STUDIES ON ANTIDIARRHOEAL POTENTIAL OF SELECTED MEDICINAL PLANTS OF MIZORAM

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STUDIES ON ANTIDIARRHOEAL POTENTIAL OF SELECTED MEDICINAL PLANTS OF MIZORAM

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CERTIFICATE

This is to certify that the thesis entitled **"Studies on Antidiarrhoeal Potential of Selected Medicinal Plants of Mizoram"**, submitted by **TBC Laldingliani** to Mizoram University for the award of the degree of Doctor of Philosophy in Botany, is an original record of research carried out under my supervision during 2020–2025.

I further certify that this work is the scholar's independent investigation and has not been submitted, in part or in full, for any degree, diploma, or other academic distinction in this or any other university or institution.

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DECLARATION

Mizoram University

August, 2025

I, **TBC Laldingliani**, 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 do 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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TBC Laldingliani

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

ABTS	2,2'-Azino-Bis(3-Ethylbenzothiazoline-6-Sulfonic Acid)
ADMET	Absorption, Distribution, Metabolism, Excretion, And Toxicity
AE	Alkaloid Equivalent
ALSG	Average Loose Stool Grade
ALT	Alanine Amino Transferase
AMR	Antimicrobial Resistance
ANOVA	Analysis Of Variance
AST	Aspartate Aminotransferase
BCG	Bromocresol Green
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CFU	Colony Forming Unit
CV	Cultural Value
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
E2	Estradiol
EAEC	Enteroaggregative Escherichia Coli
EHEC	Enterohemorrhagic Escherichia Coli
ELISA	Enzyme-Linked Immunosorbent Assay

EP2	Prostaglandin Receptor 2
EPEC	Enteropathogenic Escherichia Coli
ESI	Electrospray Ionisation
ESI	Electrospray Ionisation
ETEC	Enterotoxigenic Escherichia Coli
FDA	Food And Drug Administration
FL	Fidelity Level
FTIR	Fourier Transform Infrared Spectroscopy
GAE	Gallic Acid Equivalents
GC-MS	Gas Chromatography – Mass Spectrometry
GID	Gastro Intestinal Diseases
Hb	Haemoglobin
HIV	Human Immuno Virus
IBD	Inflammatory Bowel Diseases
ICF	Informant Consensus Factor
IEC	Information Education and Communication
IL	Interleukin
IL-1β	Interleukin-1 Beta

KLSC	Kelisha Capsule
LC-HRMS	Liquid Chromatography-High Resolution Mass Spectrometry
LC-MS	Liquid Chromatography – Mass Spectrometry
LD_{50}	Lethal Dose, 50%
LPM	Loperamide
LSG	Loose Stool Grade
LSIR	Loose Stool Incidence Rate
LTB	Labile Toxin B Subunit
MBC	Minimum Bacteriocidal Concentration
MDR	Multidrug Resistant
METLIN	Metabolite And Tandem Mass Spectral Database
MHA	Muller Hinton Agar
MIC	Minimum Inhibition Concentration
MPP	Modified Pulsatilla Powder
MTCC	Microbial Type Culture Collection And Gene Bank
NFHS	National Family Health Survey
OECD	Organisation For Economic Co-Operation And Development
OmpC	Outer Membrane Protein C

ORS	Oral Rehydration Solution
PDB	Protein Data Bank
PGE2	Prostaglandin E2
QE	Quarcetin Equivalent
RBC	Red Blood Cells
RI	Relative Importance
RT	Retention Time
SAR	Specific Absorption Rate
SD	Standard Deviation
SE	Standard Error
SEM	Scanning Electron Microscope
SGOT	Serum Glutamic-Oxaloacetic Transaminase
SGPT	Serum Glutamate Pyruvate Transaminase
STEC	Shiga Toxin-Producing Escherichia Coli
TAE	Tannic Acid Equivalent
TNF-α	Tumor Necrosis Factor Alpha
TPC	Total Plate Count
UHPLC	Ultra-High-Performance Liquid Chromatography

UNICEF	United Nations International Childern’s Emergency Fund
UV	Use Value
WBC	White Blood Cells
WHO	World Health Organisation
ZOI	Zone Of Inhibition

General Introduction

Diarrhoeal Disease: A Global Health Challenge

Diarrhoeal disease is one of the most serious public health challenges globally, especially impacting children under five years old. According to the World Health Organisation, diarrhoeal disease ranks as the third leading cause of death in children aged 1-59 months, causing about 494,683 deaths each year in this age group. The worldwide burden extends beyond this primary age group, with roughly 443,832 deaths annually in children under 5 years and a further 50,851 in children aged 5-9 years (WHO, 2024).

Epidemiological data show a staggering global incidence, with nearly 1.7 billion cases of childhood diarrhoeal disease each year. Children who are malnourished, have weakened immune systems, or live with HIV are most vulnerable to severe, life-threatening diarrhoea (WHO, 2024). The burden is heaviest in low- and middle-income countries, where children under three experience an average of three diarrhoea episodes annually (Liang et al., 2024).

National scenario

Based on UNICEF data, it was estimated that 250,000–300,000 young children die annually in India due to diarrhoeal diseases. However, during 2019-2021, the National Family Health Survey (NFHS-5) indicates a marginal decline in the prevalence of diarrhoea among children under five in India. While the overall (13.7%), Meghalaya (10.5%), and Ladakh (8.5%) experienced an increase in diarrhoeal prevalence, while states like Jammu & Kashmir (5.6%), Himachal Pradesh (4.7%), Mizoram (4.3%), and Goa (3.2%) reported lower prevalence (Ghosh et al., 2023).

Subsequently, the Ministry of Health and Family Welfare launched the National STOP Diarrhoea Campaign 2024 on 24 June 2024, with the ambitious goal of achieving "zero child deaths due to childhood diarrhoea." Mizoram follows the national strategy, which includes door-to-door distribution, ORS-zinc corners, hygiene-based IEC, and frontline training, all aimed at eliminating diarrhoeal child deaths. Although comprehensive state-level death figures for recent years are not openly published, Mizoram’s very low infant mortality and high immunisation

coverage suggest it performs better than many states in managing the diarrhoeal disease burden. However, the prevalence of diarrhoea in older adults (~33.5%) has been noted as particularly high in Mizoram. (NFHS-5, 2019-2021; Prakash et al., 2021; Srivastava et al., 2022; UNICEF, 2024).

Diarrhoeal diseases are clinically classified into three distinct types based on duration and characteristics (Dubois et al., 2023; Guandalini & Frye, 2024).

1. *Acute watery diarrhoea* - Lasts from several hours to days and includes conditions such as cholera. Profuse, watery stools characterise it without blood and is mainly caused by viruses (60% of cases), bacteria (Vibrio cholerae, enterotoxigenic *E. coli*), or parasites.

2. *Acute Bloody Diarrhoea (Dysentery*) - Presence of blood in stools, typically caused by bacterial pathogens such as *Shigella* (50% of cases), *Campylobacter jejuni*, and enteroinvasive *E. coli*, can also result from parasitic infections like intestinal amoebiasis

3. *Persistent Diarrhoea* - Duration of 14 days or longer, can be either watery or bloody, often associated with different etiologies and management challenges compared to acute forms.

The link between diarrhoea and malnutrition creates a harmful cycle that sustains both conditions. Diarrhoea is recognised as a key cause of malnutrition in children under 5 years old. Research indicates that about 35% of children with moderate-to-severe diarrhoea are malnourished, compared to 24% of those with mild disease. Each diarrhoeal episode strips children of essential nutrition needed for growth, while malnourished children are more susceptible to future diarrhoeal episodes (Ferdous et al., 2013; Adam et al., 2024).

The severity of this relationship is quantified through specific risk factors: malnourished children have 1.53 times higher odds of developing moderate-to-severe diarrhoea compared to well-nourished children. Furthermore, the mortality risk is significantly elevated, with acutely malnourished children experiencing considerably higher risk of death from moderate-to-severe diarrhoea compared to children with better nutritional status (Tickell et al., 2020; Ferdous et al., 2013).

The data clearly show that, although there has been notable progress in reducing deaths from diarrhoeal diseases in recent decades, many challenges persist. The overlap of malnutrition, antimicrobial resistance, and disease burden results in complex clinical situations that necessitate innovative, tailored strategies to improve treatment outcomes and prevent future complications.

The emergence of drug-resistant pathogens poses a significant challenge in managing diarrhoeal diseases. Antimicrobial resistance (AMR) occurs when bacterial, viral, fungal, or parasitic pathogens evolve to become resistant to previously effective medications. This phenomenon is accelerated by several factors, including the overuse and misuse of antimicrobials, inadequate access to clean water and sanitation, and limited diagnostic capabilities (Zang et al., 2018; Ashenafi et al., 2024).

Specific Resistance Patterns (Afum et al., 2022; Neupane et al., 2023):-

Shigella isolates demonstrate 100% resistance to tetracycline and significant resistance to commonly prescribed antibiotics, such as norfloxacin and ciprofloxacin.

Escherichia coli isolates show high resistance rates to tetracycline (79.1%) and cefazolin (58.3%).

Vibrio species exhibit alarming resistance to cefotaxime (87.5%) and ceftriaxone (83.3%), with emerging ciprofloxacin resistance at 25%.

Multi-drug-resistant strains are widely prevalent in cases of diarrhoea, with resistance to inexpensive first-line antibiotics being particularly prevalent.

The renewed interest in phytotherapy—using plants as medicine—has gained significant backing from global health bodies like the World Health Organisation (WHO). The WHO promotes the incorporation of ethnopharmacological knowledge into contemporary medical practices. However, both the WHO and regulators, such as the Food and Drug Administration (FDA), stress the importance of thorough scientific testing to verify the safety, effectiveness, and correct dosages of these natural treatments (Newman, 2020).

Relevance of the work

Ethnomedicinal plants have long served as a cornerstone in the traditional treatment of various ailments across cultures and civilisations. Their therapeutic applications are deeply embedded in indigenous knowledge systems, particularly in regions with rich biodiversity and limited access to conventional pharmaceuticals (Singh et al., 2021). In recent years, the global resurgence of interest in natural therapeutics has prompted a renewed focus on the scientific validation of traditional medicinal knowledge. However, prior to validating their therapeutic efficacy, comprehensive toxicological evaluations are imperative to determine safety margins and appropriate dosing parameters (Parasuraman, 2011). Despite their widespread use, the pharmacological mechanisms underpinning their efficacy remain poorly understood and often unvalidated by scientific inquiry. The application of *in silico* techniques such as molecular docking offers a powerful and cost-effective strategy for evaluating the bioactivity of plant-derived compounds. Molecular docking facilitates the prediction of binding affinities between phytochemicals and biological targets, enabling the identification of potential lead compounds with desirable pharmacological profiles (Daina et al., 2017).

In many rural and low-resource settings, medicinal plants are the primary form of healthcare due to limited access to modern pharmaceuticals. Ethnomedicinal knowledge, passed down through generations, has led to the use of numerous plants for treating gastrointestinal disorders, such as diarrhoea (Gashe, 1992; Giday et al., 2007). Medicinal plants are rich in bioactive compounds, including flavonoids, tannins, alkaloids, phenolics, and terpenoids, which are known to possess antimicrobial, anti-inflammatory, and antisecretory properties. These compounds act by reducing intestinal motility, inhibiting enterotoxins, or restoring electrolyte balance, which are key in managing diarrhoea (Palombo, 2006; Prieto-Martínez et al., 2019).

Investigating anti-diarrhoeal plants offers potential for developing new drugs, especially for multidrug-resistant (MDR) diarrhoeagenic pathogens. Screening plant extracts supports the pharmacological validation of traditional knowledge and may lead to the creation of safe, effective, and affordable anti-diarrhoeal agents (Rates, 2001; Owolabi et al., 2020). The excessive use of antibiotics in treating diarrhoea has

contributed to the growing problem of antimicrobial resistance (AMR). Medicinal plants present an alternative or supplementary approach to decrease antibiotic reliance by targeting resistant strains (Newman & Cragg, 2020; WHO, 2014). Exploring anti-diarrhoeal activity helps bridge the gap between traditional medicine and modern pharmacology. It verifies the therapeutic claims of traditional healers, ensures safety and effectiveness, and encourages evidence-based integrative medicine (Heinrich et al., 2020).

This study highlights the documentation of ethnomedicinal plants used by Mizoram's indigenous people, with a focus on those employed to treat diarrhoea. Selected anti-diarrhoeal plants were examined for their phytochemical constituents, and their effectiveness was tested through in vitro and in vivo analyses against diarrhoeal-causing pathogens, as well as their safety profiles. Additionally, the chemical compounds identified via LC-MS were used as ligands targeting the EP2 and opioid protein receptors, offering promising therapeutic potential for the management of diarrhoeal diseases. This *in silico* approach provided more profound insights into the antidiarrhoeal mechanisms and helped identify potential leads or novel compounds.

Objectives:

- Ethnomedicinal study of medicinal plants used by Mizo tribes in Champhai district of Mizoram, India
- Phytochemical profiling and *in vitro* anti-diarrhoeal evaluation of selected medicinal plants against *Escherichia coli* and *Salmonella* Typhimurium.
- Safety and therapeutic assessment of selected medicinal plant extracts in experimental models of diarrhoea
- LC-MS-based phytochemical characterisation and *in silico* insights into novel dual-target anti-diarrhoeal agents

Based on the objectives, the whole work has been divided into four chapters.

CHAPTER – 1

"Ethnomedicinal study of medicinal plants used by Mizo tribes in Champhai district of Mizoram, India"

1.1 Introduction

Plants have been recognised as a significant source of diverse chemical compounds, possessing both medicinal properties and commercial value. There have been several reports on medicinal plants as a source for drug discovery. However, new diseases are likely to continue emerging, along with drug-resistant pathogens. The dynamic nature of pathogens has constantly challenged researchers to seek alternatives. The past few decades have witnessed a surge in ethnomedicinal plant research (Ayyanar & Ignacimuthub, 2011) partly due to the fact that natural products have played a crucial role in the development of drugs, contributing more than 50% of clinical drugs in the pharmaceutical industry (Ghimire et al., 2012). Furthermore, the rapid growth in human population has increased demand, which in turn has heightened the quest for novel plant resources, posing a threat to natural resources (Simbo, 2010).

Traditional knowledge and practices of herbal remedies have been passed down through generations over the centuries and will continue to do so, with some variations occurring with each new generation. From the 19^{th} century to the present, plants have become the primary source for therapeutic regimes, and traditional practices have been proven to have few known side effects, aside from their low cost and easy availability. India has been well known worldwide for its indigenous traditional knowledge and practices from ancient times, encompassing various systems of medicine such as Ayurveda, Siddha, and Unani (Fabricant, 2001). Although more than 427 tribal communities have a vast diversity of ancient traditions, criticism of ethnomedicines persists due to regional variation, political, and socio-economic challenges (Kadhirvel et al., 2010). Reports are stating that several plants have been increasingly utilised by the indigenous people of India (Ayyanar & Ignacimuthub, 2011). Generally, in India, it is estimated that 6,000 flora are used in traditional and herbal medicine, which represent about 75% of the needs of the third world. Meanwhile, 3,000 plants have been officially recognised for their medicinal value (Rai & Ramnghinglova, 2010).

The healthcare system in India exhibits a wide variation, encompassing both urban and rural populations, which rely on a combination of modern and traditional systems of medicine. The recently implemented Ayushman Bharat Pradhan Mantri Jan Arogya Yojana, as per the Commonwealth Fund, enables cashless secondary and tertiary care

at private facilities (Tikkanen et al., 2020). Besides, health insurance schemes also exist for institutions and factories. Catering to the vast population has its limitations and thus many of the ailments are treated either by traditional healers or through traditional knowledge and practices especially in remote areas. One such state in the North-Eastern part of India is Mizoram.

The ethnomedicinal usages (Lalramnghinglova & Jha, 1997; Lalnundanga et al., 1997; Sharma et al., 2001) and the flora (Singh et al., 2002; Sawmliana, 2003) in the state of Mizoram, though have been previously reported, there is still scope for consideration considering the ethnomedicinal knowledge of the diverse tribes within the state. Hence, this manuscript aims to document the ethnomedicinal practices involving medicinal plants in the Champhai district of Mizoram, India. Their practical knowledge has been established based on more than a century of credence and observation.

Mizoram lies within the Indo-Burma biodiversity hotspot region and shares two international borders with Bangladesh in the west and Myanmar in the east. According to Champion and Seth (1968), Mizoram forests are classified into Tropical semi-evergreen forests, tropical wet evergreen forests and mountain sub-tropical pine forests. The study area, i.e., the Champhai district is classified as a rural area where health care facilities are relatively poor which drives the people to rely on traditional medicines. The traditional healers using medicinal plant-based formulations for various ailments indicate that traditional medicines are still one of the mainstays in their contemporary health care. It is felt that prospection and research on the medicinal plants that play such an important role in the health care of Mizo tribes need a more intensified effort.

1.2 Review of Literature

Mahwasane et al. (2013) documented indigenous knowledge from 30 traditional healers in Lwamondo, South Africa. The study identified 16 medicinal plants used to treat various ailments, predominantly stomach-related issues. The Fabaceae family was the most represented, and roots were the most commonly used part of the plant. The healers primarily prepared treatments by boiling plant material. This ethnobotanical survey underscores the richness of local knowledge and suggests directions for future pharmacological research.

Mükemre et al. (2015) reported 78 plant taxa used medicinally by the residents of Çatak village, Turkey. Most belonged to the families Asteraceae, Apiaceae, and Lamiaceae. Unique contributions include the first-time documentation of 19 taxa used medicinally. The study also employed quantitative indices, such as the Informant Consensus Factor (FIC) and Use Value (UV), to assess the reliability and significance of each plant's use. High consensus was found for plants such as Malva neglecta, Mentha longifolia, and Rosa canina, which are used for gastrointestinal, respiratory, and inflammatory conditions.

Kyaw et al. (2021) conducted the first quantitative ethnobotanical survey among the Mon people of Myanmar. The study documented 158 medicinal plant species across 64 families. The most frequently treated ailments were digestive, urological, and respiratory diseases. Leaf decoctions were the most common form of preparation. Notably, 13 species were newly recorded for medicinal use in Myanmar. Species such as *Tinospora sinensis* and *Chromolaena odorata* showed high consensus among informants, suggesting potent ethnomedicinal value.

Romero-Benavides et al. (2017) focused on the anthelmintic potential of medicinal plants. The review highlighted how resistance to conventional anthelmintic drugs has intensified the search for alternative therapies. Several plants traditionally used to treat helminth infections demonstrated significant in vitro and in vivo efficacy. These included compounds such as alkaloids, flavonoids, and tannins, emphasizing the role of plant secondary metabolites. The study reinforced the potential of ethnopharmacological leads for the development of novel drugs.

Ayyanar and Ignacimuthu (2011) documented 90 plant species used by the Kani tribes of Tamil Nadu for 65 ailments. Quantitative analysis using Use Value (UV), Informant Consensus Factor (ICF), and Fidelity Level (FL) highlighted Gymnema sylvestre, Terminalia chebula, and Syzygium cumini as highly valued plants for managing diabetes and gastrointestinal issues. High ICF values for jaundice and diabetes indicated strong agreement among practitioners.

An ethnobotanical study by Poonam and Singh (2009) recorded 116 plant species among the Taungya people of the Terai Arc in India. These plants were used both internally and externally, primarily in the form of powders, pastes, and decoctions. Among them, 82 species had novel phytomedicinal claims. Common ailments treated included skin diseases, rheumatism, and fever, demonstrating the community's profound knowledge of local flora.

Rajakumar and Shivanna (2009) investigated the eastern region of Shimoga, Karnataka, where 85 plant species were reported to be used for both human and veterinary ailments. Their study used ICF and FL to assess the reliability of ethnomedicinal claims. Plants such as Phyllanthus amarus and Justicia adhatoda showed FL values of 100%, underscoring their consistent use for specific diseases. This study emphasized the dual utility of plants in both human and animal healthcare.

Parthiban et al. (2016) reported 54 plant species used in ethnoveterinary practices in Kudavasal, Tamil Nadu. Quantitative indices such as Use Value, Relative Frequency Citation, and Cultural Importance Index were applied. High ICF values for urological disorders reflected focused and effective use of specific plants like Datura metel and Azadirachta indica. This study highlights the socioeconomic and practical dimensions of ethnoveterinary medicine.

Traditional knowledge of medicinal plants remains a critical source of primary healthcare for many indigenous communities in South and Southeast Asia. Das and Nongmaithem (2019) conducted a phytochemical investigation on selected medicinal plants used by the Maring tribe of Manipur, India. Their study confirmed the presence of bioactive compounds, including alkaloids, flavonoids, saponins, tannins, and terpenoids, thereby validating the therapeutic relevance of traditional formulations.

Similarly, Rahman et al. (2007) recorded over 70 medicinal plant species used by the Chakma tribe across the Hill Tracts region. Their documentation highlights the reliance of indigenous groups on forest-derived remedies for various health conditions, and calls for further pharmacological validation. Rai and Lalramnghinglova (2010) highlighted the richness of ethnomedicinal plant resources in Mizoram, India, documenting 380 plant species used for ailments ranging from digestive disorders to respiratory and skin diseases. The study emphasised the significance of preserving traditional knowledge, which is at risk due to modernisation and habitat loss.

Mizoram, located in the biodiversity-rich Eastern Himalayan region, has long been recognised for its wealth of ethnomedicinal knowledge and plant diversity. In an early ethnobotanical study, Lalramnghinglova and Jha (1997) documented a wide range of medicinal plants used by various tribal groups in Mizoram, detailing the methods of preparation and traditional beliefs associated with healing practices. This foundational work emphasised the urgency of documenting oral traditions before they erode under the pressure of modernisation.

Complementing this, Singh et al. (2002) provided a comprehensive account of the region's flora in their publication Flora of Mizoram, listing over 2,000 species, many of which hold medicinal value. The integration of floristic inventories with ethnomedicinal documentation is critical for conservation and pharmacological validation. When considered alongside Rai and Lalramnghinglova's (2010) more recent ethnomedicinal survey, it becomes clear that Mizoram represents a significant repository of traditional health knowledge in Northeast India. These collective works not only highlight the cultural and therapeutic relevance of medicinal plants but also underscore the role of regional floristic diversity in sustaining indigenous health systems.

1.3 Methodology

1.3.1 Description of study Area

Champhai is one of the 8 districts in Mizoram, amidst the North East Region of India. It is located in the eastern part of Mizoram, internationally bordered by Myanmar and therefore becoming the main gate of trading for India and Myanmar. It lies between 23.456°N latitude and 93.328°E longitude. The average annual rainfall is approximately 1814 mm and the temperature remains around 18.6°C which is slightly colder than the rest of the state during winter. The total land area is 3185.83 sq kilometres at an elevation around 1,678 m above sea level, population density is 10 per sq kilometres (32,734). According to an official Census (2011), Champhai reported a population of 1.26 lakhs, of which male and female were 62,357 and 63,388 respectively (Champhai, 2021; Khajuria et al., 2021). The study area was divided into 15 village council areas (Vengthlang, Vengthlang North, Venglai, Vengsang, Electric veng, Kanan, Kahrawt, Bethel, New Champhai, Zotlang, Hmunhmeltha, Tualcheng, Ngopa, Khawzawl and East Lungdar) for extensive data collection (Fig. 1.1). The majority of people living in this area are Mizo tribe and use the Mizo dialect in common.

1.3.2 Investigative method

In the field study, formal questionnaires were distributed to each participant while having face to face interviews at their residence (Mukemre et al., 2015). At least 16 people were interviewed in each village council area. Only those people who were skilled in the art of preparing medicines either for their families or their neighbourhood were considered for the interaction. The interactions primarily focussed on their experience, type of dosage form, duration of usage, any adverse effects observed and the source of their knowledge about the plant and their parts used (Ayyanar & Ignacimuthub, 2011). This information was then correlated with the scientific data curated from related databases (Pubmed, SciFinder and Science Direct). In most of the cases, the voucher specimens were deposited (Herbarium, Mizoram University, Aizawl, Mizoram, India) for their authentication and archiving.

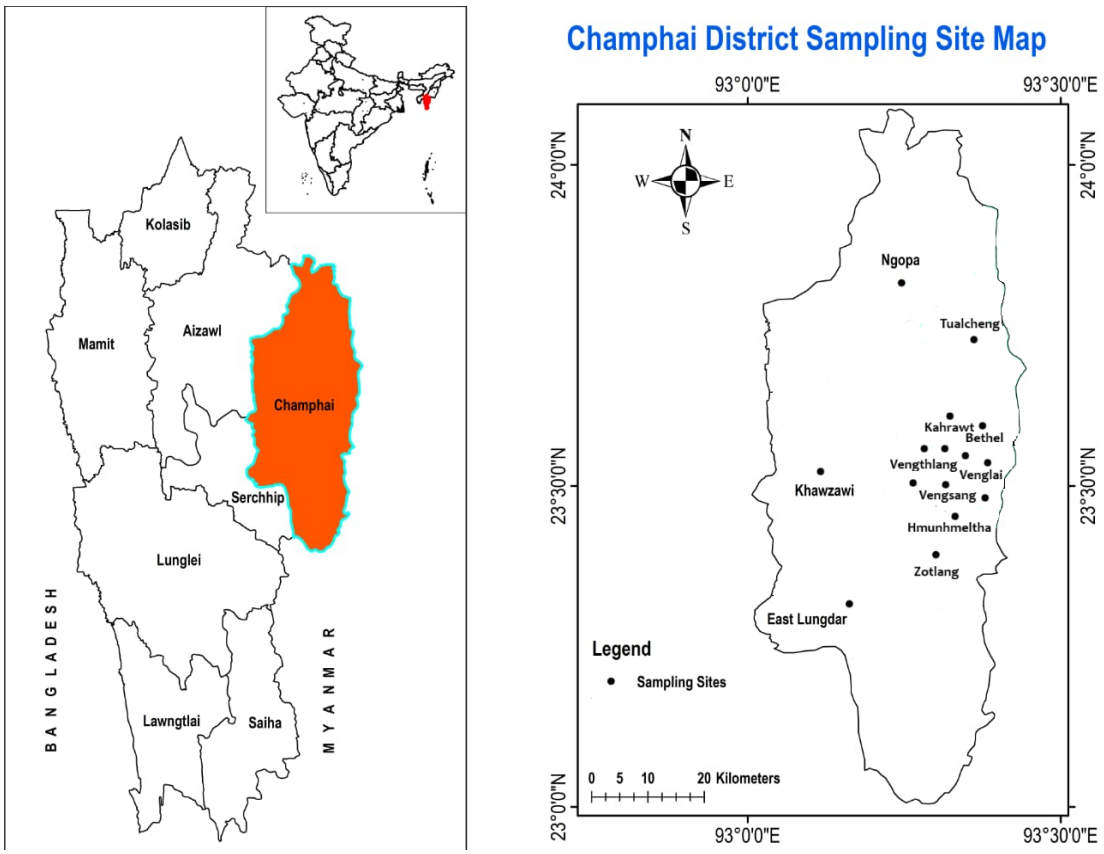


Fig. 1.1 Location of the present study area: Champhai District, Mizoram, India

1.3.3 Characteristics of demographic data

To demonstrate the socio-economic information of the informants, age, sex, education level, and occupation were recorded (Mukemre et al., 2015).

1.3.4 Quantitative analysis

1.3.4.1 *Frequency of citation (FC)*

Frequency of citation was used to further examine the primary data by finding the sum of total citations/usage reports for a particular species. The usage report is the quotation of one plant by an informant (Gazzaneo et al., 2005).

FC = Total no. of informants who convey the use of the plant

1.3.4.2 *Use Value*

Use Value or UV is used to express the correlative importance of each particular plant species locally known and was calculated by the following equation (Heinrich et al., 1998).

$$UV = \sum \frac{U_i}{n}$$

Where 'U_i' represents the number of citations of each species by the informants and 'n' represents the total number of informants in the study area. The larger the number of citations, the greater is the use-value.

1.3.4.3 *Informant Consensus Factor*

F_{ic} or ICF is used to represent the consistency of the information among the informants, indicating whether there was shared knowledge and concurrence in the use of plants for treating the ailment category amongst the plant's users in the study area. It was calculated by the following equation (Albuquerque et al., 2006).

$$F_{ic} = \frac{\mathrm{N}_{ur} - N_t}{\mathrm{N}_{ur} - 1}$$

Where 'N_{ur}' refers to the number of users reports in each illness category and 'N_t' refers to the number of plant species used for a particular illness category by all the informants.

1.3.4.4 *Relative importance*

When calculating RI, both the informants who mentioned the useful plant species and their various kinds of uses are considered. So, it was calculated by the following equation (Albuquerque et al., 2006).

$$\mathrm{RI} = \mathrm{NUC} + \mathrm{NT}$$

where 'NUC' refers to the number of illnesses use category number of illness types of uses of each species divided by the total number of most use categories among the species and 'NT' refers to the number of illness types of uses of each species divided by the total of most types of uses among the species.

1.3.4.5 *Cultural values*

In this index, the use category is taken into account and it was calculated by using the following equation (Sujarwo & Caneva, 2016).

$$CVs = UCs \times ICs \times \Sigma IUCs$$

where 'UCs' is the number of the used reports for each species divided by the total number of use categories of that species. 'ICs' is the number of informants who mention each plant as effective divided by the total number of informants, and ΣIUCs is the number of informants who report the use of each species divided by the total number of informants.

1.3.4.6 Used Report (UR)

UR is the total number of uses of the plant species mentioned by informants for each illness (Whitney et al., 2012; Reang et al., 2023)

UR = Total no. of uses mention by informants in each ailment of the plant species.

This survey is intentionally conducted to document and study ethnomedicinal plants in Mizoram used by the indigenous people, while also helping to screen and select anti-diarrhoeal plants.

1.4 Results

1.4.1 Demographic characteristics

All the 200 respondents were randomly selected from 15 village council areas (Fig. 1.1) interviewing at least 16 persons in each area with no equal separation of male-female ratio. Among them, the elderly aged 70 and above accounted for only 6.5%, while people aged 31-50 accounted for 34.5%. The average age among the informants was 54 years. Mizoram is the second most literate state in the country (2011 census) and all the informants were literate having at least primary school level education. Out of the total informants, 32.5% were engaged in government jobs like teachers, officers, while 35% were self-employed like farmers, carpenters, skilled workers, small businesses and the rest 32.5% of the informants were unemployed including students and housewives (Table 1.1).

Table 1.1 Demographic characteristics of informants (n = 200)

Age	Number	In percent (%)
18-30	42	21
31-50	69	34.5
51-70	76	38
71 above	13	6.5
Sex		
Male	112	56
Female	88	44
Educational level		
Primary	30	15
Middle	44	22
High School	56	28
Higher Secondary	32	16
University	38	19
Occupation		
Self Employed (farmer, carpenter, bussiness)	70	35
Govt. Employed (teacher, bank, officer)	65	32.5
Unemployed (Student, housewives)	65	32.5

1.4.2 Taxonomy Enumeration

In the present study, 93 medicinal plant species belonging to 53 families have been reported for treating various kinds of ailments (Fig. 1.2). The most prominent families were Euphorbiaceae with 6 plant species followed by Asteraceae with 5 plant species and 4 species each among Cucurbitaceae and Zingiberaceae. Liliaceae, Fabaceae, Verbenaceae, Solanaceae, Rutaceae, Anacardiaceae are with 3 species each while Orchidaceae, Combrethaceae Theaceae, Arecaceae, Apocynaceae, Musaceae, Rubiaceae, Schrophulariaceae, Lamiaceace, Mimosaceae, Smilacaceae are with 2 species each and other 34 families with one species each as shown in Table 1.2. The high usage report of this large family like Euphorbiaceae (6 species), Asteraceae (5 species) and Zingiberaceae (4 species) occupied 10.8%, 9.2% and 8.35% of the total used report respectively indicating that most people in the study area are inclined to use plants that are easily available and abundant around them (Table 1.2).

Table 1.2 Family with their used report and in percent

Family	No of species	No of used report	%
Liliaceae	3	76	2.8
Apocynaceae	2	53	1.95
Bromoliaceae	1	49	1.8
Ochidaceae	2	14	0.52
Combretaceae	2	55	1.66
Euphorbiaceae	6	294	10.8
Meliaceae	2	54	1.99
Begoniaceae	1	37	1.36
Cucurbitaceae	4	82	3.02
Amarathaceae	1	15	0.55
Betuliaceae	1	7	0.26
Fabaceae	3	56	2.06
Verbenaceae	3	78	2.87
Theaceae	2	28	1.03
Cannabinaceae	1	36	1.32
Apiaceae	1	29	1.07
Solanaceae	3	83	3.05
Caricaceae	1	53	1.95

Fagaceae	1	17	0.63
Zingiberaceae	4	227	8.35
Asteraceae	5	250	9.2
Rutaceae	3	120	4.42
Araceae	1	2	0.07
Dilleniaceae	1	22	0.81
Discoreaceae	1	27	0.99
Caryophyllaceae	1	28	1.03
Eleagnaceae	1	31	1.14
Areaceae	2	86	3.17
Musaceae	3	80	2.94
Rubiaceae	2	38	1.4
Protiaceae	1	15	0.55
Malvaceae	1	4	0.15
Saurauraceae	1	16	0.59
Convulvulaceae	1	37	1.36
Rosaceae	1	14	0.52
Schrophulariaceae	2	48	1.77
Campanulaceae	1	25	0.92
Myrsinaceae	1	4	0.15
Magnoliaceae	1	18	0.66
Anacardiaceae	3	79	2.91
Lamiaceae	2	40	1.47
Clusiaceae	1	18	0.66
Mimosaceae	2	53	1.95
Moraceae	1	2	0.07
Bignoliaceae	1	37	1.36
Pandanaceae	1	24	0.88
Phyllanthaceae	1	48	1.77
Plantaginaceae	1	36	1.32
Myrtaceae	1	98	3.61
Polypodiaceae	1	12	0.44
Punicaceae	1	31	1.14
Smilacaceae	2	26	0.96
Poaceae	1	15	0.55

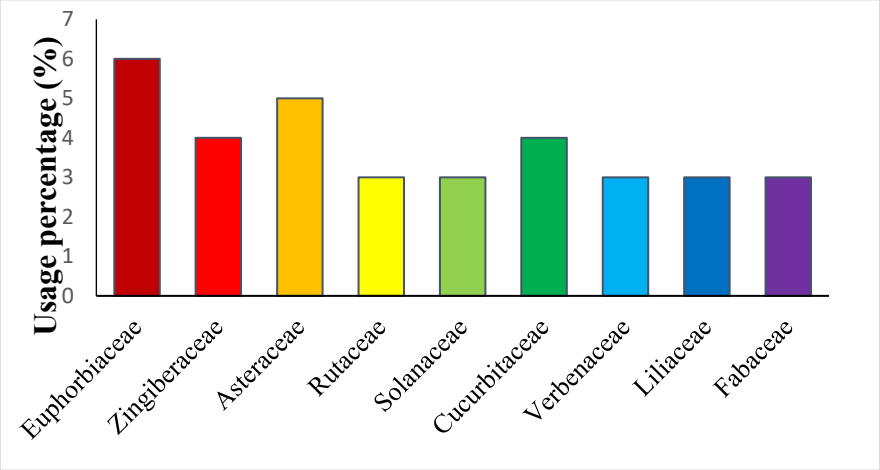


Fig. 1.2 Distribution of plant usage based on the family

1.4.3 Distribution of plant habitat and parts used

The most commonly used medicinal plants fell into the categories of herbs (35.5%), followed by trees (33.3%), shrubs (18.3%), and creepers (12.9%), as shown in Fig. 1.3. Among the parts, leaves, fruits, and barks were mainly utilised by the informants. (Fig. 1.4) A detailed analysis concluded that leaves (47%) followed by fruits (14%), barks (11%), seeds (10%), rhizomes (6%), stems (4%), young shoot (2%), oil (1%) and in some cases the whole plant (3%) were used for ethnomedicinal purposes.

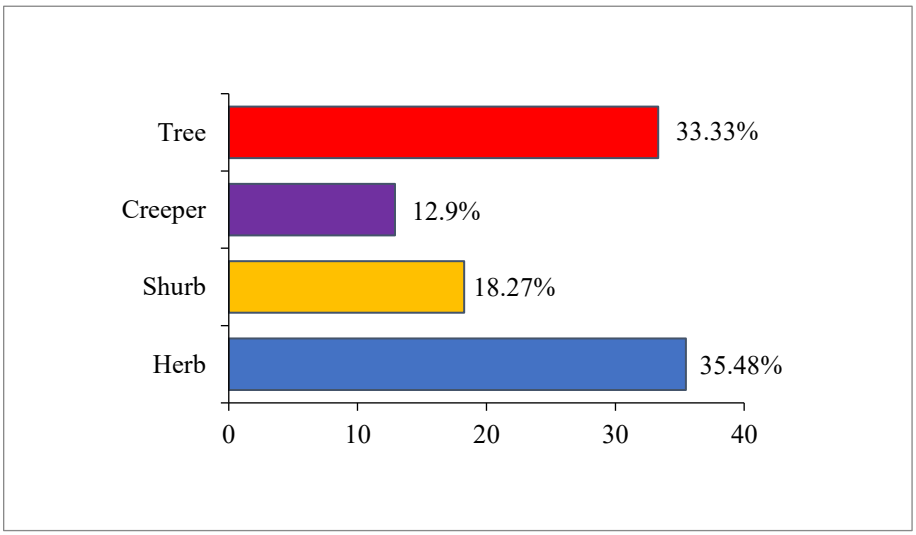


Fig. 1.3 Distribution of plant habit

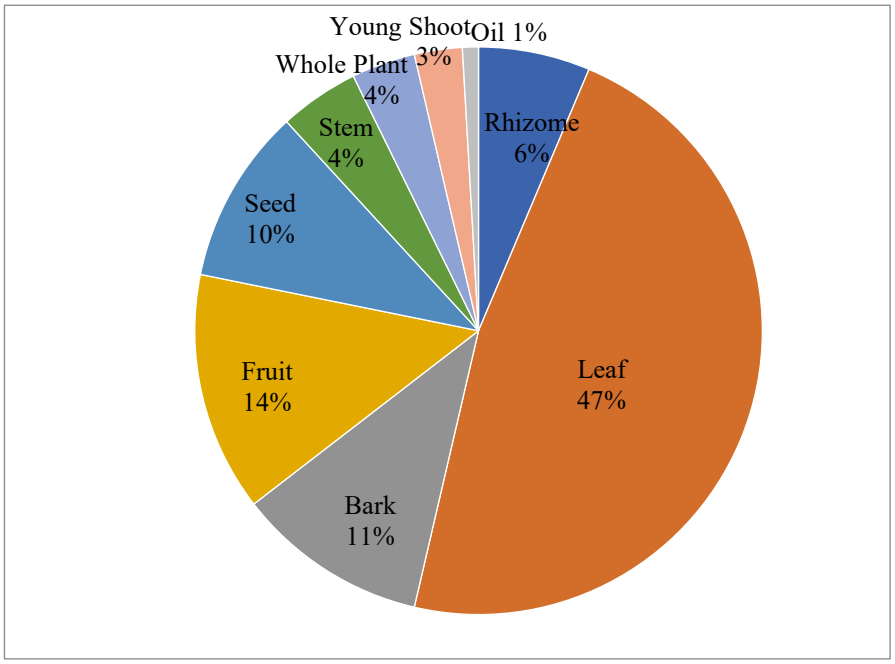


Fig. 1.4 Plants part used

1.4.4 Mode of preparation

The mode of formulation preparation or administration was observed to be in the form of decoction (44.2%) followed by paste (23%), raw (19.5%), juice (9.73%), powder (1.77%) and others like maceration and oil (1.77%) (Fig. 1.5).

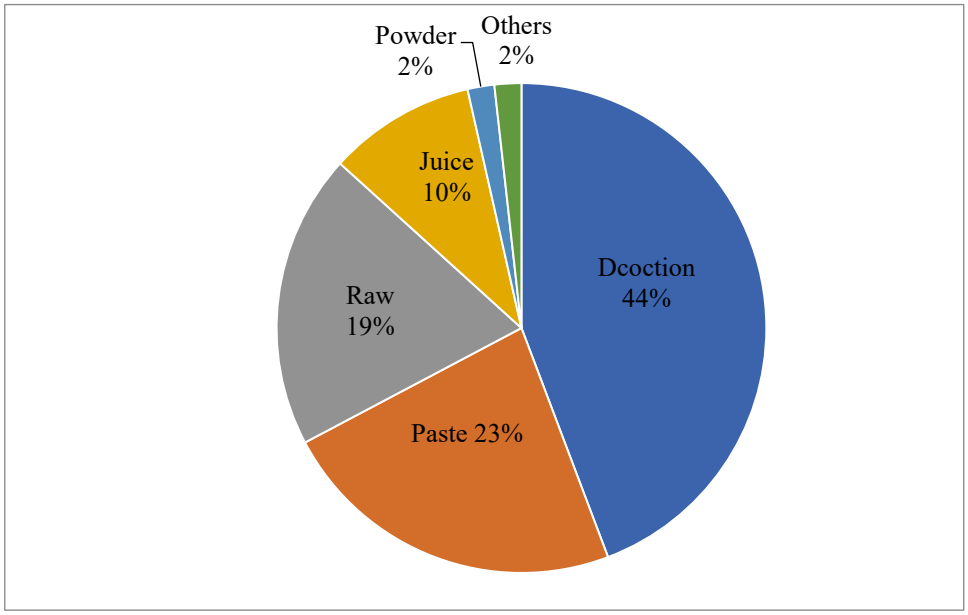


Fig. 1.5 Distribution of formulation usage

Table 1.3 List of documented ethnomedical plants with their related information

Sl. No.	Species Name; Voucher No	Family Name	Local Name	[a]Habit	[b]Part Use	Mode of preparation	Ailments & UR	FC	UV	Isolated Chemical Compounds	Bioactivity
1	*Allium cepa* L. (HAMP20045)	Liliaceae	Purun sen	H	Rt (Bulb)	Raw	Hair care (12), headache (1)	13	0.065	S-alk(en) S-alkyl-substituted cysteine sulphoxides, quercetin, kaempferol (Kundan & Anupam, 2006)	Antioxidant, appetizer, Antimicrobial, Hypertension, hair regrowth (Sharquie & A-Obaidi, 2002; Teshika et al., 2019)
2.	*Allium sativum L.* (HAMP20046)	Liliaceae	Purun var	H	Rt (Bulb)	Raw	Tension (10), toothache (2), cold (12), pimple (7)	31	0.155	S-allyl-cysteine, Allyl thiosulfinate (Allicin), S-ally-mercapto cysteine (Batiha et al., 2020; Miron et al., 2000)	Fever, hypertension, antitumor, antioxidant, antimicrobial, tooth disease, immune boost, etc. (Harris et al., 2001; Ishtaq et al., 2007; Singh et al., 2008)
3	*Aloe vera* (L.) Burm.f. (HAMP20047)	Liliaceae	Rul lei	H	Lf	Raw	Burn (21), Stomachache (2), skin care (9)	32	0.16	Vitamin E, Sulfuric acid, butyl heptadecylic ester, 1-Tetradecyne (Arunkumar et al., 2009)	Antimicrobial, anti-inflammatory, laxative, antidiarrhoea, wound healing, antiaging, antioxidant, etc. (Christaki et al., 2010; Sahu et al., 2013)
4	*Alstonia scholaris* (L.) R. Br. (HAMP20006)	Apocynaceae	Thuamriat	T	Br	Decoc-tion	Ulcer (15)	15	0.075	Alstonine, picrinine, akuammicine, echitamine (Kalaria et al., 2012)	Antimicrobial, anti-asthmatic, antioxidant, anti-ulcers, anticancer, rheumatic, inflammatory, wound healing, etc (Arulmozhi et al., 2007; Baliga, 2012)

5	*Ananas comosus* (L.) Merr. (HAMP20019)	Bromeliaceae	Lakhuihthei	Sh	Lf, Fr	Raw, paste	Ulcer (12), seizure (1), Hypertension (10), urinary infection (16), lung disease (10)	49	0.245	1-O-feruloylglycerol, tricin, 2, 4-dichlorobenzoic acid, etc (Huang et al., 2015)	Anti-inflammatory, anti-thrombotic, antioxidant, antiedematous, anthelminthic, diuretic, rheumatoid, anticancer, antimicrobial, etc (Pavan et al., 2012; Rathnavelu et al., 2016)
6	*Anoectochilus brevilabris* Lindl. (HAMP20062)	Orchidaceae	Hnah mawi	H	Lf	Paste	Pile problem (12)	12	0.06	Not reported	Not reported
7	*Anogeissus acuminata* (Roxb. ex DC.) Wall. Ex Guill. & Perr. (HAMP20023)	Combretaceae	Zairum	T	Br	Decoc-tion	Ulcer (26)	26	0.13	Castamollilin, Grandinin, (-)-Secoisolariciresinol, 2,3-bis-(4-Hydroxybenzyl butadiene (Lin et al., 1991)	Hypoglycemic, wound healing, cytoxicity, antibacterial, etc (Monali &Padhy, 2018; Panda et al., 2017)
8	*Aporosa octandra* (Buch-Ham. ex D. Don) Vickery (HAMP20035)	Euphorbiaceae	Chhawntual	T	Lf, Br	Decoc-tion	Ulcer (5), uterus problem (11)	16	0.08	2-Methyl-3-en-butyl-cyclohexyl Phthalate, (R)-Coclaurine (AO-5) (Panda et al., 2018)	Antioxidant, anthelmintic, oxidative stress, D-galactose induce protectivity, etc (Vabeiryureila et al., 2014; Sahu et al., 2016)
9	*Azadirachta indica* A. Juss (HAMP20052)	Meliaceae	Neem	T	Lf	Decoc-tion	Malaria (16), jaundice (8)	24	0.12	Diepoxyazadirol, flowerine, flowerone, O-methylazadir-onolide ((Siddiqui et al., 2003)	Immunostimulant, antiviral, analgesic, anti-inflammatory, antiulcer, antioxidant, antimicrobial, hepatoprotective, antimalarial,

											antipyretic, hypoglycemic, etc (Biswas et al., 2002)
10	*Begonia inflata* C.B. Clark. (HAMP20016)	Begoniaceae	Sekhupthur	H, Cr	Lf	Paste, juice	Pile problem (12), diarrhoea (11), dysentery (14)	37	0.185	Not reported	Not reported
11	*Benincasa hispida* (Thunb.) Cogn. (HAMP20028)	Cucurbitaceae	Maipawl	H, Cr	Fr	Decoc-tion	Stomach problem (4), digestion (10), diarrhoea (15)	29	0.145	Pentanoic acid, 5-hydroxy-2,4- dibutyl phenyl esters, Palmitic acid, 9,12-Octadeca-dienylchloride (Doshi et al., 2015)	Anti-obesity; anti-ulcer; anti-inflammatory; antioxidant; anti-diarrhoeal; anxiolytic; antidiabetic, antinociceptive, etc (Al-Snafi, 2013; Qadrie et al., 2009)
12	*Beta vulgaris* L. (HAMP20001)	Amaranthaceae	Beet root	H	Rh	Raw, juice	Anaemias (3), immunobooster (8), cancer (4)	15	0.075	Apigenin, Luteolin, Isoscutellarein 7-O-glucosyl, 8-O-xylosid, Caffeoyl-6-(3,4-dihydroxy benzoyl) β-D-glucoside (El-Hawary et al., 2017)	Antioxidant, anticancer, hepatoprotective, anti-inflammatory, antimicrobial, anti-hypertension, hypoglycemic (El-Beltagi et al., 2018; Ninfali & Angelino, 2013)
13	*Betula alnoides* Buch-Ham. ex D. Don (HAMP20017)	Betulaceae	Hriang	T	Lf	Paste	Toothpaste (7)	7	0.035	α-pinene; a-terpineol; limonene; camphor; β-pinene (Dung et al., 1995)	Cytotoxicity, anti-inflammatory, antioxidant, antimicrobial, etc. (Thu et al., 2010; Yang et al., 2013)

14	*Bischofia javanica* Blume (HAMP20036)	Euphorbiaceae	Khuang thli	T	Lf	Paste	Toothache (13)	13	0.065	3,4-dihydroxyphenylethyl alcohol, isotachioside, catechin, epicatechin, gallocatechin (Khan et al., 2001)	Antimicrobial, antiulcer, cytoxicity, antidysentry, anthelminthic, etc. (Lingadurai et al., 2011)
15	*Cajanus cajan* (L.) Millsp. (HAMP20041)	Fabaceae	Behliang	Sh	Lf	Decoc-tion	Jaundice (25), intestinal worms (3)	28	0.14	3,5-bis1,1-dimethylethyl, Tetradecanoic acid, allyl hexadecyl, ester, n-Hexadeca noic acid (Anadebe et al., 2017)	Antimicrobial, antidiabetic, antioxidant, glycemic, anthelmintic, hepatoprotective, etc (Pal et al., 2011)
16	*Callicarpa arborea* Roxb. (HAMP20087)	Verbenaceae	Hnah kiah	T	Lf, Br	Decoc-tion	Stomach ache (9), diabetes (16), convulsion (7)	32	0.16	Martynoside, Isomartynoside, Ursolic acid, Antiarol rutinoside (Tu et al., 2013)	Antioxidant, antimicrobial, analgesic, anti-inflammatory, neuroprotective, antidiabetic, etc. (Tu et al., 2013)
17	*Camellia sinensis* (L.) Kuntze (HAMP20082)	Theaceae	Thingpui fe	Sh	Lf	Raw, Decoc-tion	Toothache (7), itchy eye (7)	14	0.07	Caffeine, Theobromine, Gallic acid, Ampelopsin, epicatechin-3-O-gallate, catechin-3-O-gallate (Zhu et al., 2013)	Antimicrobial, tooth decay, antioxidant, anticancer, anti-obesity, antidiabetic, anti-inflammatory, etc (Hamilton-Miller, 1995; Sarangi, 2009)
18	*Cannabis sativa* L. (HAMP20021)	Cannabinacae	Trip/ Kanza	H	Lf	Raw,	Stomach ache (12), diarrhoea (24)	36	0.18	Cannabigerol, cannabichromene, cannabidiol, cannabisativa, delta 9-	Antioxidant, anti-allergic, anti-inflammatory, analgesic, anti-tumour, anti-diabetic, antibiotic (Nuutinen, 2018; Siddique et al., 2007)

										Tetrahydrocannabinol (Grutenherman et al., 2002)	
19	*Centella asiatica* (L.) Urb. (HAMP20005)	Apiaceae	Darbeng bur /lambak	H	WP	Decoc-tion	Stomach ache (8), urinary infection (17), kidney disease (2), eye pain (2)	29	0.145	Centellin, asiaticin, centellicin (Kalshetty et al., 2012)	Ati-ulcer, neuroprotective, antidepressant, anticonvulsion, immunostimulating, antioxidant, antibacterial, kidney injury protectivity, etc (Punturee et al., 2005)
20	*Capsicum annuum* L. (HAMP20084)	Solanaceae	Hmarhch a	H	Fr	Paste	Wasp sting (9), burn (3), toothache (4), boil (2)	18	0.09	Ascorbic acids, quercetin, luteolin, chrysoeriol, hydroxycinnamic acids (Marin et al., 2004)	Anti-inflammatory, antimicrobial, antiviral, anticancer, analgesic, etc (Khan et al., 2014)
21	*Carica papaya* L. (HAMP20026)	Caricaceae	Thingfan ghma	T	Lf, Fr sap	Paste, raw	Tonsil (1), face pack (3), cancer (16), diabetes (11), dog bite (17), milk booster in mother (5)	53	0.265	Benzyl-β-d glucoside, β-sistosterol, β-sistosterol, oleic acids (Yogiraj et al., 2014)	Antimalaria, antidiuretic, antimicrobial, anthelminthic, hepatoprotective, immunomodulatory, etc (Adam et al., 2013; Prakash et al., 2012; Krishna et al., 2008)

22	*Castanopsis tribuloides* (Sm.) A. DC. (HAMP20044)	Fagaceae	Thingsia	T	Br	Paste	Toothache (17)	17	0.085	Not reported	Antioxidant [77]
23	*Catharanthus roseus* (L.) G. Don (HAMP20007)	Apocynaceae	Kumtlua ng	T	Lf	Decoc-tion	Hypertension (18), diabetes (20)	38	0.19	4-O-caffeoylquinic acid, quercetin-3- O-(6-O-rhamnosyl-glucoside), kaempferol-3-O- (6-O-rhamnosyl-glucoside) (Ferreres et al., 2008)	Antihypercholesterollemi, anti deuretic, antibacterial, antimalaria, antiviral, cytotoxic, antidiabetic, antihyperglycemic, antidiarrhoeal, antioxidant, anthelminthic, etc (Aslam et al., 2010; Gajalakshmi et al., 2013)
24	*Cheilocostus speciosus* (J. Koenig) C.D. Specht (HAMP20090)	Zingiberaceae	Sumbul	Sh	Rh	Decoctio n	Kidney disease (5), urinary problem (16), stomach ache (18)	39	0.195	24-hydroxytriacontan-26-one, sitosterol, cyloartanol, methyl triacontanoate (Hassan et al., 2016)	Antifungal, insecticidal, antioxidant (Ayam et al., 2018; Beneli et al., 2017)
25	*Chromoleana odorata* (L.) R.M. King & H. Rob. (HAMP20011)	Asteraceae	Tlangsa m	H	Lf	Decoc-tion, raw	Kidney disease (18), diarrhoea (13), stomach, ache (8), jaundice	87	0.435	α & β-pinenes,1,8-Cineole, β-Calacorene, β-Copaen-4α-ol (Owolabi et al., 2010)	Antipyretic, analgesic, anti-inflammatory, antispasmodiac, antimalaria, antioxidant, antimicrobial, etc (Taiwo et al., 2000; Vaisakh &Panday, 2012)

							(14), wound (34)				
26	*Citrus aurantiifolia* (Christm.) Swingle (HAMP20074)	Rutaceae	Champara	T	Fr	Juice	Stomach problem (7), digestion (9)	16	0.08	Pinene, Sabinene limonene, Myrcene, Telinene (Abyaneh et al., 2008)	Antimicrobial, antioxidant, antiobesity, anthelminthic (Taur et al., 2009; Pathan et al., 2012)
27	*Citrus maxima* (Burm.) Merr. (HAMP20075)	Rutaceae	Sertawk	Sh	S	Raw	Hypertension (36)	36	0.18	Naringenin,5,7-dihydroxylcoumarin, 1, 3,5-trihydroxyhenzene, xanthotoxol (Xu et al., 2015)	Antimicrobial, analgesic, antioxidant, anti-obesity, anti-inflammatory, etc (Abirami et al., 2013; Shivananda et al., 2013)
28	*Citrus limon* (L.) Osbeck (HAMP20076)	Rutaceae	Nimbu	T	Fr	Juice	Stomach problem (24), digestion (44)	68	0.34	Ascorbic acid, y-Aminobutyric acid, alanine, aspartic acid, arginine (Kefford, 2016)	Anticancer, antiparasitic anti-inflammatory, antimicrobial (Szcykutowicz et al., 2020)
29	*Clerodendrum glandulosum* Lindl. (HAMP20088)	Verbenaceae	Phuihnam	Sh	Lf	Decoc-tion	Hypertension (44)	44	0.22	Strongyloster, lupeol, n hentriacontane, palmitic acid, 2-pentadecyn-1-ol, hexacosane, vitamin E (Jadeja et al., 2012)	Antidiabetic, antimicrobial (Jadeja et al., 2012)

