

**MOLECULAR CHARACTERISATION AND EVALUATION
OF BIOACTIVE COMPOUNDS OF SELECTED MEDICINAL
PLANTS FROM FABACEAE IN MIZORAM**

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY**

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**DEPARTMENT OF BOTANY
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In partial fulfilment of the requirement of the Degree of Doctor of Philosophy in
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CERTIFICATE

This is to certify that the thesis entitled, “**Molecular Characterisation and Evaluation of Bioactive Compounds of Selected Medicinal Plants From Fabaceae in Mizoram,**” submitted by **Laldinliana Khiangte (MZU/Ph.D./1548 of 18.11.2020)** 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 and guidance.

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
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I **LALDINLIANA KHIANGTE**, 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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PREFACE

Plants have served as the foundation of human healthcare for millennia, representing abundant reservoirs of diverse bioactive molecules including alkaloids, flavonoids, glycosides, lignins, phenolic compounds, and tannins. These secondary metabolites, particularly flavonoids and phenolic compounds prevalent in fruits, vegetables, and medicinal or aromatic plant species, possess powerful antioxidant capabilities that help neutralize oxidative stress and support overall wellness. The therapeutic potential of these bioactive molecules originates from their complex chemical structures and diverse biological activities, encompassing anti-inflammatory, antimicrobial, anticancer, and neuroprotective effects. These naturally occurring compounds have established plant-derived products as integral components of both traditional healing systems and contemporary medical approaches.

The global reliance on plant-based therapeutic systems reflects profound recognition of medicinal plants as irreplaceable components of contemporary healthcare. Approximately 85% of the world's population depends on plant-derived medicines for primary healthcare, while approximately 80% of synthetic pharmaceutical drugs originate from natural plant sources, underscoring the irreplaceable role of plant secondary metabolites in modern drug discovery and development. This global therapeutic dependence is particularly evident in developing nations where traditional medicine practices remain culturally significant and economically accessible, representing centuries of accumulated indigenous knowledge and empirical validation.

Despite this widespread therapeutic utilisation, many medicinal plants remain scientifically under-characterised, particularly those utilised by indigenous and traditional communities in biodiversity-rich regions such as Northeast India. Authentic species identification, comprehensive phytochemical profiling, and rigorous bioactivity assessment are critical prerequisites for validating traditional claims and advancing promising candidates toward pharmaceutical development. Molecular authentication technologies, particularly DNA barcoding, have emerged as

powerful tools for accurate species identification and discrimination at phylogenetic levels, addressing limitations inherent in morphological taxonomy and enabling systematic exploration of medicinal plant biodiversity.

The Fabaceae family, commonly called the legume, pea, or bean family, represents the third-largest plant family in terms of species diversity, containing approximately 765 genera and 19,500 known species, and is recognized as one of the most economically and ecologically important plant families. Members of this family demonstrate remarkable morphological diversity, ranging from huge rainforest trees and woody lianas to desert shrubs, ephemeral herbs, and herbaceous climbers. Across traditional medicine systems in Africa, Asia, and other regions, Fabaceae species have been extensively employed for managing diverse health conditions including gastrointestinal disorders, infectious diseases, reproductive health conditions, and respiratory ailments. Beyond their ecological and cultural significance, Fabaceae species demonstrate considerable pharmaceutical potential, with numerous members producing complex secondary metabolite profiles characterised predominantly by flavonoids, terpenoids, tannins, and phenolic compounds that confer diverse biological activities including anticancer, antioxidant, antimicrobial, and anti-inflammatory effects.

Mizoram, a state in the Northeastern region of India represents a biodiversity hotspot with rich traditional knowledge systems and endemic plant species of substantial medicinal value. However, many Fabaceae species from this region remain scientifically under-characterised with regard to their bioactive potential, molecular authentication, and phytochemical composition. To address this knowledge gap, the present study represents the first comprehensive systematic evaluation of methanolic pod extracts from five Fabaceae species- *Parkia timoriana*, *Leucaena leucocephala*, *Albizia odoratissima*, *Entada phaseoloides*, and *Senna siamea* collected from Aizawl, Mizoram, India. This investigation integrates molecular authentication and phylogenetic characterisation through DNA barcoding with qualitative and quantitative phytochemical characterisation using Fourier-transform infrared spectroscopy and Liquid chromatography-mass spectrometry, complemented by in vitro evaluation of antioxidant, antibacterial, and anticancer

activities alongside in silico molecular docking analysis targeting bacterial FtsZ proteins and human AKT1 kinase to validate therapeutic mechanisms. This integrated approach aims to provide scientific validation for traditional medicinal uses while identifying promising candidates for further pharmaceutical development and contributing to the global effort to harness plant biodiversity for drug discovery.

