcus faecalis* were more prevalent, reflecting the dysbiotic nature of the UC microbiota. Treatment with *T. palmata*, especially at the TPF20 dose, led to a significant restoration of beneficial species such as *Faecalibacterium prausnitzii* and *Bacteroides fragilis*. This suggests that *T. palmata* can help restore the populations of microbes that are critical for immune modulation and intestinal barrier function. By promoting the growth of these beneficial species, *T. palmata* may reduce the pathogenic overgrowth seen in UC and help restore gut homeostasis. Thus, the treatment with *Trevesia palmata* flower buds, particularly at higher doses (TPF20), significantly alters the gut microbiota in UC rats, promoting microbial diversity, restoring beneficial bacterial populations, and reducing harmful microbial taxa. The restoration of *Firmicutes*, *Bacteroidetes*, *Clostridia*, and *Bacteroidia*, along with the reduction in *Proteobacteria* and *Enterobacteriaceae*, suggests that *T. palmata* plays a key role in rebalancing the gut microbiota and mitigating UC-related inflammation. These findings highlight the therapeutic potential of *T. palmata* as a microbiome-targeted intervention for UC, offering a natural approach to restoring gut health and preventing disease progression. Further studies, particularly human clinical trials, are needed to confirm these results and explore the mechanisms underlying these effects.

6.4.3 Alpha diversity indices

Alpha diversity assesses changes in microbial diversity within individuals' gut microbiomes before and after dietary interventions. For species richness, alpha

diversity was measured using metrics such as the Abundance-based Coverage Estimator, Chao1 index, Chao1 Confidence Interval, and Margalef's Richness Index. For species abundance, it was assessed using metrics like Faith's Phylogenetic Diversity, Fisher's Index, Gini Index, and Good's Coverage. Species evenness was evaluated using the Shannon Index, Simpson's Index, Pielou's Evenness, and Simpson's Evenness.

Figure 6.7 show detailed analysis of the effect of *Trevesia palmata* flower buds on the alpha diversity of gut microbiota, using four different diversity indices: the Abundance-based Coverage Estimator (ACE), Chao1 index, Chao1 confidence interval, and Margalef's richness index. The Abundance-based Coverage Estimator (ACE), which reflects the estimated species richness and coverage of microbiota in each group. The Control group demonstrates the highest ACE value, significantly higher than the TNBS group, suggesting that the gut microbiota in the Control group is more diverse and well-covered. The TNBS group, which likely represents an induced gut disturbance, shows the lowest ACE, with significantly reduced diversity compared to the Control. The groups treated with TNBS + SPS, TNBS + TPF10, and TNBS + TPF20 exhibit intermediate ACE values, with TNBS + TPF20 showing the highest among these treatments, significantly greater than the others, indicating that *Trevesia palmata* flower buds, particularly TPF20, help restore or enhance gut microbiota diversity after the TNBS-induced disruption (**Figure 6.7 A**). The Chao1 index provides a measure of species richness. Here, the Control group again shows the highest Chao1 index, which is significantly higher than all the treatment groups, suggesting that the Control group harbors the most species-rich microbiota. In contrast, the TNBS group has the lowest Chao1 index, significantly lower than all other groups, indicating that TNBS treatment substantially reduces gut microbiota richness. The groups treated with TNBS + SPS, TNBS + TPF10, and TNBS + TPF20 show intermediate Chao1 values, with TNBS + TPF20 demonstrating the highest Chao1 index among the treatments, similar to the Control group, suggesting that the *Trevesia palmata* flower buds, especially TPF20, have a positive impact on species richness, enhancing it back toward baseline levels (**Figure 6.7 B**)

The Chao1 confidence interval highlights the variability in species richness estimates. The TNBS + TPF20 treatment has the highest confidence interval, indicating greater variability in species richness within that group, while still showing the highest Chao1 value overall. The groups TNBS + TPF10 and TNBS + SPS also show wider confidence intervals than the TNBS group, indicating that these treatments also contribute to higher species richness compared to TNBS, but not as markedly as TPF20. The Control group has the narrowest confidence interval, indicating the highest consistency in the species richness estimates, and is consistent with the observation that it has the highest alpha diversity across all indices (**Figure 6.7 C**). Finally, the Margalef's Richness Index, another indicator of species richness. The Control and TNBS + SPS groups have the highest Margalef's index values, both significantly greater than the TNBS group, suggesting that these treatments support higher species richness compared to the TNBS alone. The TNBS + TPF10 and TNBS + TPF20 groups also show higher Margalef's index values than the TNBS group, with TNBS + TPF20 exhibiting the highest value among all the treatment groups, further emphasizing the positive impact of *Trevesia palmata* flower buds, particularly TPF20, on gut microbiota diversity (**Figure 6.7 D**).

Figure 6.8 illustrates the effect of *Trevesia palmata* flower buds on species diversity, with a focus on species abundance. The analysis utilizes four different diversity indices: Faith's Phylogenetic Diversity, Fisher's Index, Gini Index, and Good's Coverage. Faith's Phylogenetic Diversity, a metric that assesses the phylogenetic diversity of microbiota by considering the total branch length of a phylogenetic tree. The Control group demonstrates the highest phylogenetic diversity, followed closely by the TNBS + TPF20 treatment. These groups significantly outperformed the TNBS group, which shows the lowest phylogenetic diversity, suggesting that *Trevesia palmata* flower buds (particularly TPF20) contribute to the restoration or enhancement of phylogenetic diversity after TNBS-induced disturbance. The TNBS + TPF10 and TNBS + SPS treatments also show intermediate levels of phylogenetic diversity, with TNBS + TPF10 and TNBS + SPS both having lower diversity than TNBS + TPF20, but higher than TNBS, indicating the potential positive impact of *Trevesia palmata* flower buds on restoring phylogenetic diversity to levels closer to the Control group (**Figure 6.8 A**).

Fisher's Index, measures the species diversity by accounting for the number of species and their relative abundances. The Control group has the highest Fisher's Index, significantly greater than all other treatments, suggesting the greatest species diversity in this group. The TNBS group, in contrast, has the lowest Fisher's Index, significantly lower than all treatments, indicating a reduction in species diversity. The groups treated with TNBS + SPS, TNBS + TPF10, and TNBS + TPF20 exhibit intermediate values. TNBS + TPF20 has the highest Fisher's Index among these treatments, closely approaching the Control group's value, indicating that *Trevesia palmata* flower buds, particularly TPF20, have a marked positive effect on the diversity and abundance of species, helping restore microbiota diversity after TNBS-induced dysbiosis (**Figure 6.8 B**).

The Gini Index, which measures the inequality of species abundances, with values closer to 1 indicating greater inequality (i.e., dominance of a few species), and values closer to 0 indicating more even distributions. The TNBS group has the highest Gini Index value, indicating a highly uneven distribution of species abundance, with a few dominant species. In contrast, the Control group has a significantly lower Gini Index value, suggesting a more even distribution of species. The TNBS + SPS group shows a slight reduction in inequality compared to TNBS, but not as much as the TNBS + TPF10 and TNBS + TPF20 groups. Among the treatments, TNBS + TPF20 exhibits the lowest Gini Index, indicating that *Trevesia palmata* flower buds, especially TPF20, help create a more balanced distribution of species, moving the microbiota toward a more even and healthy community structure. (**Figure 6.8 C**). The Good's Coverage, estimates the proportion of species that are sampled in the microbiota, with values closer to 1 indicating greater sampling coverage. All groups, including the Control, TNBS, and treatments with TNBS + SPS, TNBS + TPF10, and TNBS + TPF20, show similar Good's Coverage values, all near or above 0.985. This indicates that the sampling coverage is relatively consistent across all groups, suggesting that the differences observed in other indices, such as phylogenetic diversity and Fisher's Index, are not due to discrepancies in sampling but are instead reflective of actual differences in microbiota diversity and distribution (**Figure 6.8 D**).

Figure 6.9 highlights the impact of *Trevesia palmata* flower buds on species evenness and diversity, assessed using four indices: the Shannon Index, Simpson Index, Pielou's Evenness, and Simpson's Evenness. The Shannon Index is a measure of both species richness and evenness, representing the diversity within a community. In this panel, the Control group exhibits the highest Shannon Index, indicating a balanced and diverse microbial community. The TNBS group, on the other hand, has the lowest Shannon Index, which is significantly reduced compared to the Control, suggesting that TNBS treatment leads to a loss of microbial diversity. The groups treated with TNBS + SPS, TNBS + TPF10, and TNBS + TPF20 all show intermediate Shannon Index values, with TNBS + TPF20 demonstrating the highest value among these, significantly greater than the others. This suggests that *Trevesia palmata* flower buds, particularly TPF20, have a beneficial effect in restoring the microbial diversity that is diminished due to TNBS-induced dysbiosis (**Figure 6.9 A**). The Simpson Index measures the probability that two individuals randomly selected from a community will belong to the same species, with lower values indicating higher diversity (more even distribution). In this panel, the Control group shows the highest Simpson Index, which means it has a more even species distribution. Conversely, the TNBS group has the lowest Simpson Index, indicating a highly uneven distribution of species, where a few species dominate. The treatment groups, TNBS + SPS, TNBS + TPF10, and TNBS + TPF20, all show intermediate Simpson Index values, with TNBS + TPF20 showing a value significantly closer to the Control, suggesting that *Trevesia palmata* flower buds, especially TPF20, help restore species evenness after TNBS treatment. (**Figure 6.9 B**).

Pielou's Evenness measures the evenness of species distribution in the community, with higher values indicating a more balanced distribution. The Control group exhibits the highest Pielou's Evenness, reflecting a well-balanced distribution of species. The TNBS group shows the lowest Pielou's Evenness, suggesting that TNBS leads to a highly imbalanced community structure. The treatment groups show intermediate values, with TNBS + TPF20 exhibiting the highest Pielou's Evenness among the treatments, significantly greater than TNBS and approaching the Control group's level. This demonstrates that *Trevesia palmata* flower buds, particularly TPF20, contribute to improving the balance of species within the microbiota, bringing the

evenness closer to that seen in the Control group (**Figure 6.9 C**). Simpson's Evenness is another metric for assessing species evenness, with higher values indicating a more even distribution of species. In this panel, the Control group again shows the highest Simpson's Evenness, suggesting a well-distributed microbial community. The TNBS group exhibits the lowest Simpson's Evenness, reflecting a more uneven distribution. The groups treated with TNBS + SPS, TNBS + TPF10, and TNBS + TPF20 all show intermediate values, with TNBS + TPF20 demonstrating the highest Simpson's Evenness among the treatments. This suggests that *Trevesia palmata* flower buds, particularly TPF20, have a positive effect on restoring a more even distribution of species after TNBS-induced disruption (**Figure 6.9 D**).

The Venn diagram (**Figure 6.10**) depicts the global overlap of entities among five experimental conditions. The non-overlapping sections of each ellipse represent entities uniquely detected in each group: 729 for Control, 505 for TNBS, 640 for TNBS + SPS, 626 for TNBS + TPF10, and 626 for TNBS + TPF20, each reflecting condition-specific signatures. The intersecting regions show shared entities between two or more treatments, with the central overlap of 408 elements representing a core set that is common to all five conditions. Notably, the enriched overlap between the TNBS + SPS or TNBS + TPF groups and the Control suggests that both SPS and TPF co-treatments partially normalize the TNBS-induced alterations, restoring some control-like entities while also introducing treatment-specific changes.

6.5 DISCUSSION

Ulcerative colitis (UC) remains a significant challenge in modern medicine due to its chronic, relapsing nature and the substantial morbidity it inflicts on affected individuals. While conventional treatments offer some relief, they are often burdened with side effects, limited efficacy, and high recurrence rates, underlining the pressing need for novel therapeutic approaches. This study aimed to investigate the therapeutic potential of *Trevesia palmata* (*T. palmata*), particularly its effects on gut microbiota modulation in a TNBS-induced ulcerative colitis model using Wistar albino rats. As highlighted in previous research, dysbiosis, an imbalance in the gut microbiota is a pivotal factor in the development and exacerbation of UC. Our findings confirm that

UC, induced by TNBS, is characterized by significant shifts in the gut microbial composition. Specifically, UC rats exhibited a notable decrease in beneficial bacterial phyla such as *Firmicutes* and *Bacteroidetes*, which are essential for intestinal homeostasis and the production of anti-inflammatory metabolites like short-chain fatty acids (SCFAs). This imbalance was accompanied by an increase in *Proteobacteria*, a phylum often associated with inflammation and pathogenicity in UC. These results align with those reported by Lopez-Siles et al. (2017) and Zhao et al. (2021), who also identified dysbiosis in UC patients, with a reduction in key microbial taxa that contribute to the maintenance of a healthy gut barrier. The decrease in microbial diversity observed in the TNBS group further supports the notion that UC is marked by both compositional and functional disturbances in the microbiota.

The treatment with *T. palmata* flower buds, particularly at higher doses (TPF20), showed promising results in restoring gut microbiota balance. At the phylum level, *T. palmata* treatment effectively enhanced the abundance of *Firmicutes* and *Bacteroidetes*, while reducing *Proteobacteria* levels. These changes are crucial, as *Firmicutes* and *Bacteroidetes* are associated with anti-inflammatory functions and the production of beneficial metabolites. At the class and order levels, the treatment with *T. palmata* also led to a significant restoration of microbial communities that are essential for gut health. The increase in *Clostridia* and *Bacteroidia*, which are key for SCFA production and immune modulation, suggests that *T. palmata* not only supports microbial diversity but also enhances the functional capacity of the gut microbiota to mitigate inflammation. Interestingly, *Faecalibacterium prausnitzii*, a genus well-known for its anti-inflammatory properties, was significantly restored in the *T. palmata*-treated groups, particularly at the TPF20 dose. This finding aligns with studies that have identified *Faecalibacterium* as one of the most abundant and beneficial genera in a healthy gut microbiota.

The analysis of alpha diversity provided additional insight into the effects of *T. palmata* treatment on microbial richness and evenness. The *Chao1* index and *Abundance-based Coverage Estimator (ACE)* demonstrated that *T. palmata* (TPF20) significantly enhanced microbial diversity, bringing it closer to baseline levels observed

in healthy controls. These findings are consistent with previous studies on microbiome-modulating therapies, which often report a restoration of microbial richness following treatment with probiotics or plant-based therapies. The improvement in species evenness, as measured by indices like the *Shannon* and *Simpson's Evenness*, further highlights the potential of *T. palmata* in promoting a more balanced microbial community. The *Gini Index* also supported these findings, indicating a shift towards a more even distribution of species, which is often associated with improved gut health. Thus, these results underscore the therapeutic potential of *T. palmata* as a microbiome-targeted intervention for UC. By restoring gut microbiota balance, particularly by enhancing beneficial taxa and suppressing pathogenic overgrowth, *T. palmata* may offer a natural, sustainable alternative to traditional pharmacological treatments. The effects observed in this study, particularly the restoration of *Faecalibacterium prausnitzii* and *Bacteroides fragilis*, suggest that *T. palmata* could help alleviate UC symptoms by promoting anti-inflammatory mechanisms and enhancing gut barrier integrity.

However, while the results from this animal model are promising, clinical studies in humans are necessary to confirm these findings and determine the optimal dosage, treatment duration, and safety profile of *T. palmata*. Additionally, metagenomic and metabolomic analyses could provide deeper insights into the specific microbial pathways and metabolites influenced by *T. palmata*, which may further guide therapeutic strategies for UC.

6.6 KEY FINDINGS

➤ Dysbiosis in the TNBS-Induced UC Model

- TNBS-induced ulcerative colitis (UC) caused significant dysbiosis.
- There was a reduced abundance of beneficial gut microbial phyla, including Firmicutes and Bacteroidetes, which are vital for gut health and production of anti-inflammatory short-chain fatty acids (SCFAs).
- A notable increase in Proteobacteria, a phylum associated with gut inflammation and UC exacerbation, was observed.

- This dysbiosis involved both compositional and functional disturbances in the gut microbiota.

➤ **Restoration of Gut Microbiota Composition by *T. palmata* Treatment**

- Treatment with *T. palmata* flower buds, particularly at the higher dose (TPF20), significantly restored microbial balance in the UC model.
- *T. palmata* increased the abundance of beneficial phyla Firmicutes and Bacteroidetes.
- It reduced the overgrowth of Proteobacteria.
- These changes are crucial for re-establishing gut homeostasis and supporting anti-inflammatory functions.

➤ **Reinforcement of Anti-Inflammatory Genera**

- A key finding was the restoration of *Faecalibacterium prausnitzii*, a genus with strong anti-inflammatory properties.
- *F. prausnitzii* supports gut barrier integrity and modulates immune responses.
- Its significant increase in TPF20-treated rats suggests that *T. palmata* exerts anti-inflammatory effects via promotion of beneficial gut microbiota, aiding in UC symptom alleviation.