30	*Colocasia esculenta* (L.) Schott (HAMP20008)	Araceae	Dawl	H	St sap	Juice	Vaginal discharge/ Lochia (2)	2	0.01	14α-methyl-5α-cholesta-9, 24-diene-3b, 7α-diol; 9, 12, cyanidin 3-glucoside; 9, 12, 13-trihydroxy-(E)- 10-octadecenoic acid (Prajapati et al., 2011)	Antihepatotoxicity, anthelminthic, anticancer, antimicrobial, antioxidant, anti-inflammatory, etc. (Agyare et al., 2016; Kubde et al., 2010)
31	*Combretum wallichii* DC (HAMP20024)	Combretaceae	Leihruisen	Sh	Sh	Raw	Tonsil (19)	19	0.095	Not reported	Anthelminthic (Kone et al., 2012)
32	*Crassocephalum crepidioides* (Benth.) S. Moore (HAMP20012)	Asteraceae	Buar thau	H	Lf	Paste	Wound bleeding (14)	14	0.07	(E)-β-farnesene, α-humulene, cis-β-guaiene, α-bulnesene (Joshi et al., 2014)	Anti-tumour, cytoxicity, antidiabetic (Tomimori et al., 2012; Bahar et al., 2017)
33	*Cucurbita maxima* Duchesne (HAMP20029)	Cucurbitaceae	Mai	H, Cr	S	Raw	Instestinl worm (15)	15	0.075	Oleic acid, linoleic acid, palmitic acid, caffeic, syringic, vanillic, p-coumaric (Rezig et al., 2012)	Antidiabetic, anticancer, antiobesity, antihelmintic, cytotoxicity, antibacterial, anti-inflammatory, anti-parasitic (Md, 2017)
34	*Curcuma ceasia* Roxb. (HAMP20091)	Zingiberaceae	Ailaidum	H	Rh	Raw, juice	Stomach ache (4), diarrhoea (18)	22	0.11	α, β-pineneeucalyptol, camphor, camphene, gallic acid, quercetin (Borah et al., 2018)	Antimicrobial, analgesic, antioxidant, antiulcer, antimutagenic, anti-asthmatic, anthelminthic, etc (Baghel et al., 2013)
35	*Curcuma longa* L. (HAMP20092)	Zingiberaceae	Aieng	H	Rh	Powder, juice	Ulcer (67), diarrhoea (4),	136	0.68	Curcumin, ar-turmerone, β-sesquiphellandrene,	Antimicrobial, anticancer gastrointestinal and respiratory

							derma care (30), stomach ache (35)			curcumenol (Lateef et al., 2016)	disorder, anti-inflammatory, anti-diabetic, anti-allergic, hepatoprotective, anti-dermatophytic, neuroprotective, etc. (Krup et al., 2013)
36	Cucumis sativus L.h (HAMP20030)	Cucurbitaceae	Fanghma	H, Cr	Lf	Decoc-tion, raw	Malaria (8), derma care (17)	25	0.125	Myristic acid, karounidiol, avenasterol, palmitoleic acid, alpha-linolenic acid (Wu et al., 2012)	Antimicrobial, antiulcer, antitumour, wound healing, hypoglycemic, hypolipidemic, anti-inflammatory, antioxidant, etc (Saeedi et al., 2020)
37	*Dichrocephala integrifolia* (L.f.) Kuntze (HAMP20013)	Asteraceae	Vawkek tumtual	H	Lf	Decoc-tion	Kidney disease (24)	24	0.12	Stearic acid, stigmasta-7,22-dien-3-ol, epifriedelanol, Methyl stearate, tritetracontane (Zhu et al., 2010)	Antimicrobial, cytotoxity, antidiarhoeal, antioxidant, anti-inflammatory, neuroprotectivity (Emegam et al., 2017; Kouemouet al., 2017; Lee et al., 2015)
38	*Dilllenia pentagyna* Roxb. (HAMP20032)	Dilleniaceae	Kaihzawl	T	Lf, Br	Decoc-tion	Diarrhoea (21), kidney disease (1)	22	0.11	Dillenetin, betunaldehyde, betulinic acid, quercetin, kaempferol glucoside, lupeol (Gandhi, 2013)	Antimicrobial, antiviral antioxidant, anticancer, antidiabetic, anti-inflammatory (Gandhi, 2013)
39	*Dioscorea alata* L. (HAMP20033)	Discoreaceae	Bachhim	H, Cr	Fr	Decoc-tion	Cancer (27)	27	0.135	Hydro-Q9 chromene, γ-tocopherol-9, 1-feruloylglycerol, RRR-α-tocopherol (Cheng et al., 2007)	Antimicrobial, antioxidant, anti-inflammatory, antidiabetic, etc (Das et al., 2012; Maithili et al., 2011)

40	*Drymaria cordata* (L.) Willd. ex Schult. (HAMP20027)	Caryophyllaceae	Changkalrit	H, Cr	Lf	Decoc-tion, paste	Rheumatism (24), dysentery (4)	28	0.14	Stigmasterol, cerebroside (Nono et al., 2014)	Analgesic, anxiolytic, antipyretic, antitussive, antibacterial, anti-inflammatory (Nono et al., 2014)
41	*Dysoxylum excelsum* Blume. (HAMP20051)	Meliaceae	Thingthupui	T	YS	Decoc-tion	Diarrhoea (18), hyper tension (12)	30	0.15	Isodauc-6-ene-10β,14-diol; 4-epi-isodauc-6-ene-10β,14-diol; 4-epi-6α,10β-dihydroxy-artabotrol (Liu et al., 2012)	Not reported
42	*Elaeagnus caudata* Schltdl. ex Momiy. (HAMP20034)	Elaegnaceae	Sarzuk	T	Lf	Decoc-tion	Vaginal discharge/ Lochia (31)	31	0.155	Not reported	Expelling placenta, miscarriage, jaundice (Jayashree et al., 2012; Sharma et al., 2016)
43	*Elaeis guineensis* Jacq. (HAMP20009)	Arecaceae	Oil palm	T	Oil	Oil	Wound (1), burn (1), hair care (3)	5	0.025	3-isobutyl-2-methoxypyrazine; acetoin; 2-acetyl-1-pyrroline; ethyl hexanoate; 3-methylbutyl xacetate (Lasekan et al., 2007)	Antioxidant, wound healing, antimicrobial, anti-inflammatory, cardiovascular effect, antidiabetic, anticancer, etc (Owoyele et al., 2014; Yin et al., 2013)
44	*Embelia vestita* Roxb. (HAMP20010)	Arecaceae	Tling	Sh	Lf	Decoc-tion	Measles (16), chickenpox (65)	81	0.405	Not reported	Not reported

45	*Ensete glaucum* (Roxb.) Cheesman (HAMP20057)	Musaceae	Saisu	H	St	Decoc-tion	Nephrolithiasis (34)	34	0.17	Not reported	Not reported
46	*Ensete superbum* (Roxb.) Cheesman (HAMP20058)	Musaceae	Changel/ Tumbu	H	St sap, Fr	Juice	Snake bite (6), kidney disease (1), diabetes (4), WBC deficiency (23)	34	0.17	Pentadecanoic acid; 4H-Pyran-4-one, 2,3-dihydro-3, 5-dihydroxy-6-methyl-; 2-Furancarboxaldehyde, 5-(hydroxymethyl) (Shivprasad & Varsha et al., 2018)	Kidney stone preventivity, antioxidant, etc (Sethiya et al., 2016)
47	*Erythrina stricta* Roxb. (HAMP20042)	Fabaceae	Fartuah	T	Br	Decoc-tion	Ulcer (8), kidney disease (2)	10	0.05	β-caryophyllene; δ-cadenine; alpinum isoflavone; obovatin; isovanillin (Akter et al., 2016)	Antimicrobial, epilepsy, antioxidant, leprosy, anti-inflammatory, etc (Araujo-Junior et al., 2012; Subhashini et al., 2011)
48	*Eulophia nuda* Lindl. (HAMP20062)	Orchidaceae	Nauban	H	WP	Decoc-tion	Diarrhoea (2)	2	0.01	Eulophiol;3,4-dihydroxy-3,5,5- trimethoxybibenzyl; nudol; lupeol (Hada et al., 2020)	Antimicrobial, anti-inflammatory, cytotoxic, antioxidant, anticancer, antiasthmatic/ antibronchitis (Jain et al., 2006)
49	*Euphorbia millii* Des Moul. (HAMP20037)	Euphorbiaceae	Hlingluk hum	H	Lf	Raw	Diarrhoea (52)	52	0.26	Abruquinone B; eremopetasitenin A; ellagic acid; isopetasoside; 7,8-	Antimicrobial, antioxidant, cytoxicity, antinociceptive, anthelmintic, etc Rauf et al., 2012; Saleem et al., 2019)

										Dihydroxycoumarin (Pascal et al., 2017)	
50	*Euphorbia royleana* Boiss. (HAMP20038)	Euphorbiaceae	Chawng	Sh	Lf sap	Juice	Otorrhoea (38)	38	0.19	Antiquorine A; Eurifoloid D; sandaracopimaradienolal; 15-isopimaradien-18-a (Wang et al., 2019)	Antitumour, anti-arthritic, antimicrobial, antioxidant, cytotoxicity, anti-inflammatory, etc (Ashraf et al., 2015; Bani et al., 2000)
51	*Flueggea virosa* (Roxb. ex Willd.) Royle (HAMP20039)	Euphorbiaceae	Saisiak	Sh	Lf	Decoc-tion	Diabetes (59), stomach ache (17), chicken pox (50)	126	0.63	11-O-acetyl bergenin; gallic acid; virosecurinine; kaempferol; β-sitosterol; quercetin (Wang et al., 2008)	Anti-inflammatory, anti-pyretic, anti-hepatitis C, etc. (Chao et al., 2016; Ezeonwumelu et al., 2012)
52	*Gomphogyne cissiformis* Griff. (HAMP20031)	Cucurbitaceae	Lalruang a dawi bur	H, Cr	Fr	Juice	Hypertension (6), diabetes (7)	13	0.065	Not reported	Not reported
53	*Hedyotis scandens* Roxb. (HAMP20072)	Rubiaceae	Kelhnam tur	H	Lf	Decoc-tion	Kidney disease (4), pain relief (7), diabetes (10)	21	0.105	Hedyotoside A; hedyotoside B; hedyotoside C, D & E (Wang et al., 2013)	Antimicrobial, abdominal pain (Rahman et al., 2007; Subba & Basne, 2014)
54	*Helicia robusta* (Roxb.) R.Br. ex Blume (HAMP20069)	Protiaceae	Pasal-taka-za	T	Lf	Decoc-tion	Placenta discharge (6), stomach pain (8), kidney disorder (1)	15	0.075	Not reported	Not reported

55	*Hibiscus sinensis* Mill. (HAMP20052)	Malvaceae	Midum par	Sh	Lf	Paste	Boil (4)	4	0.02	Quercetin-3,5-diglucoside, undecanoic acid, cyanidin chorides, rachidic acid, cyanin (Jadhav et al., 2009)	Antioxidant, antipyretic, antimicrobial, antifertility, anticonvulsive, etc (Jadhav et al., 2009; Mak et al., 2013)
56	*Houttuynia cordata* Thunb. (HAMP20077)	Saurauraceae	Ui thinthang	H	Lf	Juice	Sainus problem (16)	16	0.08	Quercetin-3-O-β-D-galactoside-7-O-β-D-glucoside, aristolactam A & B, β-Sitosterol, N-phenethyl-benzamide (Fu et al., 2013)	Antiviral, antitumour, antioxidant, anti-inflammatory, antimicrobial, anti-SARS, immunomodulator, etc. (Fu et al., 2013)
57	*Ipomoea batatas* (L.) Lam. (HAMP20025)	Convovulacea e	Kawl-ba-hra	H, Cr	YS	Raw	Digestion (37)	37	0.185	3-mono-O-caffeoylquinic acid; caffeic acid; vitamin C; kaempferol (Mohanraj et al., 2014)	Antidiabetic, anticancer, antioxidant, antiulcer, cardiovascular effect (Mohanraj et al., 2014)
58	*Lablab purpureus* (L.) Sweet (HAMP20043)	Fabaceae	Bepui	Sh	Lf	Paste	Vaccine pain relief (18)	18	0.09	Phytic acid, linoleic acid, linolenic acid (Hossain et al., 2016)	Antidiabetic, antiinflam-matory, analgesic, cyto-toxicity, antimicrobial, antioxidant, hypolipidemic (Hossain et al., 2016; Al-Snafi, 2017)
59	*Laurocerasus undulata* (Buch-Ham. ex D. Don) M. Roem. (HAMP20071)	Rosaceae	Thei-ar-lung	H	Lf	Decoc-tion	Heart disease (14)	14	0.07	Not reported	Not reported

60	*Lindernia ruellioides* (Colsm.) Pennell (HAMP20078)	Schrophulariaceae	Thasuih	H	WP	Paste	Sciatica (24)	24	0.12	Linderruelliosides A & B; plantainoside A; acteoside, desrhamnosylverbascoside (Wei et al., 2018)	Anti-inflammatory, antitumour, antiulcer, analgesic (Das et al., 2019)
61	*Lobelia angulata* G. Forst. (HAMP20020)	Campanulacea	Choak-a-thi	H	WP	Decoc-tion	Tonsil (9), gallstone (16)	25	0.125	Not reported	Not reported
62	*Maesa indica* (Roxb.) A. DC. (HAMP20060)	Myrsinaceae	Arngeng	Sh	Lf	Paste	Toothache (4)	4	0.02	2 5 dihydroxy-6-methyl 3 (hemeos 16-enyl)-1 4benzoquinone (Kuruvilla et al., 2010)	Hypoglycemic (Patil et al., 2014)
63	*Magnolia champaca* (L.) Baill. ex Pierre (HAMP20050)	Magnoliaceae	Ngiau	T	Lf	Micerati on	Itchy eyes (18)	18	0.09	Butanoic acid, 2-methyl-3-oxo-, ethyl ester; Camphorsulfonic acid (Wei et al., 2011)	Anti-inflammatory, antipyretic, antimicrobial, anticancer (Wei et al., 2011)
64	*Mangifera indica* L. (HAMP20002)	Anacardiaceae	Theihai	T	YS	Decoc-tion	Diarrhoea (5), diabetes (10), hypertension (10), asthma (6)	31	0.155	Quercetin-3-O-β-L-rhamnopyranoside; maclurin-3-C-β-glucoside; amentoflavone; mangiferin (Vimala et al., 1997)	Antidiabetic, antiviral, antiulcer, antimalarial, hepatoprotective, anti-inflammatory, antibacterial (Dan et al., 2011; Pravez et al., 2016)

65	*Mentha arvensis* L. (HAMP20048)	Lamiaceae	Pudina	H	Lf	Decoc-tion	Stomach problem (38)	38	0.19	Menthol, p-menthone, neo-menthone, iso-menthone (Pandey et al., 2003)	Antioxidant, analgesic, antibacterial, cytotoxic, antifertility, anti-inflammatory, antiallergic (Thawkar et al., 2016)
66	*Mesua ferrea* L. (HAMP20022)	Clusiaceae	Herhse	T	Lf	Paste, decoc-tion	Wound (8), stomachache (7), diarrhoea (3)	18	0.09	1,5-dihydroxyxanthone; euxanthone, 7-methyl ether; β-sitosterol (Chow, 1968)	Antimicrobial, anti-inflammatory, antiulcer, anti-arthritis, analgesic, analgesic, hepatoprotective, antioxidant (Chahar et al., 2013)
67	*Mikania micrantha* Kunth (HAMP20014)	Asteraceae	Japan Hlo	H, Cr	Lf	Paste, decoc-tion	Wound (52), diarrhoea (8), dysentery (6), stomach ache (16)	82	0.41	β-cubebene; 1H-inden-1-one, 5-(1, 1-dimethylethyl)-2, 3-; allo-aromadendrene; β-caryophyllene (Shao et al., 2001)	Antimicrobial, antioxidant, anthelminthic (Dev et al., 2015; Lentz et al., 1998)
68	*Mimosa pudica* L. (HAMP20054)	Mimosaceae	Hlonuar	H	Lf	Decoc-tion	Kidney disease (37)	37	0.185	7, 8, 3', 4'-tetrahydroxyl-6-C [alpha- L-rhamnopyranosyl-(1 --> 2)]-beta- D-glucopyranosyl flavone; catcher; 5, 7,3', 4'-tetrahydroxyl-6-C [alpha-L-rhamnopyranosyl-(1 --> 2)]-beta- D-glucopyranosyl flavone (Yuan et al., 2006)	Wound healing, antimicrobial, analgesic, antimalaria, anti-inflammatory, hepatotoxicity, antidiarrhoeal, anthelminthes (Joseph et al., 2013)

69	*Morus macroura* Miq. (HAMP20056)	Moraceae	Lung-li	T	Lf	Raw	Cut / wound (22)	2	0.01	Gallic acid, catechin, p-hydroxy benzoic, ellagic acid,3,4,5- methoxy cinnamic (Ferrag et al., 2017)	Anti-inflammatory, antioxidative, antiulcer (Ferrag et al., 2017)
70	*Musa acuminata* Colla (HAMP20059)	Musaceae	Balhla	H	Fr, sap	Paste, Decoc-tion	Diabetic wound (6), lung disease (2), snake bite (4)	12	0.06	Pantothenic acid (B5), ferulic acid-hexoside, Vitamin C, provitamin A, lycopene (Sidhu & Zafar, 2018)	Antioxidant, antidiarrhoea, immunomodulatory, anti-HIV, antidiabetic, hypolipidemic, anticancer, antimicrobial (Jyothrmayi &Rao, 2017 Mathew & Negi, 2017)
71	*Mussaenda macrophylla* Wall. (HAMP20072)	Rubiaceae	Va-kep	Sh	Lf	Decoc-tion	Internal bleeding (17)	17	0.085	3-O-β-d-glucopyranosyl-28-O-α-l-rhamnopyranosyl-16α-hydroxy-23-deoxyprotobassic acid; 16α-hydroxyprotobassic acid; 3-O-acetyldaturadiol; rotundic acid (Kim et al., 1999)	Antimicrobial (Chowdhury et al., 2013)
72	*Ocimum americanum* L. (HAMP20049)	Lamiaceae	Runhmui	H	Lf, St	Paste	Pile problem (2)	2	0.01	á-pinene; farnesene; terpineol; farnesol; limonen (Shadia et al., 2007)	Antioxidant, antimicrobial (Hakkim et al., 2008; Thaweboon et al., 2009)

73	*Oroxylum indicum* (L.) Kurz (HAMP20018)	Bignoniaceae	Ar-chang-kawm	T	Br, Lf	Paste, Decoc-tion	Ulcer (6), arthritis (13), diarrhoea (3), dysentry (6), hepatitis C (9)	37	0.185	Ellagic acid; oroxylin-A; 7-o-methyl chrysin; palmitoleic; linoleic acids (Ahad et al., 2012)	Anticancer, antimicrobial, hepatoprotective, antiulcer, amtiarthritic, anti-inflammatory, antioxidant, immunostimulatant, cardioprotective (Singh & Chaudary, 2011)
74	*Pandanus odorifer* (Forssk.) Kuntze (HAMP20064)	Pandanaceae	Ram-la-kuih	Sh	Lf	Decoc-tion	Kidney disease 24	24	0.12	Not reported	Anticancer, antimicrobial (Hussain et al., 2019)
75	*Parkia timoriana* (DC.) Merr. (HAMP20055)	Mimosaceae	Zawngta h	T	Fr, peel, Br	Paste, raw	Diabetes (10), baby umblical care (2), hypertension (4)	16	0.08	β-sitostero; javanicoside A; epigallocatechin gallate; ursolic acid; hyperin (Angami et al., 2018)	Antioxidant, antidiabetic, antiproliferative, antibacterial, anti-insecticidal (Angami et al., 2018)
76	*Phylanthus emblica* L. (HAMP20065)	Phyllanthacea e	Sunhlu	T	Fr	Raw, juice	Diarrhoea (2), skincare (12), stomach problem (8), energybooster (7), diabetes (9), hypertension	48	0.24	Ascorbic acids, gallic acids, quercetin, punigluconin, emblicanin A&B, citric acids (Gaire & Subedi, 2014)	Antimicrobial, analgesic, antioxidant, antipyretic, hepatoprotective, antiulcer, antitumour, anti-inflammatory, immunostimulant (Gaire & Subedi, 2

							(6), jaundice (4)				
77	*Picria fel-terrae* Lour. (HAMP20079)	Scrophulariaceae	Khatual	H	Lf	Decoc-tion	Hypertension (24)	24	0.12	Rosmarinic acid; apigenin-7-O-β-D-glucuronide; picfeltarraegenin IV; acteoside; apigenin (Lin et al., 2010)	Antioxidant, anthelmintic, antiproliferative (Kumarasingha et al., 2016; Satria et al., 2017)
78	*Plantago major* L. (HAMP20066)	Plantaginaceae	Kelba-an	H	Lf	Decoc-tion	Kidney disease (2), urinary problem (34)	36	0.18	Plantaginin; plantagonin; ursolic acid; aucubin; palmitic acid, ascorbic acid (Samuelsen, 2000)	Immunomodulator, antioxidant, analgesic, antiulcer, antibiotic, antiviral, cytotoxicity, hepatoprotective (Samuelsen, 2000)
79	*Psidium guajava* L. (HAMP20061)	Myrtaceae	Kawl-thei	T	Lf	Decoc-tion	Diarrhoea (97), hair fall (1)	98	0.49	β-sitosterol; guajanoic acid; oleanolic acid; uvaol; ursolic acid (Begum et al., 2004)	Anti-allergy, antioxidant, antimicrobial, anticough, antinogenetic, analgesic, cytotoxicity, antidiabetic, anti-inflammator (Martha et al., 2008)
80	*Pseudodrynaria coronans* (Wall. ex Mett.) Ching (HAMP20067)	Polypodiaceae	Awmvel	H	Lf	Paste	Herpes (12)	12	0.06	Kaempferol-3-O-(6" -O-acetyl)-β-D-glucopyranoside; astragalin; isoquercitrin; kaempferol3-O-(6" -O-feruloyl-4" -O-acetyl)-β-D-glucopyranoside (Tai et al., 2012)	Antioxidant (Tai et al., 2012)

81	*Punica granatum* L. (HAMP20068)	Punicaceae	Thei-buh-fai	T	Fr	Raw, juice	Anemia (5), immuno-booster (21), cancer (5)	31	0.155	Ellagic acid; kaempferol; anthocyanins; punicalagin; quercetin; luteolin; ellagitannins (Jasuja et al., 2012)	Antiepileptic, antimicrobial, antiviral antidiabetic, antioxidant, anticancer, anti-inflammatory, hepatoprotective (Jasuja et al., 2012)
82	*Rhus chinensis* Mill. (HAMP20003)	Anacardiaceae	Khawmh ma	T	Fr	Raw, powder	Diarrhoea (27), stomach ache (18)	45	0.225	Moronic acid; gallicin; 3-oxo-6β-hydroxyolean-12-en-28-oic; aciddimethyl caffic acid; phytol (Djakpo & Rao, 2010)	Antidiarrhoeal, anticancer, anti-HIV, antidiabetic, hepatoprotective (Djakpo & Rao, 2010)
83	*Sarcococca pruniformis* Lindl. (HAMP20040)	Euphorbiacea	Pawhrual	H	Lf	Raw, Decoc-tion	Tonsil (49)	49	0.245	Not reported	Not reported
84	*Schima wallichii* Choisy (HAMP20083)	Theaceae	Khiang	T	Br/ St	Paste	Insect bite (9), wound (5)	14	0.07	Phenylpropanolamine; rotenone; glycidol; 2, 3-benzofurandione (Rishi et al., 2014)	Anti-inflammatory, antioxidant, antimicrobial, analgesic, anti-pyretic (Das &Gosh, 2013)
85	*Senecio scandens* Buch- Ham. ex D. Don (HAMP20015)	Asteraceae	Sai-ek-hlo	H, Cr	Lf	Decoc-tion, paste	Ulcer (17), diabetes (13), Hypertension (8), toothache (3), wound (2)	43	0.215	Quercetin; kaempferol; luteolin; rutin; phytol, palmitic acid; β-Amyrin; β-Sitosterol (Wang et al., 2013)	Anti-inflammatory, analgesic, mutagenic, antimicrobial, antiviral, anti-tumour, anti-leptospirosis, antioxidant (Wang et al., 2013)

86	*Smilax globra* Roxb. (HAMP20081)	Smilacaceae	Kai-tluang	H, Cr	Lf	Paste	Arthritis (20)	20	0.1	Palmitic acid; β-sitosterol; quercetin; apigenin; 3-methoxygallic acid; lignoceric acid (Hua et al., 2004)	Antimicrobial, antioxidant, cytotoxicity, anti-inflammatory, hepato-protective, antiviral (Hua et al., 2004)
87	*Smilax perfoliata* Lour. (HAMP20080)	Smilacaceae	Kai-ha	Sh, Cr	Lf	Decoc-tion	Dysentry (6)	(6)	0.03	Rutin,1; 6-O-diferuloyl-(3-O-p-coumaroyl)-b-D-fructofuranosyl-(2→1)-2-O-acetyl-a-D-glucopyra noside; cassiamin A & B; narcissin (Biao et al., 2004)	Antioxidant, antimicrobial (Biao et al., 2004)
88	*Solanum americanum* Mill. (HAMP20085)	Solanaceae	Anhling	Sh	Lf	Decoc-tion	Nephrolithiasis (15), urinary retention (6), Kidney disease (11)	32	0.16	Pinoresinol; tetracosanoic acid; syringaresinol; β-sitosterol; scopoletin; medioresinol (Zhao et al., 2010)	Antimicrobial, hepatoprotectivity, anti-inflammatory, anti-seizure, antioxidant, antipyretic, antitumour (Hameed et al., 2017; Jain et al., 2011)
89	*Solanum violaceum* Ortega (HAMP20086)	Solanaceae	Tawkte	Sh	Fr	Decoc-tion, paste	Hypertension (20), diabetes (6), burn (4), boil (1), herpes (2)	33	0.165	7-oxositosterol; yamogenin; 7-oxostigmasteroldiosgenin (Chang et al., 2010)	Antioxidant, antiobesity. anthelminthic, cytotoxity, anti-inflammatory, analgesic, antinociceptive, antipyretic (Karim et al., 2017; Mahaldar et al., 2016)

90	*Spondias pinnata* (L. f.) Kurz (HAMP20004	Anacardiaceae	Tawi-taw	T	Br	Decoc-tion	Diarrhoea (3)	3	0.015	Ellagitannins, galloylgeranin, lignoseric acid, β -carotein, oleanic acid (Bora et al., 2014)	Antimicrobial, cytoxicity, anti-cancer, antioxidant, anthelmintic, thrombolytic activity, antidiarrhoeal (Bora et al., 2014)
91	*Tectona grandis* L.f. (HAMP20089)	Verbenaceae	Teak	T	Br, Lf	Paste	Wound bleeding (2)	2	0.01	Gallic acid; β-sitosterol; betulinic acid; tectoquinone; squalene; lauric acid (Goswami et al., 2009)	Antibacterial, anti-diabetic, antioxidant, antiulcer, antipyretic, anti-inflammatory, analgesic, antiviral, cytotoxic activity (Vyas et al., 2019)
92	*Zea mays* L. (HAMP20070)	Poaceae	Vaimim	H	Lf	Decoc-tion	Kidney disease (15)	15	0.075	Eugenol; cis-α-terpineol; citronellol; 6,11-oxidoacor-4-ene (El-Ghorab et al., 2007)	Antimcrobial, antioxidant, antimutagen (Mendoza-Diaz et al., 2012; Nessa et al., 2012)
93	*Zingiber officinale* Roscoe (HAMP20093)	Zingiberaceae	Sawhthing	H	Rh	Raw, Decoc-tion	Cold / cough (12), digestion (18)	30	0.15	Zingiberene; gingerols; farnesene; curcumene; zingerone; vitamins (Gupta &Sharma, 2011)	Appetizer, antimicrobial, immunostimulant, analgesic, anticancer antioxidant, antidiabetic, anti-inflammatory, antiarthritis, etc. (Rehman et al., 2011)

Habit: H-Herbs; Sh-Shurbs; Cr-Creeper; T-Tree UR-Used reports; FC-Frequency of citation; UV-Use value[b]Part used: Lf-Leaf; Br-Bark; Fr-Fruit, Rh-Rhizome; St-Stem; S-Seed; WP-Whole Plants; YS-Young Shoot

1.4.5 Usage analysis based on the treatment of ailments

The total number of user reports documented in this study was 2,717, categorised into 16 groups, encompassing various illnesses. Among the illness category, the gastro-intestinal disease has the highest usage report (940), followed by skincare (259), cardiovascular (222), kidney disease (196), hyperglycaemia (175), ENT (159), genito-urinary disease (139) and so on, as shown in Table 1.4.

Table 1.4 Usage analysis with illness category and their term.

Illness Categories	Medical Term	Local Term	Frequency of usage
Dental Care (DC)	Tooth decay Toothpaste	Ha muat / Ha nget Ha nawhna	57
Skin Care (SC)	Pimple, burn, Face pack, boil, chickenpox, Measles, Herpes,	Bawl, Kang, Sentut, Khawihli, Awmvel, Tangseh Hmai chiahna,	259
Hair Care (HC)	Growth enhances, shining, hair fall	Sam thatna, Sam tletna, Sam tla	16
Eyes/Nose/ Ears/Mouth (ENT)	Otorrhoea, eye itching, Sinusitis, Tonsilitis	Beng kherh, Mit thak, Sinus, Tonsil	159
Genito-urinary Disease (GUD)	Delivery pain, placenta discharge, urine retention,	Nau neih zawh hlam tlakna / na, Zun in	139
Kidney Disease (KD)	Nephrolithiasis, kidney failure	Kal a lungte awm, Kalna, Kal a hnai awm	196
High glucose level (HGL)	Diabetes type I & II	Zunthlum	175
Cancer Disease (CD)	Breast cancer, Leukemia, lung cancer, etc	Hnute Cancer, Thisen cancer, Chuap cancer leh dangte	75
Liver Problem (LP)	Jaundice, Hepatitis B & C, cholelithiasis, Malaria	Thinlian, Hepatitis, Malaria, Mit a lungte awm	100
Cardiovascular Problem (CP)	Hypertension, Heart problem	Bp sang leh hniam, lung thalo	222

Muscular / Bone Problem (MBP)	Rheumatoid, Arthritis, Sciatica	Ruh seh/Sehpui, thana, Scaitica	81
Respiratory System illness (RSI)	Cold, Cough, Asthma, lung disease	Hritlang, Awmna, Khuh, Thawhah	42
Gastro-intestinal Disease (GID)	Ulcer, stomach pain, Dysentry, Diarrhoea, Digestion, Hemorrhoid, Constipation, Intestinal worms, internal bleeding	Ulcer, Pumna, Ek khal, Pile na, Puar nuamlo, Khawthalo, Santen, Rul hlut, Internal bleeding	940
Wound healing (WH)	Inflammation	Pem thar /Pilh damdawi, Pan, vung	126
Poisonous Bites (PB)	Snake bite, Scorpion bite, dog bite, Wasp sting	Rul chuk, Ui seh, Khuai zuk, Khawmual kaikuang seh	45
General Health (GH)	Fever, Headache, cold, Immuno-booster, Energy booster, etc	Luna, Khawsik, Taksa chakna, Hriselna	81
		Total	**2717**

1.4.6 Data analysis

1.4.6.1 *Frequency of citation*

Among the total number of user reports (UR) cited, *Curcuma longa* L. (136 FC), *Flueggea virosa* (Roxb. ex Willd.) Royle (126 FC), *Psidium guajava* L. (98 FC), *Chromoleana odorata* (L.) R.M. King & H. Rob. (87 FC), *Mikania micrantha* Kunth (82 FC), *Citrus limon* (L.) Osbeck (68 FC), *Carica papaya* L. (53 FC), *Ananas comosus* (L.) Merr. (49 FC), *Sarcococca pruniformis* Lindl. (49 FC), *Phylanthus emblica* L. (48 FC), *Rhus chinensis* Mill. (45 FC), *Clerodendrum glandulosum* Lindl. (44 FC), *Senecio scandens* Buch- Ham. ex D. Don (43 FC) were those species having the highest FC (Table 1.3).

1.4.6.2 *Plant use value*

From the UV value evaluation, *Curcuma longa* L. (0.68), *Flueggea virosa* (Roxb. ex Willd.) Royle (0.63), *Psidium guajava* L. (0.49), *Chromoleana odorata* (L.) R.M. King & H. Rob. (0.43), *Mikania micrantha* Kunth (0.41), *Citrus limon* (L.) Osbeck (0.34),

Carica papaya L. (0.26), *Ananas comosus* (L.) Merr. (0.24), *Sarcococca pruniformis* Lindl. (0.24), *Phylanthus emblica* L. (0.24), *Cleradendrum glandulosum* Lindl. (0.22), *Rhus chinensis* Mill. (0.22), *Senecio scandens* Buch- Ham. ex D. Don (0.21) were reported to have the highest use value (UV).

1.4.6.3 *Informant Consensus Factor*

We calculated the informant consensus factor by categorising the reported illness into 16 ailment groups along with the number of users report and taxa as shown in (Table 1.5) and in our study F_{ic} values ranged from 0.866 to 0.962 which were all close to 1. F_{ic} value with 1 or either close to 1 indicates that a large number of informants had agreed on using a few plants for curing an illness category, while a low F_{ic} value signified that there was an argument on using medicinal plants to treat illness amidst the category. Among the illness categories, Gastro-intestinal disease (GID) scores the highest Fic value, with 940 used reports and 42 plants reported.

1.4.6.4 *Relative importance and cultural value*

Results of top-ranking species in terms of both indices of relative importance and cultural value are given in Table 1.6. This study elucidates the highest cultural valued species and relative importance species utilised by the inhabitants of the study area. In general, the evaluated values were relatively high in case of CVs and an average value of RI (0.607 ± 0.38) clarified that the versatile species, i.e. *Phyllanthus emblica* (RI = 2) was 3.3 times more relevant than the rest of the listed species.

Table 1.5 Informant consensus factor with the used report in each of the ailment categories

Illness Categories	**No of used report**	**No of taxa**	**F_{ic}**
Dental Care (DC)	57	8	0.875
Skin Care (SC)	259	13	0.953
Hair Care (HC)	16	3	0.866

Eyes/Nose/ Ears/Mouth (ENT)	159	9	0.949
Genito-urinary Disease (GUD)	139	9	0.942
Kidney Disease (KD)	196	15	0.928
Endocrinal Disorder (ED)	175	12	0.936
Cancer Disease (CD)	75	5	0.945
Liver Problem (LP)	100	7	0.939
Cardiovascular Problem (CP)	222	14	0.941
Muscle/Bone Problem (MBP)	81	4	0.962
Respiratory System illness (RSI)	42	5	0.902
Gastro-intestinal Disease (GID)	940	42	0.956
Wound healing (WH)	126	10	0.928
Poisonous Bites (PB)	45	5	0.909
General Health (GH)	81	10	0.887
	2717		

1.4.7 Correlation and Validation studies

An attempt was made to compare the use of all the medicinal plants reported by the informants with the previous papers published for their biological activity or ethnomedicines (Table 1.3). According to the studies conducted by Cakilcioglu et al., 2011, it was stated that if a medicinal plant has been reported for similar use in other parts of the world, its pharmacological effect could be more easily known.

The use of crude juice of *Allium cepa* L. showed a significantly higher hair growth rate than tap water when applied twice a day for two months which corroborated the present report of hair regrowth (Sharquie & Al-Obaidi, 2009). Metallothionein, an antioxidant protein present in *Aloe vera* (L.) gel has been reported to have a protective effect against UV and gamma radiation damage to the skin. It scavenges free radicals by preventing the suppression of glutathione peroxidase and superoxide dismutase in the skin (Christaki & Florou, 2010). So, this validated the used of *A. vera* for skin care and burning by the Mizo tribes. In the present study, *Betula alnoides* has been used as toothpaste for

whitening teeth, while it was proved that 80% methanolic bark extract had the potential α-glucosidase inhibitory effect that prevents the (98.4%) at 40 µg/mL concentration (Ghimire et al., 2012). *Cajanus cajan* (L.) Millsp is used effectively in Champhai district to treat jaundice and intestinal worms. To certify this, the methanolic extracts showed hepatoprotective activity in Swiss albino mice by inducing carbon tetrachloride (CCl_4), which causes liver damage. It lowers the serum levels of glutamate pyruvate transaminase (SGPT), also known as alanine aminotransferase (ALT), aspartate aminotransferase (AST), or serum glutamate oxaloacetate transaminase (SGOT) significantly (Nuutinen, 2018).

When the aqueous extracts of *Carica papaya* and *Ananas comosus* L. were given to Spraque Dawley rats orally at doses of 5 and 10 mg/kg. Both possessed mild to vigorous diuretic activity. Careful measures should be taken when using these plants as an increase in the level of urinary K+, serum BUN and creatinine was mentioned (Adam et al., 2013). This validated the used of *C. papaya* and *A. comosus* in kidney disease and urinary infection. The contemporary reports showed that *Drymaria cordata* was used as an instant pain killer for rheumatism meanwhile the scientific study also demonstrated that the aqueous whole plant extract exhibited analgesic and antipyretic properties at doses of 100, 200, and 400 mg/kg p.o mediated through peripheral and central mechanisms (Akindele et al., 2012). The latex water-soluble fraction of *Euphorbia royleana* Boiss. showed dose-dependent anti-arthritic and anti-inflammatory effects in rats and mice administered through gavage at doses of 50–200 mg/kg having more than 1500 mg/kg oral LD_{50} in both (Ashraf et al., 2014). Dose-dependent and significant decline in the number of abdominal constrictions induced by intraperitoneal administration of acetic acid were observed in methanol extract of *Lablab purpureus* Sweet. at a dose of 200 mg and 400 mg exhibit far better analgesic activity than 200 mg aspirin per kg of body weight (Promo et al., 2014).

Colocasia esculenta and *Elaeagnus caudata* were declared to be used to discharge placenta after birth and to treat vaginal discharge (Lochia) for women in the present study. Besides this record, in Cachar hills district of Assam, India 5ml of *Elaeagnus caudata* fresh root extract diluted in 10ml of fresh water was also administered orally once a week

to prevent miscarriage during pregnancy although there is no scientific study to backup this claim (Shivprasad & Varsad, 2018). Apart from present report in Jamaica, *Mikania micrantha* Kunth. was most popularly used too for wound healing and its extract showed anti-inflammatory and antimicrobial activity against common pathogens, namely *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus* sp. (Bakir et al., 2004). In the animal study of anti-urolithialic activity of *Solanum nigrum*, the fruit hydroalcoholic extract elicited potent activity against calcium oxalate urolithiasis effected by ethylene glycol through tumour necrosis factor adiponectin stimulation and alpha inhibition, also maintained the balance between stone promoter as well as inhibitor such as calcium and magnesium respectively (Salama et al., 2019). Thus, this authenticated the used of *S. nigrum* for removing/forming kidney stone by the Mizo tribes in India.

Anoectochilus brevilabris, *Begonia inflata*, *Dysoxylum excelsum*, *Embelia vestita*, *Ensete glaucum*, *Gomphogyne cissiformis, Helicia robusta*, *Laurocerasus undulata* and *Lobelia angulata*, *Sarcococca pruniformis* were the plants that did not have biological activity reported previously which means that there is no scientific validation to support their application. Therefore, these plants were especially recommended in carrying out further investigation. In addition, we compiled the secondary metabolite isolated chemical constituents done by several researchers for all the documented plants in the present study. Further investigation revealed that secondary metabolites from 13 plant species that have neither less nor none chemical compound isolated or identified-*Anoectochilus brevilabris*, *Begonia inflata*, *Castanopsis tribuloides*, *Combretum wallichii*, *Elaeagnus caudata*, *Embelia vestita*, *Ensete glaucum*, *Gomphogyne cissiformis, Helicia robusta*, *Laurocerasus undulata, Lobelia angulata*, *Pandanus odorifer*, *Sarcococca pruniformis* (Table 1.4) which will surely have great potential on ethnopharmacological study.

In this study, 17 plants were reported for treating diarrhoea, such as *Begonia inflata* and *Benincasa hispida, Cannabis sativa*, *Chromoleana odorata*, *Curcuma ceasia, Curcuma longa*, *Dysoxylum excelsum*, *Eulophia nuda*, *Euphorbia millii, Mangifera indica, Mesua ferrea*, *Oroxylum indicum*, *Mikania micrantha, Phylanthus emblica*, *Psidium guajava*, *Rhus chinensis*, *Spondias pinnata*. Among them, the decoction of

Psidium guajava leaf has been effectively used to treat diarrhoea, demonstrating antidiarrhoeal and protein-conserving effects in diarrhoeal rats at doses of 50 and 100mg/kg of body weight. It notably increased kidney weight and the levels of sodium, potassium, and chloride (Addo-Fordjour et al., 2008).

Table 1.6 Species with high cultural values and relative Importance

CVs	RI
Curcuma longa (27.28)	*Phyllanthus emblica* (2)
Flueggea virosa (16.0)	*Carica papya* (1.85)
*Embelia vestita (*13.12)	*Senecio scandens* (1.54)
Psidium guajava (11.64)	*Ananas comosus* (1.54)
Citrus limon (7.16)	*Oroxylum indicum* (1.38)
Mikania micrantha (6.72)	*Chromolaena odorata* (1.37)
Chromolaena odorata (3.40)	*Allium sativum* (1.23)
Euphorbia milii (3.17)	*Ensete superbum (*1.23)
Sarcococca pruniformis (2.82)	*Centella asiatica* (1.23)
Clerodendrum glandulosum (1.93)	*Measia indica* (1.23)
Rhus chinensis (1.82)	*Solanum violaceum* (1.21)
Mimosa pudica (1.129)	*Capsicum annuum* (1.07)

CVs, caltural value; RI, relative importance

1.5 Discussion

According to our findings, women practitioners (44%) were fewer than men (56%), which may be partly explained by the low sex ratio of the district; however, it can be assumed that women play a lesser role in ethnomedicinal practices (Yirga et al., 2012; Prathiban et al., 2016). Among the self-employed, farmers account for 58.5%, businesspersons account for 34.2%, and carpenters account for 7.3%. Farmers represented the highest percentage, as they often lack access to modern healthcare facilities due to various issues, including financial, transportation, and educational barriers. These issues forced them to rely on traditional medicines, cultivating and utilising them more regularly than others, and somehow playing a significant role in conservation as well. Through this study, we observed that young informants, such as students around 18 to 25 years old, have limited expertise in practising ethnomedicine and use it rarely compared to elder informants. This may be due to change in mentality brought by education to rely only on prescribed medicines. Furthermore, the results of the usage of plants dominated by these families were examined. They confirmed the work done by some researchers, stating that the greater the plants grew in the study area, the more they would be favourably and commonly used (Jadid et al., 2020). This supports the non-random plant selection hypothesis by Moerman, 1979.

Large families, such as Asteraceae and Euphorbiaceae, were most utilised, while Orchidaceae and Poaceae were underutilised (as indicated by the low usage report). However, due to non-random selection, small families like Cucurbitaceae and Zingiberaceae became over-represented (high used report). Thus, this implies that medicinal plants are not selected randomly by the inhabitants of Champhai district but are utilised based on their cultural and traditional knowledge (Kutal et al., 2021). In the present study, we listed the accepted botanical names by 'The Plant List' along with their family, local name, habit, mode of preparation, and ailments, as illustrated in Table 4. Out of 93 species, 40 were cultivated species, whereas 53 were found in the wild. There were also 6 invasive alien species most notably *Chromolaena odorata* and *Mikania micrantha*

which were commonly used to treat wounds topically. This is because wounds are the most common form of injury and these two species can be found almost everywhere (Kutal et al., 2021). The frequent use of herbaceous plants as medicines among the informants was due to their richness, abundance as well as their ability to grow easily in nature. Meanwhile, many parts of the world have been commonly using herbs as their medicinal ingredients due to their wide range of medicinal properties (Addo-Fordjour et al., 2008).

Leaves are the most utilised part of the plants due to their ease off collection as compared to their underground part. It is also the active site of photosynthesis accompanied by the production of metabolites (Ayyanar & Ignacimuthub, 2011; Ghorbani, 2005). In addition, leaves can be easily prepared and stored. It can be dried quickly under the sun in a lesser amount of time than other parts like the stem, bark and rhizome. Similarly, it is also reported that decoction was the most common preparation method for herbal medicine, while in some other tribal communities (Simbo, 2010). Preparation of paste was the most common method applied (Poonam & Singh, 2009). For decoction, the plant part was washed thoroughly and boiled in water, and the resulting juice was administered orally. For paste, the materials were crushed or rubbed between the palms and applied topically. To produce a delicate powder, plant parts were shade-dried and then ground. The primary modes of administration used in traditional herbal medicines, as previously reported by Luseba et al. (2014) were oral administration and external topical formulations. Regarding the duration of consumption of herbal medicine, it depends on the illness, whether it is short-term or long-term. For instance, short-term illnesses such as colds, flu, stomach upset, and skin problems, the consumption period did not last more than 1 week.

On the contrary, for long-term illnesses like diabetes, kidney failure and heart diseases, the consumption period of plants (e.g., *Flueggea virosa*) was much longer and lasted more than a month and so on. The inhabitants of the study area extensively exploited medicinal plants to treat various illnesses and other needs, which have not been previously reported. For instance, *Anoetochilus brevilabris* was used for pile treatments,

Betula alnoides as toothpaste, *Capsicum annuum* to soothe and prevent scars from skin burns. *Colocasia esculenta* to expel lochia, *Euphorbia milii* as antidiarrhoea, Lablab purpureus as a pain reliever, *Mussaenda macrophylla* to stop internal bleeding and *Parkia timoriana* for treating baby umbilical cord. From this study it was clear that among the informants, stomach problems like ulcer, indigestion, diabetes, hypertension and kidney problems were common illness resulting in high user rate of consuming herbal medicines and similar record was reported by Mahwasane et al. (2013). Further, skin problem like dermatitis, which was the second highest usage report, was the highest ailment in most other tribal communities, like Malda district in West Bengal, reported by Saha et al. (2014).

Generally, the majority of the informants did not consume the medicines prescribed by the Doctor's prescribed medicines along with their herbal medicine and claimed that many plants like *Sarcococca pruniformis* (tonsil), *Psidium guajava* (diarrhoea), *Mikania micrantha* (cut/ wound), *Flueggea virosa* (chicken pox), and *Elaeagnus caudata* (veginal discharge) were really effective. Most importantly, none of them reported any adverse effect such as vomiting, headache, nausea, allergic reactions and/or skin rashes. Moreover, regarding the expenditure on buying medicines, 38% of the informants usually purchased their herbal medicines either in raw form (*Allium sativum, Allium cepa, Beta vulgaris*) or in processed form like juice (*Citrus limon, Phyllanthus emblica, Citrus aurantiifolia*), fruits (*Punica granatum, Phyllanthus emblica, Cucumis sativus*), and powder (*Curcuma longa*). Concerned about the source of their knowledge, all the informants reported that they have heard and learned some of their information from their elders, family and/or acquaintances. Besides these, 30% of the informants have also gathered additional information through social media and 10% through books, magazines and newspapers.

This documentation clearly showed that knowledge and cultural practices of herbal medicines had been shared through the indigenous community through word of mouth. The frequency of citation indicates the sociocultural importance of medicinal plants in identifying their therapeutic value (Khajuria et al., 2021). The FC value is

directly proportional to the use value (UV); the more the FC value increases, the more the used value will increase significantly. *Curcuma longa* L. is one of the main commercially grown plants as a seasoning in India. In Southeast Asia, including India and China, turmeric powder has been used extensively for spice and colouring food material. It had a wide range of medicinal value, with curcumin being the main bioactive chemical constituent (Verma et al., 2018). C. longa was a mandatory spice that every household kept, which is why the reason used report (UR) for medicinal value and cultural value (CVs) were high among the informants. In the case of CVs, the high value was due to a greater number of reports used, with lesser-used categories. The informants in the present study reported that *Flueggea virosa* have a prominent effectiveness against diabetes (59 UR) and chickenpox (50 UR). The Mizo tribes extensively used *F. virosa* and *Embelia vesta* Roxb. plant to treat chickenpox and measles by bathing once a day with the decoction of the leaves mixed with water. Apart from the degree of the report used, this index is also attributed to the effectiveness of its use and importance. Higher in the UV value indicates a higher rate of agreement and sharing their knowledge and practices of the medicinal plants among the informants (Prathiban et al., 2016). Among the Terai forests of western Nepal, Curcuma longa L. was also reported to have the highest value (Singh et al., 2012) similar to this result.

The plants with low UV value were *Colocasia esculenta*, *Eulophia nuda* and *Ocimum americanum, Maesa indica, Morus macroura*, *Tectona grandis, Hibiscus sinensis*, *Elaeis guineensis*, *Smilax perfoliata* with less than 0.05 UV as shown in Table 4. *Tectona grandis* was also described with a very low UV value by Ayyanar and Ignacimuthu, 2011 as relevant to this result. According to Chaudhary et al. 2006, the plants with low used value were in at risk of misrecollecting and passing on to the young generation which might be gradually disappearing. On the other hand, the relevance of knowing the plant used value was for the convenience of pharmacological study and their used reliability (Cakilcioglu et al., 2010). However, Rajakumar and Shivanna, 2009 had mentioned that the value of Fic depends on the accessibility of the taxa for the treatment of various diseases in the study area. Muscle/bone problems with 81 UR have the highest

Fic value of 0.962, followed by gastrointestinal disease (GID) with 940 UR and skin care (SC) with 259 UR. The lowest Fic value in the present study was the General Health (GH) category (Cold, fever, immunity boost) with 0.887, which was still more than the previous maximum Fic value reported in Shimoga district, Karnataka, India, i.e. 0.77 in Liver complaints. Most RI value (*Phyllanthus emblica*) is considered to be versatile in its uses, which would also increase the importance of the plant when it is used to treat more illnesses. The high RI values of some species may be attributed to their abundance and availability in the study area (Albuquerque et al., 2006).

Overall, the quantitative analysis revealed that *Curcuma longa* was the most relevant species, with the highest used value, frequency of citation, and cultural value, except in terms of relative importance. This is because the RI value is independent of the number of informants used in the report. Regarding the conflict between these reports, our study indicated a high consistency in the indigenous informant's knowledge of ethnomedicines and their use of the same plants to treat them.

Conclusions

The present study concluded that the native people in the study area have their unique way of utilising medicinal plants to treat different kinds of ailments. We documented 93 valuable medicinal plants belonging to 55 families and 85 genera in which Euphorbiaceae and Asteraceae family were the most widely used in the area. This study supported the non-random selection of medicinal plants hypothesis. Among the plant parts, leaves were the most commonly used. No new medicinal taxa were reported; however, this study is the first quantitative report of ethnomedicine in this region, and no informant had reported an adverse effect from herbal medicines. Their traditional pieces of knowledge had been passed on from their elders, mostly through word of mouth. This study also revealed that younger generations between the ages of 18 and 30 have little to no knowledge of the preparation of herbal medicines and their use, as compared to the older age groups. This is primarily due to the availability of modern clinical drugs in the villages.

Therefore, the traditional knowledge and practices related to medicinal plants in the study area are somewhat at risk of extinction. This is why it is important to document the valuable knowledge, as well as for the conservation of the taxa. The use of quantitative indices was essential in the field of ethnobotany to determine the most valuable plants, along with their role played in a particular culture, and to develop conservation initiatives. Plants with high usage reports and citation frequency were known to possess numerous phytochemical compounds. The calculated informant consensus factor was extremely high, indicating that the acquired data can serve as a reliable reference for future ethnopharmacological studies. Although the remedial value of many highly cited plants has already been verified, there are still some plants that require validation. Hence, they are strongly recommended for further studies to develop alternative drugs.

Plants frequently reported to have high usage and citation rates are known to contain numerous phytochemical compounds. Therefore, based on 17 plants reported to treat diarrhoea in the informant consensus factor for gastrointestinal diseases, they will serve as a plant sample for the next chapter - *in vitro, in vivo*, or *in silico* analysis of their anti-diarrhoeal properties.

CHAPTER – 2

"Phytochemical profiling and in vitro anti-diarrhoeal evaluation of selected medicinal plants against *Escherichia coli* and *Salmonella* Typhimurium"

2.1 Introduction

From the previous ethnobotanical survey conducted in Champhai district. In Mizoram, India, it is clear that the indigenous people have been using medicinal plants to treat various illnesses, with gastro-intestinal diseases scoring the highest Informant Consensus Factor (ICF), having the highest use report and taxa reported among the informants. Through this survey and available secondary data, 14 plants particularly used for treating diarrhoea, were initially selected for the study of their preliminary phytochemical constituents and *in vitro* anti-diarrhoeal activity. The plants, along with their local names and parts used, are listed below.

Table 2.1 Selected Plants list with part used

S. No	Scientific Name	Local Name	Part Used
1.	*Aguilaria malacensis* Lam.	Thingrai	Bark
2.	*Blumea lanceolaria* (Roxb.) Druce	Buarze	Leaf
3.	*Bergenia pacunbis* (Buch.-Ham. ex D.Don) C.Y.Wu & J.T.Pan	Kham damdawi	Leaf, Rhizome
4.	*Curcuma caesia* Roxb.	Ailaidum	Rhizome
5.	*Dillenia indica* L.	Kawrthindeng	Bark, Fruit
6.	*Dysoxylum excelsum* Blume	Thingthupui	Young shoot
7.	*Euphorbia milii* Des Moul.	Hlinglukhum	Leaf
8.	*Jasminum nervosum* Lour.	Hruikha	Whole plant
9.	*Lagerstroemia speciosa* (L.) Pers.	Thla do	Bark, Leaf
10.	*Melastoma malabatricum* L.	Builukham	Leaf
11.	*Mikania micrantha* Kunth	Japan hlo	Leaf
12.	*Myrica esculenta* Buch.-Ham. ex D. Don	Keifang	Bark
13.	*Phyllanthus emblica* L.	Sunhlu	Bark, Fruit
14.	*Quercus serrata* Murray	Sasua	Bark

Medicinal plants have long been recognised as a rich reservoir of bioactive compounds, many of which have laid the foundation for modern drug discovery and pharmaceutical innovation. These plants produce a wide array of secondary metabolites—non-essential compounds that play critical roles in defence and ecological interactions. Among these, alkaloids, flavonoids, tannins, saponins, phenolic compounds, terpenoids, glycosides, and steroids are of particular interest due to their well-documented pharmacological activities, including antimicrobial, antioxidant, anti-inflammatory, anti-diarrhoeal, and anticancer properties (Pandey & Rizvi, 2009; Cragg & Newman, 2013).

Across various traditional systems of medicine, such as Ayurveda, Siddha, and Traditional Chinese Medicine, plant-based remedies have been utilized for centuries to manage a broad spectrum of diseases. In South and Southeast Asia, medicinal plants such as *Aquilaria malaccensis, Curcuma caesia,* and *Phyllanthus emblica* are commonly used to treat a range of ailments, including gastrointestinal disturbances, infections, and chronic inflammatory conditions. These practices, passed down through generations, continue to play a vital role in rural and indigenous healthcare settings, particularly where access to conventional medicine is limited.

In recent years, the global resurgence of interest in natural therapeutics has prompted a renewed focus on the scientific validation of traditional medicinal knowledge. Among the primary objectives is the phytochemical profiling of ethnomedicinal plants, which involves both qualitative screening (to identify the presence of key compounds) and quantitative analysis (to measure their concentrations). Such studies not only offer insights into the pharmacological potential of these plants but also contribute significantly to the bioprospecting of novel therapeutic agents.

Antimicrobial agents play a pivotal role in mitigating the global impact of infectious diseases. However, the rise and proliferation of multidrug-resistant (MDR) strains among pathogenic bacteria have emerged as a critical public health concern, often leaving few or no effective therapeutic options available for combating these infections. Given the alarming rate at which resistant clinical isolates are spreading worldwide, there is an urgent need to discover and develop novel antimicrobial agents. Nonetheless,

historical patterns reveal that resistance to newly introduced antibiotics tends to emerge swiftly and broadly, suggesting that even innovative drug classes may have limited longevity. In this context, medicinal plants have garnered significant attention as promising reservoirs of bioactive antimicrobial compounds, offering potential alternatives for managing infections caused by resistant bacterial pathogens (Manandhar et al., 2019).

The present study aims to investigate the phytochemical composition and activity of fourteen selected medicinal plants traditionally used in Mizoram, India, particularly for their role in managing diarrhoeal diseases. Since diarrhoea remains a primary global health concern, especially in developing nations, where it is a leading cause of morbidity and mortality, particularly among children. The rise of antibiotic resistance in common enteric pathogens like *Escherichia coli* and *Salmonella* Typhimurium has further complicated treatment strategies (Kaper et al., 2004). Many traditional remedies employed for diarrhoea rely on plant-based preparations known to possess astringent, anti-secretory, anti-motility, and antimicrobial properties-effects that are often attributed to secondary metabolites such as tannins, flavonoids, and alkaloids. These bioactive compounds can disrupt microbial cell membranes, interfere with enzymatic activities, and induce oxidative stress within pathogenic bacteria, leading to their inhibition or death.

By examining the qualitative presence and quantitative abundance of these key phytochemical classes and evaluating the antibacterial efficacy of 14 selected medicinal plants traditionally used by the Mizo people in Mizoram against *E. coli* and *S.* Typhimurium through in vitro assays, this study seeks to provide a scientific basis for the traditional use of these plants. Moreover, it aims to identify promising candidates for further pharmacological exploration, including bioassay-guided fractionation and *in vivo* validation of anti-diarrhoeal efficacy. Ultimately, such efforts contribute to bridging the gap between traditional knowledge and modern medicine, facilitating the development of safe, affordable, and effective plant-based therapeutics.

2.2 Review of Literature

Plants are widely recognized as invaluable sources of medicinal compounds and therapeutic agents. For centuries, phytochemicals have been utilised in traditional medicine systems worldwide for treating a range of human ailments. These biologically active compounds, including alkaloids, flavonoids, phenols, tannins, terpenoids, glycosides, and saponins, are typically classified as secondary metabolites. Although not directly involved in the plant's growth or reproduction, these compounds play a vital role in defence mechanisms, aiding in plant survival against pests, pathogens, and environmental stressors (Harborne, 1998).