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ABBREVIATIONS

%	Percent
°C	Degree Celsius
±	More or less
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
CTAB	Cetyl trimethylammonium bromide
dNTPs	Deoxynucleotide triphosphates
DPPH	2,2-diphenyl-1-picrylhydrazyl
EGFR	Epidermal growth factor receptor
FBS	Fetal bovine serum
FTIR	Fourier-transform infrared spectroscopy
GAE	Gallic acid equivalents
GTR+G+I	General Time Reversible model with gamma distribution and proportion of invariant sites
HCT-116	Human colon carcinoma cells
HeLa	Henrietta Lacks cervical cancer cells
HepG-2	Human liver carcinoma cells
IC₅₀	Half-maximal inhibitory concentration
ITS2	Internal transcribed spacer 2
LC-MS	Liquid chromatography-mass spectrometry
matK	Maturase K gene
MCF-7	Human breast cancer cells
MEGA	Molecular Evolutionary Genetics Analysis
MEM	Minimum Essential Medium
mg	Milligram
mL	Millilitre
MHB	Mueller-Hinton broth
MIC	Minimum inhibitory concentration
ML	Maximum Likelihood

MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MZU	Mizoram University
NCBI	National Center for Biotechnology Information
PCR	Polymerase chain reaction
QE	Quercetin equivalents
rbcL	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene
RNaseA	Ribonuclease A
ROS	Reactive oxygen species
rpm	Revolutions per minute
rpoC1	Chloroplast RNA polymerase gene
RSA	Radical scavenging activity
SEM	Standard error of the mean
TE	Tris-Ethylene tetra acetic acid buffer
TFC	Total flavonoid content
TPC	Total phenolic content
trnH-psbA	Transfer RNA-photosystem II P700 chlorophyll A-binding protein spacer region
UV-Vis	Ultraviolet-visible spectrophotometer
w/v	Weight in volume
µg	Microgram
µL	Microlitre

Chapter 1

Introduction

1.1. Medicinal Plants

Plants represent abundant reservoirs of diverse bioactive molecules, encompassing alkaloids, flavonoids, glycosides, lignins, phenolic compounds, and tannins. The global reliance on plant-based therapeutic systems reflects the profound recognition of medicinal plants as irreplaceable components of contemporary healthcare. Medicinal plants are particularly essential in developing nations, where traditional medicine practices remain culturally significant and economically accessible (Phuyal et al., 2020). The quantitative significance of this reliance is substantial; it is estimated that approximately 80-85% of the world's population depends on plant-derived medicines for primary healthcare (Fitzgerald et al., 2020). Moreover, approximately a quarter of all modern pharmaceutical drugs originate from natural plant sources, underscoring the irreplaceable role of plant secondary metabolites in modern drug discovery and development (Fitzgerald et al., 2020; Thalia, 2025). This enduring legacy underscores the importance of plant-derived products as essential components of both traditional healing systems and modern medical practices.

Mizoram, located within Northeast India is a key part of the Indo-Burma biodiversity hotspot and stands out as a unique repository of traditional knowledge. This region is one of the most biologically diverse areas globally, renowned for its immense flora and rich ethnobotanical heritage. Indigenous tribes such as the Mizo, Mara, and Lai have relied on local plant resources for centuries to treat ailments ranging from dermatological infections to gastrointestinal disorders (Laldingliani et al., 2022). Ethnomedicinal surveys have documented over 300 medicinal plant species used by various ethnic groups in Mizoram, yet a significant portion of this knowledge remains anecdotal and confined to local healers. However, this rich biodiversity is increasingly threatened by anthropogenic pressures, making the

scientific documentation and validation of medicinal plants an urgent priority to safeguard both the cultural and biological heritage for future generations (Laldingliani et al., 2022).