➤ **Enhanced Microbial Diversity with *T. palmata* Treatment**

- *T. palmata* treatment significantly enhanced alpha diversity of the gut microbiota.
- Indices such as Chao1 and Abundance-based Coverage Estimator (ACE) showed increased species richness and diversity.

- The microbial composition shifted closer to that of healthy control animals.
- Higher diversity is associated with a more resilient gut environment, improved immune regulation, and reduced risk of inflammatory conditions like UC.

➤ **Improved Species Evenness in Treated Groups**

- Beyond richness, *T. palmata* improved species evenness.
- Indices such as the Shannon index, Simpson's Evenness, and the Gini Index reflected a more balanced microbial community.
- In TNBS-induced UC rats, certain pathogenic species dominated, leading to uneven communities.
- After *T. palmata* treatment (especially TPF20), species distribution became more even, indicating a healthier microbiome that better supports gut health and immune function.

➤ **Restoration of Specific Beneficial Species**

- Besides *Faecalibacterium prausnitzii*, *T. palmata* also restored other beneficial species such as *Bacteroides fragilis*.
- These species are important for maintaining gut barrier integrity and modulating immune responses.
- Their restoration suggests that *T. palmata* not only increases microbial diversity but also enhances the functional capacity of the gut microbiota to alleviate UC-related inflammation and tissue damage.

➤ **Potential for *T. palmata* as a Therapeutic Agent for UC**

- Overall, the study indicates that *T. palmata* has strong potential as a microbiome-targeted therapeutic for UC.
- It restores gut microbial balance, increases beneficial and anti-inflammatory species, and promotes anti-inflammatory effects.
- *T. palmata* may serve as a novel, natural alternative to conventional UC treatments.
- Its ability to modulate gut microbiota composition without typical pharmacological side effects makes it a promising candidate for future clinical research and UC management.

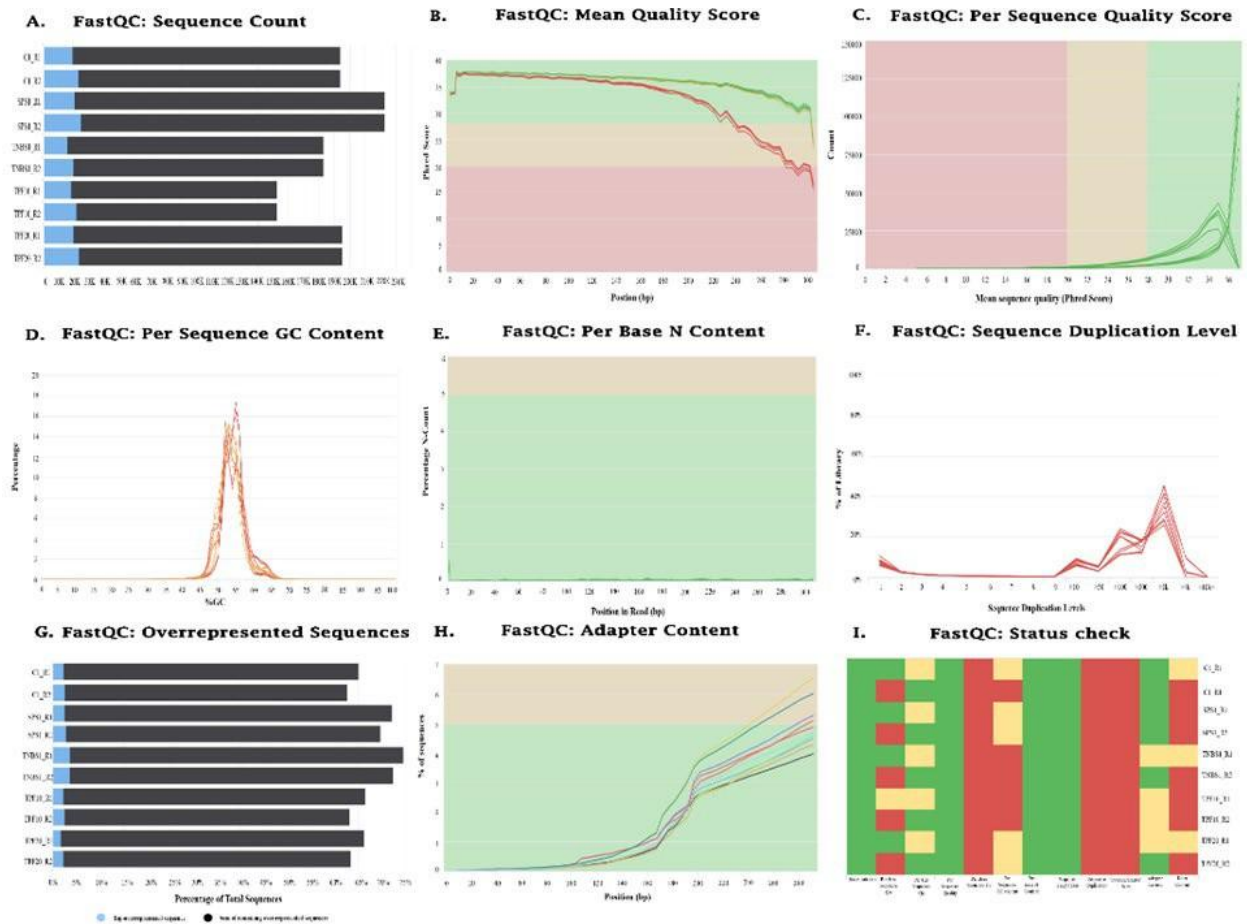


Figure 6.1 MultiQC report on the fecal composition of swiss albino female rats. **A** (Sequence count), **B** (Mean Quality Score), **C** (Per sequence quality score), **D** (Per sequence GC content), **E** (Per base N content), **F** (Sequence duplication level), **G** (Overrepresented sequences), **H** (Adapter content), **I** (Status check).

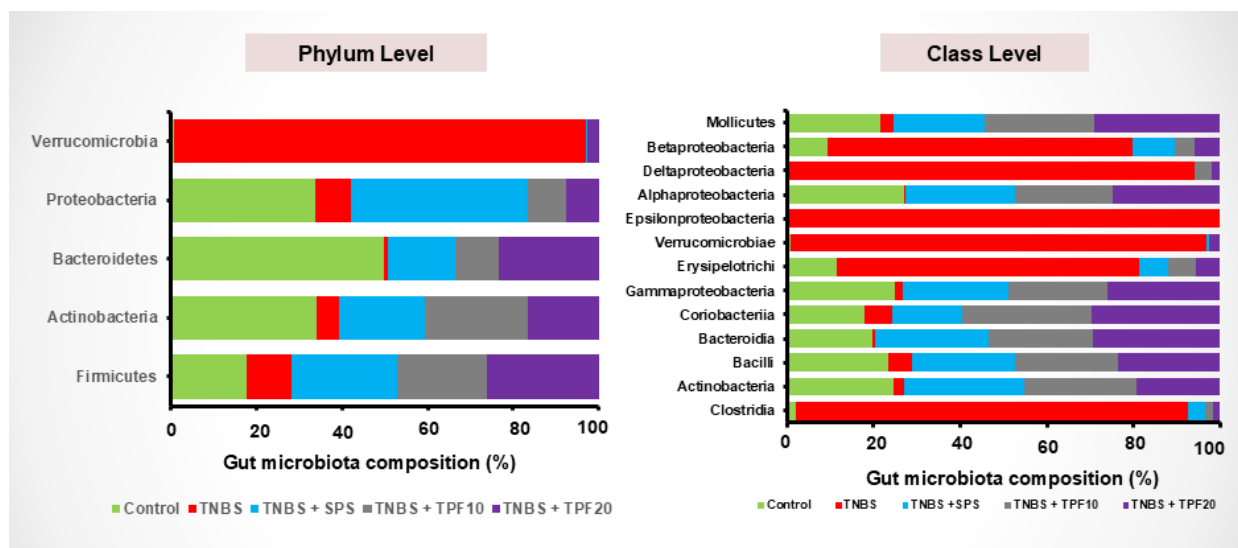


Figure 6.2 Bar graph showing taxonomic distributions at the (a) Phylum level, (b) Class level