Tian et al. (2018) explored the chemical constituents of *Ptychopetalum olacoides*, a medicinal plant used extensively in South America. Fourteen compounds were isolated, including alkaloids such as magnoflorine and menisperine, which accounted for approximately 76% of the total analytes. Although phenolic content was relatively low (less than 1.04 mg/g), the high concentration of alkaloids suggests a predominant role in the plant's pharmacological activity. These results not only affirm the traditional claims associated with *P. olacoides* but also highlight its potential for application in modern drug development.

In a comprehensive study conducted by Odeyemi et al. (2023), the stem bark of *Napoleona imperialis* was evaluated for its qualitative and quantitative phytochemical composition. The researchers reported that both aqueous and methanolic extracts contained various bioactive compounds in moderate to high concentrations. Quantitative analysis revealed the presence of flavonoids (58.24 mg/kg), tannins (52.5 mg/kg), and alkaloids (13.53 mg/kg), among other compounds. These findings suggest that *N. imperialis* possesses significant pharmacological potential, aligning with its traditional medicinal uses in southeastern Nigeria. The authors concluded that the plant could serve as a valuable candidate for the development of herbal therapeutics due to its broad-spectrum phytochemical profile.

In Nigeria, Ezeonu and Ejikeme (2016) conducted an extensive phytochemical screening of 24 indigenous softwood species. The study reported notable quantities of tannins, flavonoids, saponins, alkaloids, and phenolic compounds across various species. For instance, *Sterculia oblonga* and *Barteria nigritiana* exhibited high tannin content (1240 mg/100 g and 1230 mg/100 g, respectively), while *Cordia millenii* had the highest alkaloid content (11.2%). These findings support the therapeutic potential of these softwoods, which are often overlooked in favour of leaves and fruits in medicinal research.

Gracelin et al. (2012) focused on the Pteridaceae family, analyzing five fern species including *Pteris biaurita* and *Pteris vittata*. The qualitative tests revealed the presence of key phytochemicals, including flavonoids, alkaloids, phenols, and tannins, in all five species. Among these, *Pteris biaurita* had the highest concentration of bioactive compounds. Quantitative assays indicated significant levels of alkaloids, saponins, flavonoids, and phenolics, supporting their use in traditional healthcare practices. The study advocates for further pharmacological investigations and isolation of active components from these underutilized pteridophytes.

A related investigation by Chandran and Indira (2016) on *Strobilanthes kunthiana*, a shrub native to the Western Ghats, measured the total phenolic, flavonoid, tannin, and chlorophyll contents. The aqueous extracts of the plant leaves showed significantly higher phenolic and tannin content compared to ethanolic extracts. The study concluded that *S. kunthiana* holds potential as a source of natural antioxidants, supporting its use in traditional medicine systems in South India.

Yadav and Agarwala (2011) investigated seven medicinal plants from northeastern India, using both aqueous and organic extracts. Their findings revealed that all tested species, including *Tinospora cordifolia* and *Terminalia bellerica,* contained proteins, carbohydrates, phenols, tannins, and flavonoids. Quantitative assessments revealed that *T. cordifolia* had the highest phenolic content (71.6 mg/g), while T. bellerica exhibited significant flavonoid concentrations (42.8 mg/g). The authors emphasized the importance of such phytochemicals in antioxidant defense, thus providing scientific validation for their traditional use in treating inflammatory and degenerative diseases.

In Mizoram, India, Mazumder et al. (2018) evaluated *Ageratina adenophora*, an invasive plant species, for its phytochemical potential. The study confirmed the presence of various bioactive constituents, including carbohydrates, alkaloids, phenols, tannins, steroids, glycosides, and flavonoids. Quantitative analysis of the methanolic extract revealed high tannin and phenol content, supporting the therapeutic potential of the species. Given its invasive nature and easy availability, *A. adenophora* could serve as a cost-effective source of pharmaceutical compounds (Mazumder et al., 2018).

Vabeiryureilai et al. (2014) conducted an extensive phytochemical investigation of *A. octandra*, a shrub traditionally used in Mizoram for various ailments. Qualitative analysis revealed the presence of key secondary metabolites such as tannins, saponins, flavonoids, terpenoids, and cardiac glycosides, while phlobatannins and alkaloids were absent. Quantitative estimations demonstrated that the ethanolic extract contained a significantly higher concentration of phenolics (1010.67 ± 58 mg/10 g) compared to the chloroform extract (282.67 ± 26 mg/10 g), suggesting ethanol as a more efficient solvent for polyphenol extraction. Interestingly, the flavonoid content was slightly higher in the chloroform extract (668.8 ± 3.94 mg/10 g) than in ethanol (635.61 ± 4 mg/10 g), possibly due to the medium polarity of the compounds present.

Manandhar et al. (2019) evaluated the antimicrobial activity of methanolic extracts from four medicinal plants - *Oxalis corniculata*, *Cinnamomum tamala,* Artemisia vulgaris, and *Ageratina adenophora*-against 12 human pathogens, including MDR strains of *E. coli, S. aureus,* and *S. typhi.* Among them, *O. corniculata* exhibited the most potent antibacterial effect, especially against Gram-negative bacteria, with MICs as low as 25 mg/mL. *A. adenophora* showed broad-spectrum activity, including antifungal effects, while *C. tamala* and *A. vulgaris* were more effective against S. aureus. The study supports the potential of traditional plants as alternative sources of antimicrobials. Despite the promising antibacterial effects of these crude plant extracts, the study emphasised the variation in efficacy, which may be attributed to differences in phytochemical composition, extraction method (cold percolation), or solvent polarity. Moreover, while some plant extracts showed alignment with traditional medicinal claims, others did not

display the expected broad-spectrum activity, suggesting that further investigation with more refined extraction techniques or purified compounds may yield more definitive results.

Srivastava et al. (2013) further demonstrated the antimicrobial effectiveness of bark extracts from *Zanthoxylum armatum* against pathogens such as *Staphylococcus aureus, E. coli*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*. The acetone and methanol extracts were particularly effective against *S. aureus*, indicating that solvent selection significantly influences phytochemical extraction and bioactivity.

Similarly, Mudzengi et al. (2017) reported the antibacterial potential of *Salvadora persica*, *Colophospermum mopane*, and *Dichrostachys cinerea*, which are traditionally used in ethnoveterinary medicine in Zimbabwe. The methanol extracts of these plants were found to be more effective than aqueous extracts, with inhibitory effects observed against both *Staphylococcus aureus* and *E. coli*. The MIC values ranged from 1.03–14.6 mg/mL, confirming their substantial antimicrobial potency.

Investigations by Kumar et al. (2012) provided additional insights into the efficacy of essential oils extracted from *Micromeria biflora* and *Citrus reticulata*. These oils showed promising antibacterial activity against a range of waterborne pathogens, including *E. coli, Salmonella* Typhimurium, and *Shigella dysenteriae*. The synergistic interaction of different essential oil components was also highlighted, suggesting their potential as combinatory therapeutic agents.

The mechanisms of action of these plant extracts often include altering the permeability of bacterial membranes, lowering intracellular pH, and causing membrane depolarisation (Gonelimali et al., 2018). Such multifaceted mechanisms provide an advantage over single-target synthetic drugs, making plant extracts promising candidates for overcoming antimicrobial resistance.

In a study conducted by Lalremtluangi et al. (2025), *Pilea symmeria*-a herbaceous plant native to Mizoram, India-exhibited significant antibacterial activity against *E. coli, Bacillus subtilis*, and *Klebsiella pneumoniae*. Different solvent extracts (ethanol, chloroform, and aqueous) of the plant demonstrated concentration-dependent antibacterial

activity in both disc diffusion and microdilution assays. The ethanol extract showed a strong radical scavenging capacity, attributed to its high phenolic and flavonoid content.

These studies collectively emphasise the critical importance of both qualitative and quantitative phytochemical analyses in validating the anti-diarrhoeal activity of traditional medicinal practices. They not only provide a scientific basis for the continued use of plant-based remedies but also serve as a foundation for the development of novel pharmaceuticals derived from natural sources.

2.3 Methodology

2.3.1 Plant Materials – Collection, extraction and identification

Fourteen medicinal plants were collected from various locations in Mizoram for identification and extraction. The plant parts used were primarily leaves and barks, depending on traditional usage. The collected plant parts were washed thoroughly to remove dirt and then shade-dried for several days. After that, it was extracted using the cold maceration process with methanol as the solvent for 72 hours. Then, using a rotary evaporator, the methanol was recovered, and the crude extract was stored in tightly closed vials at 4°C for further investigation.

For authentication, all plants were identified using standard botanical references by the Botanical Survey of India, Shillong and cross-checked with the only accepted botanical names by 'The World Flora Online (WFO), an open-access database.

2.3.2 Qualitative Phytochemical Screening

Standard procedures described by Harborne (1998) and Sofowora (1993) were used to detect the presence of alkaloids, glycosides, flavonoids, saponins, tannins, terpenoids, triterpenoids, phenolics, proteins, resins, and steroids.

2.3.2.1 *Alkaloid Detection – Dragendorff's Reagent Test:*

Combine 2 ml of the crude extract with 1% hydrochloric acid and heat the mixture by steaming for approximately 10 minutes. After cooling, add six drops of Dragendorff's reagent. The formation of a reddish-brown precipitate confirms the presence of alkaloids.

2.3.2.2 *Carbohydrate Detection – Benedict's Test:*

Mix 2 ml of the plant extract with an equal volume of Benedict's reagent and boil the mixture. The formation of a reddish-brown precipitate confirms the presence of carbohydrates.

2.3.2.3 *Flavonoid Detection – Sodium Hydroxide Test:*

Mix equal volumes (2 ml each) of the plant extract and 10% sodium hydroxide solution. A change in colour from yellow to orange indicates the presence of flavonoid compounds.

2.3.2.4 *Glycosides Detection – Keller-Kiliani Test:*

Add 2 ml of glacial acetic acid containing 1–2 drops of 2% ferric chloride solution to 2 ml of the crude extract. Carefully layer this mixture over 2 ml of concentrated sulfuric acid in a separate test tube. A brown ring at the interface suggests the presence of cardiac glycosides.

2.3.2.5 *Protein Detection – Xanthoproteic Reaction:*

Add 2 ml of concentrated nitric acid to 2 ml of the crude extract and heat the mixture in a water bath. The appearance of an orange colour suggests the presence of proteins.

2.3.2.6 *Resin Detection – Turbidity Test:*

Dissolve the plant extract in distilled water and add a few drops of 4% hydrochloric acid. The development of turbidity or cloudiness indicates the presence of resins.

2.3.2.7 *Saponin Detection – Froth Formation Test:*

Shake 2 ml of the crude plant extract vigorously with 5 ml of distilled water in a test tube. Persistent foam or froth indicates the presence of saponins.

2.3.2.8 *Steroid Detection – Liebermann-Burchard Reaction:*

To detect steroids, mix 2 ml of the crude extract with 2 ml of acetic anhydride. Add a few drops of concentrated sulfuric acid. A blue-green colouration indicates the presence of steroidal compounds.

2.3.2.9 *Tannin Detection – Ferric Chloride Method:*

To identify tannins, mix 2 ml of the plant extract with a few drops of 5% ferric chloride solution. The development of a blue colouration suggests the presence of hydrolysable tannins.

2.3.2.10 *Terpenoid Detection – Salkowski Test:*

Shake 2 ml of the extract with 1 ml of chloroform, then carefully add concentrated sulfuric acid along the side of the test tube. A red-brown colouration at the interface signifies the presence of triterpenoids.

2.3.3 Quantitative Phytochemical Determination

The quantitative phytochemical content of all 14 plant samples was determined using the method described by Thangjam et al. (2020) and Fazel et al. (2010), with slight modifications. Each sample was run in triplicate. Statistical significance was analyzed using Duncan's multiple range test at $p<0.05$.

2.3.3.1 *Determination of Total Phenolic Content*

The total phenolic content (TPC) of the plant extracts was estimated using a modified Folin–Ciocalteu colourimetric method. Briefly, 0.5 ml of sample solution at 1mg/ml was mixed with 4.5 ml of distilled water and 0.5 ml of Folin–Ciocalteu's reagent in a clean test tube. After allowing the reaction to proceed for 5 minutes, 5 ml of 7% sodium carbonate solution was added. The mixture was kept in a dark chamber for 2 hours with intermittent shaking. The absorbance was measured at 760 nm using a 96-well plate UV-visible spectrophotometer. The phenolic content was quantified using a gallic acid calibration curve and expressed as gallic acid equivalents (GAE) per gram of extract.

2.3.3.2 *Determination of Total Flavonoid Content*

A 0.5 mL aliquot of the extract at a concentration of 1 mg/mL was mixed with 0.15 mL of a 15% sodium nitrite solution. After 6 minutes, 0.15 ml of 10% aluminium

chloride was added, followed by the addition of 2 ml of 4% sodium hydroxide after another 6 minutes. The final volume was adjusted to 5 ml using distilled water, and the solution was mixed thoroughly. Absorbance was recorded at 510 nm. The flavonoid content was calculated from a standard curve of quercetin and reported as milligrams of quercetin equivalent (QE) per gram of dry extract.

2.3.3.3 *Determination of Total Alkaloids*

The total alkaloid content was estimated using a modified version of Harborne's (1973) method employing the Bromocresol Green (BCG) dye complexation technique. The BCG solution was prepared by dissolving 34.9 mg of the dye in 3 ml of 2N NaOH and 5 ml of distilled water, heating gently until fully dissolved, and then making up the volume to 500 ml. A phosphate buffer (pH 4.7) was prepared by dissolving 7.16 g of Na_2HPO_4 in 100 mL of distilled water, and the pH was adjusted using 4.202 g of citric acid in 100 mL of water.

A series of standard Atropine solutions (0.4 to 1.2 ml) was reacted with 5 ml phosphate buffer and 5 ml BCG solution, followed by sequential extractions with 1 to 4 ml chloroform. The pooled chloroform extracts were made up to 10 ml and their absorbance measured at 417 nm.

For the sample, 1 g of plant extract was dissolved in 2N HCl and filtered. A 1 ml aliquot was transferred into a separatory funnel and washed thrice with 10 ml of chloroform. The solution's pH was then neutralized using 0.1N NaOH. Afterwards, 5 ml of BCG reagent and 5 ml of phosphate buffer were added, and the mixture was shaken vigorously with incremental volumes (1 to 4 ml) of chloroform. The chloroform layers were combined and diluted to a total volume of 10 ml. The absorbance was recorded at 417 nm against a blank without morphine. Alkaloid content was calculated using the standard curve and expressed as Atropine equivalents.

2.3.3.4 *Estimation of Total Tannin Content*

Tannin content was measured using the Folin–Ciocalteu method with minor adjustments. A 0.1 ml volume of extract was placed in a 10 ml volumetric flask containing 7.5 ml of distilled water and 0.5 ml of Folin–Ciocalteu reagent. Subsequently, 1 ml of 35% sodium carbonate solution was added, and the volume was made up to 10 ml with distilled water. After thorough mixing, the solution was incubated at room temperature for 30 minutes. Tannic acid standards (25, 50, 75, 100, 125 and 150 µg/ml) were prepared similarly for calibration. Absorbance was measured at 700 nm using a 96-well plate UV-visible spectrophotometer. The results were expressed as milligrams of tannic acid equivalent (TAE) per gram of dried sample.

2.3.4 *In vitro* Antibacterial Investigation:

Two enteropathogenic bacteria (*Escherichia coli* MTCC 723 and *Salmonella* Typhimurium (MTCC 3224), which are responsible for causing diarrhoea, are used to determine the antibacterial potency of all the selected medicinal plants. Muller-Hinton Agar and Broth were used as the growth medium for the bacteria, and the McFarland standard was used for bacterial standardisation. *Escherichia coli* (MTCC 723) and *Salmonella* Typhimurium (MTCC 3224) were purchased from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India.

2.3.4.1 *Zone of Inhibition: Disc diffusion method*

For screening the antibacterial properties of extracts and essential oils, the disc diffusion method was used, following the protocol of Kumar et al. (2014). Each sterilised Petri plate was pre-seeded with 15ml of Mueller-Hinton Agar medium and 0.1ml of bacterial suspension containing 1.5×10^7 cfu/ml inoculum, uniformly spread over the media to form a lawn of the culture. The treatment was prepared at high concentrations, ranging from 300mg/mL to 500mg/mL, in DMSO to evaluate the most potent antibacterial activity among the selected medicinal and aromatic plants. The sterile Whatman paper discs (10 mm diameter) were placed over the bacterial lawn, and 20 µl of the respective

concentrations was dropped and incubated for 24 hours at 35 ± 2°C. In a set of controls, an antibiotic, tetracycline 10 mcg (Hi-Media disc), was used as a positive control. Furthermore, the inhibited area (zone of inhibition) of growth will be measured in millimetres and compared with the standard reference antibiotics' inhibition. Each experiment was repeated in triplicate. The zone of inhibition is calculated by using the following formula:

$$W = \frac{T - D}{2}$$

Where:

W - Diameter of clear zone of inhibition

T - Total diameter of including disc and clear zone

D - Diameter of the test Disc

2.3.4.2 *Determination of Minimum Inhibitory Concentration (MIC)*

After screening for antibacterial activity using the zone of inhibition method, the minimum inhibitory concentration (MIC) was determined using the broth microdilution method, as described by the Clinical and Laboratory Standards Institute (CLSI, 2003), with slight modifications. Flat-bottom 96-well Microtiter culture plates are used and filled according to the standard protocol of the NCCLS. Initially, 100 µL of Muller-Hinton broth was added to all the wells, and the plant extract at the same concentration used in the Zone of Inhibition test was added to the first row. Then, using the double serial dilution method, the extract was diluted and thoroughly mixed in each well until the last row. The last two column were used for both negative and positive controls. After that, 100 µl of bacterial suspensions, each containing 1.5×10^7 cfu/ml, were added to each well. The plates were then incubated in an incubator at 35 °C ± 2°C for 15-24 hours. Furthermore, bacterial growth inhibition was evaluated using the INT indicator. After adding 50 µL of INT indicator, the incubation was continued for an additional 30 minutes. During this process, if the bacteria inoculum in each well were killed and inhibited their growth, the indicator would not change colour. However, if there were any bacterial growth, the indicator would turn a dark pink colour. Therefore, the well with less pink colour was taken as MIC, and

the well with no pink colour was taken as MBC. Each experiment was repeated in triplicate. (Kumar et al., 2012)

2.3.4.3 *Determination of minimum bactericidal concentration (MBC)*

The bactericidal concentration was defined as the lowest concentration at which bacteria in at least five of the six replicates failed to grow in MHB/NB and were not cultured when planted onto MHA/NA (Kumar et al., 2012).

2.3.5 *Statistical Analysis*

All experimental data were analysed using GraphPad Prism (version 8, USA). Results are expressed as mean ± standard error of the mean (SEM). A one-way analysis of variance (ANOVA) followed by Duncan Test range (phytochemical) and Tukey's multiple comparison test (antibacterial) was performed to determine the statistical significance of differences among the plant extracts and standard drug control for both *E. coli* and *S.* Typhimurium, considering $p < 0.05$ as significant.

2.4 Results

2.4.1 Qualitative Phytochemical Screening

All plants tested positive for at least two phytochemical classes. Flavonoids and alkaloids were most frequently detected, while glycosides and steroids were least common. *M. malabathricum* and *Dillenia indica* showed a broader spectrum of phytochemicals, suggesting rich secondary metabolism.

Table 2.2 Phytochemical screening of plant samples

Plant Samples	Alkaloids	Carbohydrates	Flavonoids	Glycosides	Phenolic	Proteins	Resin	Saponins	Steroids	Tannins	Terpenoids
Aquilaria malaccensis	+	−	+	−	+	−	+	−	+	+	−
Blumea lanceolaria	+	+	+	−	+	−	−	−	−	+	+
Bergenia pacumbis	−	−	+	−	+	−	−	+	−	−	−
Curcuma caesia	+	−	+	−	+	−	−	+	−	+	−
Dillenia indica	+	+	+	−	+	+	+	+	−	+	+
Dysoxyllum excelsum	+	−	+	+	+	+	+	−	+	+	−

Euphorbia milii	+	−	+	+	+	+	−	+	−	+	−
Jasminum nervosum	+	−	+	−	+	−	−	+	−	+	+
Lagerstroemia speciosa	+	+	+	−	+	−	+	−	−	+	−
Melastoma malabathricum	+	+	+	−	+	−	−	+	−	+	+
Mikania micrantha	+	+	+	−	+	−	−	−	−	+	+
Myrica esculenta	+	+	+	+	+	+	+	−	−	+	+
Phyllanthus emblica	+	−	+	−	+	−	+	+	−	+	−
Quercus serrata	+	−	+	−	+	−	+	−	−	+	−

2.4.2 Quantitative Phytochemical Determination

2.4.2.1 *Flavonoid Content:*

The highest flavonoid concentration was observed in *Euphorbia milii* (81.6 ± 6.04 mg QE/g), followed *Melastoma malabathricum* and *Dillenia indica. Curcuma caesia* showed the lowest flavonoid content (6.403 ± 0.66 mg QE/g).

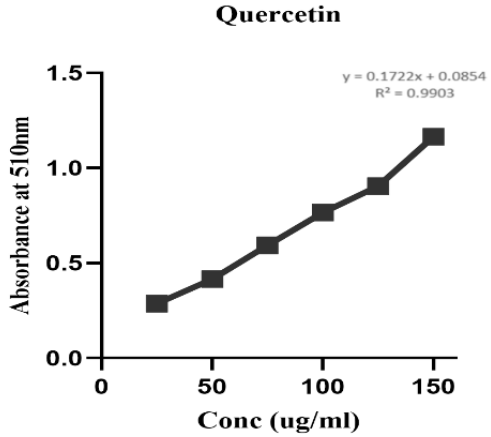


Fig. 2.1. Standard graph of Quercetin

Table 2.3 Determination of Flavonoid Content

Plant Samples	mgQE/g extract
Aquilaria malaccensis	$72.063^{b} \pm 3.70$
Blumea lanceolaria	$28.787^{e} \pm 3.83$
Bergenia pacumbis	$44.553^{c} \pm 4.69$
Curcuma caesia	$6.403^{g} \pm 0.66$
Dillenia indica	$74.84^{b} \pm 2.38$
Dysoxyllum excelsum	$23.56^{ef} \pm 1.95$
Euphorbia milii	$81.6^{a} \pm 6.04$
Jasminum nervosum	$38.033^{d} \pm 3.49$
Lagerstroemia speciosa	$44.647^{c} \pm 3.09$
Melastoma malabathricum	$77.287^{ab} \pm 4.91$
Mikania micrantha	$21.787^{f} \pm 2.01$
Myrica esculenta	$73.69^{b} \pm 2.01$
Phyllanthus emblica	$36.55^{d} \pm 4.67$
Quercus serrata	$41.773^{cd} \pm 3.15$

The mean value in the column followed by the same letter is not significantly different at significance level of 0.05

2.4.2.2 *Phenolic Content:*

Dillenia indica had the highest phenolic content (68.08 ± 1.73 mg GAE/g), followed by *Euphorbia milii* and *Phyllanthus emblica*. The lowest value was found in *Aquilaria malaccensis* (26.583 ± 2.69 mg GAE/g).

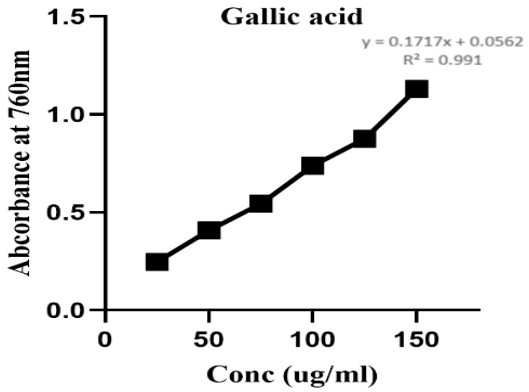


Fig. 2.2 Standard graph of Gallic acid

Table 2.4 Determination of Phenolic Content

Plant Samples	**mgGAE/g extract**
Aquilaria malaccensis	$26.583^{h} \pm 2.69$
Blumea lanceolaria	$29.61^{gh} \pm 1.01$
Bergenia pacumbis	$47.067^{e} \pm 3.22$
Curcuma caesia	$30.047^{gh} \pm 1.26$
Dillenia indica	$68.08^{a} \pm 1.73$
Dysoxyllum excelsum	$33.41^{g} \pm 1.09$
Euphorbia milii	$64.187^{b} \pm 2.18$
Jasminum nervosum	$31.443^{g} \pm 2.10$
Lagerstroemia speciosa	$38.843^{f} \pm 1.30$
Melastoma malabathricum	$53.03^{d} \pm 3.06$
Mikania micrantha	$45.623^{e} \pm 3.10$
Myrica esculenta	$57.793^{c} \pm 2.17$
Phyllanthus emblica	$63.223^{b} \pm 2.27$
Quercus serrata	$38.41^{f} \pm 2.74$

The mean value in the column followed by the same letter is not significantly different at a significance level of 0.05

2.4.2.3 *Alkaloid Content:*

Dillenia indica (66.25 ± 0.76 mg AE/g) and *Euphorbia milii* (62.64 ± 1.06 mg AE/g) were the most alkaloid-rich, while *Curcuma caesia* (12.62 ± 1.80 mg AE/g) showed the lowest.

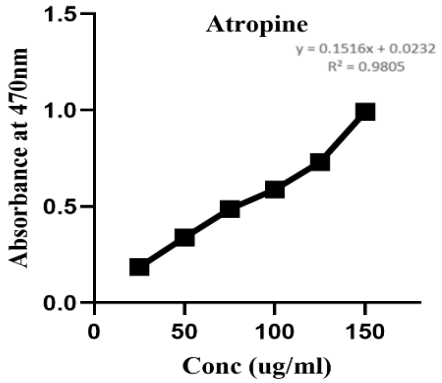


Fig. 2.3. Standard graph of Atropine

Table 2.5 Determination of Alkaloid Content

Plant Samples	**mgAE/g extract**
Aquilaria malaccensis	$53.153^{cd} \pm 6.57$
Blumea lanceolaria	$35.847^{gh} \pm 4.21$
Bergenia pacumbis	$44.637^{ef} \pm 4.21$
Curcuma caesia	$12.62^{j} \pm 1.80$
Dillenia indica	$66.25^{a} \pm 0.76$
Dysoxyllum excelsum	$30.517^{hi} \pm 3.04$
Euphorbia milii	$62.64^{ab} \pm 1.06$
Jasminum nervosum	$41.077^{efg} \pm 5.49$
Lagerstroemia speciosa	$48.14^{de} \pm 6.79$
Melastoma malabathricum	$53.743^{cd} \pm 1.97$
Mikania micrantha	$25.933^{i} \pm 3.08$
Myrica esculenta	$56.55^{bc} \pm 2.76$
Phyllanthus emblica	$38.817^{fg} \pm 5.94$
Quercus serrata	$43.667^{efg} \pm 6.12$

The mean value in the column followed by the same letter is not significantly different at significance level of 0.05

2.4.2.4 *Tannin Content:*

Tannin content was highest in *Euphorbia milii* (61.627 ± 1.06 mg TAE/g) followed by *Dillenia indica* (58.193^{ab} ± 0.76 mg TAE/g) and lowest in *Curcuma caesia* (17.063 ± 1.80 mg TAE/g).

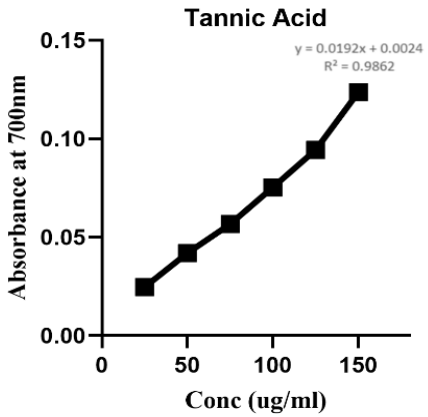


Fig. 2.4. Standard graph of Tannic acid

Table 2.6 Determination of Tannin Content

Plant Samples	**mgTAE/g extract**
Aquilaria malaccensis	47.473^{abc} ± 6.57
Blumea lanceolaria	33.317^{cd} ± 4.21
Bergenia pacumbis	42.323^{abc} ± 4.21
Curcuma caesia	17.063^{d} ± 1.80
Dillenia indica	58.193^{ab} ± 0.76
Dysoxyllum excelsum	38.767^{bcd} ± 3.04
Euphorbia milii	61.627^{a} ± 1.06
Jasminum nervosum	38.037^{bcd} ± 5.49
Lagerstroemia speciosa	45.757^{abc} ± 6.79
Melastoma malabathricum	52.62^{abc} ± 1.97
Mikania micrantha	33.32^{cd} ± 3.08
Myrica esculenta	51.763^{abc} ± 2.76
Phyllanthus emblica	36.793^{bcd} ± 5.94
Quercus serrata	38.467^{bcd} ± 6.12

The mean value in the column followed by the same letter is not significantly different at a significance level of 0.05

2.4.3 *In vitro* Antibacterial Investigation

The antibacterial activity of 14 selected medicinal plant extracts was assessed *in vitro* against two diarrhoea-causing bacteria, *Escherichia coli* (MTCC 723) and *Salmonella* Typhimurium (MTCC 3224).

2.4.3.1 *Determination of Zone of Inhibition (ZOI)*

The zones of inhibition values were observed among the plant extracts and between the two bacterial strains. *Euphorbia milii, Dillenia indica, Melastoma malabathricum* and *Myrica esculenta* exhibited significantly higher zones of inhibition in diameter (mm) against *E. coli* (25.33 ± 1.53, 24.33 ± 1.15, 21.33 ± 1.53 and 20.00 ± 4.36, respectively) and *S.* Typhimurium (24.67 ± 1.53, 22.00 ± 1.00, 20.67 ± 2.08 and 20.33 ± 1.53, respectively), indicating vigorous antibacterial activity. These values were statistically higher than those of *Curcuma caesia* and *Mikania micrantha*, which showed significantly lower inhibition zones. The standard drug (positive control), Tetracycline, showed the highest level compared to all other samples. The negative control (distilled water) did not have any effect on the tested bacteria.

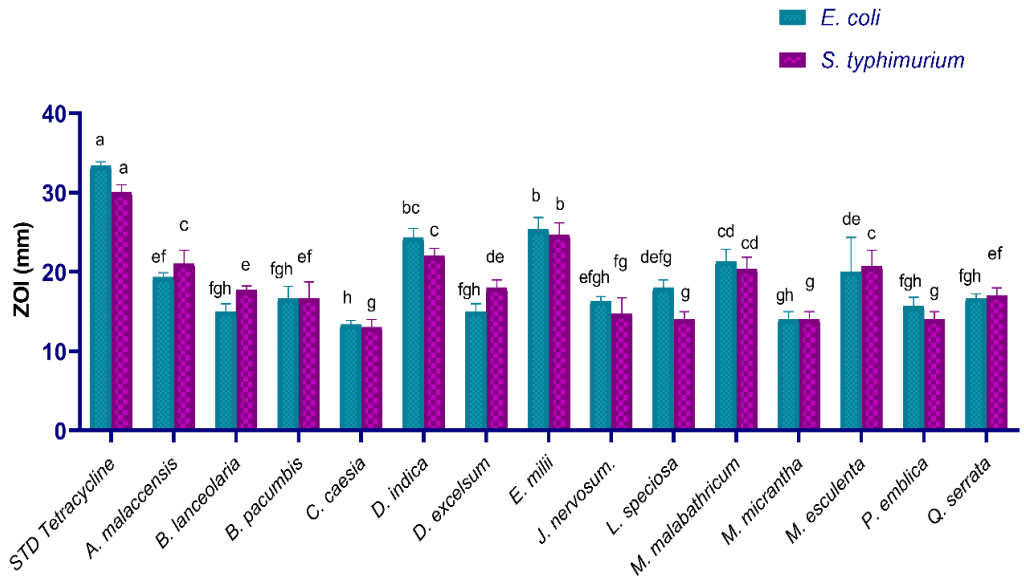
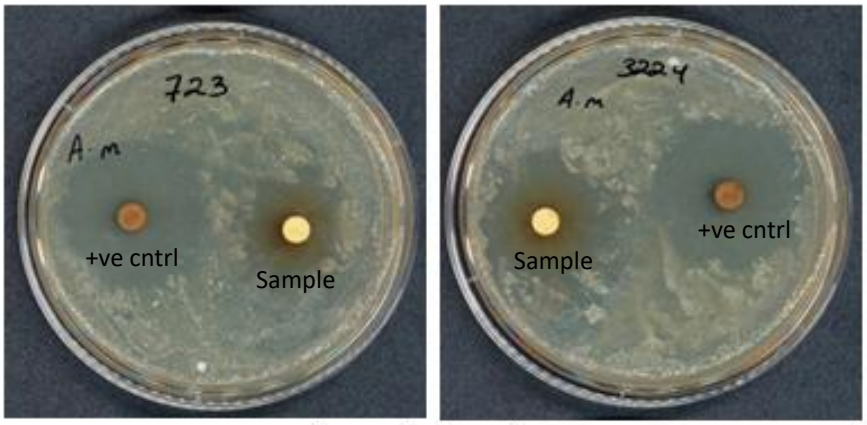
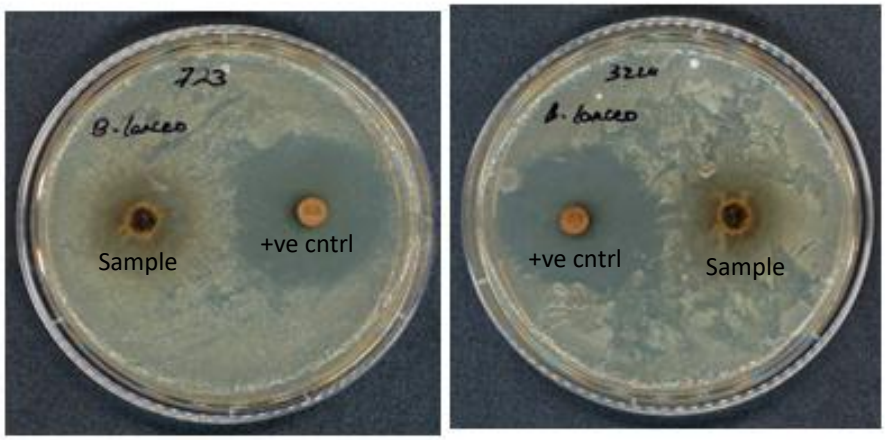


Fig. 2.5. Zone of Inhibition against tested pathogenic bacteria

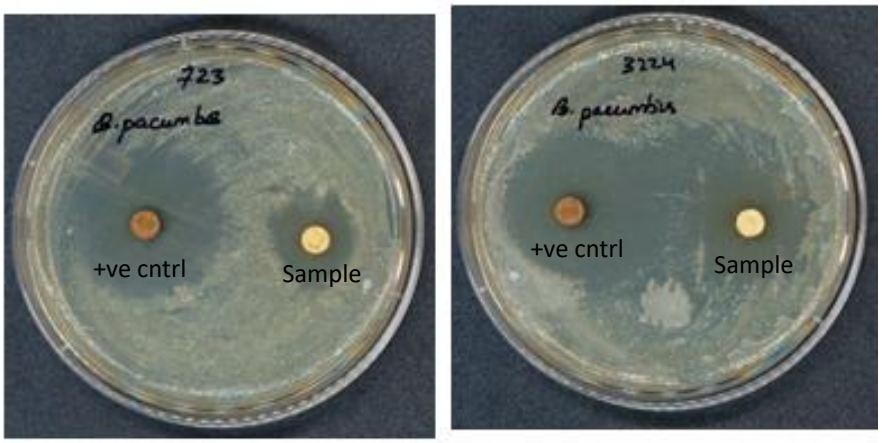
The mean value in the column bar followed by the same letter is not significantly different at significance level of 0.05



A. malacensis

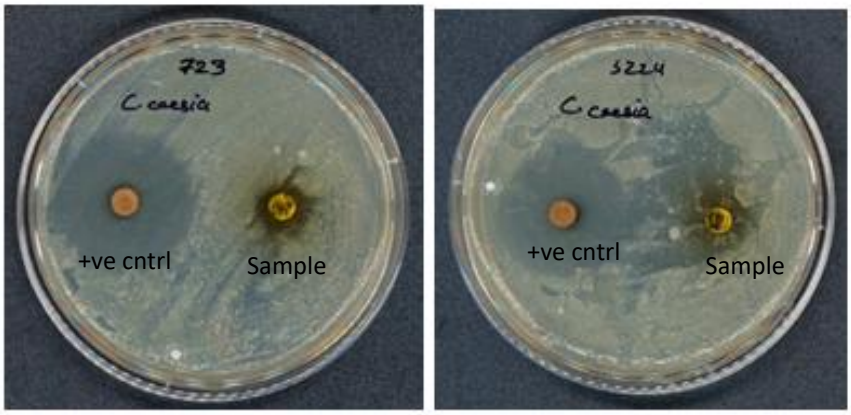


B. lanceolaria

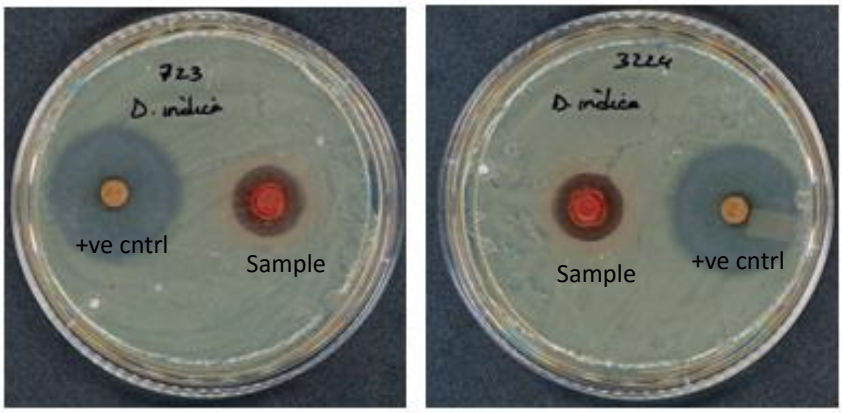


B. pacumbis

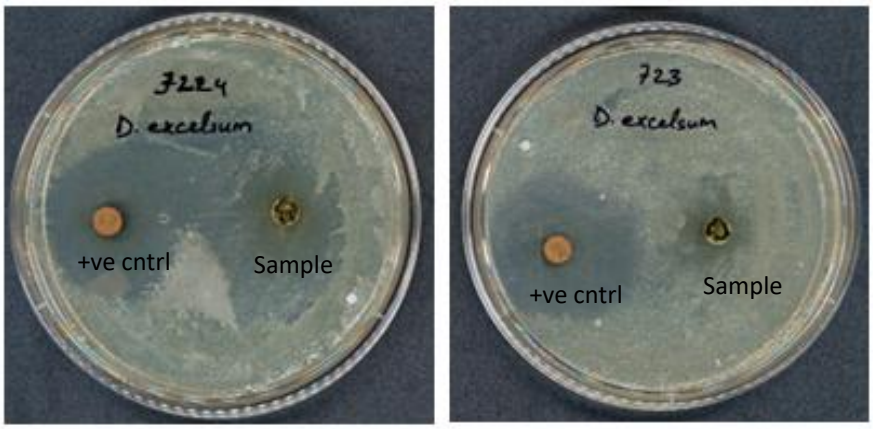
(a)



C. caesia

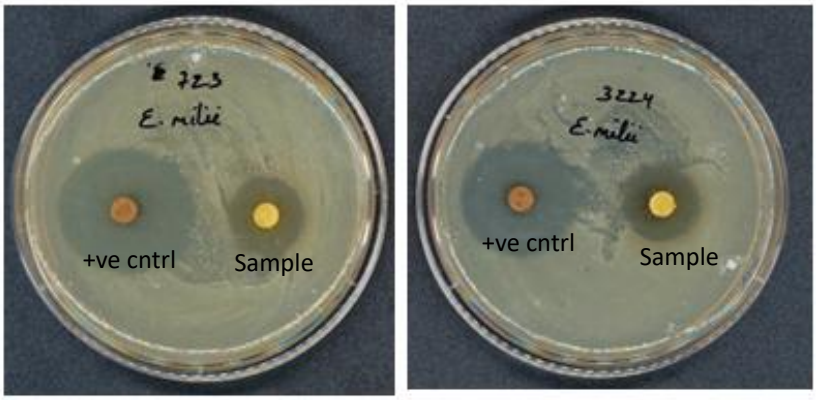


D. indica

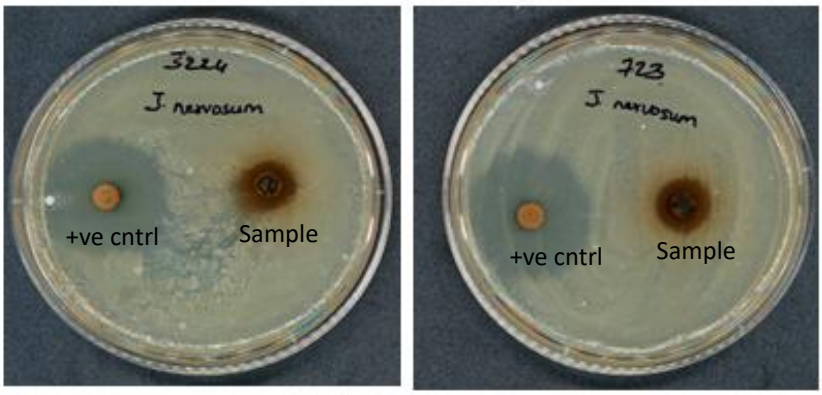


D. excelsum

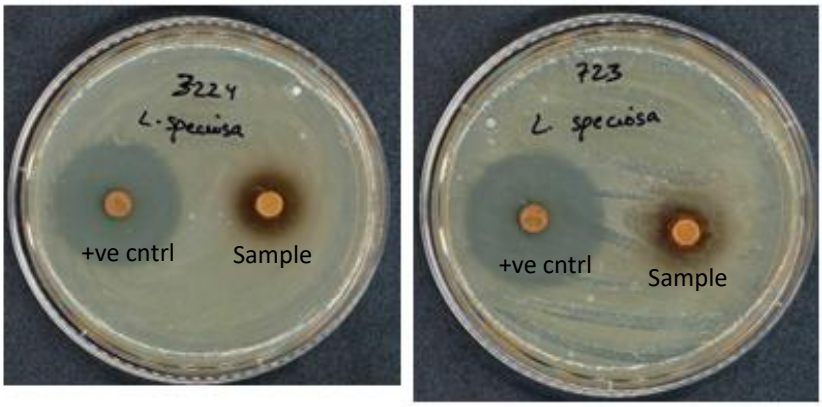
(b)



E. milii

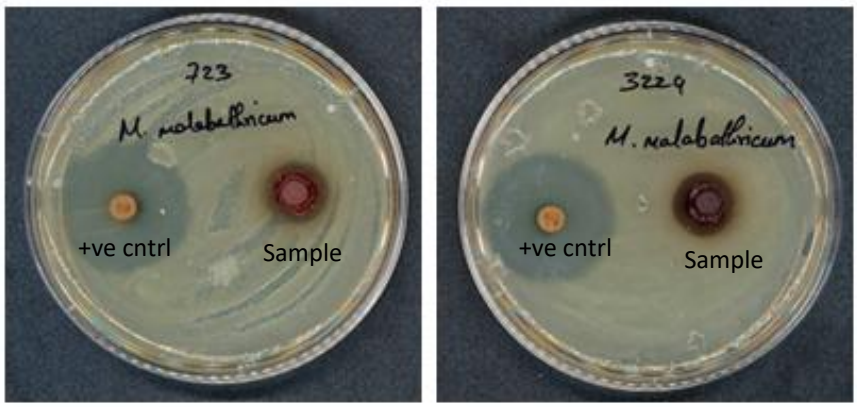


J. nervosum

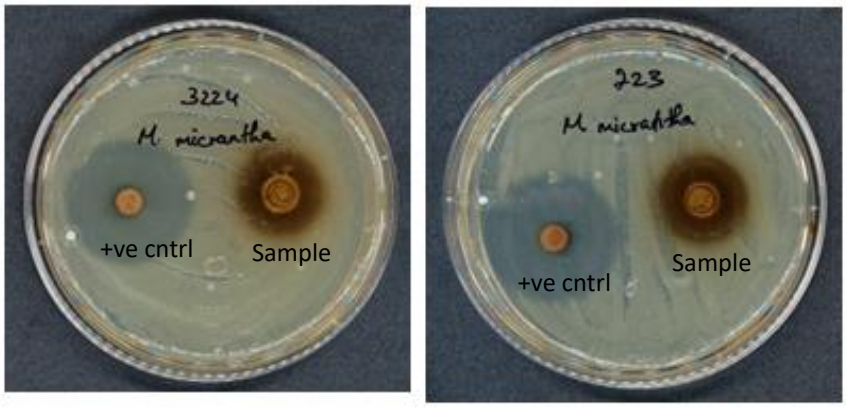


L. speciosa

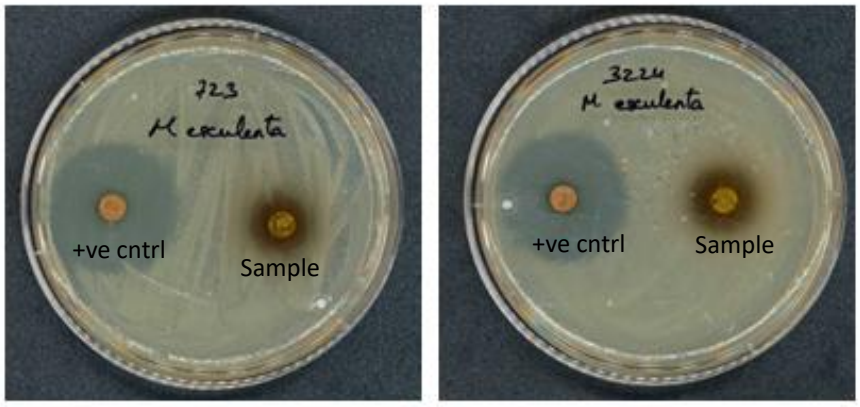
(c)



M. malabathricum

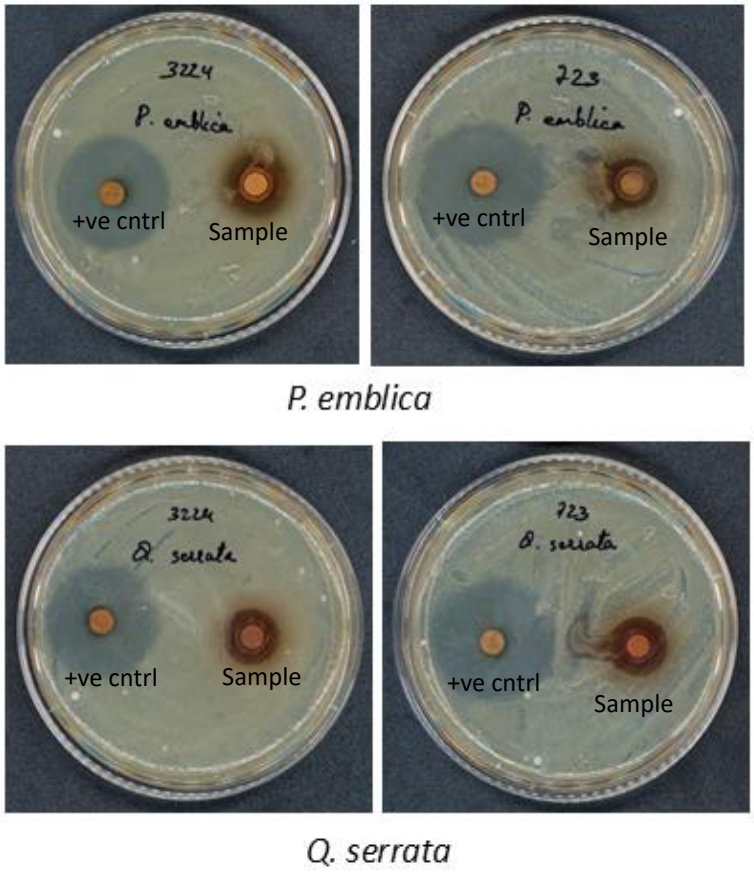


M. esculenta



M. micrantha

(d)



(e)

Fig. 2.6a-e. Evaluation of antibacterial acitivity in form of ZOI

2.4.3.2 *Determination of Minimum Inhibition Concentration (MIC)*

The MIC values also showed statistically significant variation among the plant species ($p < 0.05$). *Euphorbia milii* demonstrated the lowest MIC values (3.25 ± 0.65 mg/ml against *E. coli* and 4.55 ± 1.72 mg/ml against *S.* Typhimurium), which were significantly lower than those of most other plants tested. Conversely, *Curcuma caesia and Mikania micrantha,* despite having fewer zones of inhibition, exhibited significantly higher MICs (83.34 mg/ml), indicating that higher concentrations were needed to inhibit bacterial growth.

Table 2.7. Determination of MIC values of plant samples

Plant Samples	***E. coli*** **(mg/ml)**	***S.*** **Typhimurium (mg/ml)**
Aquilaria malaccensis	$13.02^{b} \pm 2.61$	$18.23^{ab} \pm 6.89$
Blumea lanceolaria	$52.08^{ab} \pm 10.42$	$31.25^{ab} \pm 15.63$
Bergenia pacumbis	$26.042^{ab} \pm 5.21$	$36.45^{ab} \pm 13.78$
Curcuma caesia	$83.34^{a} \pm 20.83$	$93.75^{a} \pm 31.25$
Dillenia indica	$5.208^{b} \pm 1.30$	$10.42^{b} \pm 2.60$
Dysoxyllum excelsum	$46.87^{ab} \pm 15.62$	$26.04^{ab} \pm 5.21$
Euphorbia milii	$3.25^{b} \pm 0.65$	$4.55^{b} \pm 1.72$
Jasminum nervosum	$36.34^{ab} \pm 13.87$	$41.67^{ab} \pm 10.42$
Lagerstroemia speciosa	$23.43^{b} \pm 7.81$	$51.95^{ab} \pm 36.52$
Melastoma malabathricum	$10.42^{b} \pm 2.60$	$20.84^{ab} \pm 5.21$
Mikania micrantha	$83.34^{a} \pm 20.83$	$44.27^{ab} \pm 18.23$
Myrica esculenta	$11.72^{b} \pm 3.91$	$18.22^{ab} \pm 6.89$
Phyllanthus emblica	$41.67^{ab} \pm 10.42$	$52.08^{ab} \pm 10.42$
Quercus serrata	$31.25^{ab} \pm 15.63$	$36.45^{ab} \pm 13.78$

The mean value in the column followed by the same letter is not significantly different at a significance level of 0.05

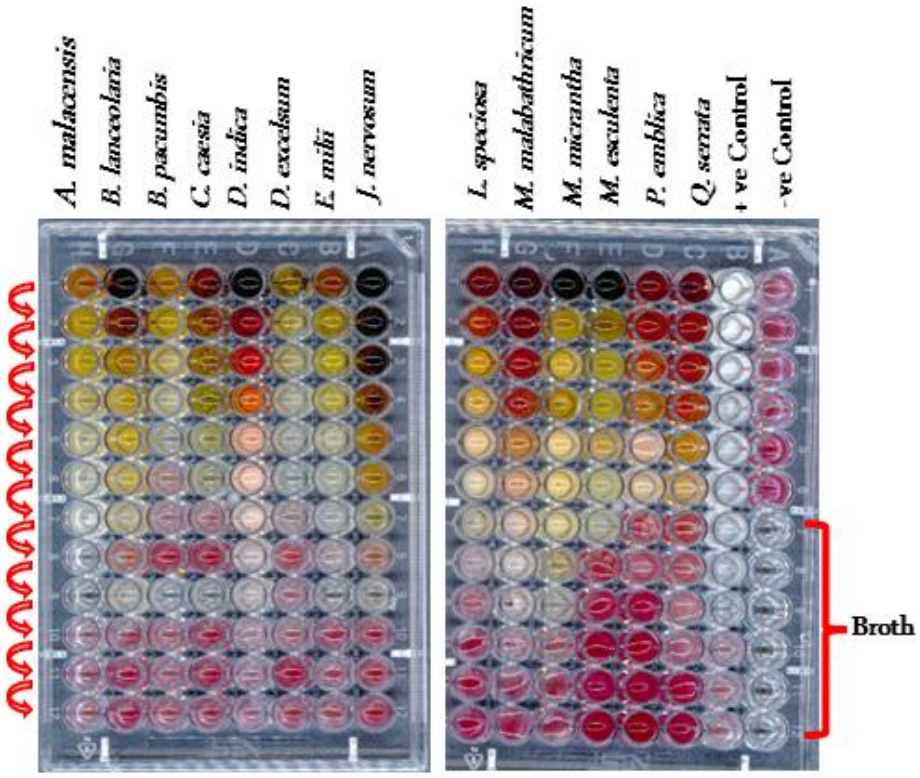


Fig. 2.7a. Determination of MIC against *E. coli*

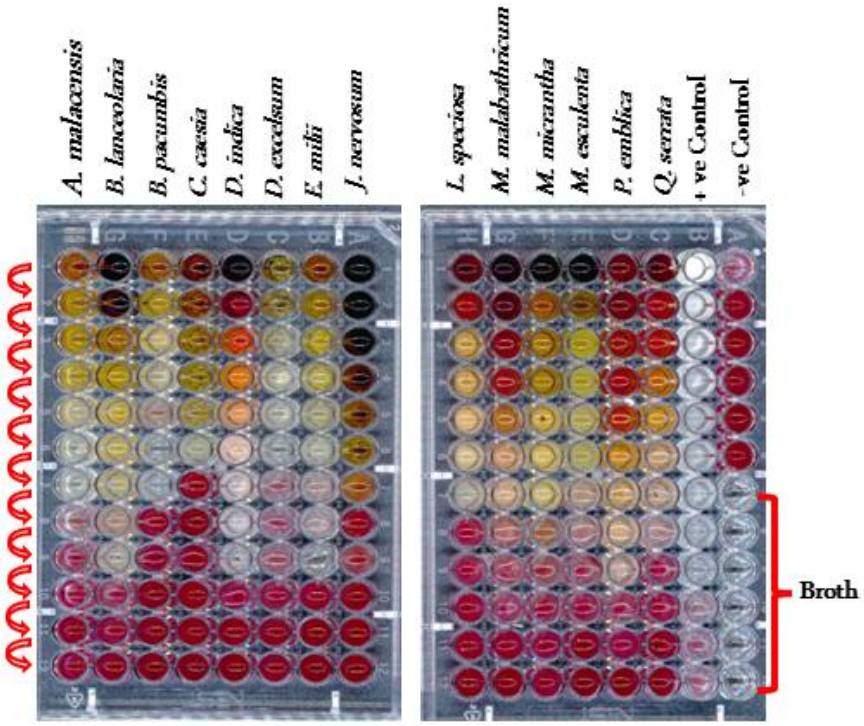


Fig. 2.7b. Determination of MIC against *S.* Typhimurium

2.4.3.3 *Determination of Minimum Bactericidal Concentration (MBC)*

MBC values showed patterns similar to MICs. *Euphorbia milii* had the lowest MBC values (6.51 ± 1.30 mg/mL and 9.11 ± 3.45 mg/mL), which were statistically lower than those of other plants ($p < 0.05$). *Curcuma caesia* and *Mikania micrantha* again showed the highest MBCs (>160 mg/ml), despite their clear inhibition zones, suggesting a discrepancy between bacteriostatic and bactericidal efficacy.

From the 96-well plate, the MBC was streaked again on the MHA plates to confirm the bactericidal, in that where no bacterial growth after 15-24 hours incubation was taken as minimum bactericidal concentration.

Tukey's post hoc comparisons helped identify specific plant pairs with statistically different responses, confirming that certain plants, such as *Euphorbia milii, Dillenia indica*, *Melastoma malabathricum*, and *Myrica esculenta*, were consistently more potent in bacteriostatic and bactericidal assays.