1.2. The Fabaceae Family

Fabaceae, commonly known as the legume, pea, or bean family, is the third-largest family of flowering plants, comprising approximately 765 genera and over 19,500 species (Zayed et al., 2016; Siddiqui et al., 2025). With a near-cosmopolitan distribution, absent only in Antarctica, Fabaceae is recognized as one of the most economically and ecologically significant plant families. It exhibits remarkable morphological diversity, with members ranging from massive rainforest trees and woody lianas to desert shrubs, ephemeral herbs, and herbaceous climbers (Zhao et al., 2021; Siddiqui et al., 2025). Ecologically, the Fabaceae family is distinguished by its unique capacity for biological nitrogen fixation through symbiotic associations with soil rhizobia. This process is essential for sustaining global soil fertility, enhancing ecosystem resilience, and reducing dependence on synthetic fertilizers, which positions Fabaceae as a cornerstone of sustainable agriculture (Del papa et al., 2024). Economically, legumes rank second only to cereals in agricultural importance and serve as a primary source of affordable plant-based protein. They contribute significantly to global food security while also providing essential fodder, timber, and industrial raw materials (Del papa et al., 2024; Kokkini et al., 2025). This combination of ecological function and economic utility underscores the pivotal role of the Fabaceae family in human societies.

Beyond their agricultural significance, Fabaceae species represent an extensive reservoir of therapeutic agents. In traditional medicine systems across Africa, Asia, and the Americas, numerous members of this family are used to manage a wide range of health conditions, including gastrointestinal disorders, infectious diseases, reproductive health issues, respiratory ailments, and inflammatory conditions (Maroyi, 2023; Sowe et al., 2025). Their therapeutic potential is largely attributed to complex secondary metabolite profiles that are predominantly

characterised by flavonoids, terpenoids, tannins, alkaloids, and phenolic compounds (Ahmad et al., 2016; Sowe et al., 2025). These metabolites, while important for plant defence, also exhibit significant pharmacological properties in humans, including anticancer, antioxidant, antimicrobial, and anti-inflammatory activities (Morante-Carriel et al., 2024; Alum et al., 2025). In the context of Northeast India, and specifically Mizoram, this family is well-represented and highly valued, with numerous wild and cultivated species serving as dual-purpose resources, providing essential nutrition while simultaneously functioning as primary therapeutic agents in local ethnomedicine.

1.3. Molecular Authentication of Plants through DNA Barcoding

The accurate identification of plant species is a fundamental challenge in botany, conservation, and global trade, where misidentification can have far-reaching ecological and economic consequences. Traditional taxonomic methods, which rely solely on morphological traits, are increasingly insufficient due to phenotypic plasticity, the existence of cryptic species, and the frequent loss of diagnostic features when plant materials are processed, dried, or fragmented (Hyder et al., 2024; Zhou et al., 2025). This taxonomic ambiguity creates significant risks that range from the unintentional loss of biodiversity to the adulteration of plant-based supply chains with inferior or unrelated species. To address these universal challenges, DNA barcoding has emerged as a robust molecular tool for the precise authentication of plant biodiversity. This technique utilizes short and standardized DNA regions, such as *ITS2*, *rbcL*, and *matK*, to discriminate species with high precision by functioning effectively as a stable "genetic fingerprint" regardless of the specimen's physical state or developmental stage (Anuja et al., 2024). The integration of DNA barcoding into systematic protocols ensures the traceability of biological resources, detects substitution, and validates botanical origins, thereby establishing a rigorous scientific basis for the reliable classification and safe utilization of plant resources globally (Anuja et al., 2024; Zhou et al., 2025).

1.4. Importance of Evaluation of Bioactive Compounds from Plant Extracts

Plants function as sophisticated chemical factories by synthesizing a vast array of secondary metabolites, including alkaloids, flavonoids, glycosides, phenolic compounds, and tannins, which serve as essential defense mechanisms against environmental stressors, herbivory, and microbial pathogens (Erb & Kliebenstein, 2020; Khan et al., 2025). These bioactive molecules represent a critical reservoir for modern drug discovery as they offer unique structural diversity and potent biological activities that underpin both traditional medicine and contemporary pharmaceutical development (Erb & Kliebenstein, 2020; Khan et al., 2025). The efficacy of retrieving these therapeutic agents is fundamentally dependent on the extraction solvent, with methanol widely recognized as a superior solvent for ensuring comprehensive phytochemical recovery (Truong et al., 2019). Due to its amphiphilic nature, which possesses both a polar hydroxyl group and a non-polar methyl group, methanol effectively solubilizes a broad spectrum of compounds ranging from hydrophilic phenolics to lipophilic steroids. This often yields higher concentrations of total bioactive constituents compared to other solvents like water or chloroform (Nobossé et al., 2014; Truong et al., 2019). This enhanced extraction efficiency directly correlates with superior biological outcomes, as methanolic extracts frequently exhibit stronger antioxidant and antimicrobial activities because the solvent efficiently penetrates plant cellular matrices to release bound metabolites (Borges et al., 2020). Consequently, methanol is the preferred choice for initial phytochemical screening, providing a "metabolic fingerprint" that is essential for identifying novel drug candidates and validating the therapeutic potential of medicinal plants (Truong et al., 2019).