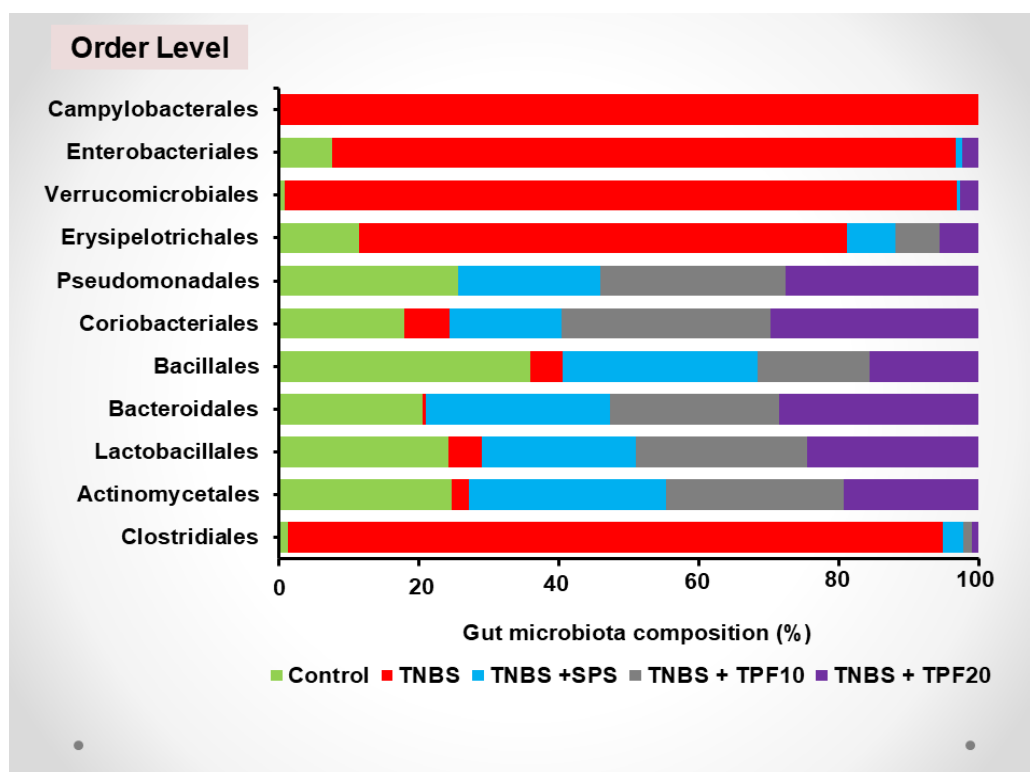


Figure 6.3 Bar graph showing taxonomic distributions at the order level

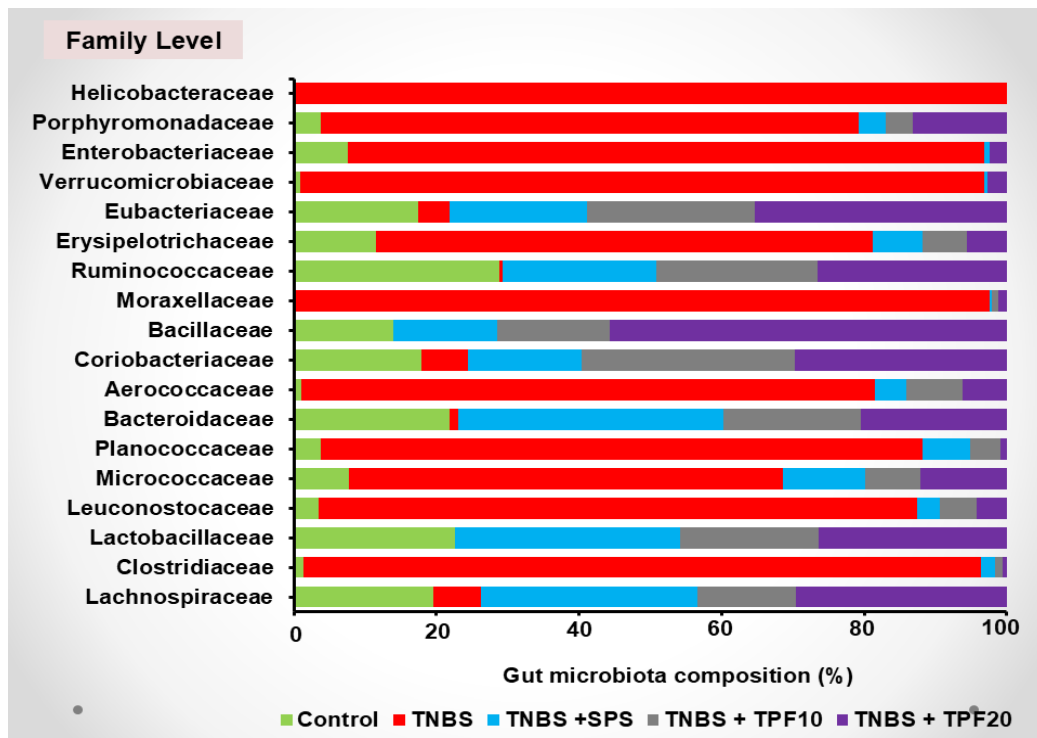


Figure 6.4 Bar graph showing taxonomic distributions at the family level

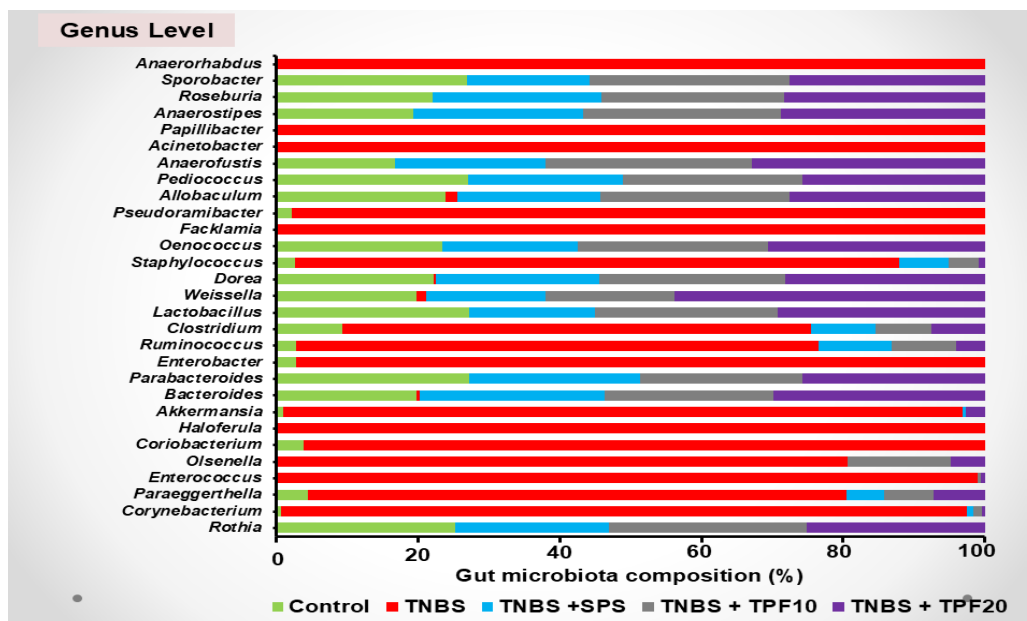


Figure 6.3 Bar graph showing taxonomic distributions at the order level

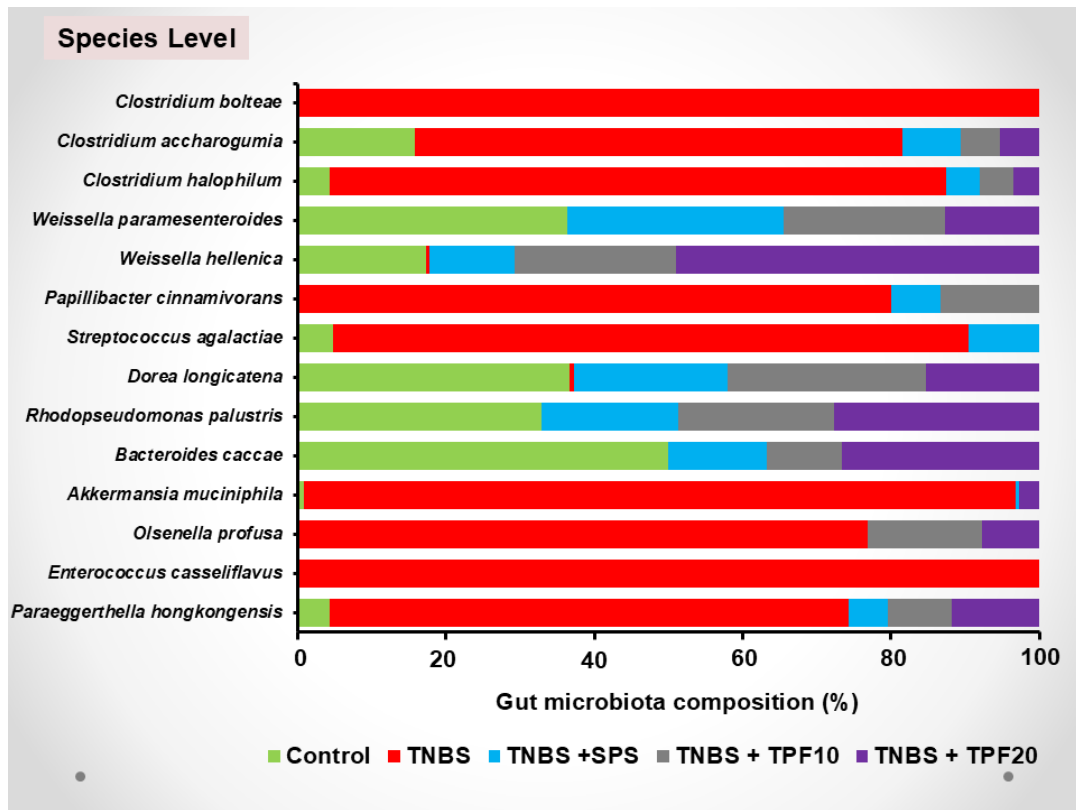


Figure 6.6 Bar graph showing taxonomic distributions at the species level

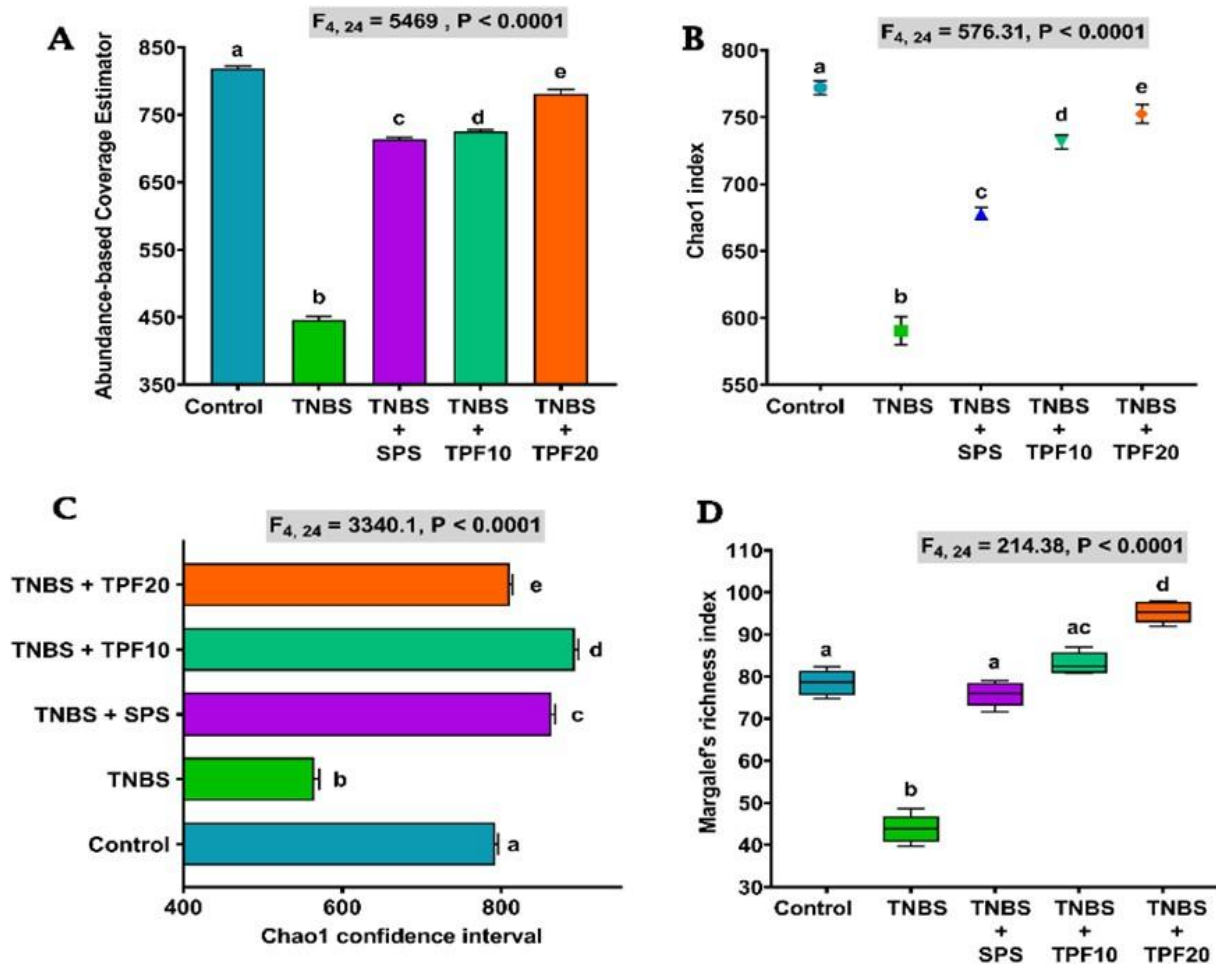


Figure 6.7 The *Trevesia palmata* flower buds modulates the species richness of gut microbiota. A (Abundance-based coverage), B (Chao1 index), C (Chao1 confidence interval), D (Margalef's richness index).

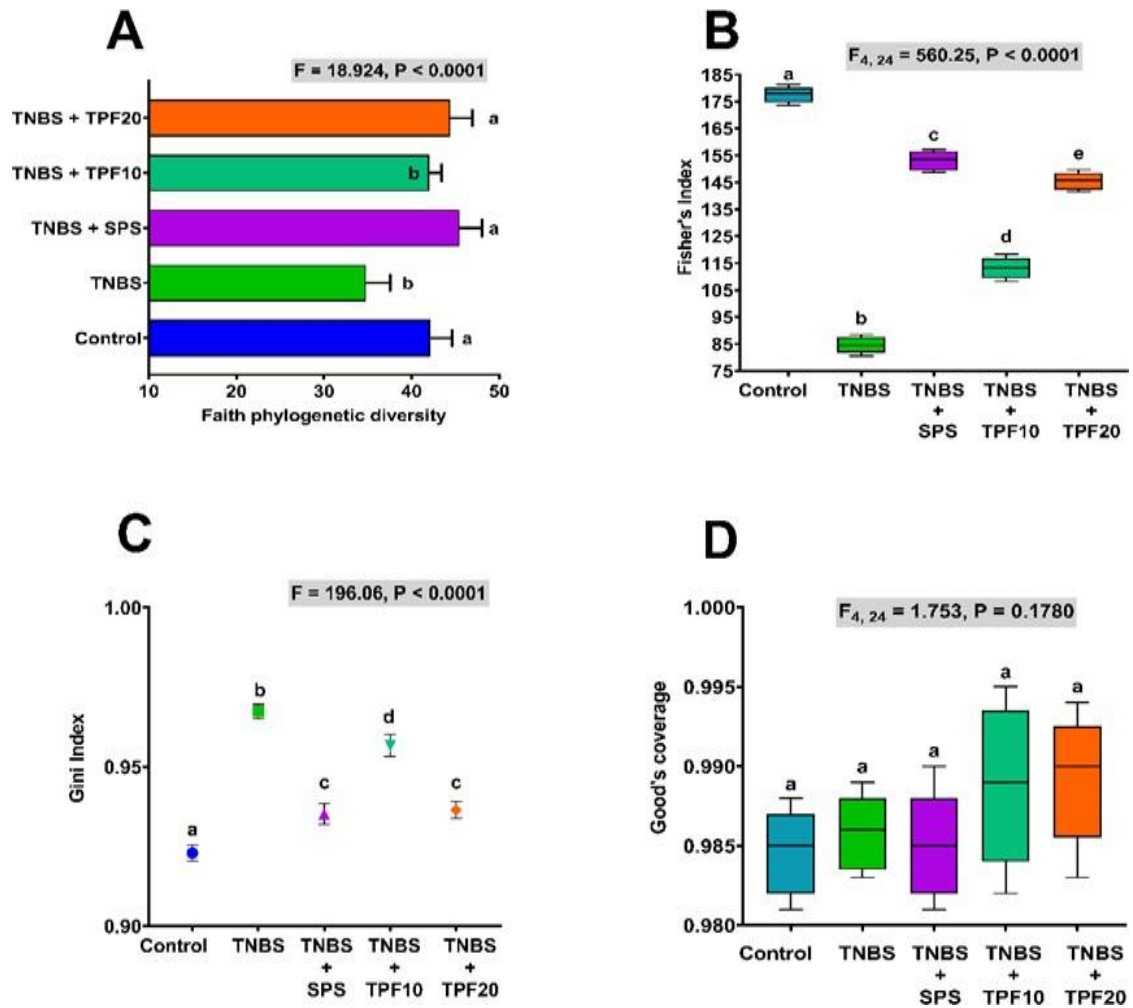


Figure 6.8 The *Trevesia palmata* flower buds modulates the species abundance of gut microbiome. A (Faith phylogenetic diversity), B (Fisher's Index), C (Gini Index), D (Good's coverage)

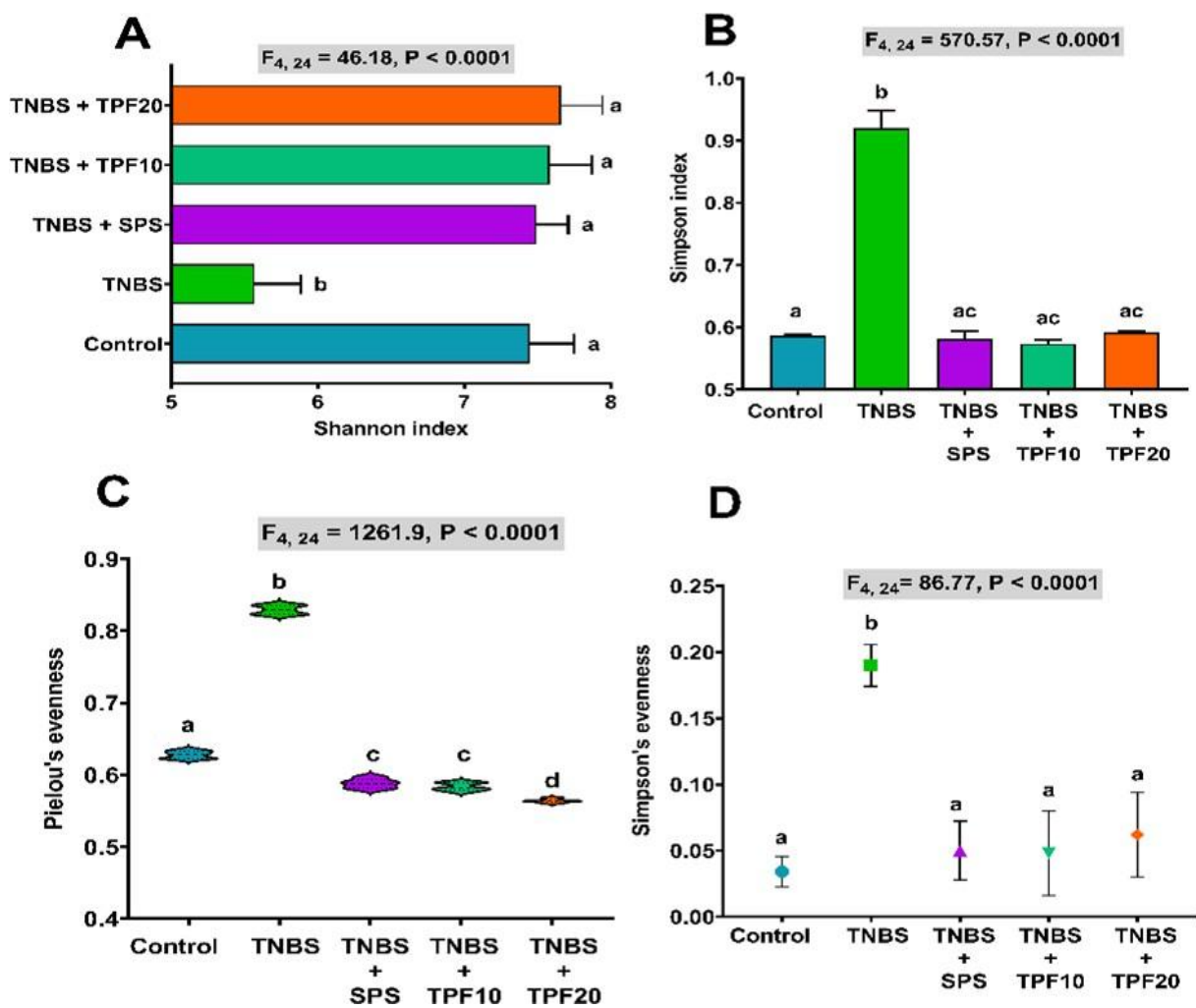


Figure 6.9 The *Trevesia palmata* flower buds modulates the species evenness of gut microbiome. A (Shannon index), B (Simson index), C (Pielou's evenness), D (Simpson's evenness).

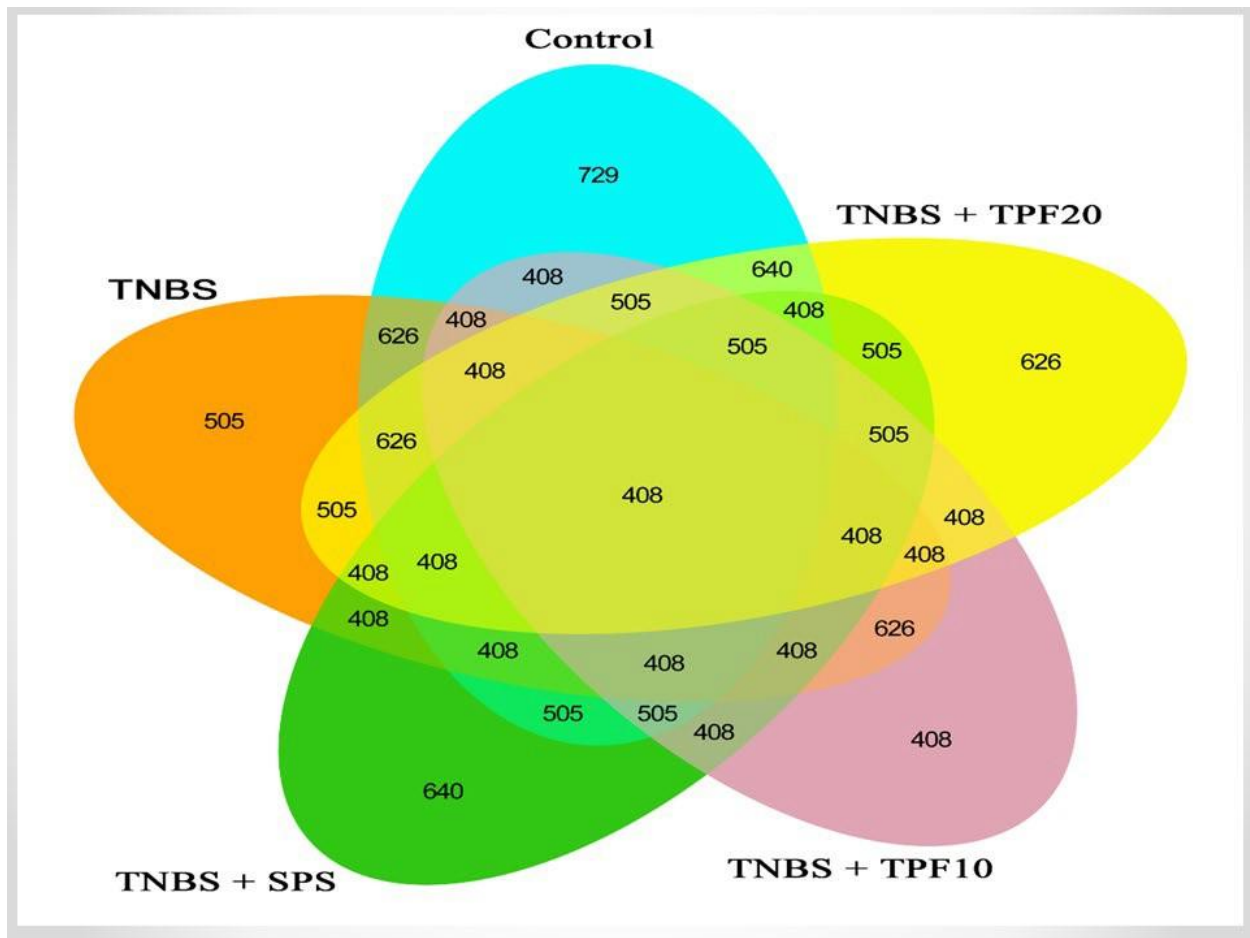


Figure 6.10 Venn Diagram operational taxonomic unit (OUT) of Microbial Overlap Across Experimental Groups

7.1 SUMMARY

The thesis systematically explores *Trevesia palmata* flower buds as a natural therapeutic for ulcerative colitis (UC), progressing from phytochemical and antioxidant profiling (Ch. 2) to in-silico target validation (Ch. 3), safety/toxicological evaluation (Ch. 4), in-vivo anti-colitic efficacy (Ch. 5), and finally microbiome-level metagenomic insights (Ch. 6).