Table 2.8. MBC of plant samples

Plant Samples	***E. coli* (mg/ml)**	***S.* Typhimurium (mg/ml)**
Aquilaria malaccensis	$26.04^{b} \pm 5.21$	$36.45^{ab} \pm 13.78$
Blumea lanceolaria	$104.16^{ab} \pm 20.83$	$62.5^{ab} \pm 31.25$
Bergenia pacumbis	$93.75^{ab} \pm 31.25$	$72.91^{ab} \pm 27.56$
Curcuma caesia	$166.66^{a} \pm 41.67$	$187.5^{a} \pm 62.50$
Dillenia indica	$10.416^{b} \pm 2.60$	$20.83^{b} \pm 5.21$
Dysoxyllum excelsum	$93.75^{ab} \pm 31.25$	$52.08^{ab} \pm 10.42$
Euphorbia milii	$6.51^{b} \pm 1.30$	$9.11^{b} \pm 3.45$
Jasminum nervosum	$72.91^{ab} \pm 27.56$	$83.34^{ab} \pm 20.83$
Lagerstroemia speciosa	$46.79^{ab} \pm 15.58$	$104.16^{ab} \pm 72.92$
Melastoma malabathricum	$20.95^{b} \pm 5.33$	$41.66^{ab} \pm 10.42$
Mikania micrantha	$166.66^{a} \pm 41.67$	$88.54^{ab} \pm 36.46$
Myrica esculenta	$23.43^{b} \pm 7.81$	$36.45^{ab} \pm 13.78$
Phyllanthus emblica	$83.34^{ab} \pm 20.83$	$104.16^{ab} \pm 20.83$
Quercus serrata	$62.5^{ab} \pm 31.25$	$72.91^{ab} \pm 27.56$

The mean value in the column followed by the same letter is not significantly different at a significance level of 0.05

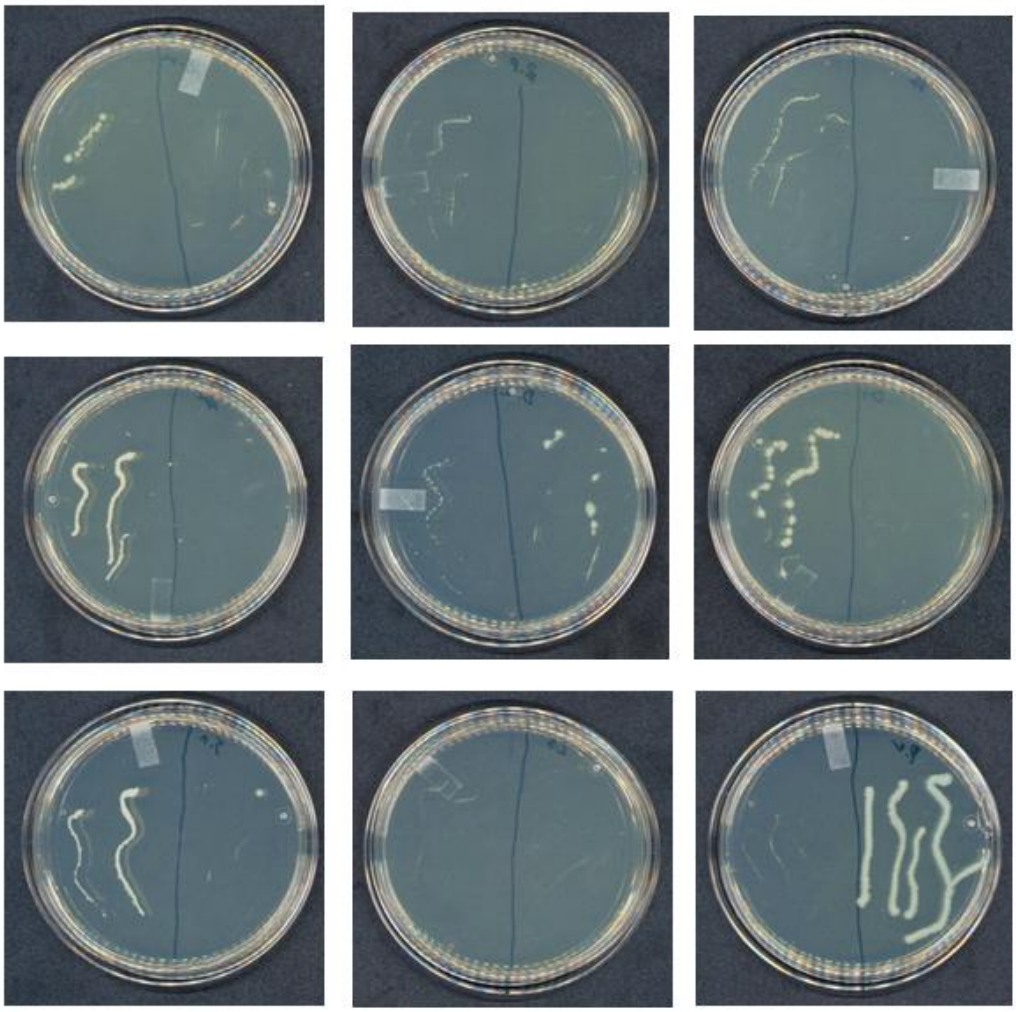


Fig. 2.8. Confirmation of Minimum Bactericidal Concentration (MBC)

2.5 Discussion

Euphorbia milii is the highest in flavonoids (81.6 mg QE/g), tannins (61.627 mg TAE/g), and high in phenolics and alkaloids. *Dillenia indica* is rich in phenolics (68.08 mg GAE/g), alkaloids (66.25 mg AE/g), and tannins (58.193 mg TAE/g). *Melastoma malabathricum* is also high in flavonoids (74.84 mg QE/g), phenolics (53.03 mg GAE/g), and tannins (52.62 mg TAE/g), while *Myrica esculenta* has significant levels of flavonoids (73.69 mg QE/g) and phenolics (57.793 mg GAE/g). *Aquilaria malaccensis* is also high in flavonoids (72.063 mg QE/g), alkaloids (53.153 mg AE/g), and tannins (47.473 mg TAE/g).

The results highlight *Euphorbia milii*, *Dillenia indica*, and *Melastoma malabathricum* as plants rich in multiple bioactive compounds. The high flavonoid content in *Euphorbia milii* supports its traditional use in wound healing and anti-inflammatory applications (Kumar & Pandey, 2013). Flavonoids are recognised for their capacity to scavenge free radicals and regulate cellular signalling (Djeridane et al., 2006). *Dillenia indica* showed notable levels of all four major secondary metabolites. Prior studies have reported its antimicrobial and hepatoprotective properties, which are likely linked to its phenolic and alkaloid profiles (Barua et al., 2014).

Melastoma malabathricum has been traditionally used to treat diarrhoea, wounds, and inflammation, which can be attributed to its high tannin and phenolic content (Sharma et al., 2010). *Myrica esculenta* and *Aquilaria malaccensis* also demonstrated a broad range of phytochemicals. *Myrica* has been associated with anti-inflammatory effects (Bhatt et al., 2011), while *Aquilaria* is valued for its aromatic resin and potential antioxidant properties (Nguyen et al., 2019).

The analysis of phytochemical constituents in medicinal plants is a cornerstone in validating traditional herbal practices and developing modern phytotherapeutic agents. Understanding their composition not only provides insights into the therapeutic potential of a plant but also helps identify the bioactive principles responsible for specific pharmacological effects. In the present study, qualitative and quantitative screenings revealed the presence of several key phytochemical groups in the tested extracts, characterised by high levels of phenols, flavonoids, tannins, and alkaloids.

These findings are significant in the context of gastrointestinal health, particularly in relation to the treatment of diarrhoea.

Tannins, for example, are known to exert anti-diarrhoeal effects through several mechanisms. They can precipitate proteins in the intestinal mucosa, forming a protective layer that reduces secretion and exudation, thereby minimising water loss during diarrhoea (Palombo, 2006). Tannins also possess antimicrobial properties, which may inhibit the growth of diarrhoea-causing pathogens such as *Escherichia coli*, *Salmonella* spp. and *Shigella* spp. (Sadiq et al., 2017).

Flavonoids have been reported to enhance the absorption of electrolytes and water by modulating intestinal motility and secretion. Their anti-inflammatory and antioxidant activities further contribute to mucosal protection and repair (Havsteen, 2002). Moreover, flavonoids such as quercetin and kaempferol inhibit intestinal chloride secretion, a process implicated in the pathogenesis of secretory diarrhoea.

Alkaloids, another primary class detected in the extracts, are known to interact with cholinergic and adrenergic receptors in the gastrointestinal tract. Some alkaloids act as anticholinergics, reducing intestinal motility and prolonging transit time, thus allowing for better absorption of fluids and nutrients. This pharmacodynamic property is central to the anti-diarrhoeal effects of several plant-based remedies (Abdullahi et al., 2001).

Saponins, although traditionally recognized for their hemolytic properties, also play a role in maintaining gut health. Their ability to form foams and emulsify fats can reduce intestinal surface tension, thereby facilitating the absorption of nutrients. At the same time, their potential to inhibit histamine release may contribute to a reduction in inflammation and secretion in the gut lining (Just et al., 1998).

The observed abundance of these bioactive compounds in the extracts suggests a strong potential for anti-diarrhoeal activity, particularly when considered in conjunction with previous studies that have linked similar phytochemical profiles to anti-diarrhoeal efficacy. For instance, extracts of *Aegle marmelos, Psidium guajava, and Terminalia chebula*-all rich in tannins and flavonoids-have demonstrated significant anti-diarrhoeal effects in both in vitro and in vivo models (Palombo, 2006).

Given that diarrhoea often involves a combination of infection, inflammation, and disrupted ion transport, the multifaceted actions of these phytochemicals-ranging

from antimicrobial to anti-secretory and anti-inflammatory-render them particularly effective in holistic gastrointestinal therapy. Therefore, the presence and concentration of these compounds in medicinal plants can serve as predictive markers for their potential as antidiarrhoeals.

The statistically validated findings from one-way ANOVA and Tukey's post hoc test revealed significant variation in antibacterial efficacy among the 14 medicinal plants against *E. coli* and *S. typhimurium*. These differences can be attributed to the distinct phytochemical compositions and concentrations of bioactive compounds in the tested plant extracts.

Remarkably, *Curcuma caesia* and *Mikania micrantha* produced the lowest zones of inhibition, suggesting that the active compounds in these plants have low diffusibility in agar-based assays. However, their high MIC and MBC values indicated that these extracts required higher concentrations to inhibit or kill the bacteria in broth conditions effectively. This discrepancy highlights the importance of using multiple parameters, as the zone of inhibition alone may not always accurately reflect the actual potency of the extract (Balouiri et al., 2016).

Statistical analysis confirmed that *Euphorbia milii* was significantly more potent than most other plants, with the lowest MIC and MBC values against both pathogens. Its consistent performance across all assays positions it as a strong candidate for further phytochemical investigation and drug development. Similarly, *Dillenia indica*, *Melastoma malabathricum*, and *Myrica esculenta* demonstrated statistically significant antibacterial activity, supporting their traditional use in treating diarrhoea.

The significant interaction effect between plant type and bacterial strain observed in the one-way ANOVA suggests that certain plants may be more effective against specific pathogens than others. This highlights the need for targeted antibacterial profiling in future work. These results validate the traditional use of several of these plants and encourage the pursuit of their active constituents for potential development into novel antimicrobial agents. However, further studies, including phytochemical characterisation and/or isolation, toxicity profiling, and *in vivo* models, are recommended to confirm their therapeutic efficacy and safety, as well as to develop them into effective anti-diarrhoeal agents.

Conclusions

This study confirms the presence of diverse and significant phytochemicals in selected medicinal plants. Quantitative analysis revealed that *Euphorbia milii, Dillenia indica, Melastoma malabathricum, Myrica esculenta* and *Aquilaria malaccensis* are rich in flavonoids and tannins, showing strong potential for antidiarrhoeal and antioxidant properties. Among the *plants, E. milii* exhibits the most potent antibacterial activity against *E. coli* (MTCC 723) and *S. typhimurium* (MTCC 3224), followed by *D. indica*, *M. malabathricum*, and *M. esculenta*. These findings highlight the therapeutic potential of these species, supporting their use in traditional medicine and providing a basis for further investigation through *in vivo* and *in silico* studies of their antidiarrhoeal activity.

Therefore, from the selected 14 plants, these 4 plants - *Dillenia indica Euphorbia milii, Melastoma malabathricum*, *Myrica esculenta* were again selected for the next chapter to study their anti-diarrhoeal activity through *in vivo* and *in silico* approach.

CHAPTER – 3

"Safety and Therapeutic Assessment of Medicinal Plant Extracts in Experimental Models of Diarrhoea"

3.1 Introduction

Diarrhoeal diseases continue to be a significant public health issue, especially in low- and middle-income countries where inadequate sanitation and limited healthcare access lead to high rates of illness and death, particularly among children under five (WHO, 2017). Pathogen-caused diarrhoea, often due to enteric bacteria such as *Escherichia coli*, damages the intestinal barrier through inflammatory and secretory processes. Meanwhile, castor oil-induced diarrhoea acts as a well-established pharmacological model that simulates secretory diarrhoea by stimulating intestinal motility and electrolyte secretion through ricinoleic acid (Brijesh et al., 2006). Together, these models provide a comprehensive framework for evaluating the anti-diarrhoeal potential of therapeutic agents.

Despite the widespread utilisation of synthetic anti-diarrhoeal pharmaceuticals, such as antibiotics, concerns remain regarding their long-term safety and potential adverse effects. As a result, the investigation into plant-based alternatives has garnered increasing scholarly attention. Medicinal plants, traditionally used in ethnomedicine for gastrointestinal disorders, are rich sources of bioactive compounds that exhibit antisecretory, antimicrobial, and anti-inflammatory properties. However, prior to validating their therapeutic efficacy, comprehensive toxicological evaluations are imperative to determine safety margins and appropriate dosing parameters (Parasuraman, 2011).

In this context, the present study examines four medicinal plants traditionally used in the treatment of gastrointestinal and inflammatory conditions: *Dillenia indica* L., *Euphorbia milii* Des Moul., *Myrica esculenta* Buch-Ham.ex D. Don, and *Melastoma malabathricum* L. based on their high flavonoid and tannin content and their notable antibacterial activity from previous studies.

***Dillenia indica* L.** commonly known as Elephant Apple, is a medium- to large-sized evergreen tree belonging to the family Dilleniaceae. It is widely distributed in tropical and subtropical regions of South and Southeast Asia, including India, Bangladesh, Nepal, and Thailand.

Botanical Features of *Dillenia indica*:

Leaves: Large, leathery, and oblong with conspicuous veins and serrated margins.

Flowers: Showy, fragrant, and creamy-white with five large petals; usually bloom in late summer.

Fruits: Large, greenish-yellow, and fleshy with a characteristic acidic taste; composed of several carpels enclosed in a thickened calyx.

Traditionally, different parts of the plant—leaves, fruits, and bark—are used in folk medicine to treat diarrhoea, inflammation, fever, wounds, and gastrointestinal disorders. The fruit pulp is often consumed in chutneys and beverages for its digestive and therapeutic properties. The fruit and leaf extracts have been shown to possess documented anti-inflammatory, antioxidant, and anti-diarrhoeal properties (Saiful et al., 2014).

***Euphorbia milii* Des Moul.** commonly known as Crown of Thorns, is a spiny, evergreen shrub belonging to the family Euphorbiaceae. Native to Madagascar, it is now widely cultivated as an ornamental and medicinal plant across tropical and subtropical regions, including India.

Botanical Features of *Euphorbia milii*:

Growth Habit: A succulent shrub that can grow up to 1–2 meters tall with thick, fleshy stems and spines along the branches.

Leaves: Oblong to spatulate, arranged mostly at the tips of branches, and deciduous in nature.

Flowers: Small, inconspicuous flowers surrounded by bright red, pink, or yellow bracts, blooming throughout the year.

Latex: Produces a milky, toxic latex, characteristic of the Euphorbia genus.

Traditionally used in folk medicine to treat skin infections, warts, gastrointestinal disturbances, and as a laxative or diuretic. Its latex and leaf extracts have been studied for wound healing, anti-inflammatory, antimicrobial, and anti-diarrhoeal properties. (Mahendiran et al., 2023).

***Melastoma malabathricum* L.,** commonly known as Indian Rhododendron, Singapore Rhododendron, or Senduduk, is a fast-growing, flowering shrub belonging

to the family Melastomataceae. It is widely distributed across South and Southeast Asia, including India, Malaysia, Indonesia, and the Philippines.

Botanical Features of *Melastoma malabathricum*:

Habit: Erect or spreading shrub or small tree, typically growing up to 1–3 meters in height.

Leaves: Simple, ovate-lanceolate, with three to five prominent longitudinal veins and covered with fine hairs.

Flowers: Large, showy, purple to pinkish-violet in colour with five petals, commonly blooming throughout the year.

Fruits: Small, berry-like capsules that split open to expose numerous tiny seeds embedded in blue pulp.

Widely used in traditional medicine to treat diarrhoea, wounds, dysentery, ulcers, haemorrhoids, and skin infections. Leaves, roots, and flowers are employed in herbal preparations due to their anti-inflammatory, antimicrobial, and astringent properties (Susanti et al., 2007).

***Myrica esculenta* Buch.-Ham. ex D. Don**, commonly known as Box Myrtle, Kaiphal, or Kafal, is a medicinal and edible evergreen tree belonging to the family Myricaceae. It is native to the subtropical Himalayas, especially found in India, Nepal, Bhutan, and parts of Southeast Asia.

Botanical Features *Myrica esculenta*:

Habit: Medium-sized evergreen tree, growing up to 12–15 meters in height, often found on hillsides and forest slopes at altitudes of 900–2,000 meters.

Leaves: Simple, lanceolate, leathery, and aromatic with serrated margins.

Flowers: Unisexual and arranged in catkin-like inflorescences; male and female flowers occur on separate plants (dioecious).

Fruits: Small, globose, deep red to purplish when ripe; edible and slightly sour-sweet, often consumed fresh or used to make juice and jams.

Traditionally used in Ayurvedic and folk medicine to treat diarrhoea, dysentery, fever, cough, asthma, throat infections, and digestive disorders. The bark and fruits are most commonly employed in decoctions and powders (Sood et al., 2018).

This study aimed to evaluate the safety of these four plant extracts by examining their acute and sub-acute oral toxicity profiles, recognising their significant ethnopharmacological importance. Furthermore, their in vivo anti-diarrhoeal efficacy was assessed using castor oil-induced and *E. coli* 078:H11-induced diarrhoea models in mice. This dual approach allows for a comprehensive assessment of the physiological and immunological mechanisms behind the anti-diarrhoeal effects, including the regulation of key pro-inflammatory cytokines such as TNF-α and IL-6, which are vital in the development of infectious and inflammatory diarrhoea.

Enterotoxigenic *Escherichia coli* (ETEC) is a diarrhoeagenic strain of *E. coli* mainly affecting infants and young children, especially in developing nations. It spreads through the faecal-oral route via contaminated food, water, or contact with infected persons. ETEC attaches to intestinal epithelial cells, causing A/E lesions that destroy microvilli and impair absorption. The bacteria inject virulence factors into host cells using a needle-like apparatus, modifying signalling, cytoskeleton, and immune responses. Microvilli loss leads to malabsorption and increased electrolyte and water secretion, causing watery diarrhoea (Kaper et al., 2004).

The growing reliance on herbal medicines worldwide has led to increased scrutiny of their safety profiles. Despite the common belief that plant-based remedies are naturally safe, many studies emphasise the need for thorough toxicological evaluations. Acute and subacute toxicity tests are essential tools for identifying potential risks and establishing safe dosage ranges. Recent research has examined the toxicological profiles of medicinal plants and formulations, aiming to connect traditional knowledge with modern pharmacological evidence. Numerous ethnomedicinal plants have been assessed for their anti-diarrhoeal efficacy and safety through *in vivo* studies, providing valuable insights into their therapeutic potential.

By bridging traditional knowledge with scientific validation, this research aims to identify safe and effective plant-based alternatives for treating diarrhoea and to elucidate their potential mechanisms of action in regulating intestinal inflammation.

3.2 Review of Literature

3.2.1 *Acute and Sub-acute Toxicity*

Obakiro et al. (2021) conducted a sub-acute toxicity study on the methanolic stem bark extract of *Entada abyssinica*, a Fabaceae family plant traditionally used in East and Central Africa. Wistar albino rats were administered oral doses of 150, 300, and 600 mg/kg daily for 28 days. The study found no significant alterations in most biochemical and haematological parameters; however, mild liver lymphocytic infiltration and focal interstitial nephritis were observed at the highest dose. The findings suggest that the extract is relatively safe at lower doses but warrants caution at elevated levels.

In another study, Maru and Belemkar (2025) evaluated the safety of a polyherbal formulation comprising *Asparagus racemosus, Tinospora cordifolia*, and *Trigonella foenum-graecum*: Acute (OECD 423) and sub-acute (OECD 407) toxicity studies were performed using Swiss albino mice. The formulation demonstrated no toxicity at doses up to 1000 mg/kg in the sub-acute setting and showed no genotoxic effects. The lethal dose (LD_{50}) was estimated to be greater than 2000 mg/kg, affirming the formulation's safety for further clinical evaluation.

The aqueous extract of *Canscora heteroclita*, a plant commonly used in Indian traditional medicine, was assessed for acute and sub-acute toxicity in mice and Wistar rats by Aiyalu and Ramasamy (2016). Following the OECD guidelines, doses up to 2000 mg/kg (acute) and 400 mg/kg (sub-acute) caused no significant toxicological symptoms or histological alterations in vital organs. These findings support the safe use of *C. heteroclita* in ethnomedicinal contexts.

Similarly, Kamnate et al. (2024) investigated the ethanol extract of *Boletus griseipurpureus*, a locally consumed mushroom in Thailand. Acute (2000 mg/kg) and sub-acute (50, 300, 2000 mg/kg/day) toxicity tests in male mice revealed no mortality or significant behavioural changes. However, histological examination at higher doses indicated mild liver inflammation, central vein congestion, and glomerular

disturbances in the kidneys. While the LD_{50} exceeded 2000 mg/kg, the extract demonstrated dose-dependent hepatotoxic and nephrotoxic effects.

In a study on *Artemisia afra*, widely used for treating respiratory and febrile illnesses, Mekonen et al. (2020) performed both acute and sub-acute toxicity assessments. Mice treated with doses up to 5000 mg/kg (acute) and 1800 mg/kg/day (sub-acute) exhibited no signs of toxicity or histopathological changes in the brain, heart, or adrenal glands. The study concludes that *A. afra* is safe for short-term use and supports its continued medicinal application.

Another investigation by Mia et al. (2022) focused on the liquid CO_2 extract of *Phaleria macrocarpa* fruit, a plant traditionally used in Indonesia and Malaysia. Acute toxicity testing up to 3000 mg/kg and sub-acute testing at doses ranging from 250 to 2000 mg/kg in mice revealed that the extract was safe at 250 and 500 mg/kg. However, higher doses resulted in histological signs of damage to the liver, kidney, heart, and lungs, highlighting a narrow safety margin at elevated dosages.

Verma et al. (2025) assessed the methanolic flower extract of *Clerodendrum infortunatum,* a plant known for its broad pharmacological activities. Acute doses up to 2500 mg/kg and sub-acute doses up to 2000 mg/kg/day in rats produced no adverse effects on haematological, biochemical, or histological parameters. The extract was well-tolerated, and the authors proposed its potential for therapeutic development, provided appropriate safety margins are established.

Finally, Singh et al. (2011) conducted short-term toxicity studies on *Eupatorium adenophorum* in Swiss albino mice. Although the study lacked detailed quantitative parameters in the available summary, it reported no notable adverse effects, suggesting that the plant's toxicity is low at the examined dosage and duration.

3.2.2 *Castor-oil Oil-Induced Diarrhoea*

Berberis aristata, a well-documented medicinal plant, has shown notable anti-diarrhoeal activity in experimental models conducted by Joshi et al. (2011). In a study using castor oil-induced diarrhoea in rats, administration of the root extract significantly reduced defecation frequency and intestinal fluid accumulation. Moreover, no toxic effects were observed at doses up to 2000 mg/kg, supporting its

safety for oral use. These results scientifically validate its traditional use in managing diarrhoeal disorders.

Similarly, Rahman et al. (2013) demonstrated that the leaf extract of *Desmodium puchellum* possessed strong anti-diarrhoeal effects. Both methanol and petroleum ether extract significantly inhibited diarrhoea and fluid accumulation induced by castor oil in rats. Among the tested fractions, the methanolic extract was found to be more potent, highlighting the importance of solvent polarity in extracting active phytochemicals. The findings support its traditional application in gastrointestinal conditions and pave the way for the development of new formulations.

Islam et al. (2013) investigated the leaves of *Pandanus foetidus* and found that different solvent fractions, particularly chloroform and methanol, possess substantial anti-diarrhoeal activity. These fractions effectively reduced enteropooling and delayed gastrointestinal motility in castor oil-induced models. The aqueous fraction was notably efficient in suppressing motility, while the chloroform extract also demonstrated cytotoxic potential in brine shrimp assays. The observed results corroborate its ethnomedicinal relevance and underscore the importance of solvent-based extraction strategies.

In another study conducted by Misra et al. (2014), *Moringa oleifera* leaves exhibited dose-dependent anti-diarrhoeal activity. Ethanol extracts at doses of 150 and 300 mg/kg significantly reduced the onset and severity of diarrhoea induced by castor oil in mice. The pharmacological effects are likely mediated by the presence of bioactive phytoconstituents, such as tannins, alkaloids, and flavonoids, which are known for their anti-motility and anti-secretory effects. These findings validate *Moringa*'s traditional usage and suggest further isolation of active moieties.

3.2.3 *Pathogen-Induced Diarrhoea*

The management of infectious diarrhoea, particularly that caused by enterotoxigenic *Escherichia coli*, remains a significant concern in both clinical and veterinary settings. Several recent studies have explored the potential of herbal, probiotic, and polyherbal interventions in mitigating diarrhoea and restoring gut homeostasis.

Yu et al. (2017) investigated the efficacy of Modified Pulsatilla Powder (MPP), a traditional Chinese herbal formulation, in alleviating *E. coli* O101-induced diarrhoea in mice. The treatment not only alleviated weight loss and normalized white blood cell (WBC) counts but also significantly reduced the levels of pro-inflammatory cytokines such as TNF-α and IL-6. Furthermore, MPP restored the balance of intestinal microflora and improved the histological architecture of the intestinal lining. These findings support the immunomodulatory and therapeutic properties of MPP in bacterial enteritis.

In a separate study, Vanjari et al. (2020) evaluated the anti-diarrhoeal effects of a probiotic formulation, SKB_Gutbiotic, comprising *Bacillus subtilis, B. vallismortis,* and *B. licheniformis.* This formulation demonstrated significant efficacy against both castor oil- and *E. coli*-induced diarrhoea in rodent models. SKB_Gutbiotic delayed the onset of diarrhoea, reduced faecal output and water content, and improved the histological integrity of colonic tissues. These outcomes were attributed to the probiotic's ability to suppress pathogenic colonisation, enhance intestinal immune responses, and restore mucosal health.

A study by Prasetia and Kesetyaningsih (2015) investigated the efficacy of Growol, a traditional Indonesian fermented food made from cassava, in preventing diarrhoea induced by enterotoxigenic *Escherichia coli* (ETEC) in *Rattus norvegicus* models. Growol, rich in probiotic strains such as *Lactobacillus plantarum*, was found to significantly alleviate clinical signs of diarrhoea, reduce stool water content, and enhance general health markers, including body weight. Both the 50% and 100% Growol treatment groups showed improved outcomes compared to the negative control and performed even better than the group treated with standard antibiotics (sulfamethoxazole-trimethoprim). The findings highlight Growol’s potential as a functional food that can modulate the gut microbiota and enhance host resistance to ETEC infections.

Likewise, Rajkumar et al. (2021) assessed the protective role of Emblica officinalis (Indian gooseberry) in a Wistar rat model of E. coli-induced diarrhoea. The extract reduced the frequency and severity of diarrhoea and improved haematological

and antioxidant parameters in treated rats. The study demonstrated the plant's potential in mitigating oxidative stress and inflammatory responses triggered by bacterial infection, making it a promising candidate for phytotherapeutic intervention.

Lastly, Song et al. (2021) investigated the anti-diarrhoeal efficacy of *Galla Chinensis*, formulated as a Galla Chinensis Oral Solution (GOS), in a murine model of castor oil-induced diarrhoea. The treatment demonstrated notable therapeutic activity, increasing the survival rate of infected mice to 75% and significantly normalising the small intestinal coefficient disrupted by enteropathogenic *Escherichia coli* (EPEC) infection. Mice administered with GOS exhibited a marked reduction in the diarrhoea index, with greater effectiveness observed compared to loperamide, a standard anti-diarrhoeal drug. Furthermore, GOS enhanced mucosal immunity by elevating immunoglobulin levels (IgG and sIgA) in the terminal ileum and significantly decreased systemic concentrations of pro-inflammatory cytokines, including IFN-γ, TNF-α, IL-1β, IL-6, and IL-8. Additionally, the formulation promoted the growth of beneficial gut microbiota such as *Lactobacillus* and *Bifidobacterium*, and mitigated histopathological damage in the colon associated with ETEC-induced lesions.

3.3 Methodology

3.3.1 Experimental Animals

Swiss albino mice, aged between 2 and 4 months and weighing 20–30 g, comprising both sexes, were procured from the Animal Facility of the Department of Zoology, Mizoram University. The animals were housed in polypropylene cages under controlled environmental conditions (25 ± 2°C temperature, 50 ± 5% relative humidity, and a 12-hour light/dark cycle). They were provided with a standard laboratory diet and free access to water. Before experimentation, all animals were acclimatised to laboratory conditions. The experimental procedures adhered strictly to the ethical guidelines set forth by the Institutional Animal Ethics Committee of Mizoram University (Approval No. MZU/IAEC/2021-22/06).

3.3.2 Toxicity Investigation

3.3.2.1 *Acute Oral Toxicity Test*

An acute oral toxicity test was initially performed following OECD Guideline 425 with six female Swiss albino mice. A single oral dose of 2000 mg/kg body weight was given. One mouse was dosed first and observed for 24 hours. If it survived, the other four mice were dosed sequentially at the same level, totalling six animals. Each mouse was monitored individually for signs of toxicity at least once within the first 30 minutes after dosing, at intervals during the first 24 hours, and daily for the next 14 days to complete the observation period.

Throughout the study, parameters like mortality, respiration, sedation, body posture, diarrhoea, drowsiness, skin colouration, fur condition, and loss of consciousness (coma) were meticulously monitored. The mice's body weight was recorded weekly from day one through day 14. At the end of these 14 days, the animals were sacrificed, and their major vital organs were removed, weighed, and checked for any morphological alterations.

3.3.2.2 *Sub-Acute Oral Toxicity Test*

The sub-acute toxicity study was conducted in accordance with OECD Guideline No. 407. Albino mice, weighing 20-30 g, were randomly divided into three

groups, each comprising six males and six females. The control group received only distilled water throughout the study. The other groups were administered the plant extract orally via gavage needles at 200 mg/kg and 600 mg/kg once daily for 28 days. Throughout the treatment period, body weight was recorded on days 1, 7, 14, and 28. Daily monitoring of food intake and water consumption was conducted across all experimental groups. After the 28-day exposure, the surviving animals were anaesthetised via inhalation of diethyl ether using a closed chamber containing ether-soaked cotton.

Blood was collected from the retro-orbital sinus for haematological analysis, including red blood cell (RBC) count, white blood cell (WBC) count, and haemoglobin (Hb) levels. A portion of the collected blood was centrifuged at 10,000 rpm for 10 minutes to separate the serum, which was subsequently analysed for key biochemical markers, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, direct bilirubin, and creatinine. Following blood collection, the animals were euthanised by cervical dislocation. Vital organs, including the liver, kidneys, lungs, and spleen, were carefully excised, weighed, and preserved for histopathological examination to assess any morphological alterations induced by the extract.

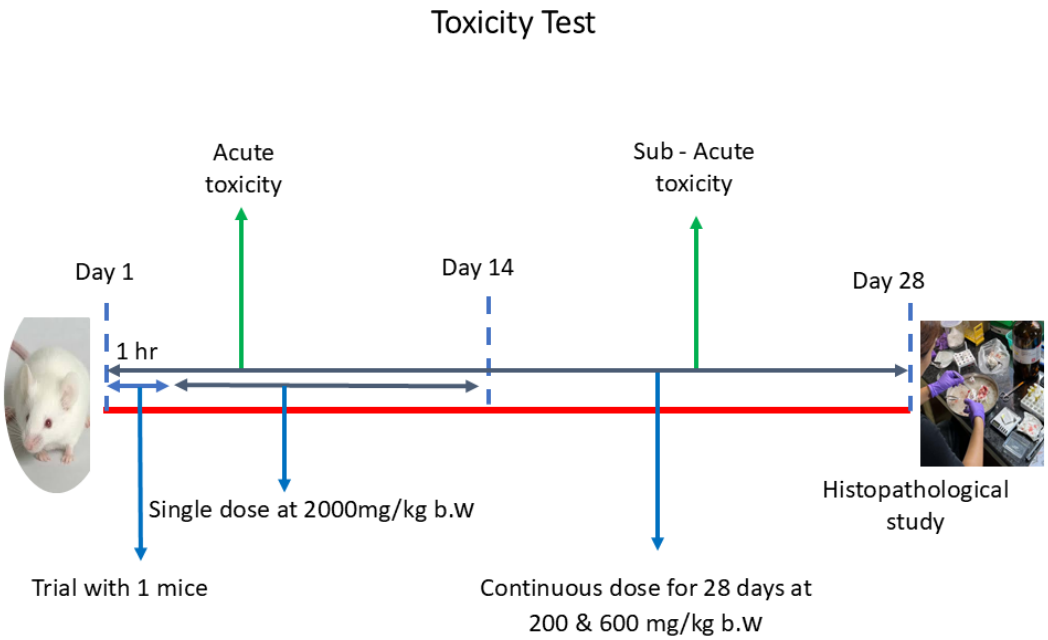


Fig. 3.1 Diagrammatic overview of Toxicity study of the plant extracts

3.3.3 In Vivo Anti-diarrhoeal Investigation

3.3.3.1 Castor Oil-Induced Diarrhoea in Mice

The experiment was conducted following the method described by Awouters et al. (1978). Thirty mice were fasted for 12 hours and randomly assigned to five groups, each consisting of six animals. Group I received distilled water (10 mL/kg b.w) and served as the negative control, while Group II received loperamide (3 mg/kg b.w) as the positive control. Groups III, IV, and V were administered the test extract at doses of 100, 200 and 400 mg/kg b.w respectively. One hour after treatment, all animals were orally administered 0.5 mL of castor oil to induce diarrhoea. The mice were then placed individually in separate cages, and the severity of diarrhoea was monitored over 6 hours. The total number of faecal outputs-both dry and wet (diarrhoeal)-was recorded for each animal and compared to the negative control group.

The diarrhoeal score of the negative control group was considered as 100%, and the percentage inhibition of total defecation and diarrhoea was calculated using the following formulas.

$$\%\ \text{of Inhibition} = \frac{\text{Mean defecation (Control} - \text{Test)}}{\text{Mean defecation of Control}} \times 100$$

3.3.3.2 Pathogen-induced diarrhoea in Mice

3.3.3.2.1 Determination of LD^{50} for *E. coli* 078:H11 in Mice

Enterotoxigenic E. coli (ETEC) strain E. coli 078:H11 was used in this study and was purchased from the Microbial Type Culture Collection and Gene Bank (MTCC), India. The median lethal dose (LD_{50}) of *Escherichia coli* 078:H11 was determined via a dose–response protocol (Song et al., 2021). A total of thirty-six male mice were randomly assigned to six groups (n = 6 per group) following a 6-hour fasting period. Each group received an oral dose of E. coli 078:H11 at concentrations ranging from 3.3 × 109 to 6.0 × 109 CFU, administered in 1 mL per animal. The specific doses administered were 3.3×10^9, 3.6×10^9, 4.2×10^9, 4.8×10^9, 5.4×10^9, and 6.0×10^9 CFU/mouse, respectively. A control group received an equivalent volume of sterile normal saline.

Post-inoculation, the mice were closely monitored for clinical symptoms, behavioural alterations, signs of systemic toxicity, and mortality—initially within the first 12 hours and subsequently twice daily over 14 days. Mice that remained alive at the end of the observation period were recorded as survivors. Mortality data from each dose group were used to compute the LD_{50} using Karber's method, and a dose–mortality curve was generated using GraphPad Prism software version 8.0.2.

3.3.3.2.2 Experimental Design for Anti-Diarrhoeal Evaluation

For the anti-diarrhoeal efficacy study, the experiment was conducted using a slight modification described by Yu et al. (2017).Mice were randomly distributed into five groups (n = 6 per group). Prior to treatment, all animals were fasted for 6 hours. The study period lasted five days. Group I (negative control) received normal saline, and Group II (positive control) received loperamide at a dose of 3 mg/kg body weight, suspended in distilled water. Groups III and IV were treated with the plant extract at doses of 100 mg/kg and 400 mg/kg body weight, respectively, administered orally.

Thirty minutes after treatment, all mice (except the normal control group) were orally administered E. coli 078:H11 at its previously determined LD50 dose for three consecutive days. Throughout the experimental period, animals were monitored daily for clinical symptoms and survival.

On day five, all animals were humanely sacrificed. Blood samples were collected for white blood cell (WBC) analysis. The colon was excised for histopathological examination, while the spleen and thymus were harvested for assessment of immune organ indices. Cecal contents were aseptically collected in sterile microtubes and preserved at −80°C for subsequent microbiological or molecular analyses (Vanjari et al., 2020).

3.3.3.2.3 Diarrhoea index

After infection, the stool counts and morphology will be observed daily. The loose stool incidence rate (LSIR) will be calculated as the ratio of the number of loose stools to the total stools within an animal. The loose stool grade (LSG) is the degree of loose stools, which will be classified into four grades according to the diameter of loose stools on the filter papers: Grade 1 (< 1 cm), Grade 2 (1–2 cm), Grade 3 (2–

3 cm), and Grade 4 (> 3 cm). The average loose stool grade (ALSG) will be calculated as the ratio of the sum of LSG of each loose stool to the total number of loose stools within an animal. The diarrhoea index will be calculated by multiplying the LSIR by the ALSG. Body weights of all mice will be monitored daily throughout the study (Yu et al., 2017; Song et al., 2021).

3.3.3.2.4 Survival rate

Animals will be observed daily throughout the experiment, and the survival rate of the mice will be recorded. The survival rate in each group will also be calculated using the following formula. (Song et al., 2021).

$$Survival\ rate = \frac{\text{Number of surviving mice after challenge}}{\text{Number of mice injected with bacteria}} \times 100\%$$

3.3.3.2.5 Organ coefficient assay

The thymus and spleen of animals were weighed. The relative weight of organs (Organ coefficient) was calculated using the following formula, as mentioned by Yu et al. (2017).

$$Organ\ coefficients = \frac{\text{Organ weight (g)}}{\text{Body weight (g)}} \times 100$$

3.3.3.4 Statistical Analysis

All experimental data were analysed using GraphPad Prism (version 8, USA). Results are expressed as mean ± standard error of the mean (SEM). A p-value of less than 0.05 was regarded as statistically significant.

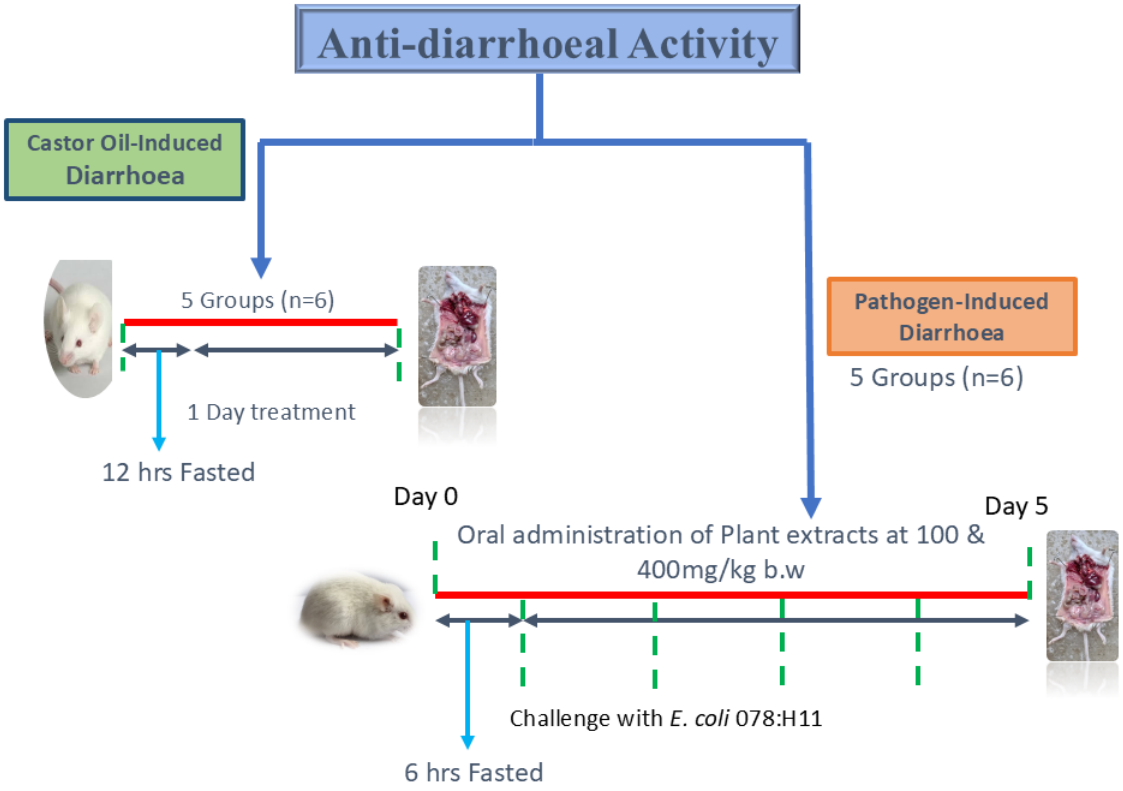


Fig. 3.2 Diagrammatic overview of anti-diarrhoeal activity of the plant extracts on mice

3.4 Results

3.4.1 *Acute toxicity study*

After administering a single oral dose of 2,000 mg/kg body weight each of plant extract of *D. indica, E. milii, M. esculenta* and *M. malabathricum*, no clinical signs of toxicity-such as abnormal respiration, excessive salivation, sleep disturbances, tremors, convulsions, aggression, piloerection, or death-were observed in the treated animals. Furthermore, there were no detectable abnormalities in the morphology, colouration, texture, or weight of vital organs, including the liver and kidneys. The high-dose treatment did not cause any significant differences in body weight loss or gain compared to the control group, except in the *M. esculenta* female group, where a gradual decrease in body weight was observed on day 14. However, it was not substantial (Fig. 3.3a and 3.3b).

Morphological examinations of vital organs revealed no structural changes in either the treated or control animals. Over the 14-day observation period, all animals survived, suggesting that the median lethal dose (LD_{50}) of the four plant extracts - *D. indica, E. milii, M. esculenta,* and *M. malabathricum* - exceeds 2,000 mg/kg body weight.

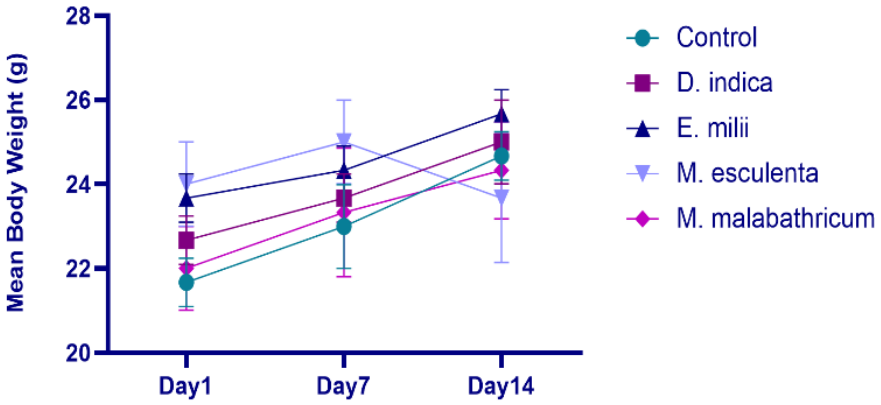


Fig. 3.3a Female Mean body weight

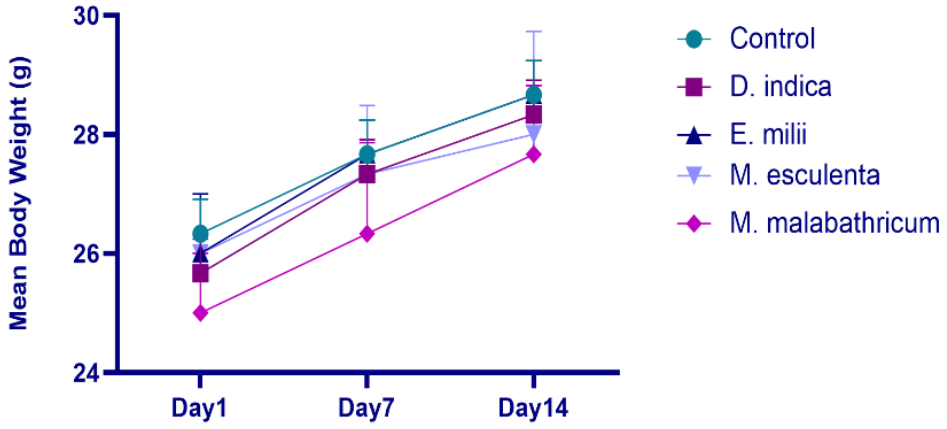


Fig. 3.3b Male Mean body weight

3.4.2 *Sub-acute toxicity study*

During the 28-day sub-acute toxicity assessment, oral administration of *Dillenia indica, Euphorbia milii, Myrica esculenta,* and *Melastoma malabathricum* extracts at doses of 200 and 600 mg/kg body weight did not elicit any observable adverse effects on the general behaviour or physiological condition of the animals when compared to the untreated controls. Behavioural observations revealed no apparent sex-related differences, except for a relatively higher level of aggression consistently noted among males across both control and treated groups. Notably, no mortality associated with treatment was recorded during the entire experimental period, suggesting that the extracts were well tolerated.

3.4.2.1 Body Weight

All experimental groups, including controls, exhibited a gradual increase in body weight over the four weeks, although the changes were not statistically significant. The absence of substantial weight loss or suppressed growth in either sex indicates that none of the plant extracts interfered with normal metabolic processes or nutrient assimilation. This pattern of progressive weight gain without deviation from control values reinforces the non-toxic nature of the administered doses (Table 3.1).

3.4.2.2 Organ Coefficient

Evaluation of organ weights after repeated dosing revealed some dose- and sex-dependent variations. In male mice, significant increases in lung weights were observed in groups administered *D. indica, E. milii,* and *M. esculenta*, compared to the groups of all plants and the control. In contrast, the liver, kidneys, and spleen showed no notable differences. Conversely, female mice exhibited significant changes in organ weights (lungs, liver, kidneys, and spleen) following administration of *D. indica, E. milii,* and *M. esculenta*, indicating potential sex-specific physiological responses. Interestingly, *M. malabathricum* did not produce any statistically significant changes in organ weights in either sex, underscoring its relatively stable toxicological profile under the tested conditions (Table 3.2).

However, these changes are all under normal range which indicates healthy organ and no sign of toxic appeared.

Table 3.1 Mean Body Weight in grams (gm)

Female	**Control**	**200mg/kg b.w**				**600mg/kg b.w**			
		D. indica	*E. milii*	*M. esculenta*	*M. malbathricum*	*D. indica*	*E. milii*	*M. esculenta*	*M. malbathricum*
Day 1	24.00±1.00	24.33 ± 0.58	23.33 ± 1.53	23.00±1.00	22.00 ± 1.00	23.33±0.58	23.00 ± 1.00	22.00 ± 1.00	23.33 ± 1.15
Day 7	24.33±1.53	25.00 ± 1.00	24.67 ± 1.53	24.00±1.00	24.33 ± 2.08	23.67±0.58	24.00 ± 1.00	23.00 ± 1.00	24.00 ± 1.00
Day 14	25.00±1.00	26.00 ± 1.00	25.33 ± 1.53	25.00±1.00	25.00 ± 2.00	24.67±0.58	25.00 ± 1.00	24.00 ± 1.00	25.00 ± 1.00
Day 28	26.00±1.00	26.33 ± 0.58	26.33 ± 1.53	25.67±0.58	26.00 ± 2.00	25.33±1.15	26.00 ± 1.00	25.00 ± 1.00	25.67 ± 0.58
Male									
Day 1	26.00±1.00	26.33 ± 0.58	26.00 ± 1.00	26.00±1.00	26.50 ± 0.71	23.33±0.58	23.67 ± 0.58	24.67 ± 0.58	25.33 ± 1.15
Day 7	26.67±1.15	27.33 ± 0.58	27.00 ± 1.00	26.67±1.15	27.00 ± 1.00	23.00±1.00	24.00 ± 1.00	25.00 ± 1.00	26.00 ± 1.00
Day 14	27.67±1.15	27.67 ± 0.58	28.00 ± 1.00	27.67±1.15	27.67 ± 0.58	22.00±1.00	23.00 ± 1.00	24.00 ± 1.00	25.00 ± 1.00
Day 28	28.33±0.58	28.67 ± 0.58	28.67 ± 1.15	28.67±1.15	28.67 ± 0.58	23.33±1.15	24.00 ± 1.00	25.00 ± 1.00	25.67 ± 0.58

Table 3.2 Organ Coefficient in 100grams b.w.

	Lung			Liver			Kidney			Spleen		
Female	**Cntrl**	**200**	**600**	**Cntrl**	**200**	**600**	**Cntrl**	**200**	**600**	**Cntrl**	**200**	**600**
D. indica	$0.63^{ab}\pm0.02$	$0.67^{a}\pm0.04$	$0.68^{a}\pm0.02$	$4.53^{a}\pm0.12$	$4.59^{a}\pm0.02$	$2.07^{b}\pm2.51$	$1.29^{ab}\pm0.15$	$1.28^{ab}\pm0.04$	$1.56^{a}\pm0.08$	$0.23^{ab}\pm0.05$	$0.23^{ab}\pm0.02$	$0.29^{ab}\pm0.01$
E. milii	$0.63^{ab}\pm0.02$	$0.70^{a}\pm0.03$	$0.56^{b}\pm0.02$	$4.53^{a}\pm0.12$	$4.56^{a}\pm0.21$	$4.46^{ab}\pm0.17$	$1.29^{ab}\pm0.15$	$1.31^{ab}\pm0.15$	$1.07^{b}\pm0.12$	$0.23^{ab}\pm0.05$	$0.30^{ab}\pm0.02$	$0.31^{a}\pm0.01$
M. esculenta	$0.63^{ab}\pm0.02$	$0.58^{b}\pm0.05$	$0.58^{b}\pm0.04$	$4.53^{a}\pm0.12$	$4.52^{a}\pm0.07$	$4.62^{a}\pm0.12$	$1.29^{ab}\pm0.15$	$1.35^{ab}\pm0.12$	$1.43^{a}\pm0.04$	$0.23^{ab}\pm0.05$	$0.27^{ab}\pm0.03$	$0.24^{ab}\pm0.02$
M. malbathricum	$0.63^{ab}\pm0.02$	$0.61^{ab}\pm0.06$	$0.65^{ab}\pm0.01$	$4.53^{a}\pm0.12$	$4.55^{a}\pm0.28$	$4.52^{a}\pm0.08$	$1.29^{ab}\pm0.15$	$1.26^{ab}\pm0.12$	$1.34^{ab}\pm0.08$	$0.23^{ab}\pm0.05$	$0.21^{b}\pm0.04$	$0.30^{ab}\pm0.03$
Male												
D. indica	$0.57^{ab}\pm0.02$	$0.57^{ab}\pm0.03$	$0.61^{a}\pm0.06$	$4.19^{a}\pm0.07$	$4.15^{a}\pm0.09$	$4.07^{a}\pm0.03$	$1.14^{a}\pm0.25$	$1.12^{a}\pm0.23$	$1.33^{a}\pm0.03$	$0.25^{a}\pm0.06$	$0.25^{a}\pm0.06$	$0.29^{a}\pm0.03$
E. milii	$0.57^{ab}\pm0.02$	$0.64^{a}\pm0.04$	$0.51^{b}\pm0.01$	$4.19^{a}\pm0.07$	$4.18^{a}\pm0.11$	$4.04^{a}\pm0.07$	$1.14^{a}\pm0.25$	$1.20^{a}\pm0.11$	$0.97^{a}\pm0.11$	$0.25^{a}\pm0.06$	$0.27^{a}\pm0.11$	$0.97^{a}\pm0.11$
M. esculenta	$0.57^{ab}\pm0.02$	$0.59^{ab}\pm0.01$	$0.65^{a}\pm0.01$	$4.19^{a}\pm0.07$	$4.12^{a}\pm0.01$	$4.10^{a}\pm0.05$	$1.14^{a}\pm0.25$	$1.25^{a}\pm0.05$	$1.25^{a}\pm0.01$	$0.25^{a}\pm0.06$	$0.26^{a}\pm0.04$	$0.26^{a}\pm0.01$
M. malbathricum	$0.57^{ab}\pm0.02$	$0.61^{a}\pm0.02$	$0.62^{a}\pm0.03$	$4.19^{a}\pm0.07$	$0.97^{a}\pm0.11$	$4.22^{a}\pm0.14$	$1.14^{a}\pm0.25$	$0.97^{a}\pm0.11$	$1.28^{a}\pm0.06$	$0.25^{a}\pm0.06$	$0.28^{a}\pm0.01$	$0.27^{a}\pm0.04$

The mean value in each row of parameter followed by the same letter is not significantly different at significance level of 0.05

3.4.2.3 *Haematological Parameter*

The haematological parameters assessed during the sub-acute toxicity study (Table 3.4) indicated no statistically significant alterations in haemoglobin (Hb) levels in either male or female mice across all treatment groups. However, a notable variation was observed in red blood cell (RBC) counts in male mice administered *Myrica esculenta* extract at a dose of 200 mg/kg body weight, suggesting a potential influence of the extract on erythropoiesis or red cell stability in males. In contrast, female mice did not exhibit any significant deviation in RBC values compared to the control group, implying a sex-specific response to the extract.

Regarding white blood cell (WBC) counts, both female and male mice showed variations following treatment; however, these changes were not statistically significant compared to the control groups, suggesting that the observed fluctuations may fall within physiological norms or lack biological relevance. Overall, the haematological findings reflect minimal systemic toxicity, with some gender-specific responses that warrant further investigation.