1.5. Antioxidant Activity of Plant Extracts

Oxidative stress, characterised by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms, has been identified as a root cause for the development of numerous chronic conditions, including cardiovascular diseases, neurodegenerative disorders, and cancer

(Chaudhary et al., 2023). While the human body possesses endogenous antioxidant systems, they are often insufficient to counteract severe oxidative damage. Supplementation with exogenous antioxidants, particularly those derived from medicinal plants, has emerged as a promising therapeutic strategy (Kasote et al., 2015). Plant-derived secondary metabolites, especially phenolic compounds and flavonoids, act as potent free radical scavengers and chain-breaking antioxidants. They function by donating hydrogen or electrons to unstable radicals, thus neutralizing them and preventing cellular damage (Chaudhary et al., 2023). The quantitative assessment of this scavenging potential through *in vitro* assays provides essential data on a plant's capacity to mitigate oxidative stress and support long-term health.

1.6. Antibacterial Activity of Plant Extracts

The rapid emergence of multi-drug resistant (MDR) bacterial pathogens represents one of the most urgent global health threats of the 21st century. The widespread misuse of conventional antibiotics has accelerated the evolution of resistance mechanisms, rendering many standard treatments ineffective and creating a critical need for alternative therapeutic agents (El-Saadony et al., 2025). Medicinal plants offer a vast and largely untapped reservoir of antimicrobial compounds that often function through novel mechanisms of action, distinct from those of synthetic antibiotics (Vaou et al., 2021). These plant-based antimicrobials can inhibit bacterial growth by disrupting cell membranes, interfering with protein synthesis, or targeting virulence factors such as biofilm formation (Vaou et al., 2021). Systematic screening of plant extracts against pathogenic strains is therefore vital not only for validating traditional remedies but also for identifying lead compounds that can act as resistance-modifying agents, potentially restoring the efficacy of existing antibiotics when used in combination (Vaou et al., 2021; El-Saadony et al., 2025).

1.7. Phytochemical Characterisation using LC-MS and FTIR

The complex chemical diversity of medicinal plants requires sensitive and high-throughput analytical platforms for comprehensive profiling. Metabolic fingerprinting using Fourier-transform infrared (FTIR) spectroscopy and Liquid chromatography-mass spectrometry (LC-MS) provides a detailed snapshot of a plant's chemical constitution (Umar et al., 2023). FTIR spectroscopy offers a rapid, non-destructive method for identifying functional groups and characterizing the overall chemical variation between samples, which is particularly valuable for establishing a preliminary chemical signature for the ethnomedicinal Fabaceae species of Mizoram (Ralte et al., 2022; Umar et al., 2023). Complementing this, LC-MS serves as a powerful tool for the separation, identification, and quantification of individual secondary metabolites, even those present in trace amounts (Waris et al., 2022). By combining the broad functional group analysis of FTIR with the precise molecular identification of LC-MS, this study aims to create a robust chemical profile for medicinal plants from the Fabaceae family. This integrated approach enables the direct correlation of specific chemical profiles with observed biological activities, facilitating the identification of active therapeutic markers and ensuring the consistency and quality of these promising herbal resources.

1.8. *In Silico* Studies and Molecular Docking

Understanding the precise molecular pathways through which plant secondary metabolites exert their biological effects is critical for substantiating their therapeutic value and enabling the development of modern drugs from traditional remedies. In the era of modern drug discovery, *in silico* molecular docking has become an indispensable tool for predicting the interactions between small bioactive molecules and macromolecular targets. This computational approach simulates the binding orientation and affinity of phytochemicals within the active sites of target proteins, such as bacterial enzymes or cancer-associated kinases (Agu et al., 2023). By predicting the binding energy and stability of these complexes, molecular docking allows researchers to screen vast libraries of compounds rapidly and cost-

effectively, reducing the reliance on extensive "trial-and-error" laboratory experiments (Kulkarni et al., 2025). Furthermore, it provides valuable mechanistic insights by revealing the specific amino acid residues involved in ligand-protein interactions, thereby guiding the selection of the most promising lead candidates for further *in vitro* and *in vivo* development (Agu et al., 2023).