- Methanolic extraction of *T. palmata* flower buds yielded a chemically rich crude extract (TPFME) containing diverse secondary metabolites as confirmed by GC-MS, LC-MS, FTIR and NMR profiling.
- Qualitative screening established the presence of alkaloids, cardiac glycosides, steroids, saponins, tannins, terpenoids and phlobatannins, supporting the ethnomedicinal use of the plant.
- Quantitative assays revealed appreciable levels of total phenolics, flavonoids and tannins, indicating strong potential for antioxidant and anti-inflammatory activity.
- NMR and FTIR spectra indicated complex aromatic and aliphatic proton environments, confirming the presence of multiple functional groups (aromatic rings, oxygenated moieties) relevant to bioactivity.
- Micronutrient analysis showed high potassium content with moderate magnesium and calcium, and trace levels of other essential elements (Mn, Na, Zn, Fe, Cu, Co), suggesting additional nutritional value.
- In-vitro antioxidant assays (DPPH, ABTS, superoxide, nitric oxide, reducing power, iron chelation) demonstrated dose-dependent free radical scavenging and antioxidant activity, with DPPH IC₅₀ of ~161 µg/mL for TPFME compared with ~143 µg/mL for ascorbic acid.
- A broad panel of identified TPFME phytocompounds was evaluated using PASS, ADMET, and toxicity modelling, revealing strong predicted anti-inflammatory, antioxidant and anti-ulcer activities, particularly for flavonoids such as isorhamnetin and kaempferol.

- ProTox-3 and related tools indicated that most phytoconstituents fall in low-toxicity classes (IV–V), with $LD_{50} \geq 5000$ mg/kg for key flavonoid glycosides, and minimal predicted organ toxicity, mutagenicity or carcinogenicity—overall suggesting a favourable safety landscape compared with sulfasalazine.
- Docking studies against COX-2 and KEAP1 showed that isorhamnetin and kaempferol exhibit stronger binding affinities and richer interaction networks than sulfasalazine, supporting a dual-target mechanism (COX-2 inhibition and KEAP1/Nrf2 modulation) relevant to UC pathophysiology.
- 100-ns molecular dynamics simulations confirmed stable binding of isorhamnetin and kaempferol in both COX-2 and KEAP1 active sites, with low RMSD/RMSF, stable Rg/SASA and persistent hydrogen bonding, indicating structurally robust ligand–protein complexes under dynamic conditions.
- Toxicity-ADMET-AI profiling showed minimal nuclear receptor and stress-response pathway activation for major phytochemicals relative to sulfasalazine, further highlighting their suitability for chronic use.
- Acute oral toxicity testing (OECD 423) established LD_{50} values of ~4400 mg/kg (males) and ~4200 mg/kg (females), classifying TPFME as “slightly toxic” but with a wide safety margin relative to projected therapeutic doses.
- Up to 3000 mg/kg in the acute study, there were no treatment-related changes in clinical signs, body weight, food and water intake or rectal temperature, indicating good short-term tolerability.
- No significant alterations were observed in absolute and relative organ weights (liver, kidney, brain, heart, spleen, colon, stomach, pancreas, lungs, reproductive organs), suggesting absence of gross organ toxicity at tested doses.
- Haematological evaluation revealed mostly normal profiles, with only modest increases in RBC, WBC and neutrophil counts at higher doses, all remaining within physiological ranges, indicating no clinically relevant haematotoxicity.
- Histopathological examination of liver, kidney, brain, colon, spleen, reproductive organs and other tissues showed no structural damage,

inflammation or degenerative changes in treated groups compared with controls.

- Intracolonic TNBS administration produced severe ulcerative colitis, evidenced by rectal bleeding, diarrhoea, body-weight loss, shortened colon length, increased colon weight/length ratio, high macroscopic damage scores, and marked histological disruption (crypt loss, inflammatory infiltration, goblet-cell depletion).
- Standard sulfasalazine (SPS50) offered only partial protection, with moderate improvement in macroscopic and histological indices but incomplete restoration of normal colon architecture and function.
- TPF at 10 and 20 mg/kg (TPF10, TPF20) produced dose-dependent amelioration of UC symptoms: reduced rectal bleeding, improved stool consistency, better body-weight trajectory and lower disease activity index (DAI) scores, with TPF20 showing near-normal clinical status.
- Morphometric and macroscopic endpoints (colon length, colon weight/length ratio, damage scores) confirmed that TPF20 markedly preserved colon structure, reduced oedema and ulceration, and outperformed sulfasalazine in overall tissue protection.
- Histology demonstrated that TPF, particularly at 20 mg/kg, restored crypt architecture, reduced inflammatory cell infiltration and preserved goblet cells, indicating structural and functional healing of the mucosa.
- Biochemical assays showed that TPF20 significantly reduced lipid peroxidation (MDA) and restored antioxidant defences (SOD, GSH, CAT) more effectively than sulfasalazine, confirming strong in-vivo antioxidant action consistent with Chapter 2.
- Inflammatory markers including MPO activity, IL-1 β , IL-6 and LTB₄ were markedly elevated in TNBS rats and were significantly suppressed by TPF, with the higher dose achieving the greatest reduction, highlighting potent anti-inflammatory effects.

- High-quality metagenomic sequencing (validated by MultiQC metrics) enabled robust taxonomic and diversity analysis of gut microbiota across experimental groups.
- TNBS-induced UC produced pronounced dysbiosis, with reduced Firmicutes and Bacteroidetes and increased Proteobacteria, along with depletion of beneficial families (Lachnospiraceae, Ruminococcaceae) and expansion of pathogenic Enterobacteriaceae.
- TPF treatment, especially TPF20, restored the Firmicutes/Bacteroidetes balance, reduced Proteobacteria overgrowth and normalised key orders (Clostridiales, Bacteroidales) and classes (Clostridia, Bacteroidia), pointing to re-establishment of a health-associated microbial configuration.
- At the genus/species level, TPF20 significantly replenished anti-inflammatory taxa such as *Faecalibacterium prausnitzii* and *Bacteroides fragilis*, known for producing SCFAs and supporting barrier integrity and immune regulation.
- Alpha-diversity indices (Chao1, ACE, Shannon, Simpson's evenness, Gini index) indicated that TPF increased species richness and evenness, shifting the community profile closer to healthy controls and reducing dominance of pathogenic species.
- These microbiome shifts align with the clinical and biochemical improvements observed in Chapter 5, suggesting that TPF exerts its anti-colitic effects partly via microbiome-mediated mechanisms enhancing beneficial SCFA-producing bacteria, dampening inflammatory taxa and promoting overall ecosystem resilience

8.1 CONCLUSION

This study systematically evaluated the therapeutic potential of *Trevesia palmata* flower-bud methanolic extract (TPFME) against ulcerative colitis using an integrated framework spanning phytochemistry, in-silico modelling, toxicity assessment, in-vivo efficacy and gut microbiome analysis. Phytochemical profiling and in-vitro assays established that *T. palmata* flower buds are rich in phenolics, flavonoids and tannins, with strong, dose-dependent antioxidant activity, supporting their ability to counteract oxidative stress-driven mucosal injury. In-silico studies highlighted isorhamnetin and kaempferol as key constituents with favorable PASS/ADMET properties and low predicted toxicity, and demonstrated stable, high-affinity interactions with COX-2 and KEAP1, consistent with combined anti-inflammatory and Nrf2-mediated antioxidant mechanisms.

Toxicity studies in Wistar rats showed a wide safety margin, with LD₅₀ values in the low-toxicity range and no relevant adverse effects at doses exceeding those used therapeutically. In the TNBS-induced ulcerative colitis model, TPF at 10 and 20 mg/kg afforded clear, dose-dependent protection, improving disease activity, colon morphology and histology, while reducing lipid peroxidation, restoring endogenous antioxidants (SOD, CAT, GSH) and suppressing MPO and pro-inflammatory cytokines, often comparable to or better than sulfasalazine. Metagenomic profiling further revealed that TPFME partially reversed TNBS-induced dysbiosis by normalising the Firmicutes/Bacteroidetes balance, limiting Proteobacteria expansion and enriching beneficial genera such as *Faecalibacterium* and *Bacteroides*.

Overall, TPFME is phytochemically rich, mechanistically supported, safe in acute and sub-acute settings, efficacious in experimental ulcerative colitis, and capable of correcting colitis-associated dysbiosis. These integrated findings support its further development as a microbiome-modulating, plant-based therapeutic or adjuvant for ulcerative colitis, warranting future work on fractionation of active constituents, chronic and relapse models, and eventual clinical translation.

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Educational Records (from Matriculation Onwards)

Sl. No	Class/Degree	Subject	Year of Passing	Board/University
1	HSLC	English, Bengali, Mathematics, Science, Social Science and Advanced Mathematics	2012	SEBA
2	HS	English, Mathematics, Chemistry, Biology, Physics, MIL (Bengali)	2014	AHSEC
3	B.Sc.	Zoology	2017	Assam University
4	PGDCA	Computer modules	2018	ASIAN INFOTECH
5	M.Sc.	Zoology	2020	Mizoram University

LIST OF CONFERENCE/WORKSHOP/SEMINAR ATTENETED

1. Poster presentation at the International Conference on **“Current Trends In Biological Sciences”** organised by the School of Life Sciences, Mizoram University, Aizawl, Mizoram on the 18-20 March 2024.
2. Oral presentation at the International Conference on **“Endocrinology, Metabolism and Reproduction: Exploring New Frontiers”** organised by the Department of Zoology, University of Jammu, Jammu (UT J&K), India on the 22-25 October 2024.
3. Participated in the National Workshop on **“Molecular phylogeny and molecular docking”** organized by the Department of Zoology, Mizoram University in January, 2021.
4. Participated in the National Workshop on **“Sequence to Database & Database to sequence”** organized by the Department of Zoology, Mizoram University in January, 2021.
5. Participated in the National Workshop on **“Bee keeping and management”** organized by the Department of Zoology, Mizoram University in January, 2021.
6. Participated in the National Workshop on **“Statistical data analysis and interpretation”** organized by the Department of Zoology, Mizoram University in January, 2021.
7. Participated in the National Workshop on **“Basic and advanced molecular techniques”** organized by the Department of Zoology, Mizoram University in January, 2021.
8. Participated in the International Workshop on **“The Value and Interconnections of Human, Animal, Plant and Microorganism: Metagenomics and Metagenomics Approach”** organized by the Department of Zoology, Mizoram University in January, 2023.
9. Participated in the International Workshop on **“Nanopore Sequencing and Data Analysis: Opportunities for Rapid Biodiversity and Biosurveillance Programs and Local Capacity Buildings”** organized by the Department of Zoology, Mizoram University in January, 2023.

10. Participated in the International Workshop on **“Introduction to data visualization using R-Studio for Biologist”** organized by the Department of Zoology, Mizoram University in January, 2023.

LIST OF RESEARCH PUBLICATION

1. Bidanchi, R. M., Lalrindika, L., Khushboo, M., Bhanushree, B., Dinata, R., Das, M., Nisa, N., Lalrinzuali, S., Manikandan, B., **Saeed-Ahmed, L.**, Sanjeev, S., Murthy, M. K., Roy, V. K., & Gurusubramanian, G. (2022). Antioxidative, anti-inflammatory and anti-apoptotic action of ellagic acid against lead acetate induced testicular and hepato-renal oxidative damages and pathophysiological changes in male Long Evans rats. *Environmental pollution (Barking, Essex : 1987)*, 302, 119048. <https://doi.org/10.1016/j.envpol.2022.119048>
2. Nisa, N., Rasmita, B., Arati, C., Uditraj, C., Siddhartha, R., Dinata, R., Bhanushree, B., Bidanchi, R. M., Manikandan, B., **Laskar, S. A.**, Abinash, G., Pori, B., Roy, V. K., & Gurusubramanian, G. (2023). Repurposing of phyto-ligand molecules from the honey bee products for Alzheimer's disease as novel inhibitors of BACE-1: small molecule bioinformatics strategies as amyloid-based therapy. *Environmental science and pollution research international*, 30(17), 51143–51169. <https://doi.org/10.1007/s11356-023-25943-4>
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acid levels mediates oxidative stress, inflammation, redox imbalance, germ cell apoptosis, disruption of steroidogenesis, and testicular dysfunction in Wistar rats. *Environmental science and pollution research international*, 30(18), 52446–52471. <https://doi.org/10.1007/s11356-023-26018-0>

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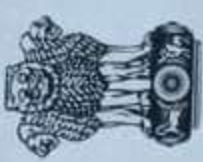
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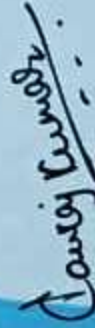


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Antioxidative, anti-inflammatory and anti-apoptotic action of ellagic acid against lead acetate induced testicular and hepato-renal oxidative damages and pathophysiological changes in male Long Evans rats^{*}

Rema Momin Bidanchi, Lalrinsanga Lalrindika, Maurya Khushboo, Baishya Bhanushree, Roy Dinata, Milirani Das, Nisekhoto Nisa, Sailo Lalrinzuali, Bose Manikandan, Laskar Saeed-Ahmed, Sanasam Sanjeev, Meesala Krishna Murthy, Vikas Kumar Roy, Guruswami Gurusubramanian^{*}

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ABSTRACT

Lead (Pb), is an environmental toxicant, causes multi-organ dysfunction including reproductive impairments. This study designed to investigate the prospective antioxidative, anti-inflammatory and anti-apoptotic effects of ellagic acid (EA) on Pb-mediated testicular and hepato-renal toxicity. Four experimental groups of five male Long-Evans rats each were used: control, Pb (60 mg/kg), EA (30 mg/kg), and Pb + EA groups. All groups were given their respective treatment orally for 30 days. Pb exposure altered body and organs weight, food and water consumption, rectal temperature, Pb residue levels in tissues, liver and kidney function, sperm quality parameters, serum metabolic and hematology profiles, and impaired the oxidative/antioxidative balance in the testicular and hepato-renal tissue, as shown by the decreased antioxidant proteins (superoxide dismutase, catalase, glutathione peroxidase, and reduced glutathione) and increased the oxidative (MDA, lipid hydroperoxides, conjugated dienes, protein carbonyl, fragmented DNA and GSH:GSSG ratio) stress and inflammatory (IL-1, IL-6, TNF- α , prostaglandin, LTB₄, NO, myeloperoxidase, LDH) markers. Moreover, a dysregulation in the stress response (HSP-70) and apoptotic-regulating proteins (BAX, BCL-2, and active Caspase-3) were recorded upon Pb exposure. Remarkably, EA oral administration reduced the Pb residue levels in tissues, improved the liver and kidney function, revived the spermatogenesis and sperm quality, restored redox homeostasis, suppressed the oxidative stress, inflammatory and apoptotic responses in the liver, kidney and testis tissue. Our findings point out that EA can be used as a phyto-chelator to overcome the adverse effects of Pb exposure due to its potent antioxidant, anti-inflammatory, and anti-apoptotic effects.