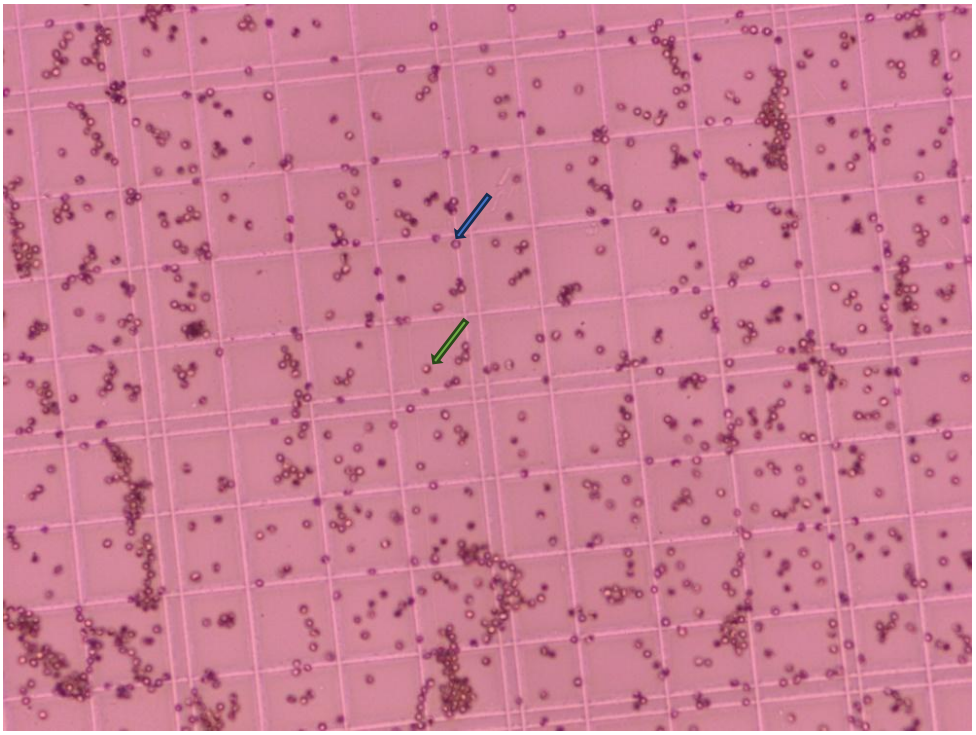


Fig. 3.4 RBC and WBC appearance under microscopic view. Green arrow indicates RBC (smaller in size, round) and blue arrow indicates WBC (larger in size, irregular shape, appear purple/pink

Table 3.3 Haematological Parameters

	Hb (g/dl)			RBC (10^6/ul)			WBC (10^3/ul)		
Female	**Cntrl**	**200**	**600**	**Cntrl**	**200**	**600**	**Cntrl**	**200**	**600**
D. indica	$12.17^{a}\pm0.29$	$12.67^{a}\pm0.29$	$12.33^{a}\pm0.58$	$8.48^{a}\pm0.18$	$8.39^{a}\pm0.28$	$8.68^{a}\pm0.30$	$5.27^{ab}\pm0.40$	$4.63^{ab}\pm0.70$	$5.47^{ab}\pm0.65$
E. milii	$12.17^{a}\pm0.29$	$12.00^{a}\pm1.00$	$13.33^{a}\pm1.15$	$8.48^{a}\pm0.18$	$8.39^{a}\pm0.28$	$8.68^{a}\pm0.30$	$5.27^{ab}\pm0.40$	$4.17^{b}\pm0.59$	$4.27^{b}\pm0.55$
M. esculenta	$12.17^{a}\pm0.29$	$12.33^{a}\pm0.58$	$12.67^{a}\pm1.15$	$8.48^{a}\pm0.18$	$8.31^{a}\pm0.16$	$9.02^{a}\pm0.66$	$5.27^{ab}\pm0.40$	$5.73^{ab}\pm0.35$	$6.03^{a}\pm0.40$
M. malabathricum	$12.17^{a}\pm0.29$	$13.00^{a}\pm1.73$	$12.50^{a}\pm0.50$	$8.48^{a}\pm0.18$	$8.66^{a}\pm0.69$	$8.30^{a}\pm0.40$	$5.27^{ab}\pm0.40$	$4.57^{ab}\pm0.65$	$5.97^{a}\pm0.67$
Male									
D. indica	$12.50^{a}\pm0.50$	$12.50^{a}\pm0.50$	$13.00^{a}\pm1.00$	$7.57^{bc}\pm0.31$	$7.93^{ab}\pm0.42$	$7.40^{bc}\pm0.50$	$4.77^{a}\pm0.42$	$4.20^{a}\pm0.46$	$4.83^{a}\pm0.70$
E. milii	$12.50^{a}\pm0.50$	$12.83^{a}\pm1.04$	$13.33^{a}\pm1.53$	$7.57^{bc}\pm0.31$	$7.90^{ab}\pm0.36$	$6.83^{c}\pm0.31$	$4.77^{a}\pm0.42$	$3.77^{a}\pm0.38$	$5.13^{a}\pm0.55$
M. esculenta	$12.50^{a}\pm0.50$	$13.33^{a}\pm1.53$	$12.50^{a}\pm0.50$	$7.57^{bc}\pm0.31$	$7.97^{ab}\pm0.40$	$7.87^{a}\pm0.65$	$4.77^{a}\pm0.42$	$3.60^{a}\pm0.46$	$4.37^{a}\pm0.45$
M. malabathricum	$12.50^{a}\pm0.50$	$12.33^{a}\pm0.58$	$12.17^{a}\pm0.29$	$7.57^{bc}\pm0.31$	$8.13^{ab}\pm0.47$	$8.8^{abc}\pm0.31$	$4.77^{a}\pm0.42$	$3.93^{a}\pm0.78$	$4.80^{a}\pm0.75$

The mean value in each row of the parameter followed by the same letter is not significantly different at a significance level of 0.05

3.4.2.4 *Biochemical Parameters*

Repeated oral administration of the four plant extracts at doses of 200 and 600 mg/kg body weight for 28 consecutive days did not result in any statistically significant alterations in aspartate aminotransferase (AST) levels in either male or female mice. However, significant elevations in alanine aminotransferase (ALT) were observed in male mice treated with *Euphorbia milii* and *Myrica esculenta* at the higher dose of 600 mg/kg, indicating a possible hepatocellular response or mild hepatic stress. In contrast, ALT levels in female mice remained within normal limits across all treatment groups, showing no significant deviations from control values.

Regarding bilirubin parameters, a notable increase in both total and direct bilirubin was observed in male mice treated with *M. esculenta* and *Melastoma malabathricum*, indicating a potential change in hepatic excretory function or mild cholestasis. Regarding renal function markers, creatinine levels increased significantly in female mice at 600 mg/kg for *Dillenia indica* and *M. malabathricum,* and at 200 mg/kg for *M. esculenta*, suggesting possible dose-dependent effects on renal clearance or glomerular function. Similarly, male mice displayed significantly elevated creatinine levels after treatment with *D. indica* and *E. milii* compared to the control group.

Despite these statistically significant alterations in hepatic and renal biomarkers, all observed values remained within clinically acceptable physiological ranges. This suggests that the biochemical changes were not indicative of overt organ toxicity or pathological damage, and the extracts, even at higher doses, did not elicit harmful effects under the conditions of this sub-acute toxicity study.

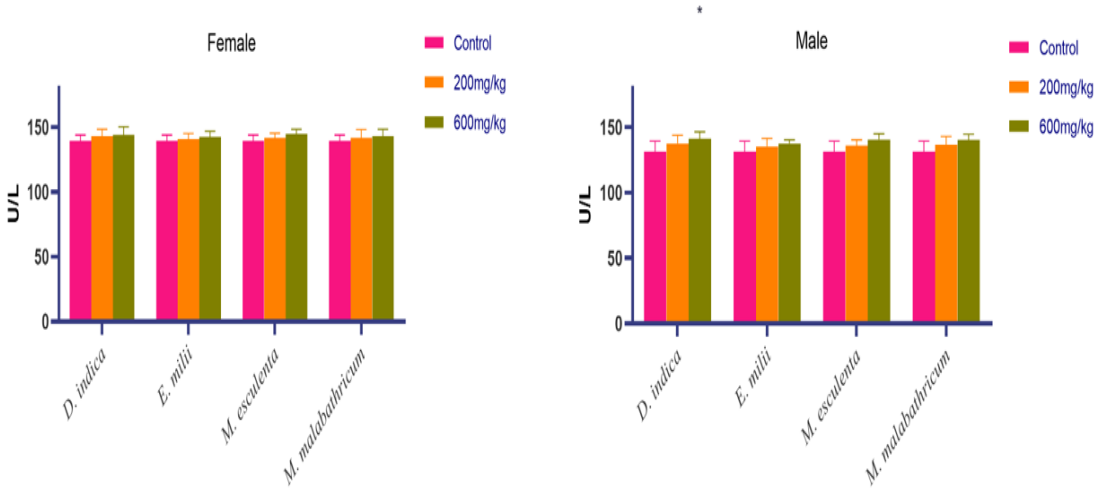


Fig. 3.5a AST Content

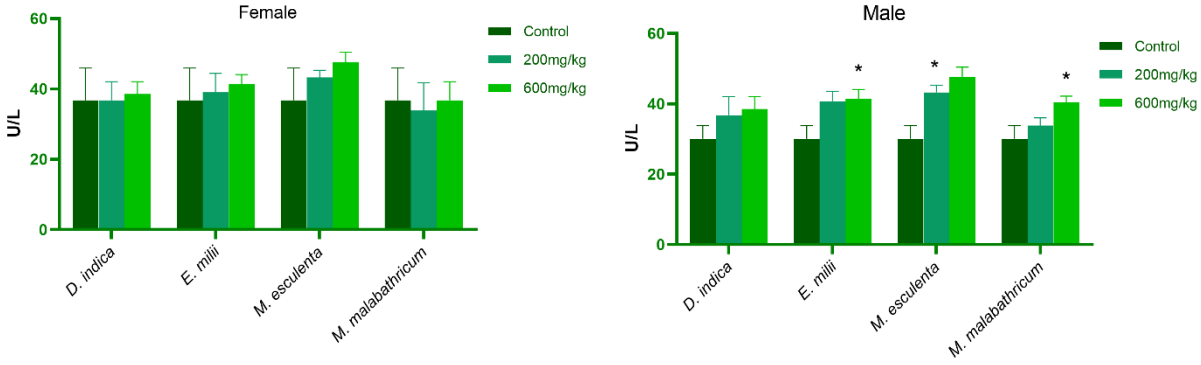


Fig. 3.5b ALT Content

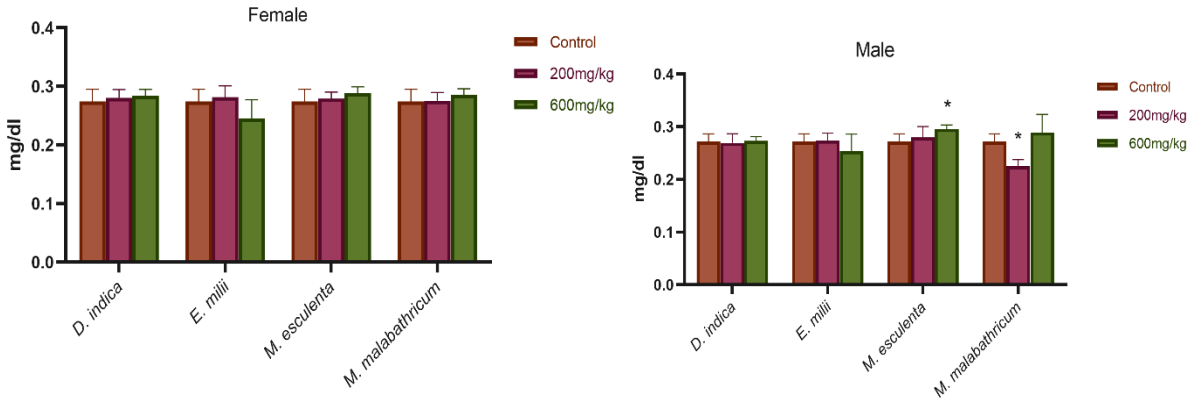


Fig. 3.5c Direct Bilirubin

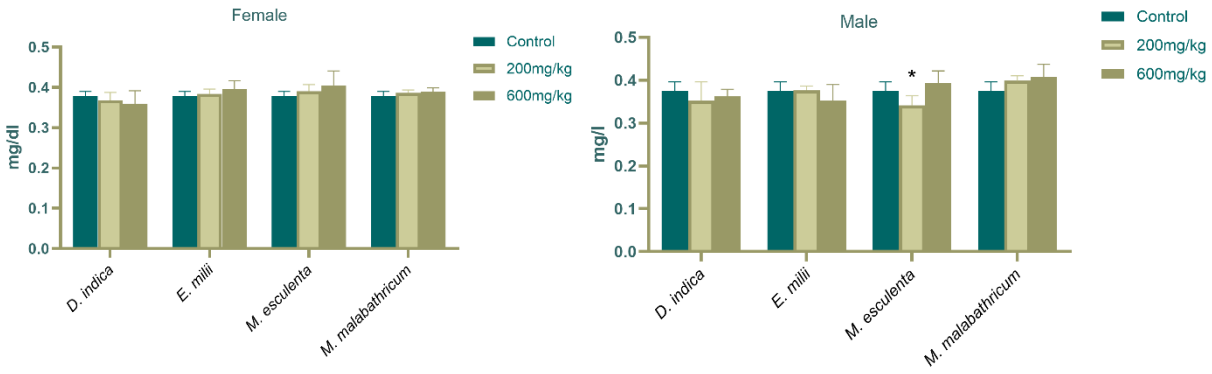


Fig. 3.5d Total Bilirubin

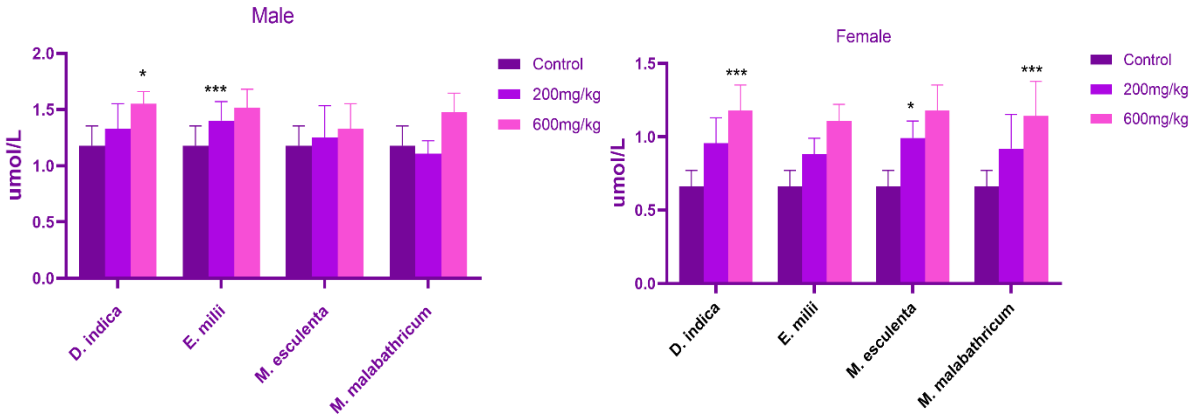


Fig. 3.5e Creatinine Content.

*Note: The graph containing * represents p<0.05, and *** represents p<0.001 compared with the control group.*

3.4.2.5 *Histopathology investigation*

3.4.2.5.1 Lungs

Histopathological examination of lung tissues from the control group revealed well-preserved alveolar architecture with normal bronchioles and alveoli at 10× magnification. Additionally, mild vascular congestion was noted in some alveolar capillaries, which is often considered a non-specific post-mortem change. In mice administered 200 mg/kg and 600 mg/kg of the four plant extracts, both male and female specimens exhibited intact pulmonary parenchyma, characterised by clearly defined alveolar spaces and bronchioles, with no evidence of pathological alterations,

including inflammation, fibrosis, or haemorrhage. These findings suggest that there is no pulmonary toxicity across all treated groups.

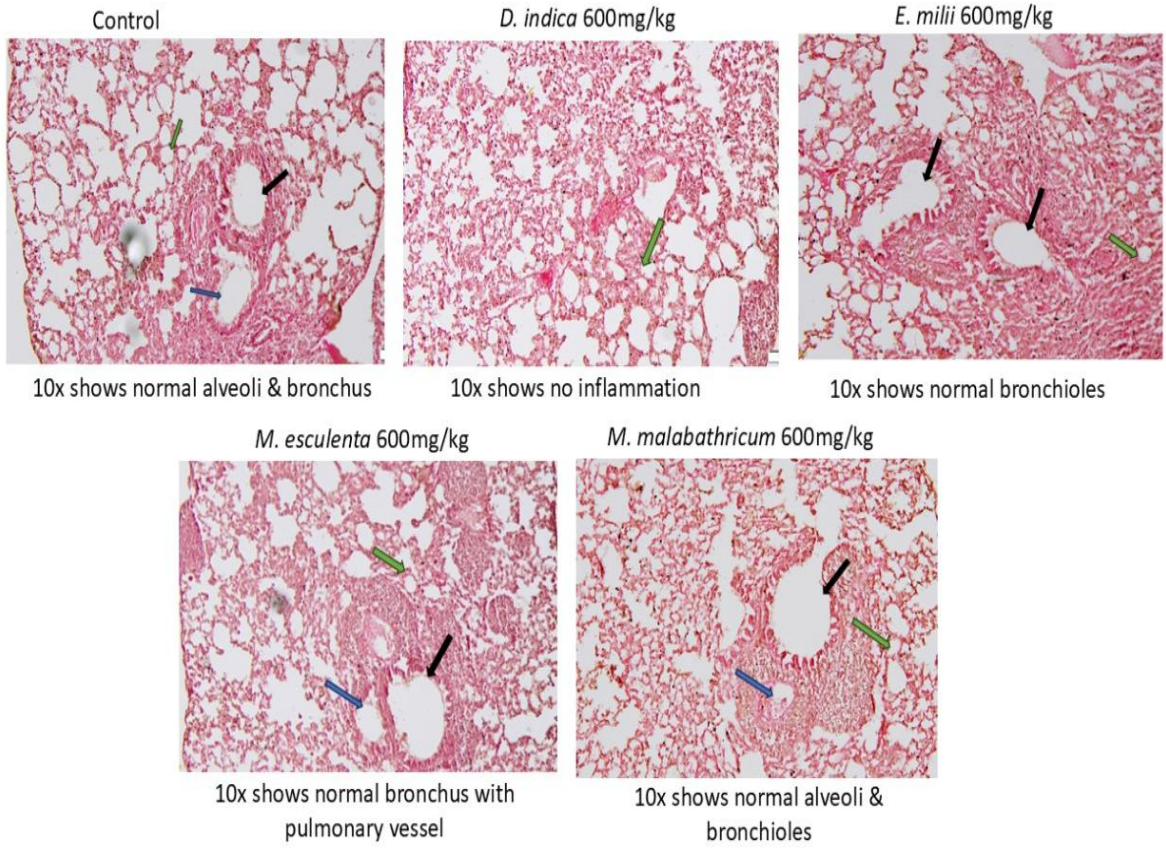


Fig. 3.6 Histology of lungs for control and treated with four plants, the black arrow shows bronchioles, the green arrow shows alveoli, and the blue arrow shows the pulmonary vessel.

3.4.2.5.2 Liver

Liver sections from the control group displayed normal hepatic lobular organisation and clearly defined portal triads under both 10x magnification. In the treatment groups receiving 200 mg/kg and 600 mg/kg of the four plant extracts, the hepatic architecture remained intact, with well-preserved central veins, sinusoids, and hepatocyte arrangement. Importantly, no histopathological features suggestive of hepatocellular injury, such as inflammatory cell infiltration, steatosis, necrosis, or fibrotic changes, were detected in either sex, indicating preserved liver function and absence of hepatotoxicity.

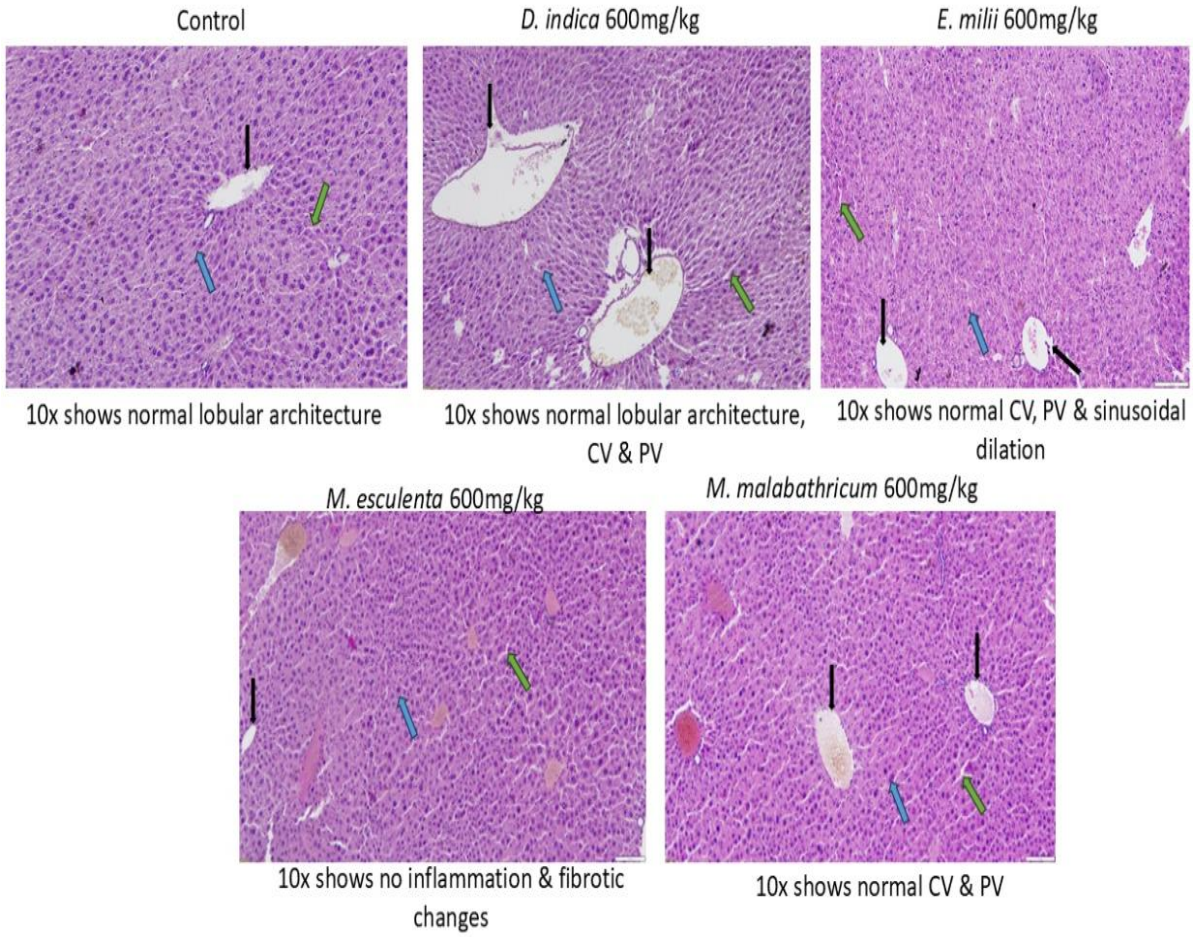


Fig. 3.7 Histology of liver for control and treatment with four plants. Central vein and Portal vein in black arrow, sinusoidal in green arrow, hepatocytes in blue arrow.

3.4.2.5.3 Kidneys.

Microscopic analysis of kidney sections from both control and treated groups demonstrated well-preserved renal histology. The renal cortex and medulla appeared normal, with intact glomeruli and tubules. The interstitial tissue showed no signs of inflammation, oedema, or necrosis. Additionally, no structural abnormalities were evident at either 200 mg/kg or 600 mg/kg doses in either sex, indicating that the four plant extracts did not exert nephrotoxic effects under the study conditions.

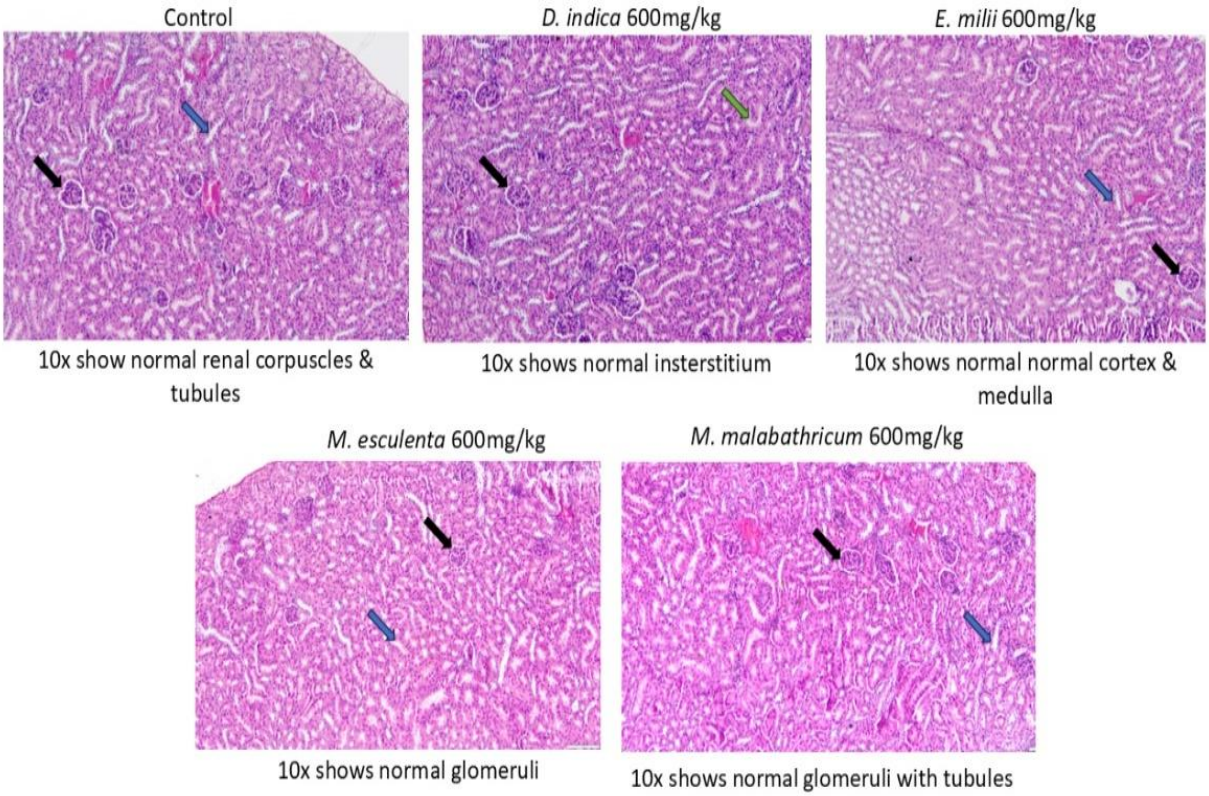


Fig. 3.8 Histology of Kidneys for control and treatments with four plants. Glomeruli in black arrow, renal tubules in blue arrow, interstitium in green arrow

3.4.2.5.4 Spleen

Histological analysis of spleen tissues showed normal morphology in the control group and the 200mg/kg groups for all plants. Both the control and extract-treated animals exhibited clear red and white pulp, including well-defined germinal centres. There were no indications of lymphoid depletion, haemorrhage, or structural distortion in either males or females, indicating that the extracts did not cause any adverse immunological or haematological effects on the spleen. However, at a dose of 600mg/kg, spleen tissue displayed signs of reactive lymphoid depletion and an expansion of the red pulp, likely due to inflammation or hemolytic stress. Despite these changes, no significant necrosis, infarction, or fibrosis was observed, suggesting a mild, reversible splenic response often associated with systemic immune activation or toxin exposure.

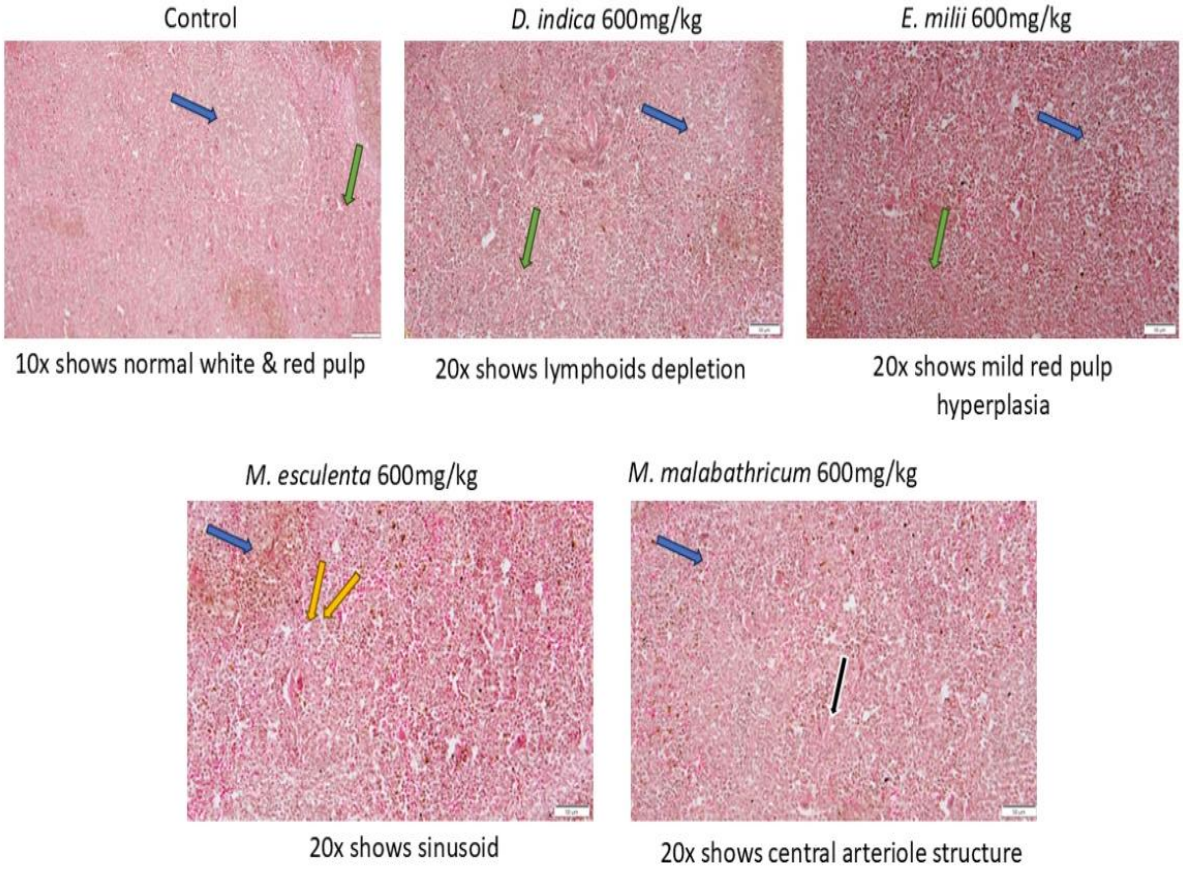


Fig. 3.9 Histology of the spleen for control and treatment with four plant extracts. The blue arrow indicates white pulp, the green arrow indicates red pulp, the yellow arrow indicates sinusoid, and the black arrow indicates the central arteriole structure.

3.4.3 *Antidiarrhoeal Activity on Mice*

3.4.3.1 *Castor oil-induced diarrhoea*

3.4.3.1.1 Onset of Diarrhoea

The onset of diarrhoea in the vehicle control group occurred approximately 41 minutes post-castor oil administration across all tested plants. In contrast, the loperamide (LPM) standard group showed a significantly delayed onset (106.67 ± 10.41 minutes; $p<0.05$). All plant extracts delayed the onset of diarrhoea in a dose-dependent manner.

At 400 mg/kg, *D. indica, E. milii, M. esculenta,* and *M. malabathricum* delayed diarrhoea onset to 86.00 ± 3.61, 94.00 ± 3.61, 79.67 ± 4.51, and 70.00 ± 5.00 minutes, respectively, all of which were significantly ($p<0.05$) delayed compared to the control, as shown in Table 3.4.

3.4.3.1.2 Total Defecation and Inhibition

The total number of fecal outputs in the control group averaged 17.00 ± 2.00. LPM significantly reduced this to 6.33 ± 1.53, corresponding to a 62.75% inhibition. Among the plant extracts at 400 mg/kg, *E. milii* showed the highest inhibition (54.90%), followed by *M. esculenta* (50.98%), *D. indica* (47.06%), and *M. malabathricum* (45.10%) as shown in Table 3.5.

3.4.3.1.3 Total Diarrhoeal Droppings and Inhibition

The control group had 16.00 ± 2.00 diarrhoeal droppings. LPM treatment significantly reduced this to 5.67 ± 1.53 (64.58% inhibition). At 400 mg/kg, *M. esculenta* showed the highest inhibition of diarrhoea (62.50%), followed by *E. milii* (58.33%), *D. indica* (52.08%), and *M. malabathricum* (41.67%) as shown in Table 3.6.

Table 3.4 Onset of Diarrhoea in minutes

Group	Treatment	Dose (mg/Kg)	*D. indica*	*E. milii*	*M. esculenta*	*M. malabathricum*
I	Vehicle Control	0	$41.00^{d} \pm 2.65$	$41.00^{e} \pm 2.65$	$41.00^{d} \pm 2.65$	$41.00^{c} \pm 2.65$
II	LPM	3	$106.67^{a} \pm 10.41$	$106.67^{a} \pm 10.41$	$106.67^{a} \pm 10.41$	$106.67^{a} \pm 10.41$
III	Extract	100	$55.00^{cd} \pm 3.61$	$63.00^{d} \pm 2.65$	$47.67^{d} \pm 2.52$	$45.00^{c} \pm 3.00$
IV	Extract	200	$67.67^{c} \pm 3.06$	$77.33^{c} \pm 2.52$	$64.00^{c} \pm 4.00$	$58.00^{bc} \pm 2.65$
V	Extract	400	$86.00^{b} \pm 3.61$	$94.00^{b} \pm 3.61$	$79.67^{b} \pm 4.51$	$70.00^{b} \pm 5.00$

The mean value in the column followed by the same letter is not significantly different at significance level of 0.05

Table 3.5 Percentage of Inhibition of Defecation

			D. indica		***E. milii***		***M. esculenta***		***M. malabathricum***	
Group	Treatment	Dose (mg/Kg)	Total Defecation	%	Total Defecation	%	Total Defecation	%	Total Defecation	%
I	Vehicle Cntrol	0	$17.00^{a} \pm 2.00$	-	$17.00^{a} \pm 2.00$	-	$17.00^{a} \pm 2.00$	-	$17.00^{a} \pm 2.00$	-
II	LPM	3	$6.33^{d} \pm 1.53$	62.75	$6.33^{e} \pm 1.53$	62.75	$6.33^{d} \pm 1.53$	62.75	$6.33^{d} \pm 1.53$	62.75
III	Extract	100	$13.67^{b} \pm 1.53$	19.61	$11.00^{b} \pm 2.00$	35.29	$13.33^{b} \pm 1.53$	21.57	$14.67^{ab} \pm 1.53$	13.73
IV	Extract	200	$11.33^{bc} \pm 2.08$	33.33	$9.00^{c} \pm 1.00$	47.06	$10.67^{c} \pm 0.58$	37.25	$12.33^{bc} \pm 1.53$	27.45
V	Extract	400	$9.00^{cd} \pm 1.00$	47.06	$7.67^{d} \pm 1.53$	54.90	$8.33^{d} \pm 0.58$	50.98	$9.33^{cd} \pm 1.15$	45.10

The mean value in the column followed by the same letter is not significantly different at significance level of 0.05

Table 3.6 Percentage of Inhibition of Diarrhoea

			D. indica		*E. milii*		*M. esculenta*		*M. malabathricum*	
Group	Treatment	Dose (mg/Kg)	Total Diarrhoea	%	Total Diarrhoea	%	Total Diarrhoea	%	Total Diarrhoea	%
I	Vehicle Control	0	$16.00^{a} \pm 2.00$		$16.00^{a} \pm 2.00$		$16.00^{a} \pm 2.00$		$16.00^{a} \pm 2.00$	
II	LPM	3	$5.67^{d} \pm 1.53$	64.58	$5.67^{d} \pm 1.53$	64.58	$5.67^{c} \pm 1.53$	64.58	$5.67^{c} \pm 1.53$	64.58
III	Extract	100	$11.33^{b} \pm 1.53$	29.17	$10.00^{b} \pm 1.00$	37.50	$12.33^{b} \pm 1.53$	22.92	$12.33^{ab} \pm 2.52$	22.92
IV	Extract	200	$10.0^{bc} \pm 2.00$	37.50	$8.33^{b} \pm 0.58$	47.92	$9.00^{c} \pm 1.00$	43.75	$11.00^{abc} \pm 1.00$	31.25
V	Extract	400	$7.67^{cd} \pm 1.53$	52.08	$6.00^{c} \pm 1.00$	62.50	$6.67^{d} \pm 1.53$	58.33	$9.33^{bc} \pm 2.08$	41.67

The mean value in the column followed by the same letter is not significantly different at significance level of 0.05

3.4.3.2 *Pathogen-induced diarrhoea*

3.4.3.2.1 Determination of LD_{50} for *E. coli* 078:H11 in Mice

During infection, mice challenged with *E. coli* 078:H11 displayed clinical symptoms between days 2 and 5 post-inoculation. These symptoms included reduced physical activity, piloerection (ruffled fur), lethargy, ataxia, tremors, and convulsions, which ultimately culminated in mortality. In contrast, no signs of illness or mortality were observed in the control group. As illustrated in Fig. 3.9, a bacterial load of 3.6×10^9 CFU was sufficient to cause 20% death, while doses exceeding 6×10^9 CFU resulted in 100% mortality. The median lethal dose (LD_{50}) of *E. coli* 078:H11 was calculated to be approximately 4.5×10^9 CFU. Consequently, this LD_{50} concentration was employed in all subsequent experimental infections.

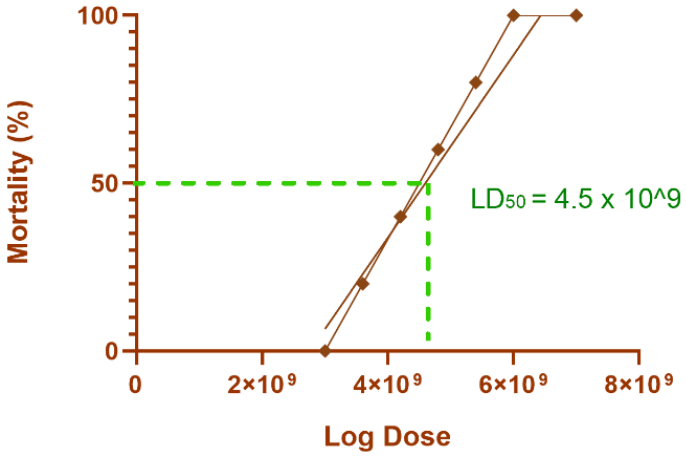


Fig. 3.10 LD_{50} of *E. coli* 078:H11

3.4.3.2.2 Diarrhoea Index

The anti-diarrhoeal efficacy of the selected plant extracts was evaluated over a 5-day treatment period by measuring the frequency of diarrhoeal feces in vehicle control and treated mice at two different doses: 100 mg/kg and 400 mg/kg body weight (b.w.). The control group exhibited persistent diarrhoeal symptoms on Days 1 through 3, with mean fecal scores decreasing slightly from 2.04 ± 0.10 on Day 1 to 1.80 ± 0.05

on Day 3 ($p < 0.05$), followed by a sharp decline to 0.02 ± 0.04 on Day 4 and complete resolution by Day 5.

Loperamide (LPM), used as the standard reference drug, showed a rapid and significant reduction in diarrhoeal feces across all time points, with values decreasing from 0.63 ± 0.04 (Day 1) to 0.21 ± 0.01 (Day 3), and complete cessation of diarrhoea (0.00 ± 0.00) by Day 4 at both doses.

Among the plant extracts, *Euphorbia milii* demonstrated the most notable antidiarrhoeal effect. At 100 mg/kg, fecal scores reduced from 1.33 ± 0.04 (Day 1) to 1.14 ± 0.04 (Day 3), and at 400 mg/kg, scores were even lower, declining from 0.94 ± 0.04 to 0.80 ± 0.03 by Day 3. *Myrica esculenta* and *Melastoma malabathricum* also showed moderate efficacy. At 400 mg/kg, *M. esculenta* reduced fecal scores from 1.14 ± 0.04 (Day 1) to 0.95 ± 0.03 (Day 3), and *M. malabathricum* from 1.23 ± 0.03 to 1.04 ± 0.03 in the same period.

Dillenia indica displayed the least effect among the extracts, particularly at 100 mg/kg, with fecal scores showing minimal reductions (1.80 ± 0.05 to 1.63 ± 0.03), though improvement was more apparent at 400 mg/kg (1.34 ± 0.04 to 1.15 ± 0.04). No diarrhoeal symptoms were recorded in any group beyond Day 3, except for the control group, which experienced symptoms on Day 4.

Table 3.7 Diarrhoea Index

Treatment	100 mg/kg b.w					400 mg/kg b.w				
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5
Vehicle Cntrol	2.04^{a}±0.10	1.90^{a}±0.04	1.80^{a}±0.05	0.02^{a}±0.04	0.0±0.0	2.04^{a}±0.10	1.90^{a}±0.04	1.80^{a}±0.05	0.02^{a}±0.04	0.0±0.0
LPM	0.63^{e}±0.04	0.45^{e}±0.03	0.21^{e}±0.01	0.00^{a}±0.00	0.0±0.0	0.63^{e}±0.04	0.45^{f}±0.03	0.21^{f}±0.01	0.00^{a}±0.00	0.0±0.0
D. indica	1.80^{b}±0.05	1.70^{b}±0.05	1.63^{b}±0.03	.00^{a}±0.00	0.0±0.0	1.34^{b}±0.04	1.25^{b}±0.03	1.15^{b}±0.04	0.00^{a}±0.00	0.0±0.0
E. milii	1.33^{d}±0.04	1.22^{d}±0.03	1.14^{d}±0.04	.00^{a}±0.00	0.0±0.0	0.94^{d}±0.04	0.85^{e}±0.05	0.80^{e}±0.03	0.00^{a}±0.00	0.0±0.0
M. esculenta	1.54^{b}±0.04	1.45^{b}±0.05	1.40^{b}±0.05	.00^{a}±0.00	0.0±0.0	1.14^{bc}±0.04	1.05^{c}±0.04	0.95^{c}±0.03	0.00^{a}±0.00	0.0±0.0
M. malabathricum	1.74^{c}±0.05	1.67^{c}±0.08	1.56^{c}±0.03	.00^{a}±0.00	0.0±0.0	1.23^{c}± 0.03	1.14^{d}±0.03	1.04^{d}±0.03	0.00^{a}±0.00	0.0±0.0

The mean value in the column followed by the same letter is not significantly different at significance level of 0.05

3.4.3.2.3 Spleen Weight Index

The spleen weight was significantly elevated in the vehicle-treated group compared to the normal control and other treatment groups. The highest spleen weight was observed in the vehicle group (approx. 0.46 %), indicating a statistically significant difference ($p < 0.05$). In contrast, treatment with plant extracts at both 100 mg/kg and 400 mg/kg doses resulted in a significant reduction in spleen weight compared to the vehicle group.

Among the plant-treated groups, *Dillenia indica, Euphorbia milii, Myrica esculenta*, and *Melastoma malabathricum* all showed a dose-dependent decrease in spleen weight. The lowest spleen weight values (approximately 0.28 – 0.30 %) were observed at the 400 mg/kg dose, particularly in *E. milii and M. esculenta*, which were significantly lower than both the control and vehicle groups (Fig. 3.9).

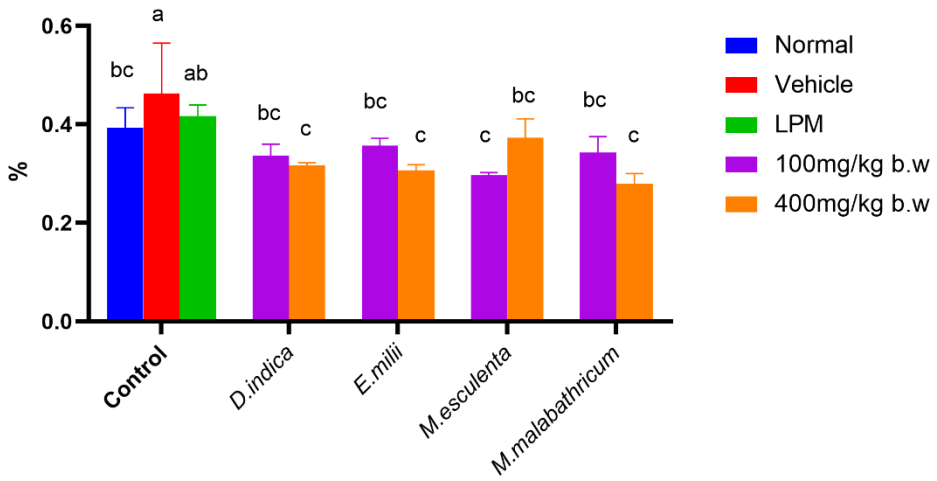


Fig. 3.11 Spleen weight

3.4.3.2.4 Thymus Weight Index

The vehicle group had a notably reduced thymus weight (approximately 0.11 mg/100 g body weight) compared to the standard control and all other treatment groups. Conversely, the Loperamide (LPM) and all plant extract-treated groups,

especially at 400 mg/kg, preserved or slightly increased thymus weight to levels similar to or higher than the normal control group (Fig. 3.10).

Thymus weight in groups treated with *D. indica, E. milii, M. esculenta,* and *M. malabathricum* at 400 mg/kg ranged between 0.21% and 0.26%, showing no signs of atrophy or immunosuppression. No significant thymic hypertrophy or toxicity was observed in any of the plant-treated groups.

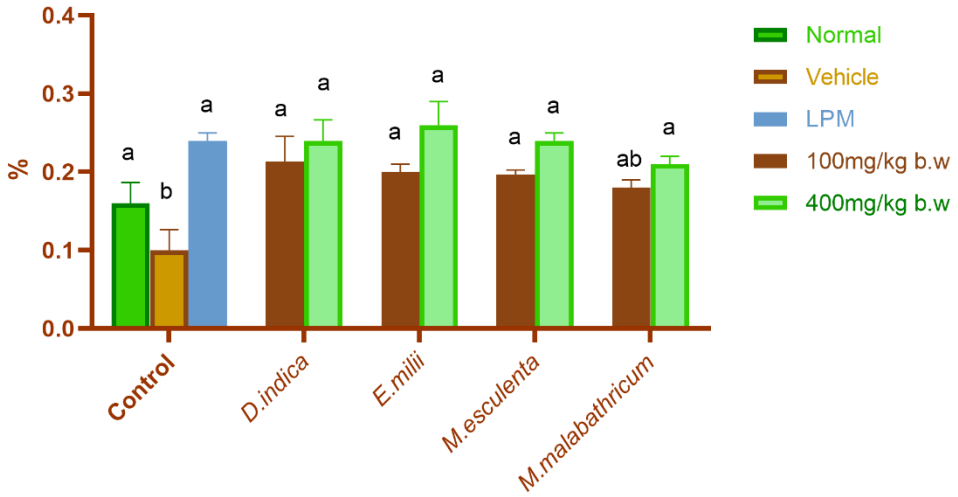


Fig. 3.12 Thymus Weight

3.4.3.2.5 White Blood Cell (WBC) Count (10^3/µL)

The vehicle-treated mice exhibited a substantial elevation in WBC count (approximately 15.7 × 10^3/µL), significantly higher than that of the normal group (approximately 5.6 × 10^3/µL), indicating a strong inflammatory or immune response to *E. coli* 078:H11 infection. Loperamide (LPM) showed moderate reduction (approx. 6.9 × 10^3/µL), but still significantly higher than the normal (Fig. 3.11). Treatment with all four plant extracts led to a significant, dose-dependent reduction in WBC count, suggesting an amelioration of inflammation. *E. milii* and *M. esculenta* at 100 mg/kg reduced WBCs to around 8.4 – 9.6 × 10^3/µL and at 400 mg/kg reduced WBCs further, approaching normal levels (approx. 7.4 – 9.5 × 10^3/µL).

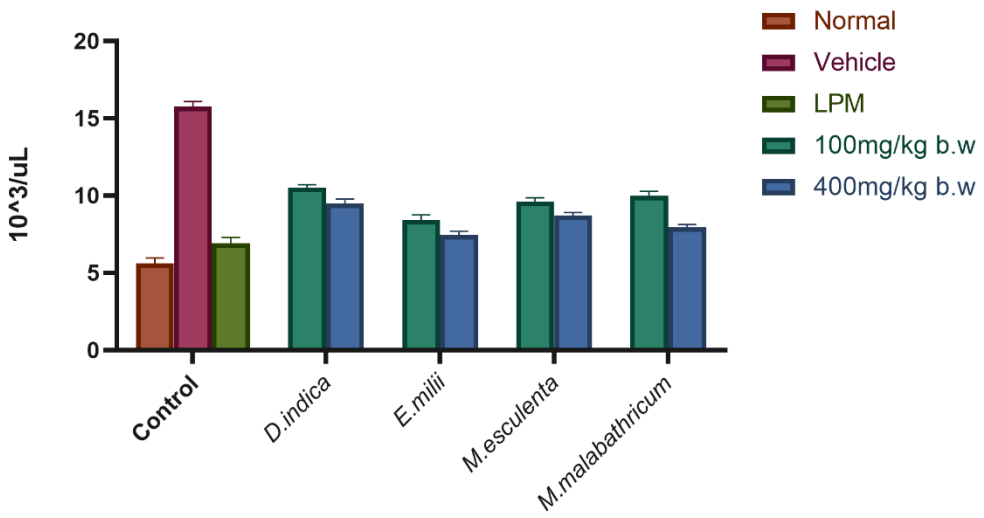


Fig. 3.13 WBC Count

3.4.3.2.6 Serum Levels of TNF-α and IL-6

The concentrations of TNF-α and IL-6 are presented in Fig. 3.12 and 3.13. Compared to the normal control group, the vehicle control group exhibited a significant increase in both cytokines, indicating an inflammatory response ($p < 0.05$). Treatment with the four plant extracts at doses of 100 and 400 mg/kg resulted in a marked reduction in TNF-α and IL-6 levels relative to the vehicle control group ($p < 0.05$).

Notably, IL-6 levels were significantly elevated in the 100 mg/kg groups of *Dillenia indica* and *Melastoma malabathricum*. In comparison, the 400 mg/kg group of *Euphorbia milii* demonstrated a significant decrease, suggesting greater efficacy at higher doses ($p < 0.05$). However, TNF-α levels in the treatment groups were all higher than those in the normal control group.

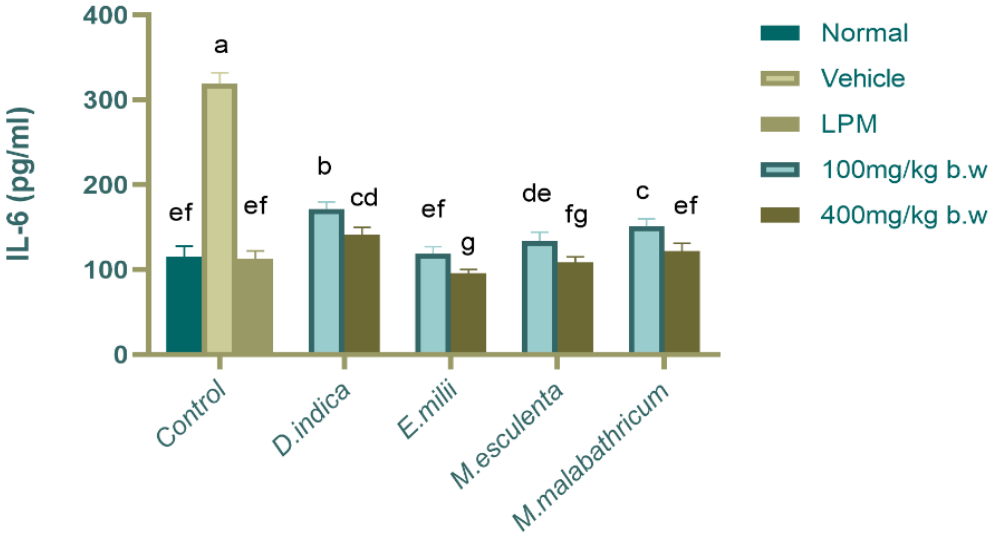


Fig. 3.14 Serum IL-6 cytokine

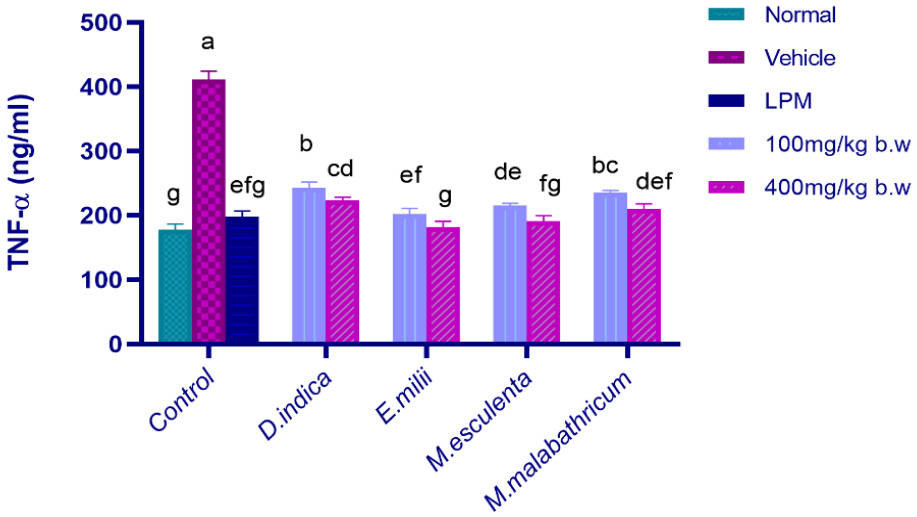


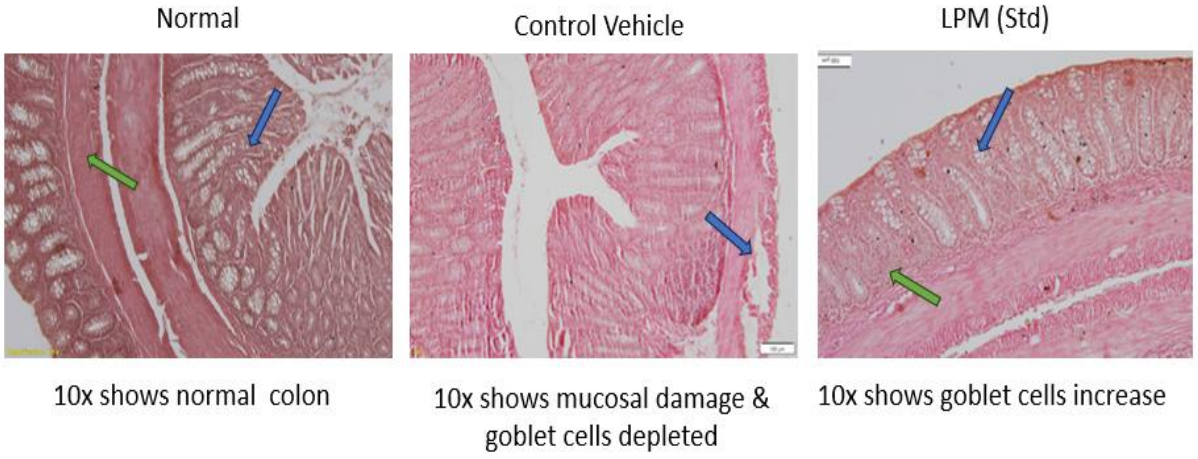
Fig. 3.15 Serum TNF-α Cytokine concentration

3.4.3.2.7 Histopathology investigation

Histological assessment of the colonic tissues is presented in Fig. 3.10, *E. coli* 078:H11-induced diarrhoea resulted in marked mucosal disruption in the vehicle control group, characterised by degradation of the mucous layer and a notable depletion of goblet cells. In contrast, mice treated with loperamide exhibited a significant restoration of mucosal integrity, as evidenced by an increase in the number of goblet cells.

Administration of plant extracts at 100mg/kg doses, particularly *Melastoma malabathricum* and *Euphorbia milii,* demonstrated protective effects on colonic architecture, with no observable pathological lesions. However, in the *Dillenia indica*-treated group, fusion of goblet cells was observed, indicating mild mucosal alteration. Mice treated with *Myrica esculenta* displayed vascular congestion and hypertrophy of goblet cells. The normal control group exhibited intact mucosal structure with some fused goblet cells, indicating near-normal histology.

Furthermore, in all plant extract-treated groups, goblet cell populations were significantly higher at 400 mg/kg compared to their respective 100 mg/kg doses, reflecting a dose-dependent enhancement in mucosal recovery and goblet cell regeneration.



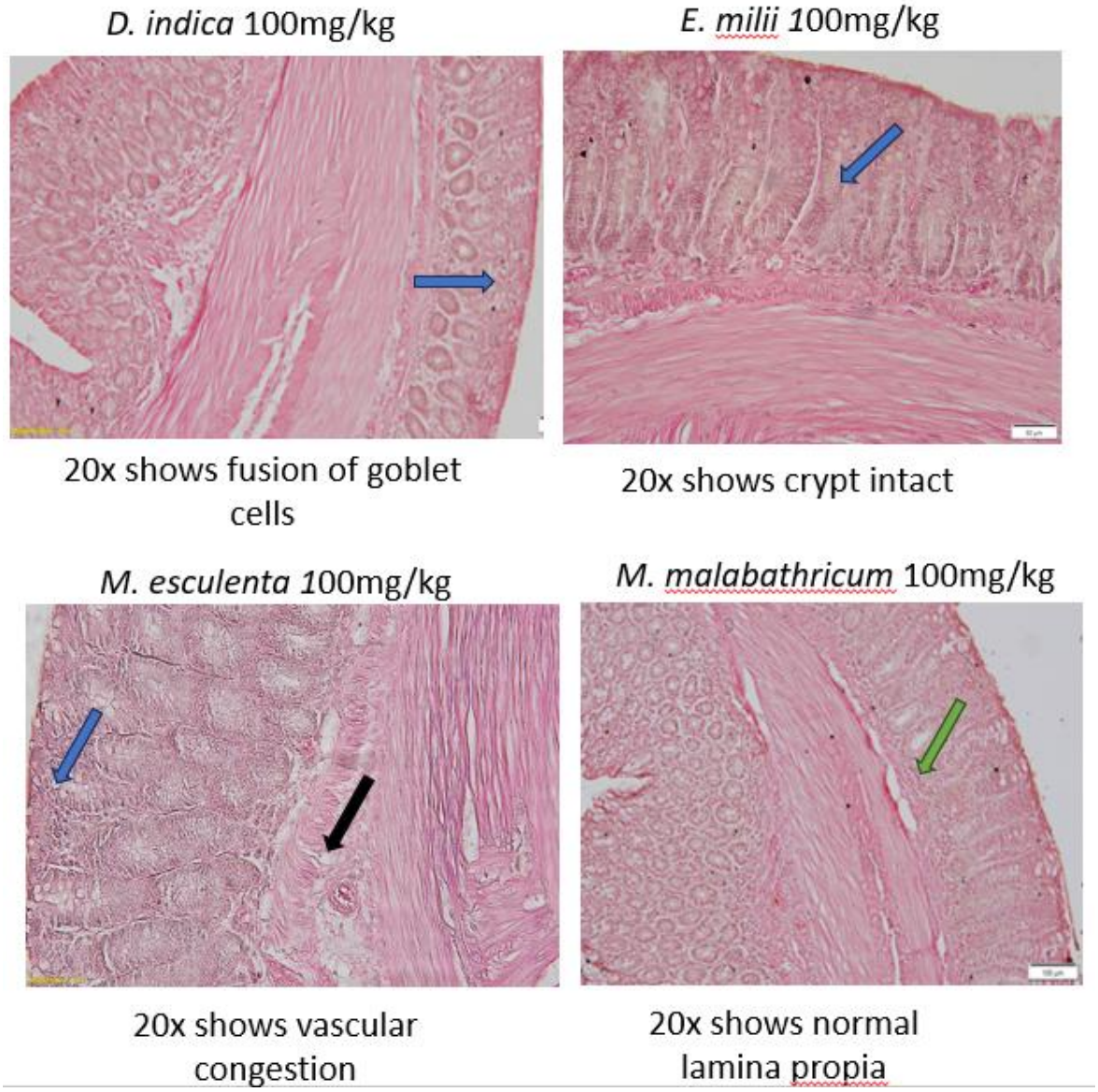


Fig. 3.16 Histology of colon for controls and treatment with four plant extracts. Blue arrow indicates goblet cells, green arrow indicates lamina propria, and black arrow indicates mucosal damage.