1.9. Significance of the Study

To the best of our knowledge, the present study represents the first comprehensive systematic evaluation of methanolic pod extracts from *Parkia timoriana*, *Leucaena leucocephala*, *Albizia odoratissima*, *Entada phaseoloides*, and *Senna siamea* from Mizoram, India. This study involves molecular authentication and phylogenetic characterisation through DNA barcoding with qualitative and quantitative phytochemical characterisation using Fourier-transform infrared (FTIR) spectroscopy and Liquid chromatography-mass spectrometry (LC-MS), complemented by *in vitro* evaluation of antioxidant, antibacterial, and anticancer activities along with *in silico* molecular docking analysis targeting bacterial FtsZ proteins and human AKT1 kinase to validate therapeutic mechanisms.

1.10. Objectives:

The specific objectives of the present study are as follows:

- i. To collect and identify the Fabaceae plants using the DNA barcoding technique.
- ii. To evaluate and characterise the bioactive compounds of the collected samples.
- iii. To study the antimicrobial and antioxidant potential of collected samples.

Chapter 2

Review of Literature

2.1. Overview of Medicinal Plant Research

The systematic exploration of medicinal plants has evolved significantly in recent decades, transitioning from purely ethnobotanical documentation to rigorous phytochemical and pharmacological validation. A comprehensive review of recent scientific literature reveals a growing emphasis on integrating traditional knowledge with advanced analytical methodologies such as metabolomics, DNA barcoding, and *in silico* modelling to authenticate species and elucidate their therapeutic mechanisms (Cavalcante et al., 2025). This shift is particularly evident in studies focusing on biodiversity-rich regions like Northeast India, where the documentation of indigenous medicinal practices is increasingly complemented by laboratory-based bioactivity assessments (Ahmad and Tamang, 2020). However, despite the abundance of ethnomedicinal data, significant gaps remain in the systematic characterisation of many species, particularly within the chemically diverse Fabaceae family. The following sections synthesize current knowledge on the phytochemical potential of Fabaceae, the specific medicinal profiles of the selected study species, and the advanced analytical techniques essential for their comprehensive evaluation.

2.2. The Fabaceae Family

The Fabaceae family has attracted considerable attention in phytochemical research due to its remarkable biosynthetic capacity to produce structurally diverse secondary metabolites, including flavonoids, alkaloids, saponins, tannins, phenolic acids, terpenoids, and lectins, which contribute to the pharmacological potential of many species within this taxonomic group (Usman et al., 2022). Phytochemical investigations across various Fabaceae genera have revealed significant concentrations of bioactive compounds responsible for diverse therapeutic activities,

including analgesic, antibacterial, anticancer, anti-inflammatory, antioxidant, antidiabetic, antirheumatic, antimicrobial, and cytotoxic effects (Agu et al., 2017; Benjamim et al., 2020; Ralte et al., 2022; Khiangte et al., 2025). These pharmacological attributes, combined with traditional ethnomedicinal knowledge, position Fabaceae as the second-largest source of medicinal plants globally, warranting systematic investigation of underexplored species for drug discovery applications (Ahmad et al., 2016; Usman et al., 2022). Within this context, several Fabaceae species from Northeast India, specifically Mizoram, remain inadequately characterised despite their traditional medicinal significance and potential pharmaceutical applications.

2.3. Ethnopharmacological Background of Selected Fabaceae Species

2.3.1. *Parkia timoriana* (DC.) Merr.

Parkia timoriana (DC.) Merr., commonly known as tree bean, is a nitrogen-fixing leguminous tree of the subfamily Mimosoideae (Fabaceae), indigenous to tropical Asian regions including northeastern India, Myanmar, Thailand, Indonesia, and Malaysia (Thangjam et al., 2019). Various parts of the plant including tender shoots, flowers, pods, seeds, bark, and leaves exhibit both traditional culinary and medicinal uses. Nutritionally, pods and seeds are consumed as vegetables or processed into fermented foods, providing substantial proteins, carbohydrates, and essential micronutrients, while ethnomedicinal applications employ bark, leaf, and seed extracts for managing diabetes, hypertension, gastrointestinal disorders, dermatological conditions, and microbial infections (Angami et al., 2018; Dinesha et al., 2022). The multifunctional utility of *P. timoriana* across nutritional, ethnopharmacological, and agroforestry domains highlights its status as an underexplored species possessing significant potential for sustainable resource utilisation and bioactive compound discovery (Ralte et al., 2022).