1. Introduction

Lead (Pb) has become a global health hazard (0.6%) because of exposure to animals and human beings with ingestion of feed and food, inhalation of industrial emissions, food chain, environment, and water resources (Ericson et al., 2016; Rehman et al., 2018). Globally, blood lead levels of 1 in 3 children (800 million) are estimated to be $\geq 5 \mu\text{g/dL}$ and over 275 million children in India suffer mild to severe effects of lead poisoning (Rees and Fuller, 2020). Pb is absorbed by the duodenum via DMT1 (divalent metal transporter 1), combined with erythrocyte

protein and later dispensed to tissues and organs (García-Niño and Pedraza-Chaverri, 2014). One third of the total absorbed Pb is stored in the liver due to hepatic conjugation, followed by the kidney and finally the residual quantity is accumulated in various tissues and organs causing biomolecules (DNA, lipid, protein, RNA) injury, cell damage and cell death (Flora et al., 2006; Chen et al., 2019). Pb is multi-organ cumulative toxicants that cause oxidative damage, hemato-biochemical alterations (Al-Omair et al., 2017) and interrupts organ functions causing various metabolic disorders (Caito et al., 2017; Gandhi et al., 2017; Park et al., 2019). Inflammation and oxidative stress that play a

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Repurposing of phyto-ligand molecules from the honey bee products for Alzheimer's disease as novel inhibitors of BACE-1: small molecule bioinformatics strategies as amyloid-based therapy

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Abstract

Alzheimer's disease (AD) is one of the neurodegenerative diseases, manifesting dementia, spatial disorientation, language, cognitive, and functional impairment, mainly affects the elderly population with a growing concern about the financial burden on society. Repurposing can improve the traditional progress of drug design applications and could speed up the identification of innovative remedies for AD. The pursuit of potent anti-BACE-1 drugs for AD treatment has become a hot boiler topic in the recent past and to instigate the design of novel improved inhibitors from the bee products. Drug-likeness characteristics (ADMET: absorption, distribution, metabolism, excretion, and toxicity), docking (AutoDock Vina), simulation (GROMACS), and free energy interaction (MM-PBSA, molecular mechanics Poisson–Boltzmann surface area) analyses were performed to identify the lead candidates from the bee products (500 bioactives from the honey, royal jelly, propolis, bee bread, bee wax, and bee venom) for Alzheimer's disease as novel inhibitors of BACE-1 (beta-site amyloid precursor protein cleaving enzyme (1) receptor using appropriate bioinformatics tools. Forty-four bioactive lead compounds were screened from the bee products through high throughput virtual screening on the basis of their pharmacokinetic and pharmacodynamics characteristics, showing favorable intestinal and oral absorption, bioavailability, blood brain barrier penetration, less skin permeability, and no inhibition of cytochrome P450 inhibitors. The docking score of the forty-four ligand molecules was found to be between −4 and −10.3 kcal/mol, respectively, exhibiting strong binding affinity to BACE 1 receptor. The highest binding affinity was observed in the rutin (−10.3 kcal/mol), 3,4-dicaffeoylquinic acid (−9.5 kcal/mol), nemorosone (−9.5 kcal/mol), and luteolin (−8.9 kcal/mol). Furthermore, these compounds demonstrated high total binding energy −73.20 to −105.85 kJ/mol, and low root mean square deviation (0.194–0.202 nm), root mean square fluctuation (0.0985–0.1136 nm), radius of gyration (2.12 nm), number of H-bonds (0.778–5.436), and eigenvector values (2.39–3.54 nm²) in the molecular dynamic simulation, signifying restricted motion of Cα atoms, proper folding and flexibility, and highly stable with compact of the BACE1 receptor with the ligands. Docking and simulation studies concluded that rutin, 3,4-dicaffeoylquinic acid, nemorosone, and luteolin are plausibly used as novel inhibitors of BACE1 to combat AD, but further in-depth experimental investigations are warranted to prove these in silico findings.

Keywords ADMET · Alzheimer's disease · BACE1 · Honeybee products · Repurposing · Molecular dynamics

Nisekhoto Nisa and Vikas Kumar Roy contributed equally to this work.

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Introduction

Alzheimer's disease (AD) is an irreversible progressive neurodegenerative disorder leading to deterioration in cognitive functions and changes in behavior and personality (Martins et al. 2018). AD causes cognitive and functional impairment, including dementia and disability in the older people and it is documented as a public health precedence by the WHO (Scott et al. 2016; WHO 2021). Aging is the main

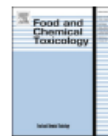
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Dietary phytoestrogen diosgenin interrupts metabolism, physiology, and reproduction of Swiss albino mice: Possible mode of action as an emerging environmental contaminant, endocrine disruptor and reproductive toxicant

Maurya Khushboo, Sanasam Sanjeev, Meesala Krishna Murthy, Maibam Sunitadevi, Roy Dinata, Baishya Bhanushree, Rema Momin Bidanchi, Nisekhot Nisa, Sailo Lalrinzuali, Bose Manikandan, Ahmed-Laskar Saeed, Giri Abinash, Buragohain Pori, Chettri Arati, Vikas Kumar Roy, Guruswami Gurusubramanian^{*}

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Steroidogenesis
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ABSTRACT

Dietary phytoestrogens are the main source of environmental contamination due to their estrogen-mimicking and endocrine-disrupting effects, posing a threat to microbial, soil, plant, and animal health. Diosgenin, a phytosteroid saponin, is used in many traditional medicines, nutraceuticals, dietary supplements, contraceptives, and hormone replacement therapies against numerous diseases and disorders. It is important to be aware of the potential risks associated with diosgenin, as well as its potential to cause reproductive and endocrine toxicity. Due to the lack of research on the safety and probable adverse side effects of diosgenin, this work evaluated the endocrine-disrupting and reproductive toxicity of diosgenin in albino mice by following acute toxicity (OECD-423), repeated dose 90-day oral toxicity (OECD-468), and F₁ extended one-generation reproductive toxicity (OECD-443) studies. Diosgenin was found to be slightly toxic, with LD₅₀ for male and female mice being 546.26 and 538.72 mg/kg, respectively. Chronic exposure of diosgenin (10, 50, 100, and 200 mg/kg) generated oxidative stress, depleted antioxidant enzymes, disturbed homeostasis of the reproductive hormones, and interrupted steroidogenesis, germ cell apoptosis, gametogenesis, sperm quality, estrous cycle, and reproductive performance in the F₀ and F₁ offspring. Long-term oral exposure of diosgenin to the mice disturbed the endocrine and reproductive functions and generated transgenerational reproductive toxic effects in F₀ and F₁ offspring. These results suggest that diosgenin should be used carefully in food products and medical applications due to its potential endocrine-disrupting and reproductive toxic effects. The findings of this study provide a better understanding of the potential adverse effects of diosgenin and the need for appropriate risk assessment and management of its use.

1. Introduction

Phytoestrogens are plant-derived polyphenolic compounds found in various foods that demonstrate a structural resemblance to endogenous human hormones and have numerous health benefits. With their promising recognition as a drug for hormonal replacement therapy, contraceptives, nutraceuticals, functional foods, and dietary

supplements, their consumption has been augmented worldwide to the tune of 15–200 mg, especially in the Asian region (Rietjens et al., 2017).

Preclinical studies have evidenced that the intake of phytoestrogens may affect the physiology (hormones and health), and reproduction in humans depending on the exposure (phytoestrogen category, milieu-food/capsule, dose, and bioavailability), race, hormone proportion (associated with age, sex, and physiological status), and health status of

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Long-term consumption of fermented pork fat-based diets differing in calorie, fat content, and fatty acid levels mediates oxidative stress, inflammation, redox imbalance, germ cell apoptosis, disruption of steroidogenesis, and testicular dysfunction in Wistar rats

Sailo Lalrinzuali¹ · Maurya Khushboo¹ · Roy Dinata¹ · Baishya Bhanushree¹ · Nisekphoto Nisa¹ · Rema Momin Bidanchi¹ · Saeed-Ahmed Laskar¹ · Bose Manikandan¹ · Giri Abinash¹ · Buragohain Pori¹ · Vikas Kumar Roy¹ · Guruswami Gurusubramanian¹

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Abstract

There is a dearth of experimental evidence available as to whether the consumption of fermented pork fat (FPF) food has any harmful effects on metabolism and reproduction due to its excessive calories, high fat content, and fatty acid methyl ester (FAME) levels. We hypothesized that exposure to a FPF-diet with excessive calories, a high fat content, and high FAME levels alters testicular physiology and metabolism, leading to permanent damage to the testicular system and its function. Thirteen-week-old male rats ($n=20$) were assigned to a high-calorie, high-fat diet (FPF-H, fat-60%, 23 kJ/g), a moderate-calorie, moderate-fat diet (FPF-M, fat-30%, 17.5 kJ/g), a low-calorie and low-fat diet (FPF-L, fat-15%, 14.21 kJ/g) compared to the standard diet (Control, fat-11%, 12.56 kJ/g) orally for 90 days. GC–MS analysis of the three FPF-diets showed high quantities of saturated fatty acids (SFAs) and polyunsaturated fatty acids- $\omega 6$ (PUFA- $\omega 6$) and low levels of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids- $\omega 3$ (PUFA- $\omega 3$) compared to the control diet. Consequently, the levels of serum FAMES of the FPF-diet fed rats were significantly increased. In addition, a high level of n-6:n-3 PUFA towards PUFA- $\omega 6$ was observed in the serum of FPF-diet fed rats due to the high content of linoleic, γ -linolenic, and arachidonic acid. Long-term consumption of FPF-diets disturbed the anthropometrical, nutritional, physiological, and metabolic profiles. Furthermore, administration of FPF-diets generated metabolic syndrome (dyslipidemia, leptinemia, insulin resistance, obesity, hepato-renal disorder and function), increased the cardiovascular risk factors, and triggered serum and testis inflammatory markers (interleukin-1 $\uparrow$, interleukin-6 $\uparrow$, interleukin-10 $\downarrow$, leukotriene B4 $\uparrow$, prostaglandin $\uparrow$, nitric oxide $\uparrow$, myeloperoxidase $\uparrow$, lactate dehydrogenase $\uparrow$, and tumor necrosis factor- α $\uparrow$). Activated testis oxidative stress (conjugated dienes $\uparrow$, lipid hydroperoxides $\uparrow$, malondialdehyde $\uparrow$, protein carbonyl $\uparrow$, and fragmented DNA $\uparrow$) and depleted antioxidant reserve (catalase $\downarrow$, superoxide dismutase $\downarrow$, glutathione S-transferase $\downarrow$, reduced glutathione $\downarrow$, glutathione disulfide $\uparrow$, and GSH:GSSG ratio $\downarrow$) were observed in FPF-diet fed rats. Disrupted testis histoarchitecture, progressive deterioration of spermatogenesis, poor sperm quality and functional indices, significant alterations in the reproductive hormones (serum and testis testosterone $\downarrow$, serum estradiol $\uparrow$, serum luteinizing hormone $\downarrow$, and follicle-stimulating hormone $\uparrow$), were noted in rats fed with FPF diets than in the control diet. Severe steroidogenic impairment (steroidogenic acute regulatory protein, StAR $\downarrow$; 3 β -hydroxysteroid dehydrogenase, 3 β -HSD $\downarrow$; and luteinizing hormone receptor, LHR $\downarrow$), deficiency in germ cells proliferation (proliferating cell nuclear antigen, PCNA $\downarrow$), and abnormally enhanced testicular germ cell apoptosis (terminal deoxynucleotidyl transferase dUTP nick end labeling, TUNEL assay $\uparrow$; B-cell lymphoma-2, BCL-2 $\downarrow$; Bcl-2-associated X protein, BAX $\uparrow$; and BAX/BCL-2 ratio $\uparrow$) were remarked in the FPF-diet administered rats in comparison with the control diet. In conclusion, the long-term feeding of an FPF-diet with excessive calories, a high fat content, and high FAME levels induced oxidative stress, inflammation, and

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Sailo Lalrinzuali, Maurya Khushboo and Vikas Kumar Roy contributed equally to this work.

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Repurposing immune boosting and anti-viral efficacy of *Parkia* bioactive entities as multi-target directed therapeutic approach for SARS-CoV-2: exploration of lead drugs by drug likeness, molecular docking and molecular dynamics simulation methods

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Physicochemical, medicinal chemistry, and ADMET characteristics of bee antimicrobial peptides as natural bio-preservatives to extend food shelf life: a roadmap for food safety regulation

Roy Dinata, Chettri Arati, Ahmed-Laskar Saeed, Bose Manikandan, Giri Abinash, Buragohain Pori, Rema Momin Bidanchi, Vikas Kumar Roy & Guruswami Gurusubramanian

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Comprehensive phytochemical characterization, antioxidant potency, and toxicological evaluation of *Paederia foetida* in Wistar albino rats: Insights into therapeutic efficacy and safety profiles

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ABSTRACT

Ethnopharmacological relevance: *Paederia foetida* has long been utilized in traditional medicine, necessitating comprehensive safety assessments and precise dosage recommendations to avert potential adverse effects in human use.

Objective: This study aimed to identify and characterize the key phytochemicals in *P. foetida*, evaluate their antioxidant and biological activities, and assess their safety through acute and sub-acute toxicity studies in a rat model, thereby establishing a scientific basis linking chemical composition, therapeutic potential, and safety.

Materials and methods: The qualitative profiling, total phenolic, flavonoid content, and antioxidant activity of aqueous (PPAE), ethanol (PFEE), and methanol (PFME) extracts were evaluated using established protocols and the PASS tool employed to predict their therapeutic potential. High-resolution liquid chromatography-mass spectrometry (HR-LC-MS) was employed for the identification and detailed characterization of bioactive constituents in the whole plant methanolic extract (PFME) of *P. foetida* based on their superior antioxidant activity. Polyphenolic compounds were quantified using high-performance liquid chromatography (HPLC). Acute (control, 500, 1000 and 2000 mg/kg, 14 days) and sub-acute (control, 500, 1000 and 1500 mg/kg, 28 days) toxicity studies were conducted on PFME at the selected doses in male and female Wistar albino rats, incorporating clinical observations, mortality tracking, hematological, biochemical, and histopathological evaluations.

Results: PFME exhibited the highest concentration of total phenols (3761.60 mg GAE/g) and flavonoids (2336.54 mg RUE/g) among the extracts analyzed. HR-LC-MS of PFME identified 36 polyphenolic compounds, with chlorogenic acid (221.84 mg/g), isoquercetin (178.47 mg/g), rutin (169.88 mg/g), scopoletin (148.99 mg/g), quinic acid (111.31 mg/g), and quercetin (71.19 mg/g) being the most abundant. Predictive analysis with the PASS tool revealed strong antioxidant, free radical scavenging, anti-inflammatory, and anti-apoptotic activities of PFME. PFME demonstrated superior antioxidant potency relative to PFEE and PPAE in assays for DPPH, ABTS^{•+}, superoxide radicals, and reducing power. Acute toxicity studies revealed no mortality at any dose level, with no significant alterations in hematological, biochemical, or histopathological profiles, confirming PFME's high safety margin with an LD₅₀ exceeding 2000 mg/kg in both male and female rats. Sub-acute toxicity studies showed no mortality at doses of 500 and 1000 mg/kg, with no significant changes in hematological, biochemical, or histological profiles of lung, brain, kidney, or liver tissues. However, at 1500 mg/kg, significant changes were observed in hematological and serum biochemical profiles, with histopathological abnormalities in liver and kidney tissues of male and female rats. Female rats showed a higher incidence of histological abnormalities.

Conclusion: The therapeutic effects of *P. foetida* are closely linked to its high levels of polyphenols, especially chlorogenic acid, isoquercetin, rutin, scopoletin, quinic acid, and quercetin. These compounds are very important for its strong antioxidant and protective effects. PFME showed superior antioxidant and free radical scavenging activity than the PFEE and PPAE. PFME exhibited a favorable safety profile, with no adverse effects detected at doses up to 2000 mg/kg in acute toxicity studies and 1000 mg/kg/day in sub-acute toxicity studies, thereby

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ABSTRACT

***TREVESIA PALMATA* ALLEVIATES INFLAMMATION AND GUT DYSBIOSIS IN ULCERATIVE COLITIS RAT MODEL**

**AN ABSTRACT SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF
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**DEPARTMENT OF ZOOLOGY
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DECEMBER, 2025**

***TREVESIA PALMATA* ALLEVIATES INFLAMMATION AND GUT
DYSBIOSIS IN ULCERATIVE COLITIS RAT MODEL**

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**In partial fulfillment of the requirement of the Degree of Doctor of Philosophy
in Zoology of Mizoram University, Aizawl.**

CHAPTER 1

General Introduction

1.1 ULCERATIVE COLITIS (UC)

1.1.1 Epidemiology

UC is a chronic inflammatory bowel disease (IBD) affecting the colon and rectum, with a rising global prevalence, particularly in high-income countries. Incidence is increasing in Asia, Africa, and South America due to urbanisation and dietary changes.

1.1.2 Pathophysiology

UC involves genetic susceptibility, epithelial barrier disruptions, microbial imbalances, and dysregulated immunity, leading to inflammation. Oxidative stress further exacerbates tissue damage.

1.1.3 Clinical Manifestations

Key symptoms include bloody diarrhea, urgency, cramping, and rectal bleeding, often with relapsing episodes. The disease typically begins in the third or fourth decade.

1.1.4 Existing Therapies & Limitations

Current treatments (5-ASA, corticosteroids, biologics) aim to induce remission but often fail due to side effects, incomplete responses, and high costs.

1.2 GUT MICROBIOTA AND UC

1.2.1 Role of Healthy Microbiota

The gut microbiota plays crucial roles in immunity, metabolism, and maintaining intestinal health, producing metabolites like SCFAs that support anti-inflammatory effects.

1.2.2 Gut Dysbiosis in UC

UC patients exhibit disrupted microbiota with reduced beneficial bacteria and increased pathogenic species, contributing to inflammation and impaired barrier function.

1.2.3 Functional Consequences

Dysbiosis leads to metabolic disruptions, including changes in SCFA production and bile acid metabolism, which further impair gut health.

1.3 TRADITIONAL MEDICINAL PLANTS AND UC

1.3.1 Overview of Medicinal Plants

Traditional plant-based remedies have been used worldwide, providing anti-inflammatory, antioxidant, and analgesic benefits, with significant bioactive compounds such as alkaloids and flavonoids.

1.3.2 Traditional Plants in Modern Medicine

Modern research validates the efficacy of medicinal plants, offering promising treatments with fewer side effects compared to synthetic drugs.

1.3.3 Medicinal Plants for UC

Plants like *Glycyrrhiza glabra*, *Curcuma longa*, and *Boswellia serrata* are being studied for their anti-inflammatory and mucosal-healing properties in UC.