3.5 Discussion

For centuries, medicinal plants have served as vital therapeutic agents in traditional healthcare systems worldwide. The resurgence of interest in phytotherapy—plant-based treatment modalities—has been strongly supported by global health authorities such as the World Health Organisation (WHO), which advocates the integration of ethnopharmacological knowledge into modern medicine. Nonetheless, WHO and regulatory agencies like the Food and Drug Administration (FDA) emphasise the necessity of rigorous scientific validation to establish the safety, efficacy, and dosage standards of these natural remedies (Newman, 2020).

Herbal drugs, while deeply embedded in cultural practices, require comprehensive pharmacological and toxicological profiling before approval for clinical or public health use. Preliminary toxicological evaluations—especially acute and sub-acute toxicity studies—play a pivotal role in determining the safety margins and potential adverse effects of such botanical agents. These assessments also aid in identifying organ-specific toxicity and dose thresholds, thereby informing further pharmacodynamic investigations.

The pharmacological efficacy of medicinal plants is primarily attributed to their diverse array of bioactive compounds, which are distributed across the roots, leaves, stems, and rhizomes. Interestingly, some plant species are categorised into distinct chemotypes due to variation in their phytochemical profiles. These chemotypic differences may result in substantially different biological effects. A case in point is the presence of ferulenol—a prenylated coumarin—found in toxic chemotypes, which can trigger ferulosis, a severe hemorrhagic condition with lethal outcomes in both animals and humans (Louvet et al., 2015).

In the present study, we assessed the acute and sub-acute oral toxicity and anti-diarrhoeal efficacy of four medicinal plant extracts-*Dillenia indica, Euphorbia milii, Myrica esculenta,* and *Melastoma malabathricum*-using a Swiss-Albino mice model. Mice are widely regarded as suitable organisms for such investigations due to their genetic and physiological proximity to humans. Moreover, their accelerated lifespan enables extrapolation of chronic effects within a relatively short observational window, with one murine year equating to approximately thirty human years (Hsu et al., 2011).

3.5.1 Toxicological Evaluation

Acute toxicity testing at a single oral dose of 2,000 mg/kg revealed no observable clinical signs, behavioural abnormalities, or mortality in any treatment group. The absence of gross pathological changes in vital organs and stable body weights post-administration strongly suggests a high safety margin for these plant extracts. According to OECD guidelines for acute oral toxicity, substances with an LD_{50} exceeding 2,000 mg/kg are considered non-toxic (OECD, 2001). These findings are consistent with earlier reports highlighting the non-toxic nature of *M. esculenta* and *M. malabathricum* in traditional medicinal use (Kumar et al., 2010; Zakaria et al., 2006).

Over the 28-day sub-acute toxicity period, none of the extracts induced statistically significant changes in body weight in either sex. The progressive but non-significant weight gain observed across both treated and control groups suggests that metabolic and nutritional absorption processes remain unaltered (Parasuraman, 2011). A slight reduction in body weight observed in female mice treated with *M. esculenta* at day 14 was transient and not suggestive of any detrimental effect.

Organ-to-body weight ratios, used as indicators of systemic toxicity, exhibited sex-specific alterations in the lungs, liver, kidneys, and spleen among groups treated with *D. indica, E. milii,* and *M. esculenta*. However, these changes were not corroborated by histological anomalies and remained within physiological reference values, implying adaptive metabolic responses rather than toxicological effects (Michael et al., 2007).

Haematological parameters, including haemoglobin and WBC counts, remained unaffected in both sexes. A minor, yet statistically non-significant, increase in RBC count in males treated with *M. esculenta* at 200 mg/kg may indicate mild modulation of erythropoiesis, potentially influenced by the extract's phytochemical constituents (Mukinda & Syce, 2007). All values remained within standard reference ranges, supporting the extract's non-toxic profile.

Serum biochemical analysis revealed a mild elevation in alanine transaminase (ALT) levels in male mice treated with higher doses of *E. milii* and *M. esculenta*. As ALT is a sensitive biomarker of hepatocellular integrity, this may suggest minimal,

reversible hepatic stress. However, as aspartate transaminase (AST) levels remained unaltered and histological analysis showed no hepatocellular necrosis or inflammation, the clinical relevance of these ALT elevations appears negligible (Giannini et al., 2005). Slight elevations in bilirubin levels in *M. esculenta*- and *M. malabathricum*-treated males may point to transient alterations in hepatic clearance or cholestasis.

Creatinine levels showed a minor rise, particularly in groups treated with *D. indica, M. esculenta*, and *M. malabathricum*. As creatinine is a renal biomarker linked to glomerular filtration efficiency, such elevations may represent early adaptive renal responses rather than overt nephrotoxicity. Importantly, all values remained within acceptable physiological limits (Bessey et al., 2015).

Histopathological examinations of the liver, kidneys, lungs, and spleen in both male and female mice revealed preserved structural integrity, with no evidence of necrosis, inflammation, or degeneration. These findings reinforce the biochemical and behavioural observations, supporting the overall non-toxic profile of all four plant extracts at the tested doses.

3.5.2 Anti-diarrhoeal Efficacy against Castor Oil-induced Diarrhoea

All four plant extracts exhibited significant, dose-dependent anti-diarrhoeal effects. At 400 mg/kg, the delay in diarrhoeal onset, reduction in frequency of defecation, and decrease in stool water content were significant and comparable to loperamide, a standard reference drug known for its opioid receptor agonist activity and suppression of intestinal motility and secretion (Awouters et al., 1978).

Among the tested plants, *M. esculenta* showed the highest efficacy, reducing diarrhoeal output by 62.5%. This finding corroborates previous studies that have cited the presence of tannins and flavonoids in *Myrica* species, which are associated with astringent and antispasmodic activities (Parveen et al., 2014). *E. milii* and *D. indica* also produced notable effects, likely attributed to their phenolic and saponin content, which can modulate prostaglandin synthesis and inhibit gastrointestinal motility (Sofowora, 1993; Palombo, 2006).

Although *M. malabathricum* exhibited relatively modest anti-diarrhoeal activity compared to the others, it still demonstrated statistically significant effects compared to the controls. Its known anti-inflammatory and antioxidant constituents

may have contributed to ameliorating prostaglandin-mediated hypersecretion and motility (Zakaria et al., 2006).

The use of castor oil-induced diarrhoea as a pharmacological model is well established, owing to the release of ricinoleic acid in the intestine, which induces prostaglandin synthesis, stimulates fluid secretion, and enhances motility (Ammon et al., 1974; Capasso et al., 1994). The plant extracts significantly counteracted these effects, reducing intestinal fluid secretion and promoting reabsorption, thereby normalising stool consistency and frequency. These actions mirror those of loperamide and may be due to anticholinergic or prostaglandin-inhibitory mechanisms (Goodman & Gilman, 2011).

3.5.3 Antidiarrhoeal Efficacy Against *E. coli* O78:H11-Induced Diarrhoea

The efficacy of these extracts was further evaluated in a more clinically relevant study using an enterotoxigenic bacteria, *E. coli* O78:H11-induced diarrhoea model. The untreated control group exhibited persistent diarrhoea, while loperamide significantly reduced faecal output and achieved complete remission by Day 4. Among the plant extracts, *E. milii* at 400 mg/kg demonstrated the most pronounced reduction in faecal score from Day 1 through Day 3. *M. esculenta* and *M. malabathricum* also showed substantial improvements. *D. indica,* though less effective at lower doses, contributed to symptom resolution by Day 5, indicating potential anti-diarrhoeal properties.

Spleen and thymus weights were assessed to monitor immune response and potential immunotoxicity. The infection-induced splenic hypertrophy observed in the vehicle group was significantly mitigated by *E. milii* and *M. esculenta*, indicating an anti-inflammatory effect. Thymus atrophy, a hallmark of infection-induced immunosuppression, was also reversed in all extract-treated groups, particularly at a dose of 400 mg/kg, implying an immunomodulatory potential (Yin et al., 2014).

E. coli O78:H11 infection significantly increased WBC counts, indicative of a systemic inflammatory response. Treatment with *E. milii* and *M. malabathricum* effectively normalised WBC levels, suggesting that the extracts exert anti-inflammatory or cytokine-modulating effects (Khayyal et al., 2001).

The inflammatory nature of *E. coli*-induced diarrhoea resembles that of inflammatory bowel diseases (IBD), wherein pro-inflammatory cytokines play pivotal roles in disease progression. Among these, interleukins (particularly IL-6) and tumour necrosis factor-alpha (TNF-α) have been extensively implicated in mediating mucosal inflammation and systemic immune responses. IL-6 is primarily produced by monocytes and activated T cells, and it contributes to the pathogenesis of diarrhoea by increasing vascular permeability, promoting leukocyte activation, and initiating acute-phase protein synthesis (Yu et al., 2017). Similarly, TNF-α, secreted by monocyte-macrophages and natural killer (NK) cells, serves as a key pro-inflammatory cytokine that modulates immune responses and amplifies the expression of other inflammatory mediators (Bradley, 2008).

Under physiological conditions, TNF-α is present at minimal levels; however, during acute infections, such as *E. coli*-induced enteritis, its production escalates, resulting in disruption of cytokine homeostasis and subsequent tissue damage (Van der Meer et al., 1995). In the current study, a significant elevation in IL-6 and TNF-α levels was observed in the vehicle control group following *E. coli* infection, confirming the onset of an inflammatory response. These findings are in line with earlier studies that reported increased serum concentrations of IL-6 and TNF-α in the acute stages of infectious diarrhoea, which correlated positively with disease severity.

Significantly, treatment with the tested plant extracts, particularly at higher doses (400 mg/kg), markedly reduced the concentrations of these cytokines. This downregulation of IL-6 and TNF-α suggests that the extracts exert anti-inflammatory effects, potentially contributing to mucosal healing and immune modulation. Notably, *Euphorbia milii* at 400 mg/kg significantly suppressed IL-6 levels below the baseline, indicating a dose-dependent immunomodulatory activity.

3.5.4 Histological Insights

Histopathological examination of colon sections provided insights into mucosal healing. On Day 1 post-infection, vehicle-treated mice exhibited severe mucosal damage, goblet cell depletion, and disrupted epithelial architecture. In contrast, treatment with *M. malabathricum* and *E. milii* preserved mucosal integrity

and supported goblet cell regeneration. *D. indica* induced mild goblet cell fusion, while *M. esculenta* caused modest congestion and hypertrophy.

By Day 5, substantial mucosal recovery was evident in all treated groups, particularly at 400 mg/kg doses. Increased goblet cell numbers indicate restored mucosal barrier function, which is essential for defence against pathogens and for maintaining intestinal homeostasis. These observations confirm the protective and reparative roles of the plant extracts.

The pathogenicity of *E. coli* in mice models is primarily mediated through its ability to colonise the intestinal epithelium, disrupt epithelial integrity, and induce toxin-mediated secretory and inflammatory responses. Upon infection, enterotoxigenic *E. coli* strains adhere to the intestinal mucosa using adhesins such as fimbriae or intimin, followed by the release of enterotoxins. Heat-labile (LT) and heat-stable (ST) toxins elevate intracellular cyclic AMP and GMP levels, respectively, resulting in excessive chloride secretion via CFTR channels and inhibition of sodium absorption, which collectively drive fluid efflux into the intestinal lumen (Nataro & Kaper, 1998).

Moreover, Shiga-like toxins from EHEC inhibit host protein synthesis by targeting the 60S ribosomal subunit, leading to epithelial cell apoptosis and mucosal erosion (Croxen & Finlay, 2010). EPEC strains also disrupt tight junction proteins such as occludin and ZO-1, compromising epithelial barrier function and increasing intestinal permeability. This breach facilitates the translocation of microbial components and subsequent immune activation. Pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α are elevated, contributing to mucosal inflammation, immune cell infiltration, and enhanced gastrointestinal motility (Chen & Frankel, 2005).

These pathophysiological processes-altered ion transport, inflammation, and increased motility-synergistically reduce water reabsorption and transit time, culminating in profuse watery or hemorrhagic diarrhoea. The observed histological features in the colon of infected mice, including goblet cell depletion, mucosal erosion, and lymphoid infiltration, are consistent with previously reported models of *E. coli*-induced intestinal pathology (Alenezi et al., 2020).

3.5.5 Conclusions

Collectively, the results of this study demonstrated that the oral administration of *D. indica, E. milii, M. esculenta* and *M. malabathricum* at a single dose at 2000mg/kg b.w and doses up to 600 mg/kg is safe over 28 days, with no significant systemic toxicity. Stable physiological, haematological, biochemical, and histological parameters support these findings. Additionally, the extracts exhibited substantial anti-diarrhoeal effects in both castor oil-induced and *E. coli* O78:H11-induced models.

Among the tested plants, *E. milii* emerged as the most promising candidate, exhibiting strong anti-inflammatory, immunomodulatory, and anti-diarrhoeal properties comparable to those of the standard drug loperamide. These findings validate the traditional use of these plants in managing gastrointestinal disturbances and support their potential for future therapeutic development. Further studies are warranted to isolate and characterise the active constituents and to elucidate their underlying mechanisms of action.

CHAPTER – 4

"LC-MS-based phytochemical characterisation and *in silico* insights into novel dual-target antidiarrhoeal agents"

4.1 Introduction

Ethnomedicinal plants have long served as a cornerstone in the traditional treatment of various ailments across cultures and civilisations. Their therapeutic applications are deeply embedded in indigenous knowledge systems, particularly in regions with rich biodiversity and limited access to conventional pharmaceuticals (Singh et al., 2021). Despite their widespread use, the pharmacological mechanisms underpinning their efficacy remain poorly understood and often unvalidated by scientific inquiry. With the increasing demand for novel bioactive agents to combat emerging health threats, particularly antimicrobial resistance and chronic inflammatory diseases, there is an urgent need to rationalise and modernise the drug discovery pipeline based on ethnopharmacological resources (Dastagir et al., 2022).

The application of in silico techniques such as molecular docking offers a powerful and cost-effective strategy for evaluating the bioactivity of plant-derived compounds. Molecular docking facilitates the prediction of binding affinities between phytochemicals and biological targets, enabling the identification of potential lead compounds with desirable pharmacological profiles (Daina et al., 2017). This approach not only allows the virtual screening of large compound libraries extracted from ethnomedicinal plants but also provides structural insights into ligand–receptor interactions at the atomic level (Ferreira et al., 2015).

Integrating molecular docking with ethnomedicinal knowledge not only validates traditional claims but also accelerates the early stages of drug development by minimising the need for exhaustive in vitro and in vivo experimentation. Furthermore, these tools support structure–activity relationship (SAR) analysis, absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiling, and target-based optimisation-all essential components for translating phytochemicals into clinically viable therapeutic agents (Lipinski, 2004). Given the increasing global interest in evidence-based traditional medicine, molecular docking provides a scientifically robust framework for investigating the pharmacological relevance of medicinal plants (Sliwoski et al., 2014).

In the context of diarrhoeal disorders, which remain a leading cause of morbidity and mortality in developing regions, identifying plant-derived modulators of pro-inflammatory signalling pathways offers a promising therapeutic avenue. Prostaglandin E2 (PGE2), acting via its EP2 receptor, is a well-documented mediator of secretory diarrhoea and intestinal inflammation. It induces fluid secretion, disrupts epithelial ion transport, and enhances gut motility, thereby contributing to the pathogenesis of diarrhoea. Targeting the EP2 receptor with plant-derived ligands thus holds significant therapeutic promise for managing diarrhoeal diseases (Mekonnen et al., 2018).

To explore this, the four plants-*Dillenia indica* L., *Euphorbia milii* Des Moul., *Myrica esculenta* Buch.-Ham. Extracts of D. Don and *Melastoma malabathricum* L. in methanol were further analysed for their phytochemical compounds and used as ligands for molecular docking analysis.

The chemical composition of their extracts was comprehensively profiled using liquid chromatography-mass spectrometry (LC-MS), a highly sensitive and robust analytical platform for the qualitative and quantitative assessment of plant metabolites. LC-MS combines the separation efficiency of liquid chromatography with the precise mass detection capabilities of mass spectrometry, enabling the identification of compounds even at trace concentrations. This technique enables the simultaneous detection of a wide range of phytochemical classes, including flavonoids, alkaloids, phenolic acids, terpenoids, and glycosides, without the need for prior derivatisation (Wolfender et al., 2015). By analysing the extracts in both positive and negative electrospray ionisation (ESI) modes, a more comprehensive metabolite profile is achieved, capturing both polar and non-polar constituents. The resulting data not only provides chemical fingerprints for each plant species but also facilitates the correlation between specific metabolites and their predicted biological activities.

The LC-MS-identified phytoconstituents were subsequently employed as ligands for molecular docking studies targeting the EP2 and opioid protein receptors responsible for causing diarrhoea. This integrated approach-linking metabolomic profiling with molecular docking-serves to validate and characterise the antidiarrhoeal potential of these plants at the molecular level. Docking analysis enables the identification of lead compounds with high binding affinity and favourable interaction

profiles, which can be further optimised through SAR-guided modifications. These findings may pave the way for the rational development of plant-based therapeutic agents for diarrhoea, offering an evidence-based bridge between traditional knowledge and modern drug discovery (Wu et al., 2012).

4.2 Review of Literature

Ahmad et al. (2023) evaluated the anti-diarrhoeal potential of *Viola canescens* through both in vivo and in silico methods. Using castor oil-induced and charcoal meal assays in mice, they demonstrated a dose-dependent anti-diarrhoeal effect, with the ethyl acetate fraction and crude alkaloids showing the most potent inhibition of defecation. Molecular docking identified emetine, quercetin, and violanthin as active compounds with high binding affinity to μ- and δ-opioid receptors, suggesting these phytoconstituents as the mechanistic basis of its activity. The study validates the traditional use of *V. canescens* for gastrointestinal disorders and provides a foundation for future phytopharmacological exploration.

In another study, Fahad et al. (2025) investigated the anti-diarrhoeal effects of ethanolic stem extract of *Operculina turpethum using castor oil-induced diarrhoea* in mice. The extract significantly reduced faecal output and increased the latency of diarrhoeal onset at 250 and 500 mg/kg. Complementary molecular docking revealed that compounds such as β-sitosterol-β-D-glucoside and dihydro-α-spinasterol glucoside exhibited strong interactions with the M3 muscarinic acetylcholine receptors, comparable to those of standard drugs. The study supports *O. turpethum* as a source of novel anti-diarrhoeal agents and demonstrates the utility of combined pharmacological and computational approaches.

Saptarini et al. (2024) explored the anti-diarrhoeal effects of *Carica papaya* leaf infusion through in vivo assays in mice and molecular docking against the M3 muscarinic receptor. The infusion significantly delayed the onset of diarrhoea, reduced total faecal count, and increased inhibition percentages in a dose-dependent manner. Among the screened phytochemicals, quercetin 3-rutinoside exhibited a notable binding affinity and favourable ADMET properties. These findings underscore the therapeutic potential of papaya leaves and support their traditional use in treating diarrhoeal conditions.

In a study, Mohawk and O'Brien (2011) provided a comprehensive review of mouse models used to study *Escherichia coli* O157:H7 and its major virulence factor, Shiga toxin. They described how these models replicate human disease symptoms such as hemorrhagic colitis and hemolytic uremic syndrome. Key virulence mechanisms

include the type III secretion system, intimin-Tir interaction, and Shiga toxin-mediated inhibition of protein synthesis. The review highlighted the utility of these models for understanding pathogen-host interactions and developing therapeutics against STEC infections.

Shi et al. (2025) investigated the anti-diarrhoeal mechanism of Kelisha Capsule (KLSC), a traditional Chinese medicine, using an *E. coli*-infected mouse model. KLSC improved gut barrier integrity, reduced pro-inflammatory cytokines (IL-6 and TNF-α), and restored microbial diversity. Molecular docking and dynamic simulations supported the involvement of bioactive compounds, such as quercetin, eugenol, and piperine, in modulating inflammatory pathways and disrupting bacterial membranes. The study affirms KLSC's potential as an effective herbal formulation for treating bacterial diarrhoea.

Similarly, SarveAhrabi and Khoei (2024) explored both in vitro and in silico antibacterial activities of newly synthesized 1,3,4-oxadiazole compounds against *Escherichia coli* O157:H7. Among the tested compounds, compound C-bearing a difluorophenyl structure-exhibited the highest antibacterial activity, with a notable inhibition zone and low MIC and MBC values. Docking studies further revealed strong binding affinities of this compound with the stx1 and stx2 toxins, critical virulence factors of EHEC. The compound formed stable hydrogen and hydrophobic interactions with active site residues of these toxins, suggesting its potential as a therapeutic candidate targeting Shiga toxin-producing *E. coli* (STEC). This study underscores the importance of molecular docking for screening novel anti-*E. coli* agents and validates oxadiazole derivatives as promising antimicrobial candidates.

Chen et al. (2007) investigated the anti-diarrhoeal effect of *Chaenomeles speciosa* (fruit extract) against heat-labile enterotoxin (LT)-induced diarrhoea caused by enterotoxigenic *E. coli* (ETEC). Using both in vivo patent gut assays and GM1-ELISA in vitro methods, the study demonstrated that oleanolic acid, ursolic acid, and betulinic acid-components from the ethyl acetate fraction of the fruit-effectively inhibited the binding of the LTB subunit to the GM1 receptor. Molecular docking revealed strong interactions via hydrogen bonding and hydrophobic forces between these bioactives and LTB. This suggests the mechanism of action lies in blocking the initial toxin-receptor interaction, preventing downstream diarrhoeal effects. These

findings highlight the therapeutic potential of C. speciosa constituents in managing ETEC-induced diarrhoea.

In a separate study, Abishad et al. (2021) employed an integrative approach combining in silico molecular docking and laboratory-based antimicrobial efficacy testing. Molecular docking was carried out using the AutoDock platform targeting the dispersin protein (aap, PDB ID: 2jvu) in EAEC and the outer membrane osmoporin (OmpC, PDB ID: 3uu2) in NTS. The results revealed that these phytochemicals displayed strong binding affinities to the protein motifs, suggesting potential mechanisms through which these compounds might exert antimicrobial effects. Complementing the docking data, in vitro assays confirmed the antimicrobial efficacy of the compounds, as reflected by their minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) against multidrug-resistant strains of EAEC and NTS.

Lastly, the study conducted by Ralte et al. (2022) revealed that *extracts from the edible parts of Parkia timoriana* possess potent antioxidant activity, as demonstrated by DPPH, ABTS, and phosphomolybdate assays. It also showed antibacterial effects against *Bacillus pumilus, Bacillus subtilis, Escherichia coli*, and *Pseudomonas aeruginosa*. FTIR and GC-MS analyses identified 49 bioactive compounds, including phenols, amines, and esters, linked to antimicrobial, anti-inflammatory, and anticancer properties. In silico docking further supported the therapeutic potential of these compounds, making this the first detailed report on the pharmacological significance of *P. timoriana.*

4.3 Methodology

4.3.1 *Liquid Chromatography Mass Spectrometry (LC-MS) Analysis*

LC-MS analysis was performed using a Thermo Scientific Vanquish UHPLC system coupled to a Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, USA). Chromatographic separation was achieved on a Hypersil GOLD™ C18 column (100 mm × 2.1 mm, 1.9 µm particle size) maintained at 40°C. The mobile phases consisted of water containing 0.1% formic acid (Solvent A) and acetonitrile with 0.1% formic acid (Solvent B). The gradient program was initiated at 5% B (0–2 min), ramped to 95% B (2–20 min), held at 95% B (20–25 min), followed by re-equilibration to 5% B (25–30 min). The flow rate was set at 0.3 mL/min with an injection volume of 10 µL.

Mass spectrometric detection was performed in both positive and negative ion modes using electrospray ionisation (ESI). Key parameters included a spray voltage of +3.5 kV / −2.5 kV, capillary temperature of 320°C, sheath gas and auxiliary gas flow rates of 35 and 10 arbitrary units, respectively, and an S-lens RF level of 50. Full MS scans were acquired over an m/z range of 100–750 at a resolving power of 70,000 (at m/z 200). Data-dependent MS/MS (dd-MS^2) was performed on the top 10 precursor ions with dynamic exclusion enabled.

Data processing was conducted using Thermo Xcalibur™ software. Compound identification was based on accurate mass measurements (mass error < 5 ppm), isotopic distribution, MS/MS fragmentation patterns, and database comparisons with public repositories including METLIN, MassBank, and mzCloud.

4.3.2 *In silico study of characterized molecules*

4.3.2.1 Protein and ligand structure preparation

The molecular structure of characterized bioactive compounds from LC-MS was downloaded from PubChem database to investigate their possible impact as inhibitors of prostaglandin EP2 (PDB ID 3wfh) and Opoid protein (PDB ID 5c1m) used as receptor proteins. 3D structures of ligand molecules were downloaded in sdf format and again converted into pdbqt file format using open babel tool on Linux

version 16.04. 3D structure of prostaglandin E2 and Opoid protein was downloaded from pdb database (https://www.rcsb.org) and the protein structure was pre-processed by removal of water molecules and addition of polar hydrogen atoms, the charges of protein were calculated using Autodock 4.2 tools.

4.3.2.2 Molecular docking of ligand molecules against E2 and opioid protein receptor

Molecular docking was carried out using Autodock vina software. The docking of bioactive compounds from the selected plants was performed on a defined grid (active site) of receptor protein. For the docking, x, y and z coordinates were depicted as 11.092, -12.955 and 37.857 for E2 receptor and x, y and z coordinates of opioid protein was depicted as 2.026, 15.919, and, whereas, 40 × 40 × 40 grid dimension of x, y and z were taken. The docking of ligands was validated in terms of binding score (Gibbs free energy-ΔG), a lesser value of ΔG indicates better binding of ligands with the receptor protein. Then the protein-ligand complexes were used for molecular interaction analysis, which was carried out using structure visualization tools i.e. Pymol and Chimera. Later, hydrophobic interactions were analyzed with LigPlus software.

4.4 Results

4.4.1 *Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis*

The LC-MS analysis of *Dillenia indica* L. plant methanolic extracts was performed using both positive and negative electrospray ionisation (ESI) modes to achieve comprehensive profiling of its secondary metabolites. A total of 49 compounds were identified: 32 in positive mode and 17 in negative mode. Flavonoids and alkaloids were mainly observed in positive mode, whereas phenolic acids were predominantly detected in negative mode.

The base peak in the positive mode (m/z 954.9062 at RT 26.65 mins) was tentatively identified as Xylobiose, followed by Peonidin-3,5-O-di-beta-glucopyranoside (m/z 619.5624 at RT 21.13). In contrast, in negative mode, the dominant ion (m/z 455.5143 at RT 23.15 mins) corresponded to Riboflavin-5′-monophosphate sodium salt hydrate (Table 1a &b).

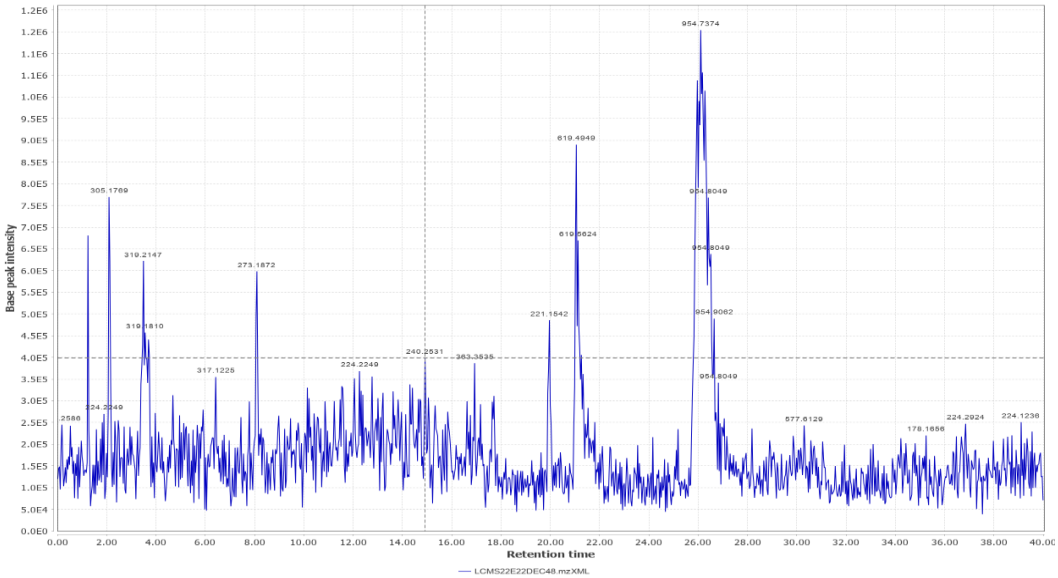


Fig. 4.1a Positive ion chromatogram of *D. indica* L.

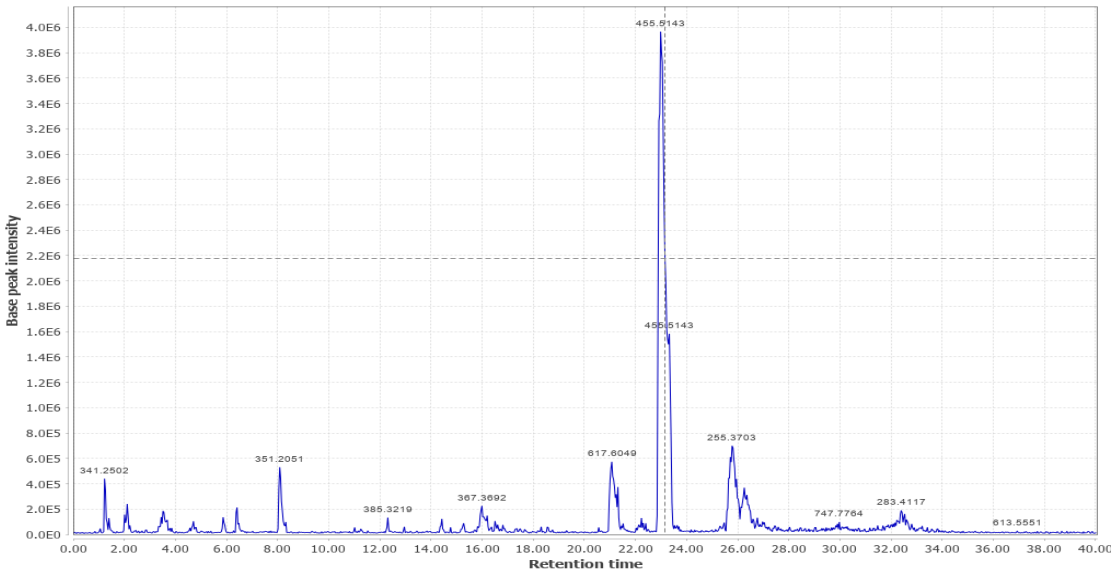


Fig. 4.1b Negative ion chromatogram of *D. indica* L.

Table 4.1a Positive ion compound identified from *D. indica* L

S.No	RT	Compound Name	Formula
1	0.19	Methyl Jasmonate	$C_{13}H_{20}O_3$
2	0.87	L-Carnosine	$C_9H_{14}N_4O_3$
3	0.97	Flavanone	$C_{15}H_{12}O_2$
4	1.24	(-)-Nicotine	$C_{10}H_{14}N_2$
5	2.10	Quercetin	$C_{15}H_{10}O_7$
6	2.34	DL-Dihydrozeatin	$C_{10}H_{15}N_5O$
7	2.95	cis-Nerolidol	$C_{15}H_{26}O$
8	3.50	zearalenone	$C_{18}H_{22}O_5$
9	3.56	Petunidin	$C_{16}H_{13}O_7$
10	5.13	Farnesol (mixture of isomers)	$C_{15}H_{26}O$
11	8.10	Naringenin	$C_{15}H_{12}O_5$
12	11.24	Cystathionine	$C_7H_{14}N_2O_4S$
13	14.92	L-Cystine	$C_6H_{12}N_2O_4S_2$
14	15.84	3-Hydroxy-DL-kynurenine	$C_{10}H_{12}N_2O_4$
15	16.93	Guanosine-5'-monophosphate	$C_{10}H_{14}N_5O_8P$
16	17.17	Isosakuranetin	$C_{16}H_{14}O_5$
17	17.72	Sinapic acid	$C_{11}H_{12}O_5$
18	19.97	DL-Dihydrozeatin	$C_{10}H_{15}N_5O$
19	20.41	4-Methoxycinnamic acid	$C_{10}H_{10}O3$
20	21.06	Folic acid	$C_{19}H_{19}N_7O_6$
21	21.13	Peonidin-3,5-O-di-beta-glucopyranoside	$C_{28}H33O16$
22	21.26	Vitexin	$C_{21}H20O10$
23	21.53	Kaempferol-3-Glucoside-3"-Rhamnoside	$C_{27}H30O15$
24	21.81	Scoulerin	$C_{19}H_{21}NO_4$

25	23.55	Fusaric acid	$C_{10}H_{13}NO_2$
26	26.65	Xylobiose	$C_{10}H_{18}O_9$
27	26.82	2-Deoxyribose-5-phosphate sodium salt	$C_5H_{11}O_7P$
28	27.06	Zeaxanthin	$C_{40}H_{56}O_2$
29	28.18	4-Hydroxy-3-methoxycinnamaldehyde	$C_{10}H_{10}O_3$
30	29.86	Procyanidin B1	$C_{30}H_{26}O_{12}$
31	30.30	Rhoifolin	$C_{27}H_{30}O_{14}$
32	32.25	D-(+)-Glucosamine hydrochloride	$C_6H_{13}NO_5$

Table 4.1b Negative ion compound identified from *D. indica* L.

S.No	RT	Compound Name	Formula
1	1.23	Galactinol Dihydrate	$C_{12}H_{22}O_{11}$
2	2.01	L-saccharopine	$C_{11}H_{20}N_2O_6$
3	2.11	1-Myristoyl-2-Hydroxy-sn-Glycero-3-Phosphate (Sodium Salt) Sodium Salt	$C_{17}H_{35}O_7P$
4	3.44	Thymidine-5'-diphosphate sodium salt	$C_{10}H_{16}N_2O_{11}P_2$
5	5.87	Xanthosine-5′-monophosphate disodium salt	$C_{10}H_{13}N_4O_9P$
6	6.41	Sodium Deoxycholate	$C_{24}H_{40}O_4$
7	8.08	Chlorogenic acid Hemihydrate	$C_{16}H_{18}O_9$
8	8.32	Prostaglandin E1	$C_{20}H_{34}O_5$
9	12.31	1-O-b-D-glucopyranosyl sinapate	$C_{17}H_{22}O_{10}$
10	15.99	Lignoceric Acid	$C_{24}H_{48}O_2$
11	21.07	isorhamnetin-3-rutinoside	$C_{28}H_{32}O_{16}$
12	22.23	D-Glucosamine-6-phosphate sodium salt	$C_6H_{14}NO_8P$
13	23.15	Riboflavin-5′-monophosphate sodium salt hydrate	$C_{17}H_{21}N_{4O9}P$
14	25.71	2'-Deoxyinosine	$C_{10}H_{12}N_4O_4$
15	25.78	D-Glucosamine-6-phosphate sodium salt	$C_6H_{14}NO_8P$
16	26.26	Acacetin	$C_{16}H_{12}O_5$
17	26.36	Xanthosine	$C_{10}H_{12}N_4O_6$

In case of *Euphorbia milii* methanolic plant extract, a total of 49 compounds were identified, of which 32 were detected in the positive mode and 17 in the negative mode.

In the positive mode, the base peak was recorded at m/z 183.1258 with a retention time (RT) of 7.11 minutes, tentatively identified as Luteolin. This was followed by 3-Indolylacetonitrile, detected at m/z 158.1900 and RT 1.28 minutes (Table 2a). Conversely, the most intense peak in the negative mode chromatogram was observed at m/z 431.2841 and RT 8.35 minutes, corresponding to Apigenin-7-O-glucoside, followed by D-Glucosamine-6-phosphate sodium salt at m/z 255.3703 and RT 26.09 minutes and Quercetin-3-Rhamnoside (m/z 447.2463 at RT 7.30 minutes) as shown in Table 2a.

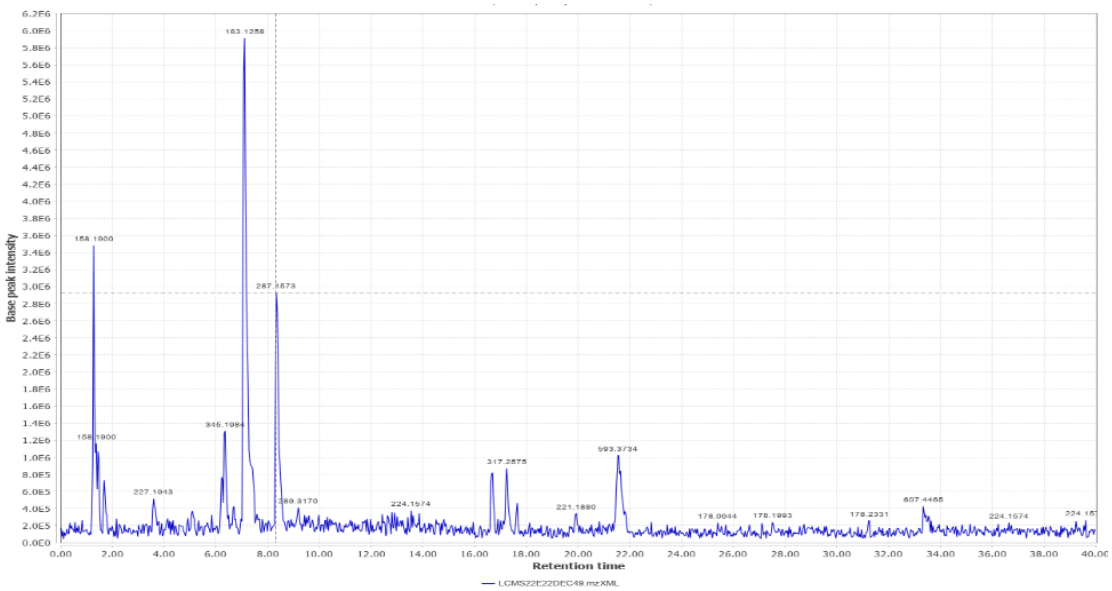


Fig. 4.2 a Positive ion chromatogram of *E. milii*

Table 4.2a. Positive ion compound identified from *E. milii*

S.No	RT	Compound Name	Formula
1	1.28	3-Indolylacetonitrile	$C_{10}H_8N_2$
2	1.38	L-Histidine	$C_6H_9N_3O_2$
3	1.69	Benzocaine solution	$C_9H_{11}NO_2$
4	3.60	L-Carnosine	$C_9H_{14}N_4O_3$
5	5.10	3-Hydroxy-DL-kynurenine	$C_{10}H_{12}N_2O_4$
6	6.22	N1-Acetylspermine Trihydrochloride	$C_{12}H_{28}N_4O$
7	6.33	Guanosine-3',5'-cyclic monophosphate	$C_{10}H_{12}N_5O_7P$
8	7.08	O-Phosphocholine	$C_5H_{15}NO_4P$
9	7.11	Luteolin	$C_5H_{11}NO_4S$
10	7.42	D-erythro-Dihydrosphingosine	$C_{18}H_{39}NO_2$
11	8.34	L-Methionine sulfone	$C_{15}H_{10}O_6$
12	8.37	Isosakuranetin	$C_{16}H_{14}O_5$
13	9.19	Argininosuccinic acid disodium salt	$C_{10}H_{18}N_4O_6$
14	9.97	Rhamnetin	$C_{16}H_{12}O_7$
15	12.43	Methyl Jasmonate	$C_{13}H_{20}O_3$
16	13.86	Cystathionine	$C_7H_{14}N_2O_4S$
17	16.66	Safranine	$C_{20}H_{19}N_4$
18	17.24	Petunidin	$C_{16}H_{13}O_7$
19	17.65	Lipoic acid, reduced	$C_8H_{16}O_2S_2$
20	19.93	DL-Dihydrozeatin	$C_{10}H_{15}N_5O$
21	21.53	Tiliroside	$C_{30}H_{26}O_{13}$
22	21.57	Keracyanin Chloride	$C_{27}H_{31}O_{15}$
23	21.64	Fortunellin	$C_{28}H_{32}O_{14}$
24	21.81	Saponarin	$C_{27}H_{30}O_{15}$
25	33.33	Neodiosmin	$C_{28}H_{32}O_{15}$
26	33.54	Diosmin	$C_{28}H_{32}O_{15}$
27	33.64	Uridine-5'-diphospho-N-acetylgalactosamine disodium salt	$C_{17}H_{27}N_3O_{17}P_2$
28	36.23	Methyl Jasmonate	$C_{13}H_{20}O_3$

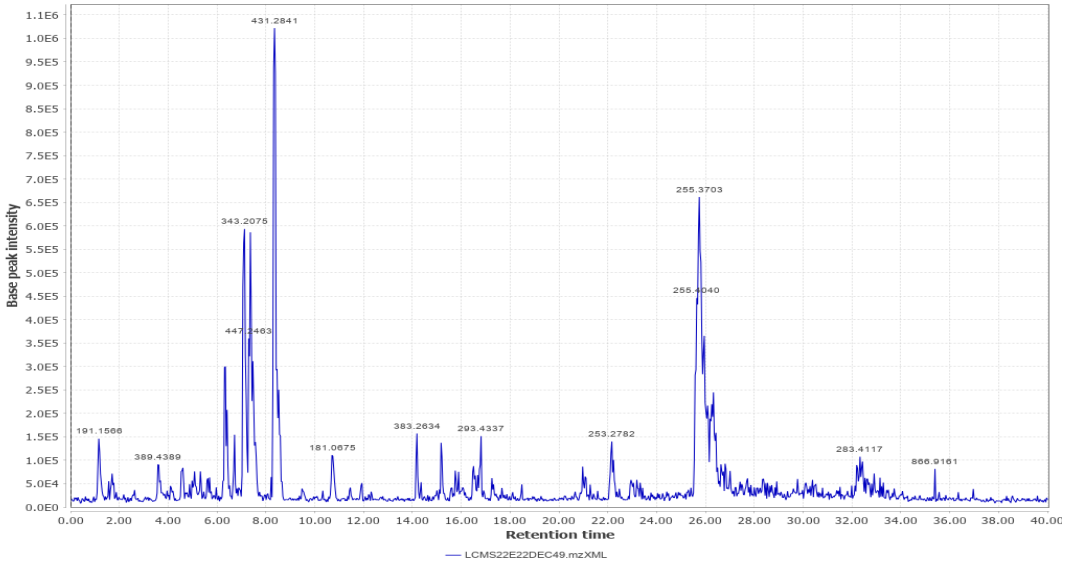


Fig. 4.2b Negative ion chromatogram of *E. milii*

Table 4.2b. Negative ion compound identified from *E. milii*

S.No	RT	Compound Name	Formula
1	1.16	Scopoletin	$C_{10}H_8O_4$
2	1.70	m-Hydroxycinnamic acid	$C_9H_8O_3$
3	3.58	(2S)-2-Hydroxybut-3-enylglucosinolate	$C_{11}H_{19}NO_{10}S_2$
4	4.54	Sinigrin	$C_{10}H_{17}NO_9S_2$
5	5.08	2'-Deoxycytidine-5'-diphosphate sodium salt	$C_9H_{15}N_3O_{10}P_2$
6	5.32	Spiraeoside	$C_{21}H_{20}O_{12}$
7	6.31	Quercetin-3-Glucuronide	$C_{21}H_{18}O_{13}$
8	6.34	isorhamnetin-3-O-glucoside	$C_{22}H_{22}O_{12}$
9	6.41	isorhamnetin-3-O-glucoside	$C_{22}H_{22}O_{12}$
10	6.72	UDP-beta-L-rhamnose	$C_{15}H_{24}N_2O_{16}P_2$
11	7.09	Esculin sesquihydrate	$C_{15}H_{16}O_9$
12	7.13	Sucrose	$C_{12}H_{22}O_{11}$
13	7.30	Quercetin-3-Rhamnoside	$C_{21}H_{20}O_{11}$
14	7.37	Homoorientin	$C_{21}H_{20}O_{11}$
15	7.47	luteolin-4'-O-glucoside	$C_{21}H_{20}O_{11}$
16	8.22	Saponarin	$C_{27}H_{30}O_{15}$
17	8.32	Kaempferol-3-O-alpha-L-rhamnoside	$C_{21}H_{20}O_{10}$
18	8.35	Apigenin-7-O-glucoside	$C_{21}H_{20}O_{10}$
19	8.39	Quercetin-3-D-xyloside	$C_{20}H_{18}O_{11}$
20	9.48	Inosine	$C_{10}H_{12}N_4O_5$

21	10.71	D-(-)-Mannitol	$C_6H_{14}O_6$
22	11.94	Cytidine-5'-diphosphocholine sodium salt dihydrate, from yeast, solid	$C_{14}H_{26}N_4O_{11}P_2$
23	14.19	1-Myristoyl-2-Hydroxy-sn-Glycero-3-Phosphate (Sodium Salt) Sodium Salt	$C_{17}H_{35}O_7P$
24	15.17	Pentachlorophenol	C_6HCl_5O
25	15.75	N-acetylneuraminic acid	$C_{11}H_{19}NO_9$
26	15.89	2'-Deoxyuridine-5'-monophosphate disodium salt	$C_9H_{13}N_2O_8P$
27	16.50	Cytidine-3'-monophosphate	$C_9H_{14}N_3O_8P$
28	16.81	(+)-Epicatechin	$C_{15}H_{14}O_6$
29	17.25	Esculin sesquihydrate	$C_{15}H_{16}O_9$
30	20.97	2'-Deoxycytidine	$C_9H_{13}N_3O_4$
31	22.23	Daidzein	$C_{15}H_{10}O_4$
32	25.58	2'-Deoxyinosine	$C_{10}H_{12}N_4O_4$
33	26.09	D-Glucosamine-6-phosphate sodium salt	$C_6H_{14}NO_8P$
34	26.26	Acacetin	$C_{16}H_{12}O_5$
35	26.33	Xanthosine	$C_{10}H_{12}N_4O_6$

The LC-HRMS analysis of *the Myrica esculenta* methanolic plant extract identified a total of 172 chemical compounds (Table 4.3). The chromatogram in positive ion mode showed that the highest peak is Skimmin at RT 14.88 with an m/z of 342.11, followed by Hydroxy matairesinol (374 m/z at RT 15.89 mins) indicating highly abundant under the given ion conditions.

In negative ion, the most intense peak was found to be Keracyanin m/z 594.15 at RT 25.75, followed by Arbutin m/z 271.08 at RT 14.88 minutes while the UV detector mainly detect the volatile compound such as 1-methyl-2-tridecylquinolin-4-one (m/z 342.27 at RT 14.88) and Peniprequinolone (m/z 382.28 at RT 14.95) and found to be the most abundant.

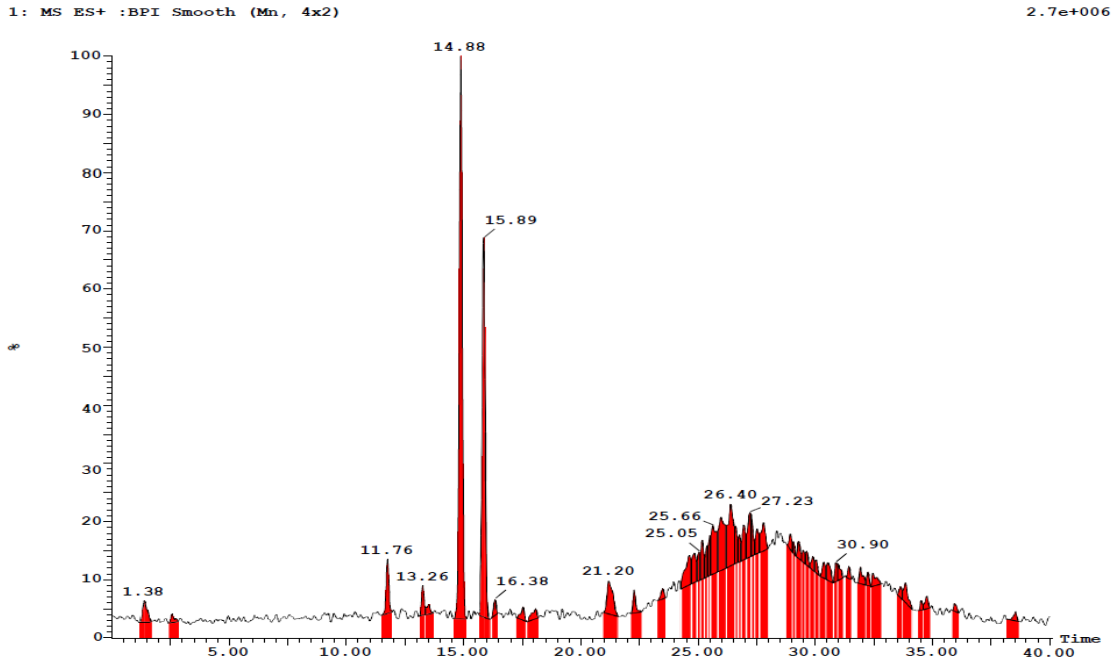


Fig. 4.3a Positive ion chromatogram of *M. esculenta*

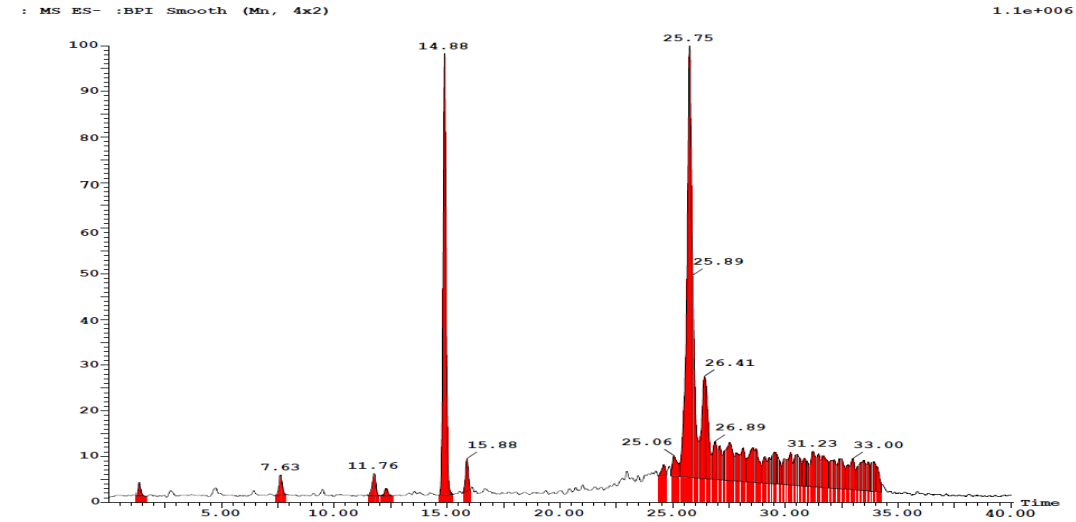


Fig. 4.3b Negative ion chromatogram of *M. esculenta*

Table 4.3 Combined compound identified from *M. esculenta*

S.No	RT	Compound Names	Formula
1	33.61	[(E,6R)-6-hydroxy... acetate (complex steroidal glycoside derivative)	$C_{38}H_{56}O_{13}$
2	33.03	2-hydroxy-… (3-methylbut-2-enyl)-6-pentylbenzoic acid	$C_{23}H_{34}O_4$
3	14.84	3-[(…2,4-dihydroxy-6-methylbenzaldehyde	$C_{17}H_{18}O_5$
4	30.33	5,7-dihydroxy-6-methoxy-2-phenylchromen-4-one	$C_{16}H_{12}O_5$
5	26.93	5-[5-hydroxy-3……-1-carboxylic acid	$C_{20}H_{34}O_5$

6	14.88	Arbutin	$C_{12}H_{16}O_7$
7	27.75	Corynanthin	$C_{21}H_{26}N_2O_3$
8	26.14	Destruxin A	$C_{29}H_{47}N_5O_7$
9	32.67	Dofetilide	$C_{19}H_{27}N_3O_5S_2$
10	35.46	Imazamox	$C_{15}H_{19}N_3O_4$
11	15.88	MMV676270	$C_{21}H_{25}FN_2O_2$
12	11.75	NCGC00169633-02!9-phenyl-1-…nonan-1-one	$C_{21}H_{26}O_4$
13	11.75	NCGC00178802-07!N……-hydroxybutanediamide	$C_{25}H_{48}N_6O_8$
14	15.87	NCGC00380210-01_C18H30O8… 2-penten-1-yl]-	$C_{18}H_{30}O_8$
16	14.96	NCGC00380391-01!2-….isochromen-3-yl)acetic acid	$C_{16}H_{16}O_8$
17	30.99	NCGC00385154-01!4……[3,2-g]chromen-7-	$C_{27}H_{34}O_{15}$
18	31.60	NCGC00385398-01!2-…. butanedioic acid	$C_7H_{10}O_7$
19	24.91	O-trimethoxy-3,4,5-benzoyl-OH-vincamajine	$C_{32}H_{36}N_2O_8$
20	32.30	Phosphoenolpyruvic Acid Trisodium Salt monohydrate	$C_3H_5O_6P$
	14.86	Pinolidoxin	$C_{18}H_{26}O_6$
22	14.88	Skimmin	$C_{15}H_{16}O_8$
23	14.84	Sweroside	$C_{16}H_{22}O_9$
24	16.39	2R,3S,4S,5R,6R)-……2-phenylethoxy)oxane-3,4,5-triol	$C_{19}H_{28}O_{10}$
25	21.18	(R, R)-TARTARIC ACID	$C_4H_6O_6$
26	32.95	[(2S,3R)-2…. -2H-chromen-7-yl] 3,4,5-trihydroxybenzoate	$C_{22}H_{18}O_{10}$
27	15.88	3,4,5-trihydroxy-…3-hydroxy-4,5-dimethoxybenzoate	$C_{15}H_{20}O_{10}$
28	33.24	3-[3-[(2E)- 4-hydroxyphenyl]-7-hydroxychromen-4-one	$C_{25}H_{26}O_4$
29	15.87	Arctigenin	$C_{21}H_{24}O_6$
30	1.328	DCA_156.0575_17.7	$C_8H_{10}C_lN$
31	34.14	Dihydromyricetin	$C_{15}H_{12}O_8$
32	15.86	Lagochilin	$C_{20}H_{36}O_5$
33	27.03	NCGC00169972-02!3,…. anthracene-1,7,12-trione	$C_{20}H_{16}O_6$
34	33.12	Nilotinib	$C_{28}H_{22}F_3N_7O$
35	32.19	(2E)-6-{13,17-dihydroxy-……..methylhept-2-enoic acid	$C_{35}H_{56}O_9$
36	11.72	Lyso-PC 18:1(9Z)	$C_{26}H_{52}NO_7P$
37	13.30	NCGC00347652-02… (14H)-hexone	$C_{35}H_{53}N_5O7$
38	15.97	Oxybutynin	$C_{22}H_{31}NO_3$
39	27.03	Pyrenophorol	$C_{16}H_{24}O_6$
40	26.50	Threonylphenylalanine	$C_{13}H_{18}N_2O_4$
41	15.80	Conessine	$C_{24}H_{40}N_2$
42	14.91	Fluorometholone	$C_{22}H_{29}FO_4$
43	28.98	Indinavir	$C_{36}H_{47}N_5O_4$
44	27.89	Mangiferin	$C_{19}H_{18}O_{11}$
45	15.87	Methyl 2…….4-hydroxy-3,6-dimethylbenzoate	$C_{19}H_{24}O_8$
46	13.48	Methyl 2……isochromen-3-yl)acetate	$C_{17}H_{18}O_8$
47	26.78	NCGC00016349-11!7-hydroxy-6-methoxychromen-2-one	$C_{10}H_8O_4$
48	9.48	NCGC00180745-02….. (2R,4aS,6aS,12bR,14aS,14bR)-	$C_{28}H_{36}O_5$
49	13.49	(E)-3-(4-methoxyphenyl)….prop-2-en-1-one	$C_{24}H_{28}O_5$