2.3.2. *Leucaena leucocephala* (Lam.) de Wit

Leucaena leucocephala (Lam.) de Wit, commonly known as white lead tree, is a fast-growing shrub widely distributed in the Northeastern states of India, and has attracted considerable ethnopharmacological interest owing to its diverse bioactive phytochemical profile and extensive traditional medicinal applications (Khangte et al., 2025). Phytochemical investigations of *L. leucocephala* have identified a wide spectrum of bioactive constituents, several of which exhibit pharmacological activities including antimicrobial, anticancer, antidiabetic, antioxidant, anti-inflammatory, and cytotoxic effects, thereby reinforcing the species' potential as a source of therapeutic agents (Sharma and Chaurasia, 2015; Zayed and Sallam, 2019).

2.3.3. *Albizia odoratissima* (L.f.) Benth.

Albizia odoratissima (L.f.) Benth., commonly referred to as black siris or fragrant albizia, is a fast-growing deciduous tree of the Fabaceae family, native to South and Southeast Asia, valued for its ecological, economic, and medicinal significance in tropical and subtropical regions (Bhoir et al., 2024). The bark, leaves, and seeds are traditionally employed in ethnomedicinal practice for wound healing, diabetes management, and gastrointestinal disorders, with pharmacological investigations confirming antimicrobial, antioxidant, and anti-inflammatory activities that substantiate its pharmaceutical potential (Banothu et al., 2017; Sonawane et al., 2023). Beyond therapeutic applications, *A. odoratissima* has demonstrated antibacterial and antifungal properties supporting its utility as a natural antimicrobial agent, while its allelopathic effects indicate potential for sustainable agricultural applications through weed management and crop productivity enhancement (Kumar et al., 2011; Sonawane et al., 2023; Wei et al., 2024).

2.3.4. *Entada phaseoloides* (L.) Merr.

Entada phaseoloides (L.) Merr., commonly referred to as St. Thomas Bean, is a large woody climber of the Fabaceae family, widely distributed across India and other tropical regions. It holds a long-standing place in traditional Indian medicine, where different parts of the plant have been employed as an emetic, anti-irritant, hair growth stimulant, analgesic, and in the treatment of conditions such as cerebral

hemorrhage, among others (Barua et al., 2014). In addition, *E. phaseoloides* has been reported to possess a broad spectrum of pharmacological activities, including anti-arthritic, anti-ulcer, anti-inflammatory, analgesic, antidiabetic, antioxidant, cytotoxic, anti-complement, antimicrobial, and neuroprotective effects (Liao et al., 2020; Mittraphab et al., 2022; Khiangte et al., 2025).

2.3.5. *Senna siamea* (Lam.) H.S. Irwin & Barneby

Senna siamea (Lam.) H.S. Irwin & Barneby, commonly known as the Kassod tree, is a fast-growing Fabaceae species native to tropical and subtropical regions of India, Thailand, Indonesia, and the Philippines (Kumar et al., 2017). The species holds significant ethnomedicinal importance in traditional Ayurvedic and Siddha medicine systems, with bark, leaves, flowers, and seeds traditionally employed for treating fever, skin infections, digestive disorders, and inflammatory conditions (Kumar et al., 2017; Oyebade et al., 2021). Phytochemical investigations have identified diverse secondary metabolites including flavonoids, alkaloids, tannins, terpenoids, and glycosides responsible for documented biological activities encompassing antibacterial, antioxidant, anti-inflammatory, antidiabetic, and anticancer effects (Oyebade et al., 2021). The antimicrobial potential of *S. siamea* has been extensively characterised, with extracts demonstrating inhibitory activity against pathogenic bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*) and fungi (*Candida albicans*) through mechanisms involving disruption of bacterial cell membranes, inhibition of enzymatic functions, and suppression of biofilm formation (Gebrehiwot et al., 2024).

Despite these documented bioactivities, molecular authentication through DNA barcoding, comprehensive phytochemical profiling of pod extracts using FTIR and LC-MS, systematic evaluation of antioxidant potential using DPPH, ABTS, and ferric reducing power assays, assessment of cytotoxic potential against HeLa cervical cancer cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, determination of antibacterial efficacy through agar well diffusion and minimum inhibitory concentration analysis, and systematic *in silico* evaluation

targeting validated therapeutic proteins remain largely unexplored for populations from Mizoram, India.