1.4 *Trevesia palmata* (SNOWFLAKE ARALIA)

1.4.1 Taxonomy & Distribution

Trevesia palmata, an evergreen tree from the Araliaceae family, is native to Southeast Asia and has applications in traditional medicine.

1.4.2 Traditional Use

Its leaves and flowers are used for treating gastrointestinal issues, pain, and inflammation in various cultures across Asia.

1.4.3 Modern Medicinal Significance

The plant contains metabolites like polyphenols and saponins with potential medicinal properties, including analgesic, antihyperglycemic, and antiproliferative effects.

1.5 TOXICITY STUDIES

Comprehensive toxicity studies (acute, sub-acute, and sub-chronic) are crucial to evaluate the safety of new drugs or extracts, with methods including in vivo, in vitro, and in silico approaches.

1.6. RATIONALE OF THE STUDY

Ulcerative colitis (UC) is a disease of the mucosal interface involving dysfunction in epithelial-barrier integrity, gut microbial ecology, and immune regulation. Current pharmacological treatments despite expanding to include biologics and targeted small molecules still face major limitations, including treatment failure, loss of response, adverse effects, and high cost. These challenges highlight the need for complementary, safe, and affordable therapeutic options.

Traditional medicinal plants, rich in anti-inflammatory and antioxidant compounds, offer potential benefits for mucosal healing but require rigorous standardisation and safety evaluation before integration into modern care. *Trevesia palmata*, an understudied ethnomedicinal plant used in South and Southeast Asia for gastrointestinal ailments, contains diverse secondary metabolites and shows promising pharmacological activities. However, scientific evidence on this species remains scarce.

This thesis proposes that a chemically standardised extract of *T. palmata* could target key pathological mechanisms in UC by reducing oxidative and cytokine-driven inflammation, strengthening epithelial-barrier structure and function, and modulating gut microbial activity. A rat model of UC provides an appropriate system to test these hypotheses through combined clinical, histological,

biochemical, and microbiome assessments, alongside toxicity and formulation studies.

Overall, evaluating *T. palmata* as an adjuvant therapy for UC is justified, addresses an explicit evidence gap, and aligns with the eco-immune understanding of UC pathophysiology.

1.7. OBJECTIVES

The objectives of the study are:

- I. Phytochemical characterisation and evaluation of in-vitro antioxidant property of methanolic extract of *Trevesia palmata* (Roxb. ex Lindl.) Vis. 1842 flower buds.
- II. Computational screening of *Trevesia palmata* flower bud phytochemicals for anti-inflammatory potential, targeting COX-2 and KEAP1 through PASS, molecular docking, molecular simulation, and integrated ADMET profiling.
- III. Evaluation of acute and sub-acute toxicity of methanolic extract of *Trevesia palmata* flower buds in Wistar albino rat model.
- IV. Evaluation of ameliorative potential of methanolic extract of *Trevesia palmata* flower buds against ulcerative colitis in TNBS-induced rat model.
- V. Metagenomics analysis of gut microbial composition to demonstrate the ameliorative potential of methanolic extract of *Trevesia palmata* flower buds in a rat model with TNBS-induced ulcerative colitis.

CHAPTER 2

Phytochemical characterisation and evaluation of in-vitro antioxidant property of methanolic extract of *Trevesia palmata* (Roxb. ex Lindl.) Vis. 1842 flower buds (*Objective 1*)

2.1 INTRODUCTION

2.1.1 Pharmacological Properties of Plants

Plants have long been a rich source of medicinal compounds, contributing significantly to both conventional and alternative medicine. Many plants possess pharmacological properties, including anti-inflammatory, anti-cancer, anti-diabetic, and antioxidant activities, which are crucial for maintaining health. Phytochemicals like alkaloids, flavonoids, and terpenoids have diverse molecular targets and are important for the development of modern drugs such as artemisinin and paclitaxel. Increased interest in natural antioxidants has emerged due to their role in combating diseases linked to oxidative stress.

2.1.2 *Trevesia Palmata* (Roxb. ex Lindl.) Vis. 1842: A Traditional Medicinal Plant

Trevesia palmata, a member of the Araliaceae family, is native to Southeast Asia. Known as the Snowflake Tree, it is used in traditional medicine across regions like China, Vietnam, and India. Various parts of the plant are used for treating conditions such as rheumatic disorders, dermatological issues, and respiratory problems.

2.1.3 Extraction of Plant Material

Effective extraction of bioactive compounds from plants is crucial for their medicinal use. Methods like cold maceration, Soxhlet extraction, and modern techniques such as microwave-assisted extraction (MAE) enhance the efficiency of isolating beneficial compounds.

2.1.4 Phyto-elements and Phytocompounds

Medicinal plants contain essential macronutrients like nitrogen and phosphorus, and micronutrients like iron and zinc. These elements, along with bioactive compounds such as polyphenols and terpenoids, contribute to the plant's health benefits. Phytochemicals play roles in antioxidant, anti-inflammatory, and metabolic regulation.

2.1.5 In Vitro Screening of Phytocompounds and Physicochemical Characterisation

Phytochemical analysis uses various techniques, including chromatography, spectroscopy, and mass spectrometry, to identify and quantify bioactive compounds in plant extracts. Methods like GC-MS, LC-MS, and FTIR are critical in characterizing complex phytocompounds.

2.2 REVIEW OF LITERATURE

2.2.1 *Trevesia Palmata* in Traditional Healing Practices

Trevesia palmata has a long history in traditional medicine. In Northeast India, it is used for treating dyspepsia, stomach aches, and high blood pressure. Ethnobotanical studies from Southeast Asia document its applications for conditions like fever, inflammation, and bone fractures.

2.2.2 Features of Elements in Medicinal Plants

Medicinal plants contain macro and microelements that enhance their therapeutic effects. These elements, such as calcium, magnesium, and zinc, contribute to bone health, immune modulation, and antioxidant defence.

2.2.3 Physicochemical Properties of Phytocompounds

Phytochemicals such as carotenoids, saponins, and flavonoids are present in high concentrations in plants and contribute to their medicinal properties. These compounds have antioxidant, anti-inflammatory, and anticancer effects, making them crucial in both traditional and modern medicine.

2.2.4 Analytical Techniques in Phytochemical Studies

Modern phytochemical studies use advanced techniques like GC-MS, LC-MS, and FTIR for analysing plant extracts. These methods allow for the identification, quantification, and characterisation of bioactive compounds, which are essential for understanding their medicinal potential.

2.3 MATERIALS AND METHODS

2.3.1 Chemicals

Chemicals used for the study were sourced from various suppliers like HiMedia Laboratories Pvt., Ltd., Merck, and Sigma-Aldrich.

2.3.2 Collection of Plant Material

Unopened flower buds of *T. palmata* were collected from Tanhril village in Mizoram, India. The plant was identified and validated at Mizoram University.

2.3.3 Preparation of Plant Extract

The dried flower buds were extracted using the cold maceration method with methanol. The extract was then evaporated to a solid form.

2.4 RESULTS

2.4.1 Determination of Extraction Yield

The yield of the methanolic extract of *T. palmata* was 8.4%, which aligns with results from other studies using similar extraction methods.

2.4.2 Qualitative Analysis

The methanolic extract of *T. palmata* flower buds tested positive for alkaloids, cardiac glycosides, steroids, saponins, tannins, and terpenoids.

2.4.3 Quantitative Analysis: Total Phenolic, Flavonoid, and Tannin Content

The total phenolic, flavonoid, and tannin content were 21.51 ± 4.25 mg GAE/g, 251.92 ± 13.77 mg QE/g, and 13.58 ± 0.35 mg TAE/g, respectively.

2.4.4 GC-MS Analysis

Seven phytocompounds were identified, including palmitic acid, oleic acid, and stigmasterol, which belong to various classes such as fatty acids and triterpenoid sterols.

2.4.5 LC-MS Analysis

Fifteen phytocompounds were detected, including several flavonoids and carotenoids, which are known for their antioxidant and anti-inflammatory properties.

2.4.6 FTIR and NMR Analysis

FTIR revealed key functional groups such as -OH , C-H , and C=O , while NMR spectra provided insights into the structural features of the compounds present.

2.5 DISCUSSION

2.5.1 Extraction and Yield of Extract

The high yield (8.4%) suggests the extraction process effectively preserved the plant's bioactive compounds.

2.5.2 Phytochemical Characterisation

The presence of alkaloids, flavonoids, saponins, and tannins supports the traditional use of *T. palmata* in treating various ailments, especially for their antioxidant and anti-inflammatory properties.

2.5.3 Total Phenol, Flavonoid, and Tannin Content

The significant quantities of phenolic compounds, flavonoids, and tannins contribute to the plant's antioxidant properties, further supporting its use in traditional medicine.

2.5.4 Characterisation of Plant Minerals

The high levels of potassium, magnesium, and calcium in *T. palmata* enhance its potential nutritional and therapeutic benefits.

2.5.5 Free Radical Scavenging Activity, Antioxidant, and Metal Chelating Power

The extract exhibited strong antioxidant activity, demonstrated by its ability to scavenge free radicals and chelate metals like iron, which are crucial for its therapeutic effects.

2.5.6 LC-MS, GC-MS, FTIR, and NMR Analysis

Advanced techniques confirmed the presence of multiple bioactive compounds, providing detailed insights into the plant's potential for drug development.

CHAPTER 3

Computational screening of *Trevesia palmata* flower bud phytochemicals for anti-inflammatory potential, targeting COX-2 and KEAP1 through PASS, molecular docking, molecular simulation, and integrated ADMET profiling (*Objective 2*)

3.1 INTRODUCTION

3.1.1 Inflammation and Molecular Targets: COX-2 and KEAP1

Inflammation is a complex biological response involving the immune system, activated by stimuli such as pathogens or tissue damage. Chronic inflammation leads to various diseases, including cancer and neurodegenerative disorders. Key molecular targets in inflammation include COX-2 (Cyclooxygenase-2) and the KEAP1-Nrf2 axis. COX-2 catalyses the production of pro-inflammatory prostaglandins, especially PGE₂, which modulates pain, fever, and immune responses. In contrast, Nrf2, activated through disruption of its interaction with KEAP1, regulates antioxidant responses to mitigate oxidative stress. These pathways interact to either promote or resolve inflammation, influencing disease progression and treatment strategies.

3.1.2 Plant-Based Drug Discovery

Plants have been a rich source of bioactive compounds, leading to successful drug discoveries like artemisinin and paclitaxel. Natural products continue to provide new leads for drugs targeting inflammatory diseases, with modern tools like metabolomics and computational biology enhancing the exploration of plant secondary metabolites for therapeutic use.

3.1.3 Computational Approaches in Drug Discovery

Computational methods, such as molecular docking, molecular dynamics simulations, and ADME/T prediction, are increasingly used in drug discovery.

These tools help predict the biological activity, stability, pharmacokinetics, and toxicity of potential drug candidates, accelerating the development of new therapies.

3.2 REVIEW OF LITERATURE

3.2.1 COX-2 and KEAP1 as Molecular Targets

COX-2 and KEAP1 are critical in regulating inflammation. COX-2 promotes inflammation through the production of prostaglandins, especially PGE₂, while the KEAP1-Nrf2 pathway plays a protective role by counteracting oxidative stress. Both are involved in the pathogenesis of conditions like **ulcerative colitis (UC)**. Inhibiting COX-2 and activating Nrf2 are potential therapeutic strategies for managing UC.

3.2.2 Computational Drug Discovery and Plant-Based Targets

Recent advancements in computational drug discovery have enabled the screening of natural compounds for their ability to modulate COX-2 and KEAP1. Natural products, particularly polyphenols and terpenoids, show promise in targeting these pathways and reducing inflammation.

3.3 MATERIALS AND METHODS

3.3.1 Phyto-Ligands from *Trevesia palmata*

The phytochemicals were extracted from the methanolic extract of *T. palmata* flower buds. A total of 21 phyto-compounds were identified using LC/GC-MS. Sulfasalazine, an FDA-approved drug for inflammatory diseases, was used as a reference ligand.

3.3.2 Computational Tools

3.3.2.1 PASS Prediction

Used to assess the anti-inflammatory and anti-ulcerative potential of the phytochemicals based on their chemical structures.

3.3.2.2 SwissADME

Evaluated the physicochemical properties and drug-likeness of the compounds.

3.3.2.3 Molecular Docking

AutoDock Vina was employed to predict the binding affinities of the compounds with COX-2 and KEAP1.

3.4 RESULTS

3.4.1 PASS Predictions

PASS predictions revealed that several compounds from *T. palmata*, including zeta-Carotene, Oleic acid, and Kaempferol, demonstrated strong anti-inflammatory and anti-ulcerative potential. These compounds were predicted to exhibit higher activity probabilities than the reference drug sulfasalazine, particularly for anti-inflammatory activity.

3.4.2 Physicochemical Properties

The physicochemical properties of the compounds were assessed using SwissADME. Compounds like palmitic acid and kaempferol exhibited favourable drug-likeness, while glycosylated flavonoids had high molecular weight and polarity, which could affect their bioavailability.

3.4.3 Molecular Docking

Molecular docking simulations revealed that isorhamnetin and kaempferol showed strong interactions with the active sites of COX-2 and KEAP1. These

compounds formed stable hydrogen bonds and hydrophobic interactions with key residues in the protein binding sites, suggesting they may serve as effective inhibitors.

3.4.4 Molecular Dynamics Simulations

MD simulations confirmed the stability of the COX-2 and KEAP1 complexes with isorhamnetin and kaempferol over 100 ns. These compounds showed minimal fluctuations in RMSD and RMSF values, indicating strong binding and stability within the protein binding sites.

3.4.5 ADMET Profile

The ADMET analysis indicated that several *T. palmata* compounds, including kaempferol and isorhamnetin, exhibited favourable absorption, distribution, and metabolism profiles. These compounds were predicted to have good oral bioavailability and minimal toxicity, making them promising candidates for further development.

3.5 DISCUSSION

3.6.1 Inflammation and Molecular Targets: COX-2 and KEAP1

Inflammation in conditions like ulcerative colitis is driven partly by COX-2, an enzyme that produces pro-inflammatory prostaglandins. While COX-2 inhibitors can reduce inflammation, some such as rofecoxib have been linked to cardiovascular risks. Separately, the KEAP1-Nrf2 pathway protects cells from oxidative stress by enabling Nrf2 to activate antioxidant genes when needed. Activating Nrf2 has shown therapeutic benefits in ulcerative colitis. Combining COX-2 inhibition with Nrf2 activation may therefore provide a more effective and safer treatment approach for chronic inflammatory diseases.

3.6.2 Plant-Based Drug Discovery and the Role of *Trevesia palmata*

Plants provide many therapeutic compounds, and *Trevesia palmata* used traditionally in Southeast Asia shows anti-inflammatory, antioxidant, and antidiabetic properties. Its flavonoids and polyphenols fit well with known anti-inflammatory pathways. Computational analysis indicates that several of its phytochemicals, including isorhamnetin and kaempferol, may both inhibit COX-2 and activate Nrf2, offering a dual anti-inflammatory and antioxidant effect. These results support the potential of *Trevesia palmata* in managing ulcerative colitis and related inflammatory disorders, complementing existing therapies.

3.6.3 PASS Predictions and Anti-inflammatory Potential

The PASS tool identified several *Trevesia palmata* compounds with strong predicted anti-inflammatory and anti-ulcerative effects. Zeta-carotene and oleic acid showed high probabilities of anti-inflammatory activity, while flavonoids like kaempferol and isorhamnetin also scored well, aligning with their known roles in reducing oxidative stress and cytokine signalling. Their predicted activity exceeded that of sulfasalazine, suggesting that these natural compounds may serve as effective alternatives to existing UC treatments.

The predicted anti-ulcerative effects of zeta-carotene and oleic acid further support the plant's therapeutic potential. Together, these compounds may help lessen oxidative injury, reduce inflammation, and promote mucosal healing in ulcerative colitis.

3.6.4 Physicochemical Properties and Drug-Likeness

SwissADME analysis showed that the *T. palmata* phytocompounds vary in drug-likeness, which affects oral bioavailability. Smaller, more lipophilic fatty acids such as palmitic and oleic acid displayed good properties for oral absorption. In contrast, large and highly polar glycosylated flavonoids like luteolin-6-C-glucoside may have poor membrane permeability and limited

bioavailability, highlighting the need for structural modification or specialized formulations and ongoing challenge in developing plant-derived therapeutics.

Flavonoids such as kaempferol and isorhamnetin had more favourable molecular profiles, with moderate polarity and higher predicted bioavailability. These characteristics make them strong candidates for further development and potential clinical use.

3.6.5 Molecular Docking and Binding Affinities

Molecular docking simulations indicated that isorhamnetin and kaempferol exhibited strong binding affinities for both COX-2 and KEAP1, forming stable interactions with critical residues within the active sites. These interactions were predominantly hydrogen bonds and hydrophobic contacts, which are essential for the stability and potency of ligand-receptor complexes. The COX-2 interactions, especially with isorhamnetin, showed the highest stability, suggesting a high potential for these compounds as COX-2 inhibitors.