50	15.91	Bisdethiobis(methylthio)gliotoxin	$C_{15}H_{20}N_2O_4S_2$
51	25.85	Maesopsin	$C_{15}H_{12}O_6$
52	25.71	Thymol-beta-D-glucoside	$C_{16}H_{24}O_6$
53	22.04	“7_,13_,17-O-triacetoxy-5_-O-benzoyloxy…..-oxopremyrsinol"	$C_{36}H_{46}O_{12}$
54	11.74	[(2R)-1-[(2S)-….3-(3,4-dihydroxyphenyl)propanoate	$C_{17}H_{20}O_6$
55	14.91	[7-(3,4-dihydroxyphenyl)-1-(4-hydroxyphenyl)heptan-3-yl] acetate	$C_{21}H_{26}O_5$
56	24.58	2-[(4S,5S,5aS,9aS)….. isoindol-2-yl]pentanedioic acid	$C_{29}H_{39}NO_8$
57	32.27	Ceramide (d18:1/18:0)	$C_{36}H_{71}NO_3$
58	25.81	2-decyl-3-hydroxypentanedioic acid	$C_{15}H_{28}O_5$
59	28.07	5-(5-methoxycarbonyl-5,…..3-methylpentanoic acid	$C_{21}H_{34}O_4$
60	11.75	Methyl (2E)- octadecan-12-yl]prop-2-enoate	$C_{29}H_{36}O_{10}$
61	28.59	Phosphatidylcholine 15:0-16:1	$C_{39}H_{76}NO_8P$
62	29.78	7-[(2S,3R,4R,5R,6S)- ….. chromen-4-one	$C_{27}H_{30}O1_2$
63	30.78	D-GULONIC ACID GAMMA-LACTONE	$C_6H_{12}O_7$
64	26.99	Tanshindiol B	$C_{18}H_{16}O_5$
65	27.11	Thaxtomin D	$C_{22}H_{22}N_4O_4$
66	15.8r	Hydroxy matairesinol	$C_{20}H_{22}O_7$
67	25.69	4,4'-Methylenebis(2,6-diethylaniline)	$C_{21}H_{30}N_2$
68	15.90	Pachyrrhizin	$C_{19}H_{12}O_6$
69	29.67	LPC 15:0-d5	$C_{23}H_{48}NO_7P$
70	25.67	Droperidol	$C_{22}H_{22}FN_3O_2$
71	13.55	Famoxadone	$C_{22}H_{18}N_2O_4$
72	26.05	Laccaic Acid A	$C_{26}H_{19}NO_{12}$
73	25.76	Ovalitenin B	$C_{19}H_{18}O_4$
74	27.62	Humulone	$C_{21}H_{30}O_5$
75	15.84	Behenic acid	$C_{22}H_{44}O_2$
76	27.76	CHOLESTANE	$C_{27}H_{48}$
77	15.87	Bavachinin A	$C_{21}H_{22}O_4$
78	11.74	Dendroamide B	$C_{21}H_{24}N_6O_4S_3$
79	15.87	Florfenicol	$C_{12}H_{14}C_{l2}FNO_4S$
80	27.03	Kinetin Riboside	$C1_5H_{17}N_5O_5$
81	14.88	NCGC00385995-01!1-methyl-2-tridecylquinolin-4-one	$C_{23}H_{35}NO$
82	21.04	Phosphatidylcholine lyso 20:3	$C_{28}H_{52}NO_7P$
83	0.386	Tropic acid	$C_9H_{10}O_3$
84	8.43	5-[(2R,3S)-6-hydroxy-……]benzene-1,3-diol	$C_{28}H_{22}O_6$
85	31.79	[(4R,5S,6R,6aS,7R,10aR,11bR)…… methyl acetate	$C_{25}H_{36}O_8$
86	11.12	Myristoleic acid (14:1(n-5))	$C_{14}H_{26}O_2$
87	11.78	Capecitabine	$C_{15}H_{22}FN_3O_6$
88	16.69	Maleamic acid	$C_4H_5NO_3$
89	15.07	MMV688771	$C_{18}H_{19}N_3OS$
90	27.30	Prolinamide	$C_5H_{10}N_2O$
91	29.21	Alpha-TOCHOPHEROL	$C_{29}H_{50}O_2$

92	32.30	D-sorbosonic acid	$C_6H_{10}O_7$
93	26.97	Vercuronium	$C_{34}H_{57}N_2O_4$+
94	11.70	N6-Aristololactam-I-deoxyadenosine	$C_{27}H_{22}N_6O_7$
95	15.91	2-{7-hydroxy-11- ….trien-13-yl}acetic acid	$C_{16}H_{14}O_7$
96	15.86	Sulpiride	$C_{15}H_{23}N_3O_4S$
97	14.89	7-hydroxy-2-(4-hydroxyphenyl)-…. chromen-4-one	$C_{16}H_{14}O_5$
98	33.90	9-phenyl-1-(2,4,6-trihydroxyphenyl)nonan-1-one	$C_{21}H_{26}O_4$
99	33.86	5,7-dihydroxy-2-(4-hydroxyphenyl…..chromen-4-one	$C_{27}H_{30}O_{14}$
100	32.37	9-(4-hydroxy-3,5-dimethoxyphenyl)-…. benzodioxol-8-one	$C_{27}H_{30}O_{13}$
101	27.40	NCGC00180261-02!.... 5-hexone	$C_{25}H_{44}N_6O_9$
102	5.58	Salsoline	$C_{11}H_{15}NO_2$
103	16.49	Phenmetrazine	$C_{11}H_{15}NO$
104	14.94	NCGC00347805-02!(2E,6E)…octol	$C_{45}H_{86}O_8$
105	14.90	Pangamic Acid Sodium	$C_{10}H_{18}NNaO_8$
106	24.40	(2R,3R,4S,5S,6R)-…. 6-(hydroxymethyl)oxane-3,4,5-triol	$C_{32}H_{54}O_{13}$
107	15.93	"2,4-Dichloronorlichexanthone"	$C_{14}H_8Cl_2O_5$
108	11.77	Desacetyl (7)Khivorinic Acid, Methyl Ester	$C_{28}H_{40}O_{10}$
109	14.84	Salicin	$C_{13}H_{18}O_7$
110	15.90	SH373-1	$C_{17}H_{31}N_3O_6$
111	13.52	Flucarbazone	$C_{12}H_{11}F_3N_4O_6S$
112	24.87	Cyclo(Val-Pro)	$C_{10}H_{16}N_2O_2$
113	14.89	NCGC00169490-02!3-….. b]quinoline-1,9-dione	$C_{16}H_{18}N_2O_2$
114	31.56	NCGC00385671-01…… pyran-1-yl ester	$C_{23}H_{36}O_{13}$
115	32.43	4-Hydroxybenzaldehyde	$C_7H_6O_2$
116	33.82	[(1S,4aS,5R,7S)-4a,-….4-hydroxyphenyl)prop-2-enoate	$C_{24}H_{30}O_{12}$
117	28.31	NCGC00384811-01_C35H56O9….L-xylopyranoside	$C_{35}H_{56}O_9$
118	14.87	C-mavacurine	$[C_{20}H_{25}N_2O]$+
119	25.75	Keracyanin	$[C_{27}H_{31}O_{15}]$+
120	33.13	(3S,4S)-5-[(3S,4S….benzo[g]isochromene-4,10-diol	$C_{32}H_{34}O_{10}$
121	33.55	Paromomycin	$C_{23}H_{45}N_5O_{14}$
122	14.95	Peniprequinolone	$C_{22}H_{25}NO_5$
123	11.75	1-[4,5-dihydroxy-6-(hydroxymethyl)….pyran-4-carboxylic acid	$C_{25}H_{28}O_{12}$
124	32.97	Eriodermin	$C_{17}H_{12}C_{l2}O_6$
125	30.19	Methyl (1R,4S,4aS,7R,8S,9R)-4……-phenanthrene-1-carboxylate	$C_{27}H_{38}O_8$
126	11.77	NCGC00381435-01!13-dodecan-…….-tetrone	$C_{28}H_{51}N_3O_7$
127	15.88	5-p-Coumaroylquinic acids	$C_{16}H_{18}O_8$
128	15.93	NCGC00380773-01_C20H25ClO6_	$C_{20}H_{25}ClO_6$
129	6.55	1,N2-6-Oxo-malondialdehyde-deoxyguanosine	
130	20.06	4-(3,4-dihydroxyphenyl)-6,7-dihydroxynaphthalene-2-carboxylic acid	$C_{17}H_{12}O_6$
131	14.87	NCGC00380612-01!3,8-dihydroxy……xanthen-9-one	$C_{18}H_{18}O_8$
132	20.83	(1R,3aR,5aR,5bR,9S,11aR)…chrysen-9-ol	$C_{30}H_{50}O$
133	32.03	NCGC00380124- …..methylethyl 2-(acetylamino)-2-deoxy-	$C_{38}H_{63}NO_{10}$

134	14.84	NCGC00381440-01!(2S,3S…..oxopropyl)butanedioic acid	$C_{18}H_{32}O_7$
135	14.90	Allopregnanolone	$C_{21}H_{34}O_2$
136	27.16	(2R,4'aR,5'S,6'R,6'aS,10'aR,10'bR)-….. 5'-yl 2-methylpropanoate	$C_{26}H_{38}O_8$
137	15.88	5-(4-acetyloxy-3-hydroxy-…..)-3-methylpentanoic acid	$C_{22}H_{36}O_5$
138	30.75	3,5-dibromo-4-chloro-2-(3,5-dibromo-2-methoxyphenoxy)phenol	$C_{13}H_7Br_4ClO_3$
139	14.84	MMV090930	$C_{16}H_{13}N_3O_4S_2$
140	26.23	Deacetoxy(7)-7-Oxokhivorinic Acid	$C_{27}H_{36}O_{10}$
141	22.25	NCGC00381110-01!13…..triazacyclopentadecane-2,5,8,11-tetrone	$C_{28}H_{51}N_3O_8$
142	22.28	(4aS,6aR,6bR,10S,12aR)….tetradecahydro-1H-picene-4a,6a-dicarboxylic acid	$C_{36}H_{56}O_{10}$
143	11.77	Sulfoluciferin	$C_{11}H_8N_2O_6S_3$
144	15.88	NCGC00180622….-hydroxyphenyl)methyl]-5-oxofuran-2-carboxylate	$C_{19}H_{16}O_7$
145	21.28	Methylchloroisothiazolinone	C_4H_4ClNOS
146	15.90	Hematein	$C_{16}H_{12}O_6$
147	31.37	NCGC00384690-01!	$C_{27}H_{22}N_2O_{10}S_4$
148	14.82	Antanapeptin B	$C_{41}H_{62}N_4O_8$
149	0.273	N-Nitrosodibutylamine (NDBA)	$C_8H_{18}N_2O$
150	31.37	[(1S,6S,7R,10R,15S,16R,18S,19R,20R……acetic acid	$C_{32}H_{40}O_{14}$
151	11.77	Methyl (2S,3R,4S)…..dihydro-2H-pyran-5-carboxylate	$C_{24}H_{30}O_{14}$
152	28.67	Nitrofen	$C_{12}H_7Cl_2NO_3$
153	14.82	Phosphatidylethanolamine 18:1-18:2	$C_{41}H_{76}NO_8P$
154	17.05	Fucoxanthin	$C_{42}H_{58}O_6$
155	15.84	Monoolein	$C_{21}H_{40}O_4$
156	11.71	Dihydrocapsaicin	$C_{18}H_{29}NO_3$
157	7.793	Chloridazone-methyl-desphenyl	$C_5H_6ClN_3O$
158	12.82	1-(3,4-dihydroxyphenyl)….dicarboxylic acid	$C_{18}H_{14}O_8$
159	14.86	Eicosenoic acid	$C_{20}H_{38}O_2$
160	28.77	NCGC00385068-….oxan-2-yl]oxybenzoate	$C_{20}H_{28}O_{13}$
161	33.56	2-O-Rhamnosylvitexin	$C_{27}H_{30}O_{14}$
162	13.52	Hexacosanoic acid	$C_{26}H_{52}O_2$
163	14.97	Cholest-4,6-Dien-3-One	$C_{27}H_{42}O$
164	32.12	Isoxaflutole	$C_{15}H_{12}F_3NO_4S$
165	14.87	MMV688411	$C_{20}H_{21}N_7$
166	33.04	Etoposide	$C_{29}H_{32}O_{13}$
167	31.84	(2R,6R)-6-[(3R,10S,12S,13R,17R)…..methylideneheptanoic acid	$C_{34}H_{52}O_7$
168	28.86	Spermine	$C_{10}H_{26}N_4$
169	19.95	Hypoprotocetraric acid	$C_{18}H_{16}O_7$
170	15.91	NCGC00016425-09!6,7-dihydroxychromen-2-one	$C_9H_6O_4$
171	14.84	NCGC00385919-01_C20H34O…methylene-1-naphthalenyl]-3-methyl-, (2E)-	$C_{20}H_{34}O$
172	13.21	N-Acetyl-D-galactosamine [M+H-H2O]+; AIF; CE30; MS2Dec	$C_8H_{15}NO_6$

The LC-HRMS analysis of *Melastoma malabathricum* methanolic plant extract identified 76 chemical compounds in total (Table 4.4). The chromatogram in positive ion mode exhibited that Flavanone as the highest peak at RT 7.64 with an m/z of 319.045, followed by N-acetyl-L-ornithine (175 m/z at RT 1.44 mins) and Luteolin-7,3'-di-O-glucoside (m/z 609.14 at RT 33.94mins), indicating a highly abundant ion under the given conditions.

In negative ion, the most intense peak was found to be Thymol-beta-D-glucoside m/z 311.14 at RT 25.78, followed by Villosinol m/z 465.09 at RT 7.65 minutes while in UV detector, 9-phenyl-1-(2,4,6-trihydroxyphenyl)nonan-1-one (m/z 381.14 at RT 1.56) and 2-chloro-1,3,8-trihydroxy-6-(hydroxymethyl)anthracene-9,10-dione (m/z 319.11 at RT 7.71) found to be the most abundant.

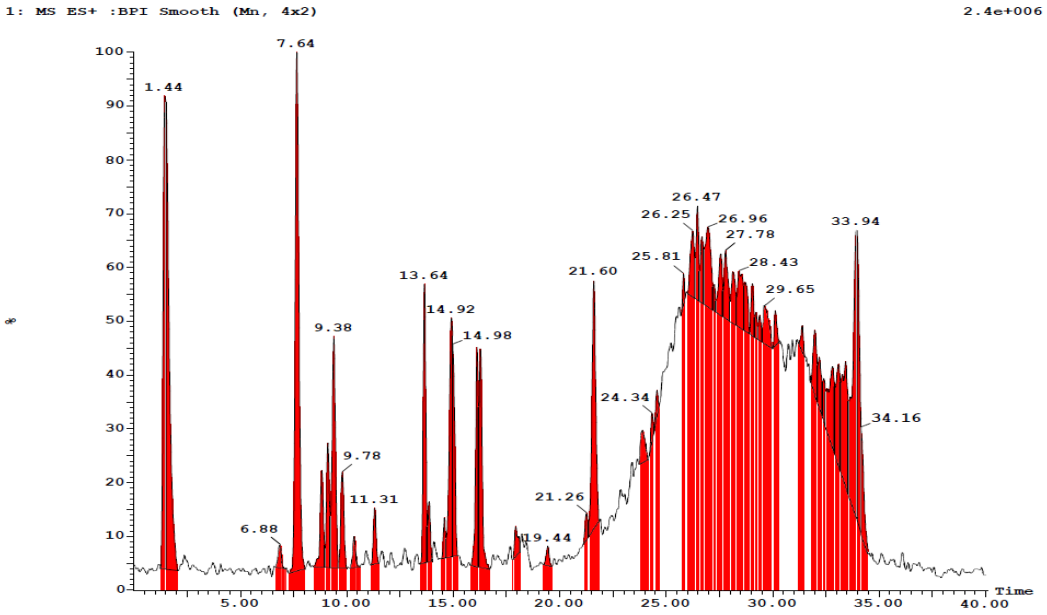


Fig. 4.4a Positive ion chromatogram of *M. malabathricum*

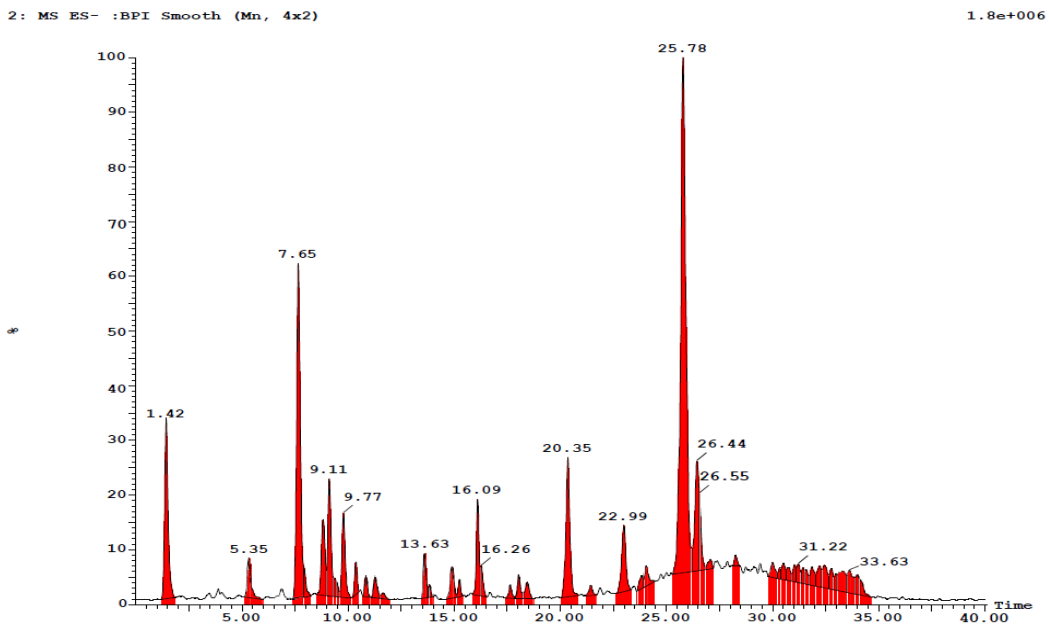


Fig. 4.4b Negative ion chromatogram of M. malabathricum

Table 4.4 Combined compound identified from positive and negative ions of *M. malabhatricum*

S.No.	RT	Compound Name	Formula
1	13.85	(1S,4aS,5S,7aS)-7-(acetyloxymethyl)...carboxylic acid	$C_{18}H_{24}O_{12}$
2	16.26	[(1R,2R,3aR,5R,6E,10R,11S,13R,13aS)...2-methylbutanoate	$C_{35}H_{48}O_{14}$
3	27.00	1,7-bis(3,4-dihydroxyphenyl)heptan-3-one	$C_{19}H_{22}O_5$
4	9.423	2,4-bis(3-methylbut-2-enyl)...chromene-3,9-diol	$C_{25}H_{28}O_4$
5	7.71	2-chloro-1,3,8-trihydroxy-6-(hydroxymethyl)anthracene-9,10-dione	$C_{15}H_9ClO_6$
6	16.47	2-methylidene-4-[(2R,3R,4S,5S,6R)...oxybutanoic acid	$C_{11}H_{18}O_8$
7	1.552	4-guanidinobutanamide	$C_5H_{12}N_4O$
8	16.29	5-[5-hydroxy-3-(hydroxymethyl)pentyl]...carboxylic acid	$C_{20}H_{34}O_5$
9	1.496	Choline chloride	$C_5H_{14}NO$
10	1.384	Cyclamate	$C_6H_{13}NO_3S$
11	16.12	Docosatetraenoic acid	$C_{22}H_{36}O_2$
12	7.638	Flavanone	$C_{15}H_{12}O_8$
13	13.64	Grandilodine B	$C_{24}H_{26}N_{27}$
14	1.398	Hinokitiol	$C_{10}H_{12}O_2$
15	18.23	Methyl 2-(2,6-dihydroxy-4-methylbenzoyl)-3-hydroxy-5-methoxybenzoate	$C_{17}H_{16}O_7$
16	22.57	MLS001201763-01!Azithromycin	$C_{38}H_{72}N_{22}$
17	1.440	N-acetyl-L-ornithine	$C_7H_{14}N_2O_3$
18	17.87	NCGC00180261-02!...hexazacyclohentriacontane...	$C_{25}H_{44}N_{69}$
19	16.28	NCGC00381425-01!...octyloxiran...octanoic acid	$C_{18}H_{34}O_4$

20	1.890	Norlichexanthone	$C_{14}H_{10}O_5$
21	7.8074	Tomelukast	$C_{16}H_{22}N_{43}$
22	21.24	(R,R)-Tartaric acid	$C_4H_6O_6$
23	16.24	15-(carbamoylmethyl)...tetracosa...carboxamide	$C_{33}H_{38}N_{60}$
24	11.264	5-hydroxy-2-(4-hydroxyphenyl)...oxychromen-4-one	$C_{27}H_{30}O_{15}$
25	1.398	Coumaroyl hexoside (isomer of 690, 692)	$C_{15}H_{18}O_8$
26	22.83	L-Proline	$C_5H_9NO_2$
27	16.11	NCGC00381419-01_C28H30N4O7_	$C_{28}H_{30}N_{47}$
28	11.377	NCGC00386086-01!...octahydropyrano...xanthen...	$C_{33}H_{46}O_{11}$
29	7.77	Picrotin	$C_{15}H_{18}O_7$
30	14.581	Camellimidazole E	$C_8H_{12}N_4O$
31	1.86213	NCGC00380331-01 methyl 11-hydroxy-10-methyl-4-oxododecanoate	$C_{14}H_{26}O_4$
32	21.5390	NCGC00385599-01 1-methyl-2-nonylquinolin-4-one	$C_{19}H_{27}NO$
33	9.817	NCGC00168939-02 8-hydroxy-3-(4-oxopentyl)-3,4-dihydroisochromen-1-one	$C_{14}H_{16}O_4$
34	14.877	NCGC00180745-02 2-Picenecarboxylic acid...	$C_{28}H_{36}O_5$
35	7.6106	NCGC00380493-014H-Pyran-4-one...glucopyranosyl]oxy	$C_{17}H_{24}O_{12}$
36	7.6528	Villosinol	$C_{23}H_{22}O_8$
37	14.090	Tricaprylin (C8:0)	$C_{27}H_{50}O_6$
38	16.156	2',4-Dihydroxy-3,4',6'-Trimethoxychalcone	$C_{18}H_{18}O_6$
39	16.085	Leucoridine A	$C_{38}H_{44}N_4$
40	21.59	methyl (2R)-2-[(...pentacyclo...)octadecan-15-yl]-2-hydroxyacetate	$C_{29}H_{36}O_{11}$
41	10.43	Phenazone / Antipyrine	$C_{11}H_{12}N_2O$
42	25.79	Thymol-beta-D-glucoside	$C_{16}H_{24}O_6$
43	9.3815	4-Fluorobenzoylpropionic acid	$C_{10}H_9FO_3$
44	18.109	Serine-Cholic Acid	$C_{27}H_{45}NO_7$
45	18.109	Apiosylskimmin	$C_{20}H_{24}O_{12}$
46	1.426	NCGC00385643-01...Phenanthrenecarboxylic acid...	$C_{20}H_{30}O_3$
47	8.833	2-Methylene-5-(2,5-Dioxotetrahydrofuran-3-Yl)...	$C_{18}H_{24}O_4$
48	1.370	2-Oxoglutarate	$C_5H_6O_5$
49	7.624	MMV676383	$C_{14}H_{13}N_{32}$
50	9.100	Thymol	$C_{10}H_{14}O$
51	27.28	Goniomedinone	$C_{40}H_{47}N_5$
52	13.626	SLCA - Sulfolithocholic acid	$C_{24}H_{40}O_6S$
53	10.39	Telmisartan	$C_{33}H_{30}N_{42}$
54	14.72	Thonizide	$C_{32}H_{55}N_4O$
55	14.84	(1S,4S,5R,10S...)...oxahexacyclo...tetracos-15-en-23-one	$C_{30}H_{46}O_3$
56	27.10	Thalsimidine	$C_{37}H_{38}N_{27}$
57	8.805	4-hydroxy-3-[(4-hydroxy-6-methyl-2-oxopyran-3-yl)methyl]-6-methylpyran-2-one	$C_{13}H_{12}O_6$
58	9.05	Caryophyllene [T(-)]	$C_{14}H_{22}$
59	33.94	Luteolin-7,3'-di-O-glucoside	$C_{27}H_{30}O_{16}$
60	15.34	(2R,3R,4S,5S,6R)-2-[(2E)...oxane-3,4,5-triol	$C_{16}H_{26}O_6$
61	29.46	NCGC00373238-02 (Delvocid)	$C_{33}H_{47}NO_3$

62	2.22	Piperacetazine	$C_{24}H_{30}N_2S$
63	33.87	Reserpine	$C_{33}H_{40}N_{29}$
64	1.412	[(2R,4S,5R)...methanol	$C_{20}H_{24}N_{22}$
65	16.36	NCGC00380990-01 (long diene acid)	$C_{19}H_{32}O_6$
66	9.283	1,2-Diphenylethanone	$C_{14}H_{12}O$
67	18.29	2-(2,6-dihydroxy-4-methoxycarbonylbenzoyl)-3-hydroxybenzoic acid	$C_{16}H_{12}O_8$
68	1.56	9-phenyl-1-(2,4,6-trihydroxyphenyl)nonan-1-one	$C_{21}H_{26}O_4$
69	24.78	Fusarenone-X	$C_{17}H_{22}O_8$
70	13.87	Isochromophilone VI	$C_{23}H_{28}ClO_5$
71	7.68	NCGC00380365-01 (C19H28O13) – 4-Hydroxy-3,5-dimethoxyphenyl glucopyranoside	$C_{19}H_{28}O_{13}$
72	1.482	N-Nitrosopiperidine (NPIP)	$C_5H_{10}N_2O$
73	14.83	5-[(2R,3S)...benzene-1,3-diol	$C_{28}H_{22}O_6$
74	14.87	Asiatic Acid	$C_{30}H_{48}O_5$
75	22.53	Famoxadone	$C_{22}H_{18}N_{24}$
76	33.99	NCGC00385384-01 – [Steroid-like heptanyl acetate]	$C_{32}H_{50}O_8$

4.4.2 *Molecular Docking analysis*

Molecular docking simulations were performed to evaluate the binding affinity of selected phytoconstituents identified in medicinal plant extracts against two key receptors associated with diarrhoeal pathophysiology—the opioid receptor and the E2 prostaglandin receptor (EP2). The top five scores were selected from each of the four plants. The binding affinity with more negative values indicates stronger predicted interactions. The docking scores, which indicate the predicted binding free energy (in kcal/mol), are summarised in Table 4.5-8.

4.4.2.1 Molecular docking of *D. indica* L.

For the Opioid Receptor, among the tested compounds, Peonidin-3,5-O-di-β-glucopyranoside exhibited the most favourable binding affinity toward the opioid receptor, with a docking score of -9.8 kcal/mol, suggesting strong receptor-ligand interaction. This is followed by Kaempferol-3-Glucoside-3"-Rhamnoside (-8.9 kcal/mol) and Zearalenone (-8.7 kcal/mol), both of which also demonstrated notable binding affinities (Table 4.5a). These scores are comparable to or better than those of the standard anti-diarrhoeal drugs Loperamide and Atropine.

The high binding affinity of Peonidin-3,5-O-di-β-glucopyranoside suggests its potential role in modulating the opioid receptor pathway, which is known to regulate

intestinal motility. Binding to this receptor mimics the antiperistaltic action of opioids, thereby contributing to anti-diarrhoeal effects.

In the case of the EP2 receptor, which mediates inflammation and fluid secretion in the gastrointestinal tract, Flavanone demonstrated the strongest binding with a score of -9.0 kcal/mol. Other compounds such as Naringenin (-8.7 kcal/mol), Isorhamnetin-3-rutinoside (-8.6 kcal/mol), Isosakuranetin (-8.5 kcal/mol), Quercetin (-8.5 kcal/mol), and Guanosine-5'-monophosphate (-8.5 kcal/mol) also showed good binding affinities (Table 4.5).

Comparatively, the docking scores for the standard drugs Atropine (–8.4 kcal/mol) and Loperamide (- 8.5 kcal/mol) were within a similar range to those of the tested phytocompounds. This suggests that selected plant-derived molecules possess a comparable or better affinity for the EP2 receptor and may potentially exert anti-diarrhoeal activity by inhibiting prostaglandin-mediated secretory and inflammatory responses.

Table 4.5 Binding affinity of *D. indica* chemical compounds

Receptor	Ligands	Docking Score kcal/mol
Opoid Protein	Isorhamnetin-3-rutinoside	-8.6
	Zearalenone	-8.7
	Peonidin-3,5-O-di-beta-glucopyranoside	-9.8
	Kaempferol-3-Glucoside-3"-Rhamnoside	-8.9
	Isorhamnetin-3-rutinoside	-8.6
	Atropine (Std)	-8.2
	Loperamide (Std)	-8.3
EP2-(prostaglandin)	Flavanone	-9.0
	Guanosine-5'-monophosphate	-8.5
	Isorhamnetin-3-rutinoside	-8.6
	Isosakuranetin	-8.5
	Naringenin	-8.7
	Quercetin	-8.5
	Atropine (Std)	-8.4
	Loperamide (Std)	-8.5

The binding interactions of *D. indica* compounds—kaempferol 3-glucoside-7-rhamnoside, peonidin-3,5-O-di-beta-glucopyranoside with the opioid receptor and flavanone, naringenin with the prostaglandin E2 (PGE2) and opioid protein receptor were shown in Table 4.5.

For the opioid receptor, kaempferol 3-glucoside-7-rhamnoside exhibited strong binding, forming hydrogen bonds with Asp147(A), Tyr75(A), Tyr326(A), and Gln124(A). Hydrophobic interactions with Val108(A), Ile144(A), Trp133(A), and Ile304(A) contributed to the stability of the ligand-receptor complex. Peonidin-3,5-O-di-beta-glucopyranoside also demonstrated multiple hydrogen bond interactions with Asp147(A), Tyr75(A), Gln124(A), Asn127(A), Ile215(A), and Ser214(A). Additionally, hydrophobic contacts with Trp133(A), Cys217(A), Val108(A), and Ile144(A) further stabilized the complex.

For the PGE2 receptor, flavanone bound within the active site primarily via hydrophobic interactions involving Tyr37(B), Tyr101(A), Trp50(A), and Phe33(A), along with polar contacts with Asp102(A) and His99(A). Naringenin showed a well-defined fit in the PGE2 receptor pocket, interacting hydrophobically with Trp47(A), His35(A), Tyr37(B), and Leu94(B), while forming polar contacts with Asp102(A) and Glu39(B).

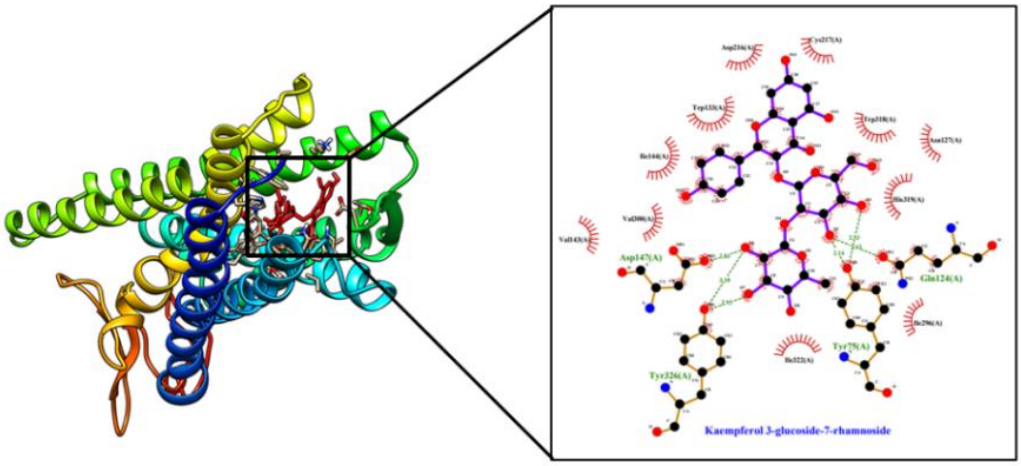


Fig. 4.5a Molecular docking interaction of kaempferol 3-glucoside-7-rhamnoside with the opioid receptor. (Left) 3D docking pose showing ligand orientation in the receptor binding site. (Right) 2D interaction diagram depicting hydrogen bonds, hydrophobic contacts, and other non-covalent interactions with key amino acid residues

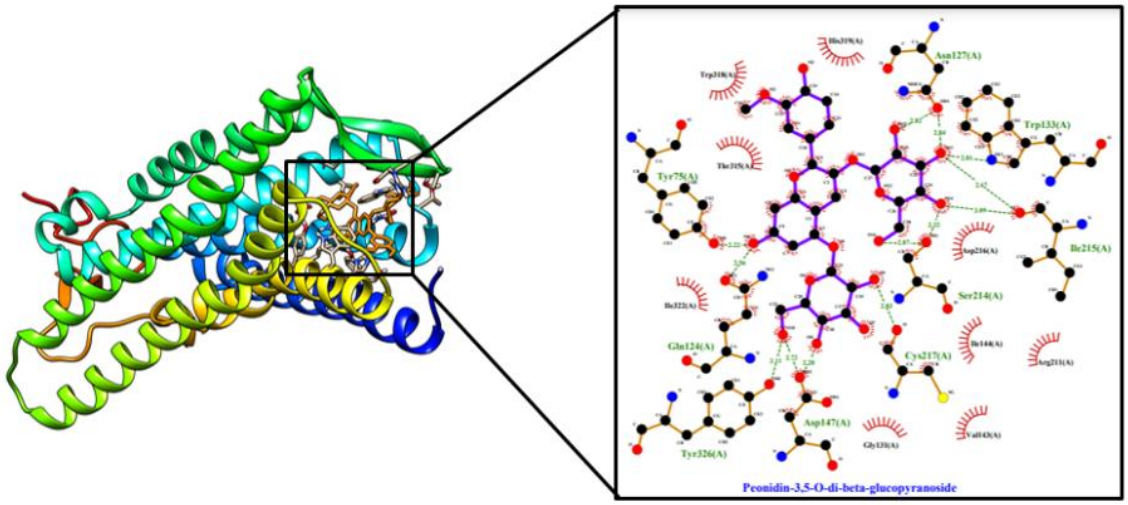


Fig. 4.5b Molecular docking interaction of peonidin-3,5-O-di-beta-glucopyranoside with the opioid receptor. (Left) 3D docking pose showing ligand orientation. (Right) 2D interaction map showing hydrogen bond donors/acceptors and hydrophobic contacts

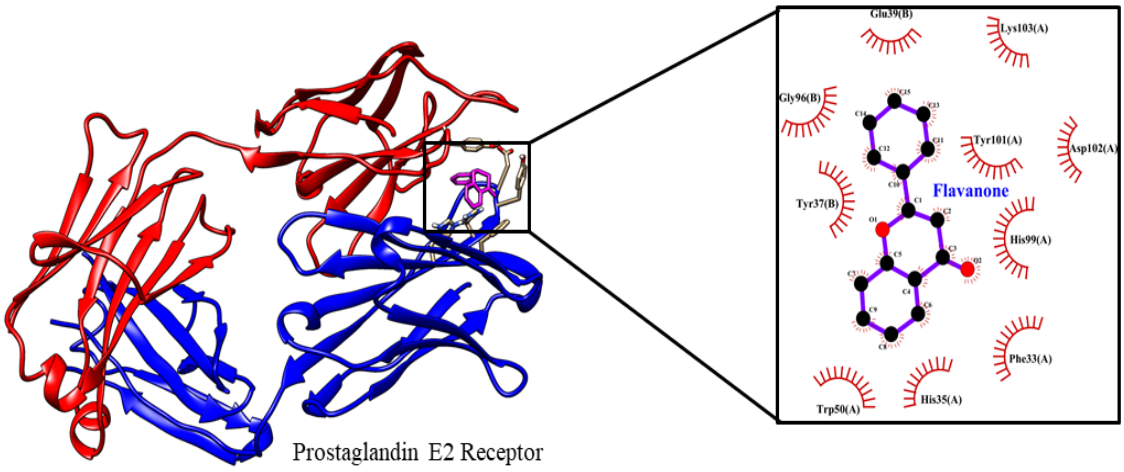


Fig. 4.5c Molecular docking interaction of flavanone with the prostaglandin E2 receptor. (Left) 3D pose showing docking orientation within the active site. (Right) 2D ligand–receptor interaction profile highlighting amino acid contacts

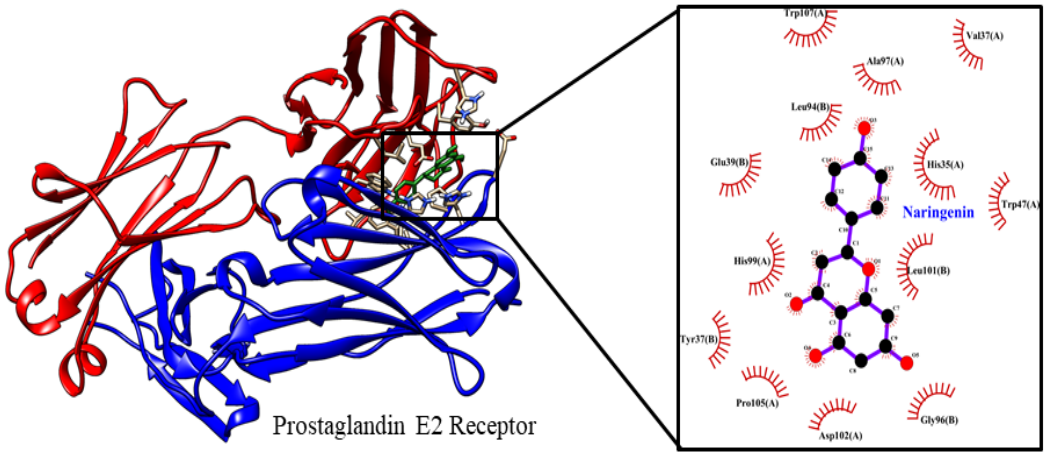


Fig. 4.5d Molecular docking interaction of naringenin with the prostaglandin E2 receptor. (Left) 3D docking pose. (Right) 2D interaction diagram indicating hydrophobic and polar interactions with active site residues

4.4.2.2 Molecular docking of *E. milii* L.

Among the tested ligands, Fortunellin demonstrated the strongest binding affinity to the opioid receptor, with a docking score of -9.2 kcal/mol, followed closely by Diosmin (-9.0 kcal/mol) and Neodiosmin (-8.8 kcal/mol). These values are superior to the standard anti-diarrh0eal agents Atropine (-8.2 kcal/mol) and Loperamide (-8.3 kcal/mol), indicating potentially stronger interactions with the opioid receptor. Additionally, Kaempferol-7-O-α-L-rhamnoside, Quercetin-3-D-xyloside, and Saponarin also exhibited favourable docking scores ranging from -8.6 to -8.7 kcal/mol, suggesting moderate-to-strong binding potential (Table 4.6). These flavonoid glycosides may exert antiperistaltic effects through partial agonism or modulation of opioid receptor activity, aligning with their ethnomedicinal use for gastrointestinal disorders.

Docking results against the EP2 receptor revealed even stronger binding interactions. Apigenin-7-O-glucoside exhibited the highest binding affinity with a docking score of -10.2 kcal/mol, followed by Tiliroside (-9.3 kcal/mol), Homoorientin (-9.2 kcal/mol), Spiraeoside (-9.0 kcal/mol), and Kaempferol-7-O-α-L-rhamnoside (-9.0 kcal/mol) as shown in Table 4.6.

These scores were all significantly more favourable than those of Atropine (-8.4 kcal/mol) and Loperamide (-8.5 kcal/mol), suggesting that these phytochemicals may act as EP2 receptor antagonists, potentially inhibiting prostaglandin E2-mediated secretory and inflammatory processes in the gut—mechanisms often implicated in pathogen- or castor oil-induced diarrhoea.

Table 4.6 Binding affinity values of *E. milii* chemical compounds

Receptor	Ligand	Docking Score (kcal/mol)
Opoid Protein	Diosmin	-9.0
	Fortunellin	-9.2
	Neodiosmin	-8.8
	Kaempferol-7-O-alpha-L-rhamnoside	-8.7
	Quercetin-3-D-xyloside	-8.6
	Saponarin	-8.6
	Atropine (Std)	-8.2
	Loperamide (Std)	-8.3
EP2-(prostaglandin)	Apigenin-7-O-glucoside	-10.2
	Homoorientin	-9.2
	Kaempferol-7-O-alpha-L-rhamnoside	-9.0
	Spiraeoside	-9.0
	Tiliroside	-9.3
	Atropine (Std)	-8.4
	Loperamide (Std)	-8.5

The interaction patterns of *E. milii* plant-derived compounds—diosmin, fortunellin, apigenin-7-O-glucoside, and tiliroside—with two pharmacologically relevant targets: the opioid receptor and the prostaglandin E2 (PGE2) receptor displayed on Table 4.6.

For the opioid receptor, diosmin displayed a stable binding conformation through multiple hydrogen bonds with Asp147(A), Tyr148(A), Tyr326(A), and Gln124(A). Hydrophobic contacts were observed with Trp133(A), Tyr75(A),

His319(A), and Val108(A), suggesting strong hydrophobic stabilisation within the receptor's binding pocket. Fortunellin exhibited a similar strong interaction profile, forming hydrogen bonds with Asp147(A), Tyr75(A), Gln124(A), Asn127(A), and Trp318(A). It also demonstrated hydrophobic interactions with Tyr326(A), Trp133(A), Val143(A), and Ile322(A).

For the PGE2 receptor, apigenin-7-O-glucoside bound to the active site via hydrogen bonds with Gly96(B) and hydrophobic interactions involving Trp47(A), His35(A), Phe33(A), Leu94(B), and Tyr37(B). Tiliroside formed hydrogen bonds with Gly96(B), His99(A), His31(B), and Ser57(A), along with hydrophobic contacts involving Phe33(A), Tyr37(B), and Trp50(A).

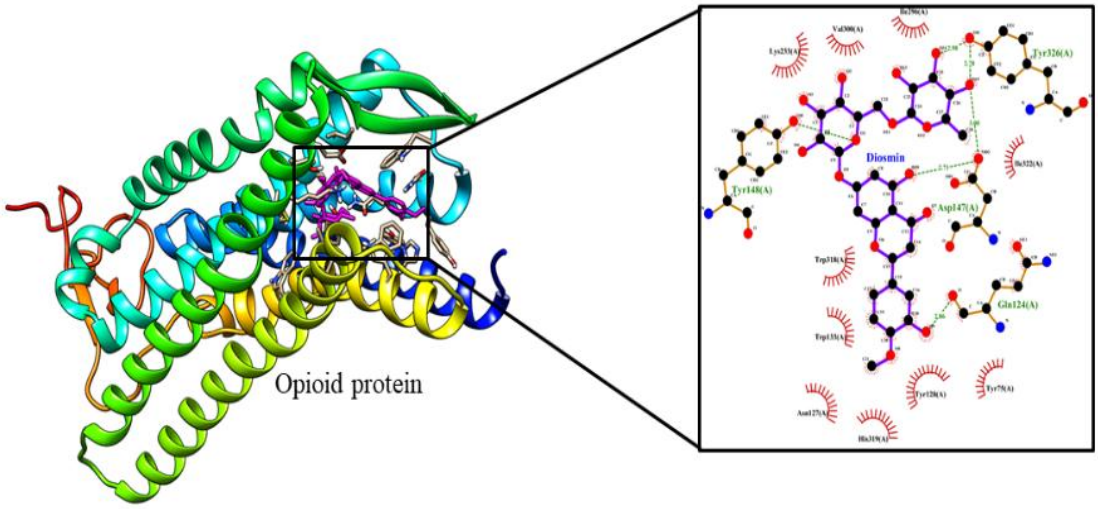


Fig. 4.6a Molecular docking interaction of diosmin with the opioid receptor. (Left) 3D docking pose showing ligand orientation within the binding site. (Right) 2D interaction diagram depicting hydrogen bonds (green dashed lines) and hydrophobic interactions (red arcs) with key amino acid residues

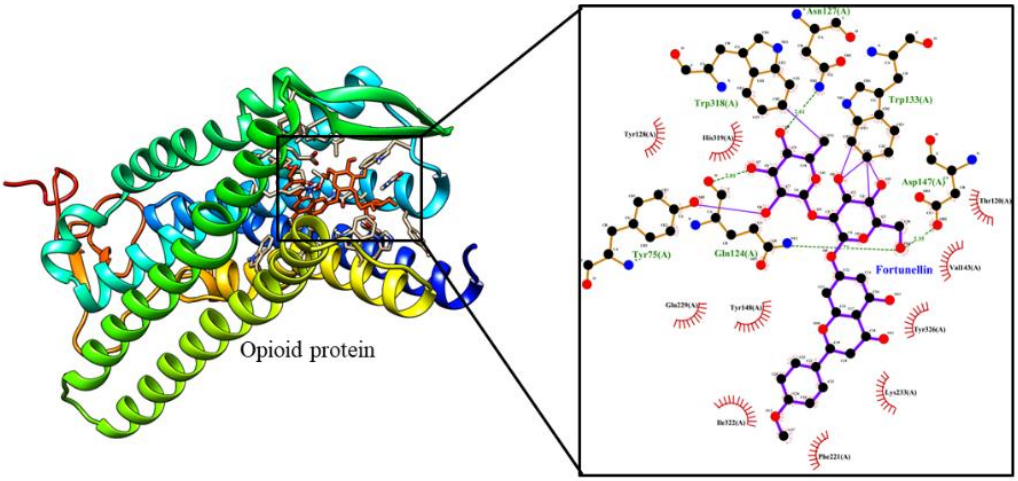


Fig. 4.6b Molecular docking interaction of fortunellin with the opioid receptor. (Left) 3D docking pose showing ligand binding conformation. (Right) 2D interaction map showing hydrogen bonds and hydrophobic contacts with active site residues

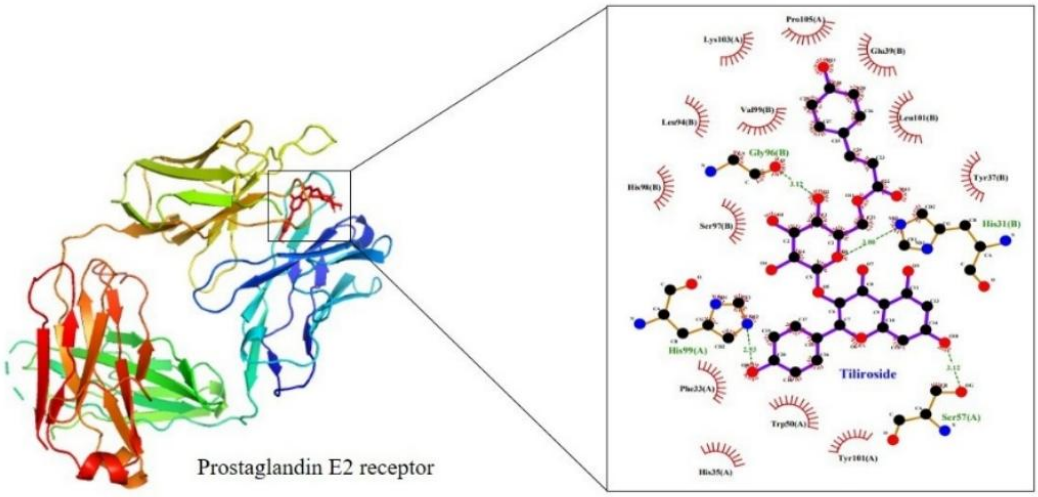


Fig. 4.6c Molecular docking interaction of apigenin-7-O-glucoside with the prostaglandin E2 receptor. (Left) 3D docking pose showing ligand within the binding cavity. (Right) 2D interaction profile highlighting hydrophobic and polar interactions

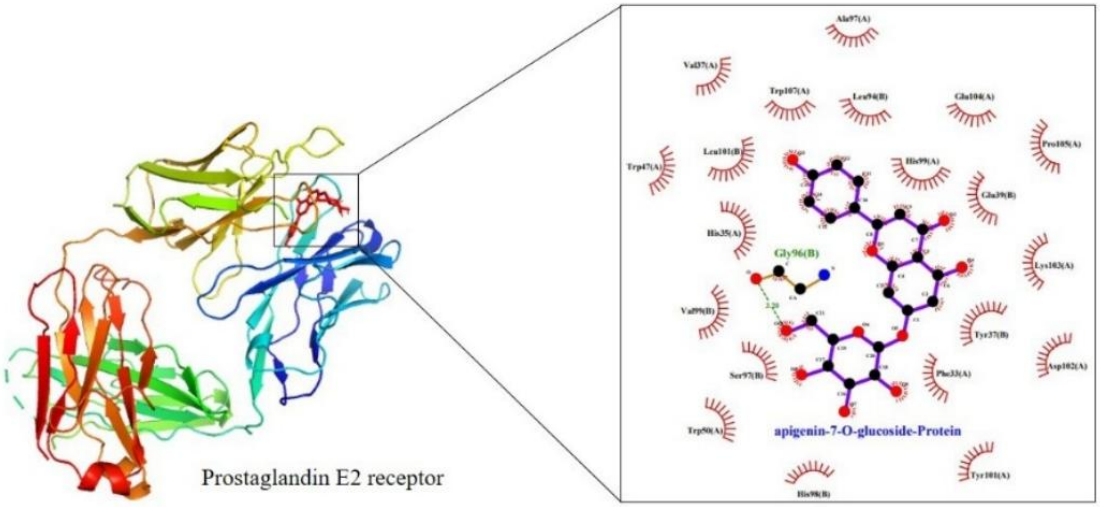


Fig. 4.6d Molecular docking interaction of tiliroside with the prostaglandin E2 receptor. (Left) 3D docking pose of the ligand in the receptor pocket. (Right) 2D interaction diagram showing hydrogen bond network and hydrophobic interactions with surrounding residues

4.4.2.3 Molecular docking of *Myrica esculenta* Buch.-Ham. ex D. Don

Among the tested compounds, NCGC00384811-01 demonstrated the highest binding affinity with a docking score of -9.9 kcal/mol, followed closely by NCGC00179898-01 (-9.8 kcal/mol). Both ligands showed significantly stronger predicted interactions compared to the standard drugs Loperamide (-8.3 kcal/mol) and Atropine (-8.2 kcal/mol), suggesting these phytoconstituents may exert potent anti-diarrhoeal effects via the opioid receptor pathway.

Other compounds such as Etoposide (-9.2 kcal/mol), Catechin gallate (-9.0 kcal/mol), and NCGC00385154-01 (-9.0 kcal/mol) also exhibited strong binding, reinforcing the potential of *M. esculenta* constituents to modulate opioid receptor activity (Table 4c). These interactions are particularly relevant, as opioid receptor agonists reduce intestinal peristalsis and enhance water absorption, mechanisms underlying the action of classic anti-diarrhoeal drugs.

Similarly, strong binding affinities were observed for several *M. esculenta*-derived ligands with the EP2 receptor. The top-performing compounds, 1-(4-Piperidylmethyl)-3-(3-quinolyl)pyrazolo[3,4-d]pyrimidin-4-amine and 5,7-

dihydroxy-6-methoxy-2-phenylchromen-4-one, both achieved a docking score of -9.7 kcal/mol, surpassing those of Loperamide (-8.5 kcal/mol) and Atropine (-8.4 kcal/mol).

Also, NCGC00385154-01 (-9.1 kcal/mol), Corynanthin (-8.9 kcal/mol), Skimmin (-8.9 kcal/mol), and Pinolidoxin (-8.8 kcal/mol) displayed significant binding affinities, suggesting possible EP2 receptor antagonism as shown in Table 4c. This could result in reduced prostaglandin-mediated secretion and inflammation in the intestinal mucosa, key contributors to diarrhoeal symptoms.

Table 4.7 Binding affinity values of *M. esculenta* chemical compounds

Receptor	Ligand	Docking Score (kcal/mol)
Opoid protein	NCGC00384811-01	-9.9
	NCGC00179898-01	-9.8
	Etoposide	-9.2
	Catechin gallate	-9.0
	NCGC00385154-01	-9.0
	Atropine (Std)	-8.2
	Loperamide (Std)	-8.3
EP2-(prostaglandin)	1-(4-Piperidylmethyl)-3-(3-quinolyl)pyrazolo[3,4-d]pyrimidin-4-amine.	-9.7
	5,7-dihydroxy-6-methoxy-2-phenylchromen-4-one	-9.7
	Corynanthin	-8.9
	Pinolidoxin	-8.8
	Skimmin	-8.9
	NCGC00385154-01	-9.1
	Atropine (Std)	-8.4
	Loperamide (Std)	-8.5

The binding interactions of NCGC00384811-01, neodiosmin, 1-(4-piperidylmethyl)-3-(3-quinolyl)pyrazolo[3,4-d]pyrimidin-4-amine, and 5,7-dihydroxy-6-methoxy-2-phenylchromen-4-one with the opioid receptor and the prostaglandin E2 (PGE2) receptor as shown in Table 4.7a-d.

For the opioid receptor, NCGC00384811-01 exhibited strong hydrogen bonding with Asn127(A), Trp133(A), Gly131(A), Ser214(A), and Arg211(A), while hydrophobic interactions involved Val208(A), Tyr326(A), His322(A), and Asp146(A). Neodiosmin formed multiple hydrogen bonds with Asp147(A), Tyr75(A), Tyr326(A), Asn127(A), Trp133(A), and Ser214(A), accompanied by hydrophobic contacts with His319(A), Glu229(A), and Ile276(A).

For the PGE2 receptor, 1-(4-piperidylmethyl)-3-(3-quinolyl)pyrazolo[3,4-d]pyrimidin-4-amine displayed hydrophobic interactions with Leu94(B), Phe33(A), Tyr37(B), Trp50(A), and Leu101(B), and polar contacts with His99(A) and Asp102(A). 5,7-Dihydroxy-6-methoxy-2-phenylchromen-4-one showed hydrophobic interactions with Leu94(B), Tyr37(B), Trp47(A), and Phe33(A), and polar contacts involving His99(A) and Gly96(B).