2.4. Molecular Authentication and the Role of DNA Barcoding

Molecular-based plant authentication has become increasingly vital, as the global trade of medicinal herbs faces growing issues of product mislabelling and adulteration, which can compromise both consumer safety and the therapeutic value of herbal preparations (Anuja et al., 2024). Conventional morphological, macroscopic, and microscopic identification techniques often fail to distinguish processed herbal products where characteristic features are missing or unclear (Santhosh Kumar et al., 2018). DNA-based molecular markers provide an accurate, reliable, and cost-effective platform for authenticating medicinal plant species from various plant parts (fruit, seed, rhizome, flower, leaves, and root), overcoming the limitations posed by age, climate factors, and varying constituent concentrations that compromise chemical-based authentication approaches (Anuja et al., 2024). Implementation of DNA barcoding technique using standardised nuclear and chloroplast loci such as ITS2, *rbcL*, *matK*, and *trnH-psbA* enables quality control institutions and regulatory agencies to create strong verification frameworks that ensure the safety, authenticity, and general quality of medicinal plant products before they are commercialized (Urumarudappa et al., 2022).

2.5. Bioactive Compounds and Synergistic Effects in Crude Extracts

Plant-derived bioactive compounds represent a diverse and chemically complex reservoir of therapeutic agents, encompassing multiple classes of secondary metabolites including alkaloids, phenolic compounds, flavonoids, terpenes, and polysaccharides each exhibiting distinct pharmacological properties such as antioxidant, antimicrobial, anti-inflammatory, and cytotoxic activities (Riaz et al., 2023). The study of crude plant extracts, rather than isolated pure compounds, has emerged as a scientifically justified approach owing to synergistic bioactivity,

wherein the combined action of multiple bioactive constituents produces a therapeutic effect greater than the sum of individual component effects, with evidence demonstrating that whole plant preparations are often more effective than isolated single compounds due to enhanced bioavailability and reduced adverse effects (Vaou et al., 2022; Caesar et al., 2019). Furthermore, crude extracts possess a clinical advantage in antimicrobial and anticancer applications: disease resistance is less likely to occur against a combination of structurally and functionally diverse bioactive compounds than against single isolated molecules, as complex plant mixtures target pathogens through multiple mechanisms that prevent the emergence of resistance (Subramani et al., 2017; Caesar et al., 2019; Vaou et al., 2022).

2.6. FTIR Spectroscopy for Phytochemical Profiling

FTIR spectroscopy represents a rapid, non-destructive, and cost-effective analytical technique for preliminary phytochemical screening and functional group characterisation of plant extracts through detection of specific molecular vibrations in the infrared region ($400\text{--}4000\text{ cm}^{-1}$) (Angami and Ramaswamy, 2014). The technique enables identification of diverse phytochemical classes based on characteristic absorption bands: hydroxyl groups (O-H stretching, $3200\text{--}3600\text{ cm}^{-1}$) indicative of phenolic compounds and alcohols, carbonyl groups (C=O stretching, $1600\text{--}1750\text{ cm}^{-1}$) characteristic of flavonoids and carboxylic acids, aliphatic C-H stretching ($2800\text{--}3000\text{ cm}^{-1}$) representing lipids and terpenoids, and aromatic C=C stretching ($1400\text{--}1600\text{ cm}^{-1}$) signifying aromatic compounds and phenolic substances (Puranik and Taranath, 2023). FTIR spectral fingerprinting facilitates quality control, authentication, and standardization of herbal medicines by generating characteristic spectral profiles that enable botanical authentication and comparative analysis across different plant populations and extraction methods (Puranik and Taranath, 2023). The technique has proven particularly effective for metabolomic profiling and rapid screening of bioactive compound classes including alkaloids, terpenoids, saponins, glycosides, and steroids that contribute to the pharmacological properties of medicinal plants (Sahoo and Umashankara, 2023). In the present research context,

FTIR analysis provides rapid qualitative phytochemical profiling of the five Fabaceae pod extracts, generating spectral fingerprints that complement the detailed compound identification performed through LC-MS analysis while facilitating comparative assessment of phytochemical variations among different species and geographical populations. In context to the present study, FTIR analysis provides rapid qualitative phytochemical profiling of the five Fabaceae pod extracts, generating spectral fingerprints that complement detailed compound identification performed through LC-MS analysis while facilitating comparative phytochemical assessment across the different species examined.