The binding affinities of isorhamnetin and kaempferol were superior to sulfasalazine, highlighting the plant-derived compounds as promising candidates for the development of new anti-inflammatory drugs. These findings also support the hypothesis that *Trevesia palmata* compounds can act through multiple mechanisms, including direct inhibition of COX-2 and activation of Nrf2, to mitigate inflammation in UC.

3.6.6 ADMET Profile and Toxicity Prediction

The ADMET analysis of the *T. palmata* phytochemicals revealed favourable absorption, distribution, metabolism, and excretion (ADME) profiles, with several compounds demonstrating optimal oral bioavailability. The toxicity profiles were also promising, with most compounds showing minimal toxicity across a range of endpoints, including organ toxicity and genotoxicity. These results suggest that *T. palmata* compounds are relatively safe and well-tolerated,

making them suitable candidates for further preclinical testing and eventual clinical development.

3.5.7 Integration of Computational and Experimental Approaches

The integration of computational drug discovery methods, such as PASS prediction, molecular docking, and molecular dynamics simulations, with ADMET and toxicity profiling, provides a comprehensive framework for screening and optimizing bioactive compounds. This approach allows for the rapid identification of promising drug candidates from complex plant extracts, which can be further validated in in vitro and in vivo studies. The findings from this study reinforce the potential of *Trevesia palmata* as a source of novel anti-inflammatory agents for UC treatment, with the added advantage of reduced toxicity compared to synthetic drugs.

CHAPTER 4

Evaluation of acute and sub-acute toxicity of methanolic extract of *Trevesia palmata* flower buds in Wistar albino rat model (*Objective 3*)

4.1 INTRODUCTION

4.1.1 Acute Toxicity Study

Acute toxicity refers to the harmful effects resulting from a single or short-term exposure to a substance. These effects can manifest in various forms, including physiological and functional impairments that reduce an organism's ability to adapt to its environment. Acute toxicity studies aim to determine the dose that causes mortality or significant toxic effects within a short exposure period. Regulatory guidelines such as the OECD Test Guidelines (OECD, 2001) provide standardized approaches, including the LD50 test, which determines the lethal dose expected to cause mortality in 50% of animals. Advances in ethical testing methods have led to the development of alternatives such as the Up-and-Down Procedure and the Fixed-Dose Method to minimize animal usage while ensuring reliable toxicological categorization.

4.1.2 Sub-acute Toxicity Study

Sub-acute toxicity studies evaluate the effects of repeated exposure over a prolonged period, typically 14 to 28 days. These studies provide essential data on the dose-response relationship and help identify the target organs affected by the substance. The OECD Test Guideline 407 outlines the methodology for these studies, which include daily administration of varying doses and comprehensive evaluation of clinical symptoms, body weight, organ health, and biochemical changes. Key parameters such as the No Observed Adverse Effect Level (NOAEL) and the Lowest Observed Adverse Effect Level (LOAEL) are critical in assessing safety.

4.1.3 Hepatic and Renal Function Tests

The liver plays a critical role in drug metabolism and biotransformation, making it a primary target for toxicity assessments. Drug-induced liver damage can manifest as a range of conditions from mild transaminitis to acute liver failure. Renal function is also a crucial parameter, with kidney markers such as creatinine and urea being routinely assessed to determine the impact of substances on renal health. Abnormal increases in these markers often indicate impaired kidney function, which necessitates close monitoring during toxicity studies.

4.1.4 Blood Parameters

Haematological parameters provide valuable insights into the impact of substances on blood components. Changes in parameters such as haemoglobin, red and white blood cell counts, and platelet levels can indicate the presence of haematological disorders. Substances that induce anaemia, leukopenia, or thrombocytopenia need to be carefully evaluated to ensure they do not pose a risk to the overall health of the organism.

4.2 REVIEW OF LITERATURE

4.2.1 Acute and Sub-acute Toxicity Study of *Trevesia palmata*

Several studies have assessed the toxicity of *Trevesia palmata* extracts. Research on the methanolic extracts of *T. palmata* leaves in rats has shown no significant adverse effects up to doses of 2000 mg/kg. However, inconsistent results were observed in sub-acute toxicity studies, where variations in toxicity outcomes were noted depending on the dose, exposure duration, and the endpoints assessed. These findings suggest that further evaluation is required to establish the safe therapeutic dose for *T. palmata*.

4.3 MATERIALS AND METHODS

4.3.1 Collection and Extraction of *Trevesia palmata* Flower Buds

The flower buds of *Trevesia palmata* were collected from Tanhril village, Aizawl, Mizoram and identified by the Botanical Survey of India. The buds were air-dried and powdered before being extracted using the cold maceration method with 100% methanol, which was then evaporated to yield a crude extract (TPFME).

4.3.2 Animal Ethics

The study followed ethical guidelines set by the ARRIVE protocol, and animal procedures were approved by the Institutional Animal Ethical Committee at Mizoram University, ensuring minimal animal suffering and distress.

4.4 RESULTS

4.4.1 Acute Toxicity Study

The acute toxicity study revealed clinical symptoms such as loose stools, salivation, and tremors at higher doses (5000 mg/kg). Mortality was recorded at this high dose, with 60% mortality in males and 80% in females. The LD₅₀ was determined to be 4400 mg/kg for males and 4200 mg/kg for females, classifying *T. palmata* as slightly toxic.

4.4.2 Sub-acute Toxicity Study

No clinical signs of toxicity, mortality, or abnormal behaviour were observed during the 28-day sub-acute toxicity study. There were no significant changes in body weight, organ weights, food or water consumption, or rectal temperature across the treatment groups (10, 100, and 500 mg/kg). Haematological and biochemical markers remained within normal ranges, further supporting the non-toxicity of the extract at these doses.

4.4.3 Histopathological Analysis

Histopathological examination revealed no significant damage to the liver, kidney, heart, lung, spleen, or other vital organs in both the acute and sub-acute toxicity studies, indicating that *T. palmata* did not induce major structural changes at doses up to 3000 mg/kg.

4.5 DISCUSSION

Medicinal plants have long supported healthcare, but their safety must be validated scientifically. *Trevesia palmata* showed a generally good safety profile in this study. In acute toxicity tests, the extract was well tolerated at 2000–3000 mg/kg, whereas a high dose of 5000 mg/kg caused toxicity and some mortality, highlighting the need for careful dose selection. Sub-acute testing showed no major organ damage or biochemical abnormalities, indicating the extract's safety at moderate doses.

These results are consistent with earlier reports on plant extract safety and support *T. palmata* as a potential therapeutic agent. Nonetheless, the dose-dependent toxicity observed at higher levels underscores the need for further research to determine safe therapeutic ranges and to assess long-term effects during chronic use.

CHAPTER 5

Evaluation of ameliorative potential of methanolic extract of *Trevesia palmata* flower buds against ulcerative colitis in TNBS-induced rat model (Objective 4)

5.1 INTRODUCTION

5.1. Reactive Oxygen Species in Inflammatory Bowel Disease

Oxidative stress and inflammation are closely connected in chronic diseases such as ulcerative colitis. Excessive ROS production, mainly from activated neutrophils and macrophages, leads to damage of intestinal tissues and intensifies inflammatory signalling. This is worsened by weakened antioxidant defenses, including reduced activity of SOD, catalase, and glutathione peroxidase, resulting in ongoing oxidative injury. Targeting these oxidative mechanisms is therefore a promising therapeutic approach. *Trevesia palmata*, which contains phenolic and flavonoid compounds, has shown strong antioxidant and anti-inflammatory potential, making it a valuable candidate for managing conditions driven by oxidative stress and inflammation.

5.1.3 Antioxidant and Anti-inflammatory Effects of Plants Containing Phenolic and Flavonoid Compounds

Plant-derived antioxidants help counter oxidative stress by scavenging ROS and limiting free radical formation. Phenolic and flavonoid compounds reduce cellular damage, modulate inflammatory mediators such as TNF- α and nitric oxide, and may strengthen immune function. *Trevesia palmata*, rich in these flavonoids, shows notable antioxidant, anti-inflammatory, and gastrointestinal protective effects. Evidence from previous studies supports its potential in treating disorders like ulcerative colitis. This work evaluates the anti-inflammatory activity of *T. palmata* flavonoids in comparison with sulfasalazine in a TNBS-induced rat model.

5.2 REVIEW OF LITERATURE

Ulcerative colitis is a chronic inflammatory condition driven by oxidative stress, immune imbalance, abnormal cytokine activity, and disrupted apoptosis. Although current therapies help many patients, maintaining long-term remission remains challenging, prompting interest in safer, natural alternatives. The TNBS-induced colitis model is commonly used because it reproduces key pathological features of UC and allows researchers to study oxidative stress, cytokine regulation, and potential treatments.

UC develops through a combination of genetic susceptibility, immune dysfunction, microbial imbalance, and environmental triggers. Standard drugs—including corticosteroids, immunosuppressants, and aminosalicylates—can be effective but often cause significant side effects or offer incomplete relief. According to the oxidative stress hypothesis, excessive ROS damage the epithelial barrier, activate inflammatory pathways, and worsen mucosal injury. Strengthening antioxidant defences or reducing ROS production may therefore slow disease progression and support new therapeutic strategies.

5.3 MATERIALS AND METHODS

5.3.1 Chemicals and Materials

2,4,6-Trinitrobenzenesulfonic acid (TNBS), sulfasalazine, and various ELISA kits for assessing biomarkers were purchased from Sigma-Aldrich, Bioassay Technology Laboratory, and Hind Biotech; DMSO and molecular-grade ethanol were used for preparing solutions.

5.3.2 Animal Ethics and Maintenance

This study adhered to the ARRIVE guidelines and was approved by the Institutional Animal Ethical Committee at Mizoram University (Approval No. MZU/IAEC/2021-22/08). Forty male Wistar albino rats (150–170 g, aged 10-12

weeks) were housed in controlled conditions, provided with a commercial pellet diet, and allowed ad libitum access to ultrapure water.

5.3.3 Induction of Ulcerative Colitis

UC was induced by intrarectal administration of TNBS (50 mg/kg) dissolved in 50% ethanol using a polyurethane feeding tube. Control animals received saline.

5.4 RESULTS

5.4.1 Clinical Parameters in TNBS-induced Colitis Rats

5.4.1.1 Body Weight

TPF (10 mg/kg and 20 mg/kg) prevented weight loss induced by **TNBS**, with the 20 mg/kg dose showing the most pronounced effect.

5.4.1.2 Food and Water Intake

TPF significantly improved food and water intake, with the higher dose (20 mg/kg) showing the most substantial improvement compared to the **TNBS-only** group.

5.4.1.3 Rectal Temperature

Treatment with TPF restored rectal temperature to normal levels, further indicating its protective role against inflammation-induced hypothermia.

5.4.2 Survival Rate

Kaplan-Meier survival curves showed that TPF treatment, especially at the 20 mg/kg dose, significantly improved survival rates (100% survival by Day 7), compared to TNBS-only (40% survival).

5.4.2 Rectal Bleeding and Colon Architecture

Rectal bleeding was significantly reduced in the TPF-treated groups, with the TPF20 group showing the most significant improvement, followed by TPF10. Colon architecture was markedly improved in TPF-treated rats, particularly at the 20 mg/kg dose, which exhibited nearly normal colon morphology.

5.4.4 Stool Consistency

The TPF20 group showed near-normal stool consistency, indicating the extract's strong ability to alleviate gastrointestinal disturbances caused by TNBS.

5.4.5 Histopathological Assessment

Histological analysis revealed reduced epithelial erosion, mucosal damage, and inflammatory cell infiltration in TPF-treated rats, with the 20 mg/kg dose showing the most significant improvement.

5.4.6 Biochemical Analysis

Oxidative stress markers like MDA, SOD, and GSH were significantly improved in TPF-treated rats, especially at the 20 mg/kg dose, indicating *T. palmata*'s strong antioxidant properties.

5.4.7 Inflammatory Cytokines and Oxidative Stress Markers

TNF- α , COX-2, iNOS, and MPO levels were significantly reduced in TPF-treated rats, with the TPF20 group exhibiting the most prominent effects.

5.5 DISCUSSION

5.5.1 Clinical Parameters

The study demonstrated that *T. palmata* flower bud methanolic extract (TPF) provided significant protection against TNBS-induced colitis, particularly

at the 20 mg/kg dose. TPF improved body weight, food and water intake, and rectal temperature, supporting its potential as a therapeutic agent for UC.

5.5.2 Survival Rate

The TPF20 dose significantly improved survival rates in TNBS-induced colitis rats, demonstrating the extract's potential to prevent mortality associated with severe colonic inflammation.

5.5.3 Rectal Bleeding and Colon Architecture

Treatment with TPF led to significant reductions in rectal bleeding and preserved colon architecture, with the 20 mg/kg dose offering the most pronounced protection.

5.5.4 Stool Consistency

TPF20 restored stool consistency to near-normal levels, suggesting its therapeutic potential in alleviating gastrointestinal disturbances associated with ulcerative colitis.

5.5.5 Biochemical and Histopathological Findings

TPF treatment significantly modulated oxidative stress and inflammation, as demonstrated by improved MDA, GSH, SOD, and CAT levels. Histological analyses confirmed that TPF protected against mucosal damage, inflammatory cell infiltration, and oxidative injury in the colon.

5.5.6 Inflammatory Cytokines and Cell Signalling Pathways

TPF exhibited anti-inflammatory effects by downregulating key pro-inflammatory cytokines and proteins like TNF- α , COX-2, NF- κ B, IL-6, NOX-2, p53, PPAR- γ , Bax, and Bcl-2 further supporting its therapeutic potential for treating ulcerative colitis.

CHAPTER 6

Metagenomics analysis of gut microbial composition to demonstrate the ameliorative potential of methanolic extract of *Trevesia palmata* flower buds in a rat model with TNBS-induced ulcerative colitis. (Objective 5)

6.1 INTRODUCTION

6.1.1 Role of Gut Microbiota in Ulcerative Colitis

Ulcerative colitis is influenced by genetics, immune function, and gut microbiota composition. Dysbiosis characterized by reduced microbial diversity and overgrowth of pathogenic bacteria like *Escherichia coli* and *Bacteroides fragilis*, along with depletion of beneficial species such as *Faecalibacterium prausnitzii* and *Roseburia hominis* promotes chronic intestinal inflammation.

Restoring microbial balance is a promising therapeutic approach. Probiotics like *Lactobacillus* and *Bifidobacterium* can reduce inflammation and improve symptoms, while plant-based therapies, including *Trevesia palmata*, offer natural alternatives to modulate the microbiota and support UC management.

6.1.4 Metagenomic Insights into Gut Microbiota

Metagenomics enables comprehensive analysis of gut microbial communities by sequencing DNA directly from samples, offering insights beyond traditional culture methods. In UC, metagenomic studies show reduced microbial diversity and increased pathogenic species.

This approach can also evaluate how interventions affect the microbiota. For instance, *T. palmata* methanolic extracts have shown anti-inflammatory effects in UC models, and metagenomic profiling can identify the microbial shifts underlying these benefits. This study uses a TNBS-induced colitis model to

explore how *T. palmata* restores microbial balance and alleviates UC symptoms through metagenomic analysis.

6.2 REVIEW OF LITERATURE

6.2.1 Ulcerative Colitis and Intestinal Microbiota

The development of UC is closely linked to gut microbiota imbalances. A healthy microbiome helps prevent the overgrowth of harmful microorganisms, while dysbiosis marked by reduced microbial diversity and increased pathogenic species impairs intestinal defences, weakens immunity, and promotes inflammation.

In UC, beneficial bacteria such as *Akkermansia muciniphila* are often depleted, while pathogenic species like *Escherichia coli* proliferate. This microbial imbalance drives chronic intestinal inflammation and tissue damage, complicating disease management.

6.2.2 Impact of Gut Microbiota Dysbiosis on Ulcerative Colitis Pathogenesis

Gut microbiota dysbiosis plays a central role in UC pathogenesis by disrupting microbial balance and triggering inflammatory responses that damage the intestinal mucosa. UC patients often show reduced microbial diversity and an increase in pathogenic taxa, such as Proteobacteria. Dysbiosis also alters key microbial metabolites, including short-chain fatty acids, which are vital for intestinal homeostasis and immune regulation. This impairs the epithelial barrier, increases permeability, and promotes inflammatory cytokine production. The resulting cycle where dysbiosis drives inflammation and inflammation worsens microbial imbalance perpetuates chronic UC, highlighting the importance of restoring microbial balance as a therapeutic strategy.

6.2.3 Impact of Gut Microbiota on Ulcerative Colitis Pathogenesis and the

Restoring gut microbial balance is a key therapeutic strategy for UC. Approaches such as probiotics, prebiotics, synbiotics, and faecal microbiota transplantation have shown benefits. *Trevesia palmata*, rich in bioactive compounds, demonstrates potential to modulate the microbiota, reduce inflammation, and support immune regulation.