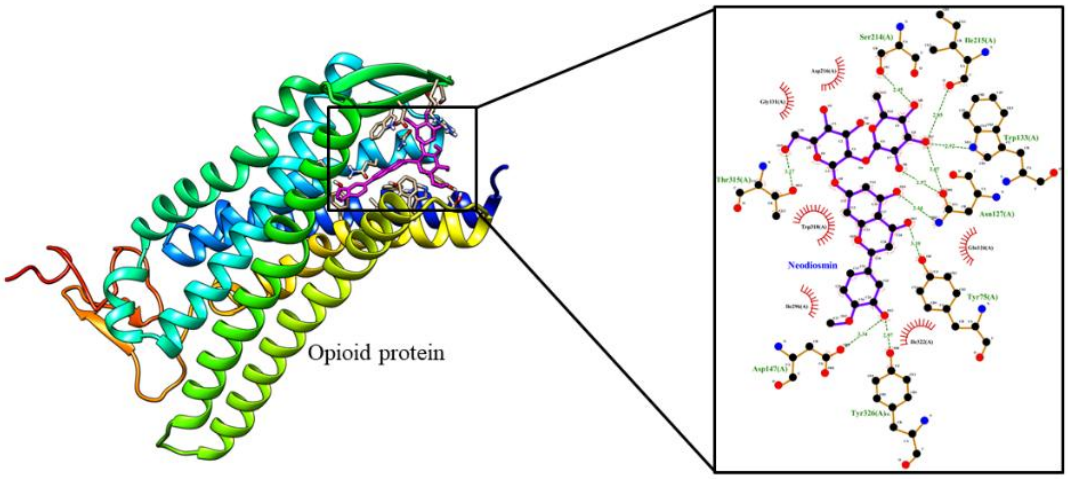


Fig. 4.7a Molecular docking interaction of NCGC00384811-01 with the opioid receptor. (Left) 3D docking pose within the receptor binding pocket. (Right) 2D interaction diagram showing hydrogen bonds and hydrophobic contacts with active site residues

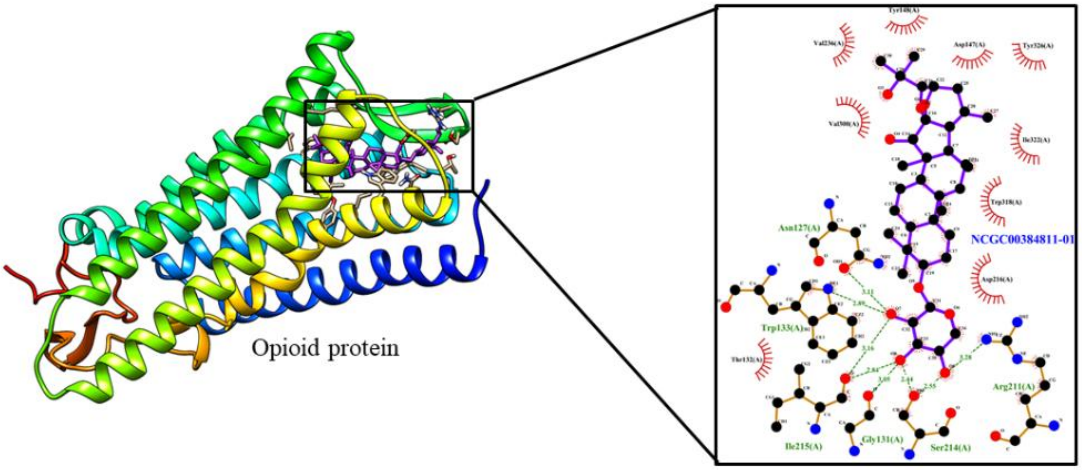


Fig. 4.7b Molecular docking interaction of neodiosmin with the opioid receptor. (Left) 3D docking pose showing ligand orientation. (Right) 2D interaction diagram with key hydrogen bonding and hydrophobic interactions

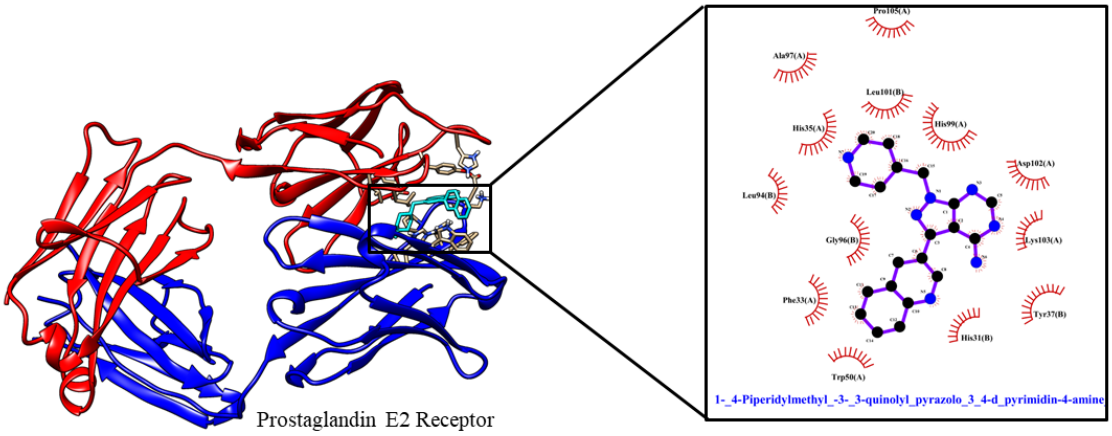


Fig. 4.7c Molecular docking interaction of 1-(4-piperidylmethyl)-3-(3-quinolyl)pyrazolo[3,4-d]pyrimidin-4-amine with the prostaglandin E2 receptor. (Left) 3D pose showing ligand within the binding cavity. (Right) 2D interaction profile highlighting hydrophobic and polar contacts

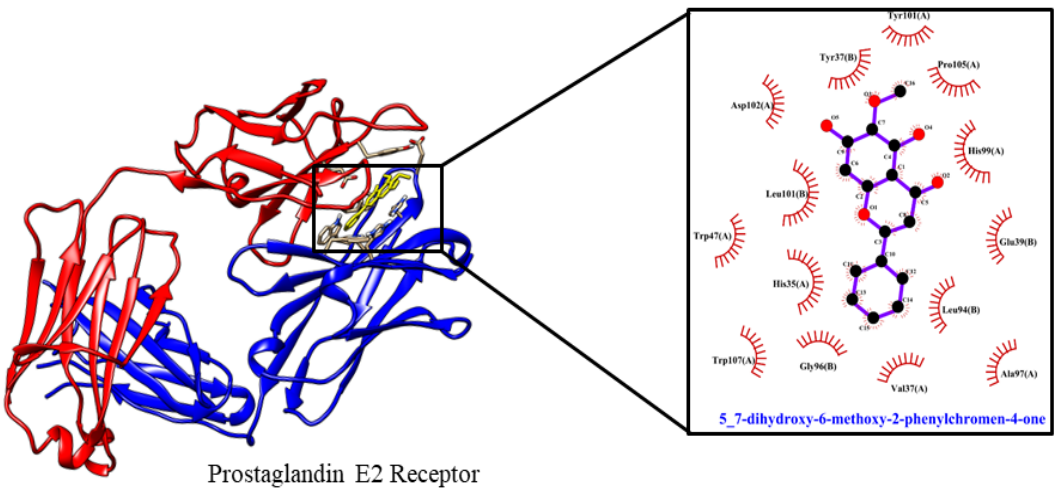


Fig. 4.7d Molecular docking interaction of 5,7-dihydroxy-6-methoxy-2-phenylchromen-4-one with the prostaglandin E2 receptor. (Left) 3D pose of the ligand in the receptor. (Right) 2D interaction diagram showing key residue contacts

4.4.2.4 Molecular docking of *M. malabathricum* L.

For the opioid receptor, the highest binding affinity was observed for goniomedinone (−11.0 kcal/mol), followed closely by 2,4-bis(3-methylbut-2-enyl)-6a,11a-dihydro-6H-[1]benzofuro[3,2-c]chromene-3,9-diol (−10.1 kcal/mol) and thalsimidine (−10.0 kcal/mol). All these compounds outperformed the standard drugs loperamide (−8.3 kcal/mol) and atropine (−8.2 kcal/mol) as shown in Table 4.8.

Against the EP2 prostaglandin receptor, the strongest binding was recorded for 2,4-bis(3-methylbut-2-enyl)-6a,11a-dihydro-6H-[1]benzofuro[3,2-c]chromene-3,9-diol with a docking score of −11.6 kcal/mol, followed by thalsimidine (−9.8 kcal/mol) and goniomedinone (−9.7 kcal/mol). Again, these phytoconstituents demonstrated superior binding compared to the standards loperamide (−8.5 kcal/mol) and atropine (−8.4 kcal/mol) as shown in Table 4.8.

Table 4.8 Binding affinity values of M. malabathricum chemical compounds

Receptor	Ligand	Docking Score (kcal/mol)
Opoid protein	2,4-bis(3-methylbut-2-enyl)-6a,11a-dihydro-6H-[1]benzofuro[3,2-c]chromene-3,9-diol	-10.1
	Goniomedinone	-11.0
	NCGC00373238-02	-9.7
	Thalsimidine	-10.0
	AsiaticAcid	-9.4
	Atropine (Std)	-8.2
	Loperamide (Std)	-8.3
EP2-(prostaglandin)	NCGC00385384-01	-9.4
	Goniomedinone	-9.7
	[(2R,4S,5R)-5-ethenyl-1-azabicyclo[2.2.2]octan-2-yl]-(6-methoxyquinolin-4-yl)methanol	-9.7
	2,4-bis(3-methylbut-2-enyl)-6a,11a-dihydro-6H-[1]benzofuro[3,2-c]chromene-3,9-diol	-11.6
	Thalsimidine	-9.8
	Atropine (Std)	-8.4
	Loperamide (Std)	-8.5

The binding interactions of 2,4-bis(3-methylbut-2-enyl)-6a,11a-dihydro-6H-1-benzofuro[3,2-c]chromene-3,9-diol, goniomedinone, and thalsimidine with two pharmacologically important targetswere—the opioid receptor and the prostaglandin E2 (PGE2) receptor was evaluated in Fig. 4.8a-d.

For the opioid receptor, 2,4-bis(3-methylbut-2-enyl)-6a,11a-dihydro-6H-1-benzofuro[3,2-c]chromene-3,9-diol formed hydrogen bonds with Gln124(A) and Asp147(A), and exhibited hydrophobic contacts with Tyr75(A), Tyr326(A), His322(A), and Trp133(A). Goniomedinone displayed hydrogen bonding with Trp133(A) and Gln124(A), along with hydrophobic interactions involving Ile322(A), Trp318(A), Cys217(A), Asp147(A), and His319(A).

For the PGE2 receptor, 2,4-bis(3-methylbut-2-enyl)-6a,11a-dihydro-6H-1-benzofuro[3,2-c]chromene-3,9-diol bound via hydrogen bonds with His31(B) and Gln39(B), while hydrophobic interactions involved Tyr37(B), Tyr101(A), Phe33(A), and Trp50(A). Thalsimidine engaged in hydrogen bonding with Ser97(B), Gly96(B), and Ser57(A), complemented by hydrophobic interactions with Phe33(A), Tyr101(A), and Trp50(A).

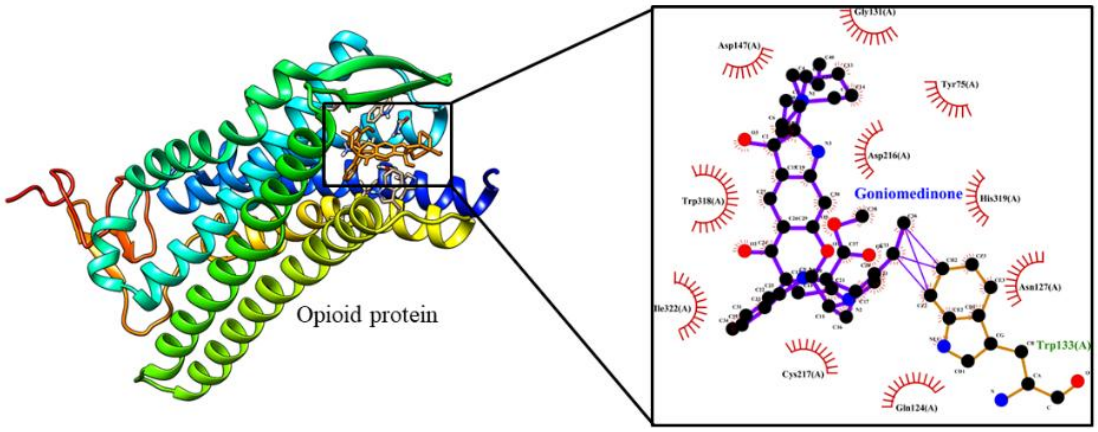


Fig. 4.8a Molecular docking interaction of 2,4-bis(3-methylbut-2-enyl)-6a,11a-dihydro-6H-1-benzofuro[3,2-c]chromene-3,9-diol with the opioid receptor. (Left) 3D docking pose within the receptor binding site. (Right) 2D interaction diagram showing hydrogen and hydrophobic interactions

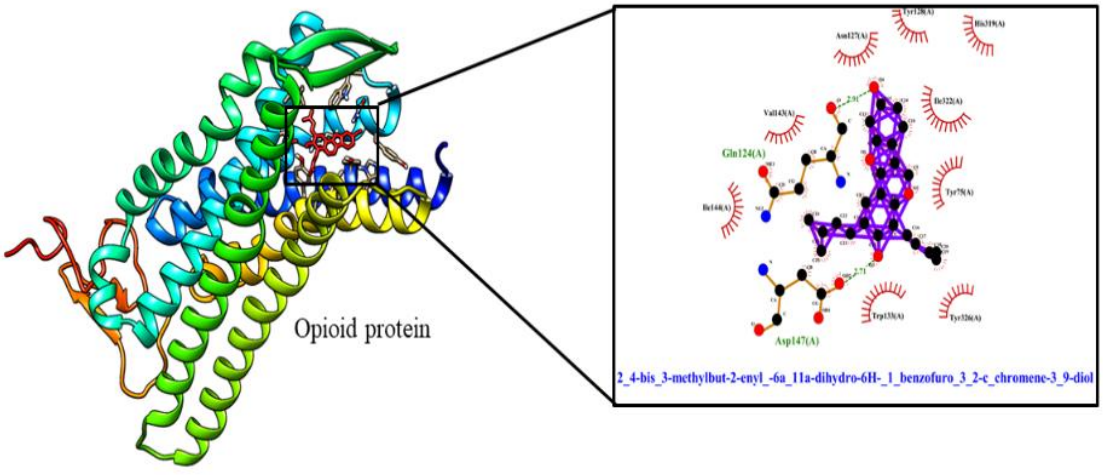


Fig. 4.8b Molecular docking interaction of goniomedinone with the opioid receptor. (Left) 3D docking pose in the active site. (Right) 2D interaction diagram highlighting key binding residues

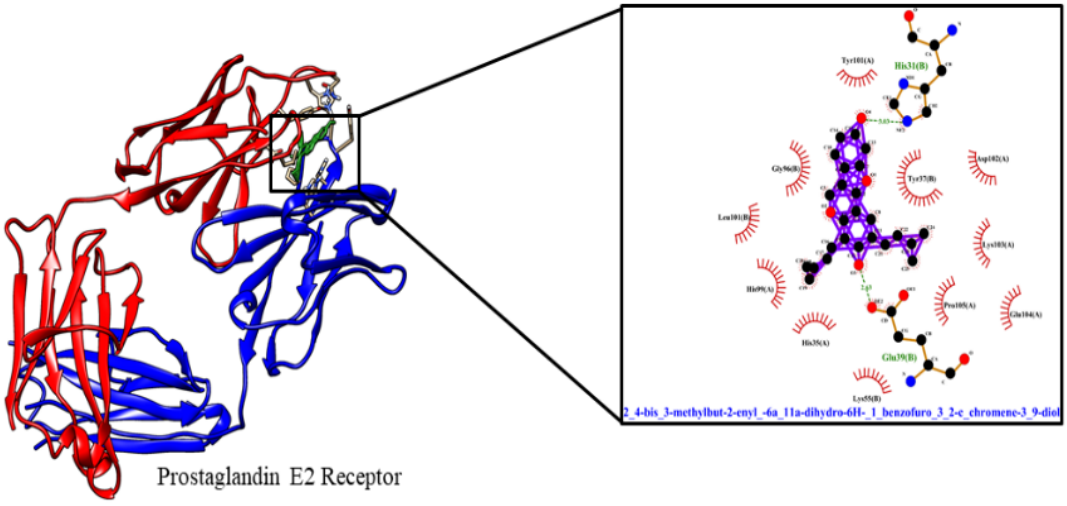


Fig. 4.8c Molecular docking interaction of 2,4-bis(3-methylbut-2-enyl)-6a,11a-dihydro-6H-1-benzofuro[3,2-c]chromene-3,9-diol with the prostaglandin E2 receptor. (Left) 3D docking pose showing the ligand in the binding cavity. (Right) 2D interaction diagram depicting hydrogen bonds and hydrophobic contacts

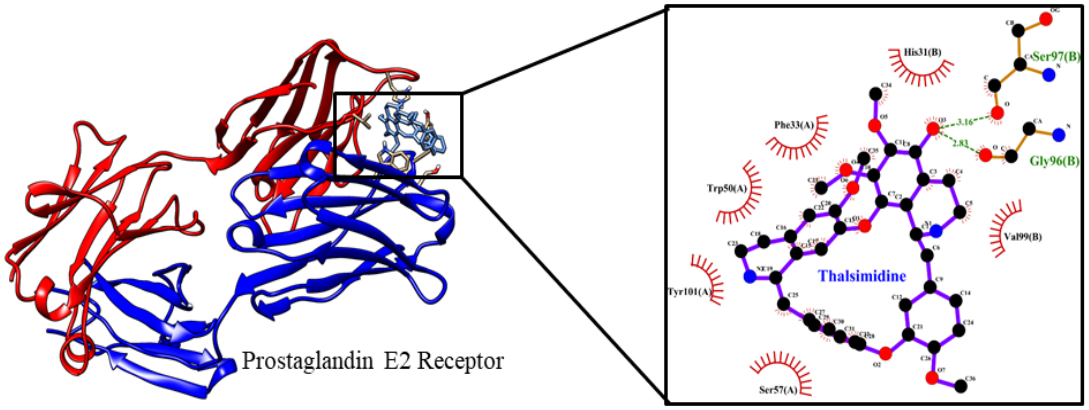


Fig. 4.8d Molecular docking interaction of thalsimidine with the prostaglandin E2 receptor. (Left) 3D docking pose within the receptor binding site. (Right) 2D interaction diagram showing hydrogen bonding and hydrophobic interactions

4.5 Discussions

The LC-MS/LC-HRMS profiling of methanolic extracts from *Dillenia indica, Euphorbia milii, Myrica esculenta,* and *Melastoma malabathricum* revealed a diverse array of bioactive secondary metabolites across both positive and negative electrospray ionisation (ESI) modes. The identification of high-intensity peaks and predominant compounds across each plant extract reflects their phytochemical richness and pharmacological relevance, particularly in the context of their traditional use as anti-diarrhoeal and antimicrobial agents.

In the methanolic extract of *Dillenia indica*, a total of 49 compounds were identified, with 32 detected in positive mode and 17 in negative mode. The predominant classes were flavonoids and alkaloids in positive mode and phenolic acids in negative mode. The base peak in positive mode, Xylobiose (m/z 954.9062 at RT 26.65 min), is a disaccharide that has been implicated in enhancing gut microbial diversity and prebiotic function (Lim et al., 2016). Its abundance may contribute to the plant's known gastroprotective effects. The presence of Peonidin-3,5-O-di-β-glucopyranoside (m/z 619.5624 at RT 21.13 min), an anthocyanin, suggests potent antioxidant and anti-inflammatory properties, potentially mitigating intestinal oxidative stress during diarrhoeal episodes (Kähkönen et al., 1999).

In the negative ion mode, Riboflavin-5′-monophosphate sodium salt hydrate (m/z 455.5143 at RT 23.15 min) emerged as the dominant compound. This phosphorylated form of vitamin B2 is crucial in cellular energy metabolism and has been associated with mucosal protection and the modulation of gut microbial homeostasis (Powers, 2003). Its high abundance further justifies the ethnomedicinal use of *D. indica* in the treatment of gastrointestinal disorders.

A similar pattern was observed in *Euphorbia milii*, with 49 compounds identified (32 in positive mode and 17 in negative mode). The base peak in positive mode was L-Methionine sulfone (m/z 183.1258 at RT 7.11 min), an oxidised sulfur-containing amino acid derivative known for its antioxidative and protective roles against oxidative stress-induced cellular injury (Stadtman et al., 2004). This supports the plant's traditional use in inflammation-linked gastrointestinal conditions.

3-Indolylacetonitrile (m/z 158.19 at RT 1.28 min), a known plant-derived nitrile, has been reported for its antimicrobial and cytotoxic effects, and its presence aligns with the therapeutic role of *E. milii* in treating infections (Talapatra et al., 1984). In the negative mode, Apigenin-7-O-glucoside (m/z 431.2841 at RT 8.35 min) was the most intense peak. Apigenin glycosides are flavonoids known for their anti-diarrhoeal and spasmolytic activities through inhibition of intestinal motility and prostaglandin synthesis (Shukla & Gupta, 2010). The presence of D-glucosamine-6-phosphate and Quercetin-3-rhamnoside further substantiates the anti-inflammatory and gut-barrier protective roles attributed to this plant (Middleton et al., 2000).

The LC-MS analysis of *Myrica esculenta* methanolic extract revealed a remarkably diverse phytochemical profile with 173 compounds identified. The positive mode chromatogram was dominated by Skimmin (m/z 342.11 at RT 14.88 min), a coumarin derivative with reported anti-diarrhoeal and hepatoprotective properties, which often act by downregulating proinflammatory mediators and preserving mucosal integrity (Zang et al., 2013).

Hydroxy matairesinol (m/z 374.00 at RT 15.89 min), a lignan, also showed high intensity and is known for its antioxidant and potential estrogenic activities, which may influence gut permeability and inflammation (Adlercreutz, 2007). In the negative ion mode, the peak corresponding to Keracyanin (m/z 594.15 at RT 25.75 min) points to a high anthocyanin content, which is consistent with the anti-inflammatory and vasoprotective potential of this plant extract (He & Giusti, 2010).

Additionally, compounds such as Arbutin (m/z 271.08) and Peniprequinolone (member of quinoline) were detected by UV absorbance, claiming to have the potential inhibitory effect on UVB-induced photoaging, the anti-inflammatory and antimicrobial activity supporting the high potential of *M. esculenta* as an anti-diarrhoeal plant (Shu & Zang, 2024; Moussa et al., 2025).

In the methanolic extract of *Melastoma malabathricum*, 76 compounds were identified. The most abundant compound in the positive mode was Flavanone (m/z 319.045 at RT 7.64 min), a core structure of many anti-inflammatory and antispasmodic flavonoids. Flavanones have been shown to exert anti-diarrhoeal activity by modulating ion transport and inhibiting smooth muscle contraction (Di Carlo et al., 1999).

N-acetyl-L-ornithine and Luteolin-7,3'-di-O-glucoside were also detected, with the latter known for its potent antioxidant and mucosal protective properties (Lopez-Lazaro, 2009). In the negative ion mode, Thymol-β-D-glucoside (m/z 311.14 at RT, 25.78 min) was the most intense peak, indicating its antimicrobial and antispasmodic properties, which are relevant in the treatment of gastrointestinal infections (Marchese et al., 2017).

Other compounds, such as Villosinol, 9-phenyl-1-(2,4,6-trihydroxyphenyl)nonan-1-one, and chlorinated anthracene derivatives identified via UV detection, are known for their bioactivity in scavenging free radicals and modulating inflammation, thereby providing a pharmacological basis for the plant's use in treating diarrhoea and related gastrointestinal disturbances (George et al., 2015).

All four plants-*Dillenia indica, Euphorbia milii*, *Myrica esculenta,* and *Melastoma malabathricum*-were assessed for their potential anti-diarrhoeal mechanisms through molecular docking against opioid and EP2 (prostaglandin) receptors. The observed docking interactions, combined with phytoconstituents identified via LC-MS, offer significant insights into the therapeutic relevance of these species.

In the present molecular docking study, Peonidin-3,5-O-di-β-glucopyranoside – a type of anthocyanin exhibited the strongest binding to the opioid receptor (–9.8 kcal/mol), surpassing the binding affinity of reference drugs like loperamide. Additionally, flavonoid compounds such as Flavanone, Isorhamnetin-3-rutinoside, and Naringenin showed strong interactions with the EP2 receptor (-0.6 to -9.0 kcal/mol). These interactions support the ethnomedicinal use of *D. indica* in modulating intestinal motility and secretory responses. However, they are all reported to possess antioxidant and anti-inflammatory activity (Cai et al., 2023; Kalai et al., 2020; Xue et al., 2023).

Docking analysis of *Euphorbia milii* revealed significant binding of Fortunellin (-9.2 kcal/mol) and Diosmin (-9.0 kcal/mol) to the opioid receptor, suggesting potential antimotility effects. Furthermore, Apigenin-7-O-glucoside and Tiliroside demonstrated high affinity for the EP2 receptor (-10.2 and -9.2 kcal/mol, respectively). These compounds are all flavonoids and are reported to possess anti-inflammatory, anti-cancer, and anti-diabetic properties. These findings align with the plant's

traditional use in treating diarrhoea (Hu et al., 2023; Huwait & Mobashir, 2022; Zhao et al., 2017).

Myrica esculenta, a financially important staple, also finds use in ethnomedicine for treating diarrhoea, fever, and skin ailments. Molecular docking identified NCGC00384811-01 – a synonym of (1S,3S,9S,12R,14S,17R,19R,21R,22S)-2-Hydroxy-22-(2-hydroxy-2-propanyl)-3,8,8,17,19-pentamethyl-23,24-dioxaheptacyclo-etracos-9-yl beta-L-xylopyranoside in PubChem and NCGC00179898-01 ((1R,3aR,5aR,5bR,9S,11aR)-3a,5a,5b,8,8,11a-hexamethyl-1-prop-1-en-2-yl-1,2,3,4,5,6,7,7a,9,10,11,11b,12,13, 13a, 13b-hexadecahydrocyclopenta[a]chrysen-9-ol) as potent ligands of the opioid receptor (-9.9 and -9.8 kcal/mol, respectively), outperforming standard medications.

For the EP2 receptor, an organic compound such as 1-(4-Piperidylmethyl)-3-(3-quinolyl)pyrazolo[3,4-d]pyrimidin-4-amine was identified. Furthermore, flavonoid compounds, such as 5,7-dihydroxy-6-methoxy-2-phenylchromen-4-one, exhibited strong interactions (-9.7 kcal/mol each), indicating potential for reducing inflammation-related intestinal secretion. Both have been reported for their anti-proliferative and memory impairment activities, but none of the top compounds have been reported for diarrhoea treatment (Liu et al., 2013).

The dual high-affinity interactions of compounds such as NCGC00385154-01 with both opioid and EP2 receptors suggest a multitarget mechanism, potentially offering synergistic anti-diarrhoeal effects by both slowing gut motility and reducing fluid secretion. The remarkable binding strength of novel phytochemicals, especially NCGC00384811-01, highlights their pharmacological relevance and prioritises them as lead candidates in the *in vitro* and *in vivo* validation.

In the docking study of *Melastoma malabathricum*, Goniomedinone, which contains flavonoids, tannins, phenolics, and triterpenes, exhibited the strongest interaction with the opioid receptor (-11.0 kcal/mol). It was followed by Thalsimidine and Chromene derivatives - specifically, 2,4-bis(3-methylbut-2-enyl)-6a,11a-dihydro-6H-[1]benzofuro[3,2-c]chromene-3,9-diol- with binding energies of approximately -10.0 to -10.1 kcal/mol. Notably, the same chromene compound achieved the highest binding score with the EP2 receptor (-11.6 kcal/mol), suggesting a promising inhibitory effect on prostaglandin-mediated secretory pathways. Goniomedinone has

been previously reported only for its antiplasmodial activity, with no records available for the other compounds (Dai & Rakotondraibe, 2018). This study is the first to suggest the potential of these compounds in treating diarrhoea.

The comparative analysis reveals that *M. malabathricum* L. exhibits the strongest binding across both targets, suggesting potent dual inhibitory action through both opioid receptor agonism and EP2 receptor antagonism. The high binding affinities of compounds from *E. milii* and *M. esculenta* also point to their potential as multitarget anti-diarrhoeal agents.

Several compounds demonstrated high binding affinity for the opioid receptor, characterized by strong hydrogen bonding networks with critical residues such as Asp147(A), Asn127(A), Gln124(A), Tyr75(A), Tyr326(A), and Trp133(A). These residues have been previously implicated in ligand stabilisation and μ-opioid receptor activation, which is known to reduce peristalsis and prolong intestinal transit time (Sobczak et al., 2014; Al-Harthy et al., 2022).

Flavonoid glycosides such as kaempferol 3-glucoside-7-rhamnoside, peonidin-3,5-O-di-beta-glucopyranoside, diosmin, fortunellin, and neodiosmin exhibited extensive hydrogen bonding and hydrophobic contacts, suggesting high ligand stability within the binding pocket. These interaction profiles are consistent with previous reports of flavonoids modulating opioid receptors to produce antinociceptive and gut motility-regulating effects (Shapiro et al., 2007).

Non-flavonoid ligands such as goniomedinone and 2,4-bis(3-methylbut-2-enyl)-6a,11a-dihydro-6H-1-benzofuro[3,2-c]chromene-3,9-diol also displayed favourable interactions, predominantly through hydrophobic stabilisation and π–π stacking with aromatic residues, indicating potential opioid receptor modulatory activity. The molecule NCGC00384811-01 formed an extensive hydrogen-bond network, further supporting its strong receptor engagement.

Collectively, these findings suggest that opioid receptor-active ligands in this study may exert anti-diarrhoeal effects by reducing gastrointestinal motility, similar to the mechanism of action of loperamide, but with potential added anti-inflammatory benefits from their polyphenolic nature.

Compounds binding to the PGE2 receptor exhibited interactions indicative of potential receptor antagonism, a mechanism that could reduce prostaglandin-mediated cAMP signalling, intestinal secretion, and inflammation (Kawabata, 2011; Smyth et al., 2009). Key polar contacts frequently involved Gly96(B), His99(A), Ser57(A), and His31(B), along with hydrophobic interactions with Phe33(A), Tyr37(B), Trp50(A), and Leu94(B).

Notable flavonoid and flavonoid-like ligands with strong PGE2 receptor binding included flavanone, naringenin, apigenin-7-O-glucoside, tiliroside, 5,7-dihydroxy-6-methoxy-2-phenylchromen-4-one, and thalsimidine. These compounds formed multiple stabilising interactions, suggesting the ability to block endogenous PGE2 from binding, thereby attenuating inflammatory and secretory responses.

Some ligands, such as 2,4-bis(3-methylbut-2-enyl)-6a,11a-dihydro-6H-1-benzofuro[3,2-c]chromene-3,9-diol, demonstrated dual receptor activity, engaging both the opioid and PGE2 receptors. This dual mechanism may be especially beneficial in diarrhoeal management by combining motility suppression with anti-secretory action. Synthetic ligands such as 1-(4-piperidylmethyl)-3-(3-quinolyl)pyrazolo[3,4-d]pyrimidin-4-amine showed predominantly hydrophobic binding within the PGE2 receptor pocket, supported by polar contacts that enhance stability, potentially offering potent antagonistic activity.

The docking results support the hypothesis that plant-derived polyphenols and related derivatives can serve as multi-target anti-diarrhoeal agents by modulating both motility and secretion pathways. The opioid receptor-binding compounds may act as gut-specific μ-opioid receptor modulators with reduced central side effects due to structural polarity, while PGE2 receptor-binding compounds could offer anti-inflammatory and anti-secretory benefits. Such dual-target modulation aligns with modern pharmacotherapy strategies for diarrhoea, where agents that can act on multiple pathways are preferable for broad-spectrum efficacy (Batiha et al., 2020; Oteiza et al., 2023; Thapa et al., 2012).

Conclusions

The LC-MS analyses of the four medicinal plant extracts revealed a wide range of bioactive compounds, including flavonoids, glycosides, phenolic acids, and lignans.

Many of these metabolites are known to have anti-diarrhoeal, antioxidant, antimicrobial, or anti-inflammatory activities, providing a mechanistic basis for the traditional use of these plants in treating gastrointestinal disorders. These findings also support their potential as candidates for natural drug development.

Several phytochemicals exhibited strong, specific binding to the opioid and EP2 receptors, indicating potential for dual modulation of intestinal motility and secretion. Among these, Goniomedinone, scoring highest binding affinity on opioid receptors (-11.0) and the chromene derivative 2,4-bis(3-methylbut-2-enyl)-6a,11a-dihydro-6H-[1]benzofuro[3,2-c]chromene-3,9-diol demonstrated the highest binding affinity among all tested ligands for the EP2 receptor (–11.6 kcal/mol) and strong binding to the opioid receptor (–10.0 to –10.1 kcal/mol), indicating potential for dual modulation of intestinal motility and also 5,7-dihydroxy-6-methoxy-2-phenylchromen-4-one possessing anti-diarrhoeal property scoring high binding affinity(-9.7).

To the best of our knowledge, this is the first report of this compound in the context of anti-diarrhoeal pharmacology, positioning it as a novel multitarget lead for further experimental validation. These results provide a robust mechanistic rationale for the traditional use of these plants in gastrointestinal disorders and underscore their promise as multi-target agents for natural drug development. Future studies should focus on *in vitro* and *in vivo* validation, pharmacokinetic profiling, and safety assessment to advance these candidates from *Melastoma malabathricum* and *Myrica esculenta* plants toward therapeutic application.

Summary

5. Summary

Overview and Rationale

Diarrhoeal diseases remain a major global health threat, disproportionately affecting vulnerable populations in low- and middle-income countries. Despite significant improvements in sanitation, vaccination, and awareness, these diseases account for substantial morbidity and mortality, especially among children under five. In India, while prevalence has declined nationally, regional disparities persist, with Mizoram reporting comparatively better infant outcomes but high diarrhoea prevalence in older populations.

The emergence of antimicrobial resistance (AMR) in diarrhoeal pathogens such as *Escherichia coli, Shigella,* and *Vibrio* has compounded the challenge, rendering many first-line antibiotics ineffective. This has revitalised interest in ethnopharmacology - the study and validation of traditional medicinal knowledge - as a viable pathway to identify novel, effective, and affordable anti-diarrhoeal agents.

This research bridges the gap between traditional knowledge and modern pharmacology by systematically documenting anti-diarrhoeal plants used by the Mizo tribes, validating their efficacy and safety through *in vitro* and *in vivo* assays, identifying bioactive compounds via LC–MS, and exploring their mechanistic potential using molecular docking (*in silico*).

Ethnobotanical Survey

An extensive ethnomedicinal survey was conducted across 15 village council areas in Champhai district, Mizoram. Two hundred informants, predominantly older and male, provided detailed accounts of medicinal plant use for various ailments.

Diversity: Ninety-three plant species from 53 families were documented, with Euphorbiaceae and Asteraceae most represented.

Plant parts used: Leaves (47%) were the primary material, followed by fruits, bark, and seeds.

Preparation methods: Decoctions dominated (44.2%), reflecting the ease of preparation and perceived potency.

Therapeutic focus: Gastrointestinal diseases, including diarrhoea, dysentery, ulcers, and digestive problems, were recorded as the highest usage reports (940), yielding the highest Informant Consensus Factor (0.962).
Key species: *Curcuma longa*, *Flueggea virosa,* and *Psidium guajava* exhibited the highest Frequency of Citation (FC) and Use Value (UV).

Seventeen species (17) were specifically used for diarrhoea, forming the basis for the following research phase. Cross-validation with the literature was done as some plants were already validated, for instance, the established anti-diarrhoeal effects of *P. guajava* leaf decoction.

Selection of Candidate Plant

From the ethnobotanical dataset, 14 plants traditionally used for treating diarrhoea were selected for phytochemical profiling and antibacterial evaluation; based on that *Dillenia indica*, *Euphorbia milii*, *Myrica esculenta* and *Melastoma malabathricum,* which were further selected for *in vivo* and *in silico* studies.

Phytochemical Profiling

Qualitative Screening of all extracts tested positive for at least two phytochemical classes. Flavonoids and alkaloids were ubiquitous, while glycosides and resins were less common. *E. milii* and *D. indica* displayed the richest phytochemical diversity. While Quantitative Analysis showed as:
Flavonoids: Highest in *E. milii* (81.6 mg QE/g) and *M. malabathricum* 77.28 mg QE/g)
Phenolics: Highest in *D. indica* (68.08 mg GAE/g).
Alkaloids: Highest in *D. indica* (66.25 mg AE/g) and *E. milii* (62.64 mg AE/g)
Tannins: Highest in *E. milii* (61.63 mg TAE/g).

These classes are known for their antimicrobial, anti-inflammatory, and anti-diarrhoeal effects, achieved through mechanisms such as protein precipitation, inhibition of enterotoxins, and modulation of gut motility.

In Vitro Antibacterial Evaluation

Using the disc diffusion method and broth microdilution assays against *E. coli* MTCC 723 and *S. typhimurium* MTCC 3224:

E. milii, D. indica, M. malabathricum, and *M. esculenta* exhibited the largest inhibition zones (>20 mm) for both pathogens.

MIC values ranged from 2.5 – 4.55 mg/mL for the most active extracts, and low MBC values confirmed bactericidal potential.

These results corroborate the traditional claims and indicate the potential to target diarrhoea causing enteric pathogens. These four potent plants were used for further analysis through *in vitro* and *in silico* studies to investigate their anti-diarrhoeal properties.

Toxicity Assessment

Acute toxicity tests at 2000 mg/kg b.w in mice revealed no mortality or behavioural abnormalities and no significant changes in body weight or organ weight.

The sub-acute oral toxicity test also revealed no mortality, no signs of significant changes in body and organ weight ratios. Histopathology studies also showed no signs of toxicity in the organs, including the lungs, liver, kidneys, and spleen.

This establishes a high safety margin for the tested extracts of the four plants.

In-vivo Antidiarrhoeal Efficacy

In castor oil-induced diarrhoea models, extract-treated groups exhibited a significant reduction in the percentage of diarrhoea inhibition and the onset of diarrhoea.

On other hand, *E. coli* 078:H11-induced diarrhoea, the four plant extracts showed a significant reduction in stool frequency and water content. Their blood parameters study also showed no significant effect. Histopathological analysis revealed the restoration of mucosal architecture and goblet cell number, particularly in the *E. milii* and *D. indica* groups, which were comparable to the loperamide standard.

This demonstrated their anti-diarrhoeal activity and validated the traditional usage for treating diarrhoea.

LC–MS Profiling

LC-MS identified a diverse secondary metabolite profile across the four lead plant extracts, including flavonoids such as kaempferol derivatives, tiliroside, diosmin, apigenin-7-O-glucoside, and quercetin. Other classes include terpenoids, phenolic acids, and alkaloids.

The most common phytochemical compounds are Flavonoids, followed by Terpenoids, alkaloids, and phenolic acids. *M. esculenta* showed the highest number of total identified compounds followed by *M. malabathricum* plant.

Some compounds, notably 5,7-dihydroxy-6-methoxy-2-phenylchromen-4-one, Goniomedinone, and 2,4-bis(3-methylbut-2-enyl)-benzofurochromene-diol, were identified for the first time in the field of anti-diarrhoeal pharmacology.

In Silico Mechanistic Insights

Molecular docking targeted the EP2 prostaglandin receptor (involved in fluid secretion) and opioid receptor (modulates gut motility):
Goniomedinone: Highest affinity for opioid receptor (–11.0 kcal/mol), suggesting potential motility suppression.
Chromene derivative: Highest EP2 receptor affinity (–11.6 kcal/mol) with additional opioid activity.

Several flavonoids exhibited multi-target potential, supporting a synergistic therapeutic mechanism. This dual-target binding profile is particularly promising for diarrhoea management, as it addresses both secretion and motility.

Integrated Interpretation

The research confirms that the ethnobotanical leads identified in Mizoram possessed high phytochemical content linked to known anti-diarrhoeal mechanisms and potent antibacterial activity against relevant enteric pathogens. *In vivo* efficacy in restoring normal gut physiology in pathogen-induced diarrhoea and favourable safety profiles in the mouse models. There is also a mechanistic plausibility via dual receptor modulation, as indicated by docking studies.

The novelty lies in:

- First-time reporting of the antidiarrhoeal relevance of specific compounds.
- Comprehensive validation pathway from field ethnobotany to mechanistic computational modelling.
- Identification of multi-target lead candidates.

Conclusions

This study exemplifies the effectiveness of a multidisciplinary strategy in the discovery of novel anti-diarrhoeal agents derived from ethnomedicinal plants of Mizoram, integrating ethnobotanical insights, phytochemical profiling, microbiological assays, and *in silico* receptor-binding analyses. Among the investigated species, *Euphorbia milii* Des Moul. emerged as particularly promising, owing to its high phytochemical diversity, pronounced antimicrobial activity, favourable safety margins, and unique receptor interaction profiles. Furthermore, the detection of bioactive constituents, notably goniomedinone, 5,7-dihydroxy-6-methoxy-2-phenylchromen-4-one, and benzofurochromene derivatives, underscores their potential as multi-target therapeutic agents capable of modulating multiple pathways associated with diarrhoeal diseases. To the best of our knowledge, this is the first report of this compound in the context of anti-diarrhoeal pharmacology, positioning it as a novel multitarget lead for further experimental validation.

Such findings represent a significant advancement in ethnopharmacological drug discovery, bridging the gap between traditional healing practices and modern pharmacological validation. By harmonising indigenous medicinal knowledge with rigorous experimental methodologies, this research not only substantiates the empirical claims of Mizo traditional healers but also enriches the global pharmacopoeia with candidates for safe, effective, and sustainable interventions against diarrhoeal diseases. This is particularly critical in the context of escalating antimicrobial resistance, where plant-derived compounds offer an invaluable reservoir of structurally novel, mechanistically diverse therapeutic options to address pressing public health challenges.

Future Prospect

While the study successfully establishes a scientific basis for selected plants, further work should include:

- Bioassay-guided fractionation to isolate pure active compounds.
- Pharmacokinetic and metabolism studies to assess bioavailability.
- Clinical trials to validate safety and efficacy in human populations.
- Synergistic interaction studies among the identified phytochemicals.

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HSLC	English, Mizo, Science, Social Studies & Mathematics	Mizoram Board of School Education	2011	I

List of Publications

1. **Laldingliani, T. B. C**., Thangjam, N. M., Zomuanawma, R., Bawitlung, L., Pal, A., & Kumar, A. (2022). Ethnomedicinal study of medicinal plants used by Mizo tribes in Champhai district of Mizoram, India. Journal of Ethnobiology and Ethnomedicine, 18(1), 22.
2. Singh, I. R., Pulikkal, A. K., Hnamte, M., **Ladingliani, T.B.C**., & Kumar, A. (2025). Assessment of Anticancer and Antibacterial Activities of Patchouli Essential Oil Nanoemulsion. The Journal of Physical Chemistry B.
3. Kumar, A., Kumar, R., **Laldingliani, T. B. C**., & Thangjam, N. M. (2024). Evaluation of four essential oils of family zingiberaceae and their antifungal activity against plant-pathogenic fungi. Biochemical & Cellular Archives, 24(2).
4. Thangjam, N. M., **Laldingliani, T. B. C**., Kumar, A., & Thangjam, L. M. (2024). Ethnobotanical documentation of medicinal plants used during COVID-19 by the Meitei Community in Manipur, India. Ethnobotany Research and Applications, 29, 1-24.
5. Thangjam, N. M., Kumar, A., **Laldingliani, T.B.C**., & Upreti, D. K. (2022). New distributional records of lichens for the state of Mizoram, Indo-Burma region of India. Trends in Sciences, 19(4), 2573-2573.
6. Zomuanawma, Hnamte, R., **Laldingliangi, T. B. C**., Lalruatsanga, H., & Lalfakzuala, R. (2021). Influence of copper contamination on soil physico-chemical properties, microbial population and enzyme activities. Science and Technology Journal, 9(2). ISSN: 2321-3388.
7. Laldingngheti, B., Bawitlung, M., **Laldingliani, T. B. C.,** Thangjam, N. M., Kumar, A., & Pal, A. (2022). Synergistic effect of Litsea cubeba and Cymbopogon flexuosus essential oils against Staphylococcus aureus: A dermal pathogen. Science and Technology Journal, 10(1). ISSN: 2321-3388.

Book Chapters

1. Kumar, A., Thangjam, N. M., **Laldingliani, T. B. C**., & Singh, C. B. (2025). Lichens in the Himalayan region: Present and future perspectives. In High-value plants: Novel insights and biotechnological advances. Chapter 12, pp. 243–262.
2. Kalita, C.K., Thawmte, L., Lalrammuanpuia., **Laldingliani, T.B.C**., Lalruatpuia., Rakesh, K., Devi, K.N., Upreti, D. K., Kumar, A. (2025). Lichen-based Biomonitoring of Air Pollution and Sustainable Forestry.Sustainable Solutions: Navigating Climate Change and Natural Resources Chapter-2; pp:13-23

Paper Presented (Oral / Poster) in Conference/Seminar/Workshop

International Conference

1. Presented the paper entitled "Anti-diarrhoeal properties of *Dillenia indica* L. through LC-MS and Molecular Docking Analysis: A promising Approach for Drug Validation" at The International Conference on Science and Technology for Innovation and Sustainable Development (STISD-2023) jointly organised by Department of Chemistry and Department of Industrial Chemistry, Mizoram University, Aizawl during 28-30th June, 2023.

2. Presented poster presentation entitled "Evaluation of the nti-diarrhoeal potential of *Dillenia indica* L. and *Euphorbia milii* Des Moul." At the International Conference on Current Trends in Biological Sciences organised by the School of Life Sciences, Mizoram University, during 18-20th March, 2024.

National Conference

1. Presented oral presentation on the topic "Evaluation of Dillenia indica L. bark extract for its anti-diarrhoeal property" at the National Conference on Recent Advances in Plant Biology with Special References to North-East, India, organised by Department of Botany, School of Life Sciences, Mizoram University, Aizawl, Mizoram, India-796004, from April 20-21st,2023.

2. Presented the paper entitled "LC-MS and Molecular docking analysis to validate potential antidiarrhoeal drug from Euphorbia milii Des Moul. and its antioxidant activity" during the National Seminar on Value addition and Marketing of Horticultural Crops for Mizoram under Aroma Mission-III on 15th and 16th May, 2023. Jointly organised by Department of Horticulture, Aromatic and Medicinal Plants (HAMP), Mizoram University, Aizawl and CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, U.P., India.

Workshop

1. Participated as a Resource Person during the training programme entitled "Establishment of seed villages for high-value crops in Tanhril and Reiek village of Mizoram" on March 27 and 29, 2025, organised by the Department of Botany, Mizoram University, Aizawl, in collaboration with ICAR-National Institute for Plant Biotechnology, New Delhi.

PARTICULARS OF THE CANDIDATE

NAME OF CANDIDATE	: TBC LALDINGLIANI
DEGREE	: Ph.D
DEPARTMENT	: Botany
TITLE OF THESIS	: Studies on antidiarrhoeal potential of selected medicinal plants of Mizoram
DATE OF ADMISSION	: 02. 11. 2020

APPROVAL OF RESEARCH PROPOSAL

1. DRC	: 12.04.2021
2. BOS	: 26.04.2021
3. SCHOOL BOARD	: 05.05.2021

MZU REGISTRATION NO.	: 1953 of 2013
Ph.D REGISTRATION NO. & DATE	: MZU/Ph.D./1523 of 02.11.2020
Extension	: NA

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ABSTRACT

STUDIES ON ANTIDIARRHOEAL POTENTIAL OF SELECTED MEDICINAL PLANTS OF MIZORAM

AN ABSTRACT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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MZU REGN. NO.: 1953 of 2013

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DEPARTMENT OF BOTANY

SCHOOL OF LIFE SCIENCES

AUGUST, 2025

STUDIES ON ANTIDIARRHOEAL POTENTIAL OF SELECTED MEDICINAL PLANTS OF MIZORAM

BY

TBC LALDINGLIANI

Department Of Botany

Name of Supervisor

PROF. AWADHESH KUMAR

Submitted

In partial fulfilment of the requirement of the degree of Doctor of Philosophy in Botany of Mizoram University, Aizawl.

Introduction

According to the World Health Organisation (WHO, 2025) diarrhoea is still the world's third leading cause of death under the age of 5. About 1.7 billion cases of childhood diarrhoeal disease were recorded, out of which more than 4 lakhs died each year, even though it is preventable and treatable. So, diarrhoeal diseases remain a significant global health threat.

Also in India, around 2-3 lakhs children die annually, accounting for 13% of all deaths/year under the age of 5. However, the latest National Health Survey (2021) indicates a decline in the rate of diarrhoeal disease from 10% to 7.3%. Some states, such as Bihar, Meghalaya, and Ladakh, were identified as high-prevalence states, while Mizoram is stated as a low-prevalence state. In 2024, the Ministry of Health and Family Welfare launched "The National STOP Diarrhoea Campaign", aiming to achieve zero child deaths due to childhood diarrhoea by distributing ORS-zinc and frontline training as well. However, they are not concerned about the causal organisms of diarrhoea, such as viruses and bacteria. Our body can heal itself minor diarrhoea but when it comes to major to severe, we need to administer a vaccine or antibiotics; however, due to the misuse of antibiotics, the emergence of drug-resistant pathogens poses a significant challenge in managing diarrhoeal diseases. Multi-drug-resistant strains are widely prevalent in cases of diarrhoea, with resistance to inexpensive first-line antibiotics being particularly prevalent.

As a result, renewed interest in phytotherapy *i.e* using plants as medicine, has gained significant backing from global health bodies, such as the World Health Organisation, which promotes the incorporation of ethnopharmacological knowledge into contemporary medical practices. However, both the WHO and regulatory bodies, such as the Food and Drug Administration (FDA), stress the importance of thorough scientific testing to verify the safety, effectiveness, and correct dosages of these natural treatments.

Therefore, this study emphasises ethnopharmacology as a viable pathway to identify novel, effective, and affordable anti-diarrhoeal agents by bridging the gap between traditional knowledge and modern pharmacology.

Objectives:

- Ethnomedicinal study of medicinal plants used by Mizo tribes in Champhai district of Mizoram, India
- Phytochemical profiling and *in vitro* anti-diarrhoeal evaluation of selected medicinal plants against *Escherichia coli* and *Salmonella typhimurium.*
- Safety and therapeutic assessment of selected medicinal plant extracts in experimental models of diarrhoea.
- LC-MS-based phytochemical characterisation and *in silico* insights into novel dual-target anti-diarrhoeal agents.

Based on the objectives, the whole work has been divided into four chapters.

Chapter 1: An extensive ethnomedicinal survey was conducted in Champhai District among the Mizo people. A random face-to-face method was used to interview at least 16 people from each village, and all the medicinal plants they had used for treating various diseases were recorded. From that, the quantitative analysis, such as Use value (UV), Frequency of citation (FC), Relative importance (RI), cultural value (CV), and informant consensus factor (Fic), was estimated for each of the plants they had mentioned. The survey data revealed information about which plants have been used to treat diarrhoeal diseases.

This survey comprises 200 respondents from 15 villages, with the majority being elderly individuals. This suggests that ethnomedicinal knowledge is decreasing in the younger generation, and men tend to have a better understanding than women. 93 plant species from 53 families were recorded, in which Asteraceae and Euphorbiaceae are commonly utilised, while Orchidaceae and Poaceae were underutilised. In terms of plant parts used and mode of preparation, leaves were the most common part used and were mainly utilised in the form of decoction, followed by paste and raw form. Also, among the plant habitats, herbs were commonly used followed by trees and shrubs. In the quantitative analysis, *Curcuma longa, Flueggea virosa* and *Psidium guajava* exhibited the highest Frequency of Citation (FC) and Use Value (UV). The most culturally valued (CVs) plant species is *Curcuma longa*, and the Relative importance (RI) plant species is *Phyllanthus emblica*.

Whereas, Gastrointestinal diseases score the highest Informant Consensus Factor according to the informants' ailments mentioned, illnesses were categorised into 16 categories. Out of which gastrointestinal diseases, such as diarrhoea, dysentery, and digestive problems, have the highest use report (UR-940), with 42 plant species being reported. From that, 17 plant species were specifically used for treating diarrhoea, and these plants were selected for cross-validation with the available literature. Because, for instance, among the plants listed here, *Psidium quajava* has the highest Frequency of citation and use value. However, this plant has already been widely studied for its anti-diarrhoeal properties. So, it was excluded from this study. Therefore, a literature review was conducted, and 14 anti-diarrhoeal plants were selected to serve as plant samples for the next chapter, examining their anti-diarrhoeal properties through an *in vitro* study.

Chapter 2: Through the previous survey and with the help of the literature review, these 14 plants were initially selected for the preliminary screening of anti-diarrhoeal plant activity. Firstly, the plants were collected from different places of Mizoram and all plants were identified using standard botanical references by the Botanical Survey of India, Shillong and cross-checked with the only accepted botanical names by 'The World Flora Online (WFO), an open-access database. After that, a crude extract was obtained using a cold maceration process using methanol as a solvent. Then, the qualitative and quantitative phytochemical analyses were conducted. The zone of Inhibition of both pathogenic bacteria was used for screening the activity of all the plant samples at 500mg/ml. After that, the minimum inhibition concentration and minimum bactericidal concentration were estimated.

Qualitative analysis confirmed the presence of at least two phytochemical classes in all species, with flavonoids and alkaloids most frequently represented, while glycosides and steroids were the least represented phytochemicals. Quantitative results revealed that *Euphorbia milii* contained the highest flavonoid (81.6 ± 6.04 mg QE/g) and tannin levels (61.63 ± 1.06 mg TAE/g), while *Dillenia indica* exhibited the highest phenolic (68.08 ± 1.73 mg GAE/g) and alkaloid (66.25 ± 0.76 mg AE/g) contents. In antibacterial assays, *E. milii, D. indica, Melastoma malabathricum,* and *Myrica esculenta* demonstrated significantly larger zones of inhibition against *E. coli* (20–25

mm) and *S. typhimurium* (20–24 mm) compared to other species tested. MIC and MBC results further supported these findings, with *E. milii* recording the lowest MIC (3.25 mg/ml for *E. coli*) (lower the MIC values, higher is the inhibition) and MBC (6.51 mg/ml), while *Curcuma caesia* and *Mikania micrantha* showed poor efficacy with MIC/MBC values exceeding 80 mg/ml and 160 mg/ml, respectively.

Collectively, findings revealed *E. milii* and *D. indica* as phytochemically rich and highly active against diarrhoea-causing bacteria, followed by *M. esculenta* and *M. malabathricum*. In contrast, *C. caesia* exhibited weak potential, likely due to the volatility of its aromatic compounds. Based on these results, the four most potent species were selected for subsequent *in vivo* and *in silico* investigations.

Chapter 3: The four most potent plants from the previous experiment, such as *D. indica, E. milii, M. esculenta* and *M. malabathricum* were selected for this study to test their safety profile with acute and sub-acute toxicity test, and their anti-diarrhoeal activity on mice by inducing diarrhoea through castor oil and pathogen *E. coli* 078:H11.

Acute toxicity tests at 2,000 mg/kg body weight revealed no mortality or significant behavioural or organ changes. In contrast, sub-acute administration (200 and 600 mg/kg for 28 days) showed no systemic toxicity, aside from mild, reversible variations in certain organ weights and haematological parameters. The biochemical parameters showed mild inflammatory and immune response, which remained within physiological limits. Histopathology study also demonstrated protective effects on the organ's architecture, with no observable pathological lesions in all four plants.

In castor oil-induced diarrhoea, all four extracts significantly delayed onset and reduced fecal output, with *E. milii* displaying the most excellent efficacy (54.9% inhibition). In *E. coli*-induced diarrhoea, extracts attenuated diarrhoeal severity, reduced elevated WBC counts, spleen weights, and pro-inflammatory cytokines (TNF-α, IL-6), while maintaining thymus stability. Histopathological examination confirmed the protective effects on the colonic mucosa, with greater preservation of goblet cells at higher doses.

Among the tested plants, *E. milii* emerged as the most promising candidate, exhibiting strong anti-inflammatory, immunomodulatory, and anti-diarrhoeal properties comparable to those of the standard drug loperamide.

Chapter 4: In this chapter, the four plant extracts were analysed by LC-MS at Cytogene, Lucknow to identify phytochemicals, which were then used as ligands for molecular docking with EP2 and opioid receptors implicated in diarrhoea. Docking was performed using Autodock Vina and validated by binding scores (ΔG), where higher negative values indicated stronger interactions. Protein–ligand complexes were further examined for molecular interactions using PyMOL and Chimera, and hydrophobic interactions were analysed with LigPlus software.

LC-MS identified a diverse secondary metabolite profile across the four lead plant extracts, including flavonoids such as kaempferol derivatives, tiliroside, diosmin, apigenin-7-O-glucoside, and quercetin. Other classes include terpenoids, phenolic acids, and alkaloids. The most common phytochemical class of compounds were Flavonoids, followed by Terpenoids, alkaloids, and phenolic acids. *M. esculenta* showed the highest number of total identified compounds, followed by *M. malabathricum* plant.

Some compounds, notably 5,7-dihydroxy-6-methoxy-2-phenylchromen-4-one, Goniomedinone, and 2,4-bis(3-methylbut-2-enyl)-benzofurochromene-diol, were identified for the first time in the field of antidiarrhoeal pharmacology.

Molecular docking targeted the EP2 prostaglandin receptor, which is involved in fluid secretion, and the opioid receptor that modulates gut motility. Goniomedinone showed the highest affinity for opioid receptor (–11.0 kcal/mol), suggesting potential motility suppression, while the chromene derivative showed the highest EP2 receptor affinity (–11.6 kcal/mol) with additional opioid activity.

Several flavonoids exhibited multi-target potential, supporting a synergistic therapeutic mechanism. This dual-target binding profile is particularly promising for diarrhoea management, as it addresses both secretion and motility.

Overall, this study highlights a multidisciplinary approach in discovering new antidiarrhoeal agents from Mizoram's ethnomedicinal plants, combining ethnobotany, phytochemistry, microbiology, and *in silico* analysis. *Euphorbia milii* Des

Moul. stood out due to its rich phytochemicals, antimicrobial effects, safety, and receptor profiles. The discovery of bioactive compounds like goniomedinone, 5,7-dihydroxy-6-methoxy-2-phenylchromen-4-one, and benzofurochromene derivatives emphasises their potential as multi-target therapies for diarrhoeal diseases. This is the first report of the relevance of this compound in antidiarrhoeal pharmacology, marking it as a promising lead for further investigation.