Probiotics like *Lactobacillus* and *Bifidobacterium* can improve microbial balance, strengthen the gut barrier, and regulate immune responses, though strain and dosage are critical. *T. palmata* offers a complementary or alternative approach by simultaneously modulating the microbiota, enhancing immune function, and providing anti-inflammatory effects, positioning it as a promising natural therapy.

6.3 MATERIALS AND METHODS

6.3.1 Sample Collection

Faecal samples from each rat group were homogenized and stored in sterile microcentrifuge tubes. Scissors were disinfected with 99% ethanol before sample handling. After homogenization, faecal samples were frozen at -80°C for further analysis.

6.3.2 DNA Extraction

DNA was extracted using the Qiagen DNA Extraction Kit following the manufacturer's protocol, with adjustments to digestion time (60 minutes at 57°C) to ensure bacterial lysis. Mechanical lysis was achieved using glass beads (0.5 mm), followed by centrifugation and purification using the kit's collection columns.

6.3.3 16S rRNA Sequencing

16S rRNA gene sequencing was performed using high-throughput DNA sequencing. DNA concentration was measured with a Nanophotometer, and libraries were prepared using the Quick-16S Primer Set V3-V4, targeting the V3-V4 region of the 16S rRNA gene. The sequencing was performed on the Illumina MiSeq platform, with DADA2 software used to assign bacterial taxa.

6.3.4 V3-V4 Amplicon Generation

DNA samples were amplified using V3-V4 primers, followed by PCR amplification. A positive control was used, and PCR products were analyzed on an agarose gel. Amplified PCR products were cleaned using AMPure XP beads for library preparation.

6.3.5 Library Preparation Protocol

The amplified products were repaired and mono-adenylated using the NEBNext Ultra DNA Library Prep Kit for Illumina, followed by ligation of NEB adapters. After ligation, a PCR step added unique barcodes for multiplexing. The Fragment Analyzer was used to assess the fragment distribution of all prepared libraries.

6.3.6 Sequencing Protocol

Libraries were evaluated for quality using the Qubit dsDNA HS Assay Kit. Pooled libraries were loaded onto the Illumina MiSeq V2 instrument, generating paired-end 300 bp reads.

6.3.7 Bioinformatics Analysis

QIIME2 was used for bioinformatics analysis, including sequence demultiplexing and feature classification. Alpha and beta diversity analyses were

performed using standard metrics like Shannon index and Bray-Curtis dissimilarity to assess microbial diversity and community structure.

6.4 RESULTS

6.4.1 MultiQC Report on Fecal Composition

The sequencing results for fecal samples were evaluated using MultiQC, confirming high-quality sequencing across all experimental groups. Key metrics, such as sequence count, mean quality scores, and GC content, indicated robust sequencing performance. The low levels of sequence duplication and overrepresented sequences further validated the integrity of the microbiome data.

6.4.2 Taxonomic Distribution

Taxonomic analysis at the phylum, class, order, family, genus, and species levels revealed significant microbial shifts in UC rats. Treatment with *T. palmata* (particularly TPF20) restored the abundance of beneficial phyla like Firmicutes and Bacteroidetes, reduced Proteobacteria levels, and enhanced the abundance of anti-inflammatory genera such as *Faecalibacterium prausnitzii*.

6.5 DISCUSSION

6.5.1 Dysbiosis in the TNBS-Induced UC Model

The TNBS-induced UC model exhibited significant dysbiosis, with a marked reduction in beneficial bacterial phyla and an increase in pathogenic Proteobacteria, consistent with findings in UC patients. The treatment with *T. palmata* (particularly at TPF20) successfully restored microbial balance, especially by enhancing Firmicutes and Bacteroidetes, which are critical for gut health and anti-inflammatory effects.

6.5.2 Restoration of Microbial Balance by *T. palmata*

T. palmata effectively modulated gut microbiota, promoting the growth of beneficial bacteria and suppressing pathogenic species. The restoration of microbial diversity and richness, as measured by the Chao1 and ACE indices, suggests that *T. palmata* could offer a promising therapeutic approach for restoring a healthy microbiota in UC.

6.5.3 Potential Mechanisms and Therapeutic Efficacy

The positive effects of *T. palmata* on microbial diversity, inflammation, and gut barrier function suggest its potential as a natural alternative to current UC therapies. The ability to restore the balance of key microbial species, such as *Faecalibacterium prausnitzii* and *Bacteroides fragilis*, highlights *T. palmata*'s role in enhancing immune regulation and gut health. Further studies are needed to confirm these results in human clinical trials.

SUMMARY

Objective 1 provides a detailed account of the key findings from the study, covering the yield, phytochemical composition, antioxidant activity, and bioactive compounds present in the *T. palmata* flower bud methanolic extract.

- The yield of the crude methanolic extract of *T. palmata* flower buds (TPFME) was 8.4%, with no significant changes in the appearance of the extract.
- Preliminary phytochemical screening of TPFME revealed the presence of alkaloids, cardiac glycosides, steroids, saponins, tannins, and terpenoids.
- The total phenol content of the extract was 21.51 ± 4.25 mg GAE/g, total flavonoid content was 251.92 ± 13.77 mg QE/g, and total tannin content was 13.58 ± 0.35 mg TAE/g.
- Micro-nutrient analysis showed the highest concentrations of potassium (K) (251.070 ± 0.447 ppm), followed by magnesium (Mg) (11.41 ± 0.115

ppm) and calcium (Ca) (8.984 ± 0.050 ppm). Other micronutrients (Mn, Na, Zn, Fe, Cu, Co) were present at concentrations below 1 ppm.

- Free radical scavenging, antioxidant, and metal chelation activities of TPFME demonstrated a concentration-dependent effect. IC₅₀ values for various activities were:
 - DPPH scavenging: 161.286 ± 0.450 µg/mL
 - ABTS•+ scavenging: 186.423 ± 1.230 µg/mL
 - SOD activity: 127.947 ± 1.251 µg/mL
 - NO scavenging: 631.472 ± 4.276 µg/mL
 - Iron chelation: 777.807 ± 1.945 µg/mL
- Highest reducing power of 0.456 ± 0.002 at 750 µg/mL.
- Phytocompound analysis using LC-MS and GC-MS identified 21 bioactive compounds, including flavonoids, fatty acids, and carotenoids. Seven compounds were detected by GC-MS, and 13 by LC-MS.
- FTIR spectral analysis identified functional groups such as O-H, CH₂, C-H, C=C, N-H, and C-O, suggesting the presence of compounds like hydroxyl groups, lipids, alkanes, fatty acids, amines, and R-CH₃.
- NMR spectrum analysis indicated the presence of alkanes, alkenes, alcohols, esters, ether groups, and carbonyl compounds, with the absence of aldehyde (R-CHO) and carboxylic acid (-COOH) groups.

Objective 2 captures the key findings of the study, highlighting the promising bioactive compounds in *T. palmata*, their therapeutic potential, and the predicted safety and efficacy profiles based on computational models and molecular dynamics simulations.

- Isorhamnetin and kaempferol were identified as the most promising bioactive compounds in *T. palmata*, exhibiting strong anti-inflammatory, antioxidant, and anti-ulcerative potential.
- Carotenoids, fatty acids, and flavonoids showed the highest predicted biological activity, highlighting their key role in *T. palmata*'s therapeutic effects.

- Zeta-carotene, kaempferol, apigenin derivatives, and quercetin derivatives had significantly higher Pa than Pi values, indicating a strong likelihood of biological activity, particularly for anti-inflammatory and anti-ulcerative effects.
- Several *T. palmata* compounds outperformed the reference drug sulfasalazine in PASS prediction models for anti-inflammatory and anti-ulcerative endpoints, suggesting their potential as effective alternatives.
- Flavonoids, especially aglycone forms like isorhamnetin and kaempferol, played a central role in modulating inflammation, enhancing antioxidant defenses, and promoting mucosal healing, underpinning the medicinal properties of *T. palmata*.
- Aglycone flavonoids exhibited favorable drug-like properties, including moderate lipophilicity, balanced polarity, and good solubility, supporting their therapeutic potential.
- Glycosylated flavonoids and procyanidins were predicted to have poor gastrointestinal absorption, limiting systemic bioavailability but suggesting potential local effects or use in prodrug formulations for enhanced absorption.
- Most metabolites, including kaempferol and isorhamnetin, demonstrated minimal predicted toxicity (carcinogenicity, mutagenicity, hepatotoxicity), indicating good safety profiles.
- Some sterol derivatives and procyanidins showed isolated toxicity alerts, highlighting the need for further in vitro validation.
- Isorhamnetin and kaempferol exhibited strong binding interactions with COX-2 and KEAP1, key targets in inflammation and oxidative stress, demonstrating potent inhibitory potential.
- Isorhamnetin showed stable binding to COX-2 and KEAP1, with low RMSD and RMSF fluctuations in molecular dynamics simulations, indicating robust dual-target modulation.
- Dual-target docking and molecular dynamics results highlight the potential of isorhamnetin and kaempferol as multi-target modulators of inflammation and oxidative stress, offering broader therapeutic benefits

compared to single-target drugs for treating inflammatory diseases like ulcerative colitis.

Objective 3 highlights the main findings from the acute and sub-acute toxicity studies, showing that *T. palmata* methanolic extract exhibits a relatively safe profile, with mild toxicity observed at high doses and no significant systemic damage at lower doses.

Acute Toxicity:

- Preparation of Extract: The methanolic extract of *Trevesia palmata* flower buds (TPFME) was prepared by cold maceration (1:4 w/v) with 100% methanol and stored at –20 °C for in vivo studies.
- Acute Oral Toxicity: Tested in Wistar albino rats (both sexes) at 2000, 3000, and 5000 mg/kg following OECD TG 423 guidelines.
- Toxicity Observations:
 - No significant signs of toxicity or mortality at 2000 and 3000 mg/kg.
 - At 5000 mg/kg, dose-related toxic signs appeared, including slow movement, loose stools, and diarrhea in females, with 60% mortality in males and 80% in females.
 - LD₅₀ values were 4400 mg/kg (males) and 4200 mg/kg (females), classifying the extract as slightly toxic.
- Clinical Parameters: No significant changes in body weight, food and water intake, rectal temperature, or organ weights at 2000 and 3000 mg/kg.
- Histopathology: Liver and kidney were normal at 2000 and 3000 mg/kg; at 5000 mg/kg, hepatic vacuolar degeneration, endothelial disruption, congestion, and renal lesions such as atrophied glomeruli and hemorrhage were observed.
- Hematology: At 3000 mg/kg, RBC, WBC, and neutrophil counts increased significantly but remained within normal ranges.

- Biochemical Analysis: Serum ALT increased at 3000 mg/kg; other markers (AST, ALP, bilirubin, creatinine) remained unchanged. Triglycerides decreased in males, total cholesterol and triglycerides decreased in females, with reduced serum glucose in both sexes.
- Oxidative Stress: No significant changes in liver or kidney MDA, SOD, CAT, or GPx at 2000 and 3000 mg/kg.

Sub-acute Toxicity:

- Oral Dosing: Daily administration of 10, 100, and 500 mg/kg for 28 days showed no clinical toxicity or mortality.
- Clinical Parameters: At 500 mg/kg, reductions in body weight, weight gain, food and water intake, and increased rectal temperature were observed; changes were mild or non-significant at 10 and 100 mg/kg.
- Organ Weights and Histology: No significant changes in organ weights except increased colon weight (10 and 100 mg/kg) and altered uterus weight (500 mg/kg); histopathology remained normal.
- Hematology: Hemoglobin, RBC, and platelet counts remained normal; WBC count increased at 500 mg/kg, suggesting mild immunomodulation.
- Biochemical and Renal Function: No significant changes in hepatic or renal biomarkers; values remained within reference ranges.
- Lipid and Electrolyte Changes: Triglycerides, HDL-C, sodium, and chloride decreased in both sexes; total cholesterol decreased and LDL-C increased in males; serum calcium and potassium reduced in females; all values within physiological limits. Blood glucose remained unchanged.
- Oxidative Stress: Kidney MDA increased modestly, indicating mild lipid peroxidation; liver MDA unchanged. Kidney SOD and hepatic CAT increased in males at 500 mg/kg, while GPx remained unchanged.
- Histopathology: Liver, kidney, heart, lungs, brain, colon, spleen, testis, and ovary showed normal architecture with no necrosis, inflammation, or degeneration.

Overall Toxicological Interpretation:

- Acute Toxicity: TPFME is slightly toxic based on LD₅₀, with a wide safety margin up to 3000 mg/kg.
- Sub-acute Toxicity: NOAEL for 28-day dosing is 100 mg/kg; LOAEL is 500 mg/kg, based on reductions in growth, food intake, rectal temperature changes, and mild oxidative stress, without overt organ damage.

Objective 4 shows that TPF (particularly TPF20) demonstrates significant anti-inflammatory, antioxidant, and anti-apoptotic effects in TNBS-induced ulcerative colitis rats. TPF20 shows strong therapeutic potential, making it a promising candidate for the treatment of inflammatory bowel diseases (IBD).

- Body Weight, Food and Water Consumption, Rectal Temperature: TPF at 10 mg/kg and 20 mg/kg prevented weight loss and improved food and water intake, with TPF20 showing the strongest effect. Both doses reversed TNBS-induced hypothermia, while SPS50 showed only partial improvement.
- Survival Rate: TPF20 improved survival to 100%, TPF10 to 80%, compared to 40% in the TNBS group and 60% with SPS50.
- Rectal Bleeding, Inflammation, and Colon Architecture: TPF reduced rectal bleeding and inflammation, with TPF20 showing the greatest restoration of colon architecture and minimal ulceration.
- Stool Consistency: TPF improved stool consistency, with TPF20 showing near-normal, well-formed stools.
- Colon Length and Colon Weight/Length Ratio: TPF, especially TPF20, restored colon length and reduced the weight/length ratio, indicating decreased inflammation and edema.
- Disease Activity Index (DAI) Score: TPF10 and TPF20 significantly reduced DAI scores, with TPF20 having the most pronounced effect.
- Gross Morphology and Macroscopic Score: TPF20 showed the greatest improvement, with minimal ulceration and inflammation.
- Histopathological Improvements: TPF preserved crypts and goblet cells, with TPF20 providing the most significant histological protection.

- Oxidative Stress Markers: TPF significantly reduced MDA and enhanced SOD, GSH, and catalase activities, particularly at TPF20, demonstrating strong antioxidant effects.
- Inflammatory Cytokines and Markers: TPF, especially TPF20, reduced MPO, IL-6, and IL-1 β levels, indicating anti-inflammatory potential.
- NF- κ B p65, TNF- α , COX-2, iNOS: TPF decreased the expression of these pro-inflammatory mediators, with TPF20 showing the most substantial reduction.
- Nrf2, HO-1, and Caspase-3 Expression: TPF, particularly TPF20, enhanced Nrf2 and HO-1 and reduced active caspase-3, suggesting antioxidant and anti-apoptotic effects.
- Apoptotic Markers (PPAR- γ , Bax, Bcl-2): TPF increased PPAR- γ , reduced Bax, and increased Bcl-2 expression, with TPF20 showing the strongest anti-apoptotic effect.

Objective 5 encapsulates the critical findings from the study, highlighting the potential of *T. palmata* in restoring gut microbiota balance, enhancing microbial diversity, and promoting anti-inflammatory effects, making it a promising candidate for treating ulcerative colitis (UC).

- Dysbiosis in the TNBS-Induced UC Model: TNBS-induced UC caused significant gut dysbiosis, with reduced beneficial phyla (Firmicutes and Bacteroidetes) and increased Proteobacteria, contributing to inflammation and impaired gut function.
- Restoration of Gut Microbiota by *T. palmata*: *T. palmata*, especially at TPF20, restored microbial balance by increasing Firmicutes and Bacteroidetes and reducing Proteobacteria overgrowth.
- Reinforcement of Anti-Inflammatory Genera: *Faecalibacterium prausnitzii*, an anti-inflammatory genus supporting gut barrier and immune modulation, was restored following *T. palmata* treatment, suggesting a mechanism for its anti-inflammatory effects.

- Enhanced Microbial Diversity: *T. palmata* treatment increased alpha diversity (Chao1, ACE), improving species richness and bringing the gut microbiota closer to healthy controls.
- Improved Species Evenness: Indices such as Shannon, Simpson's Evenness, and Gini Index indicated a more even microbial distribution after *T. palmata* treatment, countering the pathogenic dominance seen in UC.
- Restoration of Specific Beneficial Species: Beneficial species including *Bacteroides fragilis* were also restored, supporting gut barrier integrity and immune regulation.
- Therapeutic Potential of *T. palmata*: By restoring microbial balance, enhancing beneficial species, and promoting anti-inflammatory effects, *T. palmata* offers a natural, microbiome-targeted therapeutic approach for UC, with fewer side effects compared to conventional treatments.