2.7. LC-MS Based Phytochemical Characterisation

Liquid Chromatography-Mass Spectrometry (LC-MS) represents a sophisticated hyphenated analytical platform integrating the high-resolution separation capabilities of liquid chromatography with the precise structural elucidation power of mass spectrometry, enabling comprehensive identification, characterisation, and quantification of complex phytochemical mixtures in plant extracts (Göger et al, 2021). The technique facilitates identification of diverse phytochemical classes including phenolic acids, flavonoids, alkaloids, terpenoids, glycosides, and other secondary metabolites through analysis of molecular ion information, fragmentation patterns, and chromatographic retention behaviors, providing essential data for botanical authentication, quality control standardization, and discovery of bioactive compounds with therapeutic potential (Ali et al, 2021; Kaur et al, 2022; Cinelli and Wilson, 2024). In this study, LC-MS analysis provides detailed phytochemical characterisation of the five Fabaceae pod extracts, enabling identification of specific bioactive compounds that serve as candidates for subsequent molecular docking analysis and correlation with observed *in vitro* pharmacological activities.

2.8. Antioxidant Potential of Plant Extracts

The antioxidant capacity of plant extracts constitutes a primary focus of this investigation, employing a multifaceted suite of complementary assays that interrogate distinct biochemical mechanisms of free radical neutralization and electron transfer. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay measures the ability of antioxidants to donate hydrogen atoms or electrons to the stable purple DPPH radical, resulting in decolorization and measurable decrease in absorbance (Baliyan et al., 2022). The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay generates a blue/green radical cation whose reduction by antioxidants produces quantifiable absorbance change, with greater sensitivity to lipophilic antioxidants compared to DPPH (Rumpf et al., 2023). The ferric reducing power assay assesses the extract's capacity to reduce ferric iron to ferrous iron, providing a measure of electron-donating ability complementary to radical scavenging (Bahari et al., 2014). These three assays, when employed in parallel, provide a comprehensive and mechanistically robust assessment of the free radical scavenging capacity of the plant extracts.

2.9. Cytotoxicity Assessment of Plant Extracts

The evaluation of cytotoxicity from plant extracts is crucial in identifying compounds that can inhibit cancer cell proliferation while minimizing damage to normal cells. Many phytochemicals exhibit selective toxicity, making them promising candidates for anticancer drug development (Deniz et al., 2017). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay is extensively used to measure such cytotoxic effects quantitatively, providing reliable IC_{50} values for both cancerous and normal cell lines, which helps establish therapeutic safety and efficacy (Nair and Varalakshmi, 2011). This assay is integral for screening the anticancer potential of plant-derived extracts and guiding further pharmacological studies.

2.10. Evaluation of Antibacterial Potential in Plant Extracts

The determination of antibacterial potential of plant extracts represents a critical step in phytopharmaceutical research, as many medicinal plants have been traditionally employed for infectious disease management and their antimicrobial efficacy requires scientific validation through standardized methodologies. The agar well diffusion method is widely employed for qualitative screening, as it allows comparison of inhibition zones formed around wells containing test extracts to indicate antibacterial potency (Bonev et al., 2008). For quantitative evaluation, the minimum inhibitory concentration (MIC) assay establishes the lowest concentration of an extract required to inhibit visible bacterial growth, thus facilitating precise comparisons of antimicrobial efficacy across plant samples and bacterial strains (Ndezo Bisso et al., 2022). These complementary assays are routinely used in medicinal plant research to identify bioactive compounds and validate traditional antibacterial claims.

2.11. Molecular Docking in Plant-Based Drug Discovery

Molecular docking represents a sophisticated computational methodology that predicts the preferred binding orientation, interaction modes, and binding affinity of small molecule ligands with biological target proteins, serving as a cornerstone of structure-based drug design and discovery (Raval and Ganatra, 2022). The technique accelerates drug discovery by enabling high-throughput virtual screening of extensive compound libraries, substantially reducing the time, cost, and experimental resources required compared to conventional drug development approaches while facilitating rapid identification of promising therapeutic candidates (Mir et al., 2022). In medicinal plant research, molecular docking has emerged as an indispensable tool for elucidating molecular mechanisms of bioactive phytochemicals, predicting their interactions with disease-associated proteins, and supporting the development of plant-derived therapeutics for diverse conditions including cancer, diabetes, cardiovascular disorders, and infectious diseases (Ralte et al, 2022; Khiangte et al, 2025). For antibacterial drug discovery, bacterial cell division protein FtsZ represents

a validated molecular target, with docking studies identifying natural product inhibitors that disrupt bacterial cytokinesis without affecting eukaryotic systems, offering potential for developing novel antibiotics against multidrug-resistant pathogens (Mir et al, 2022). Similarly, human AKT1 serine/threonine kinase serves as an established anticancer target, with molecular docking analyses identifying phytochemicals that inhibit this survival signalling protein, providing mechanistic insights into the anticancer activities of plant extracts (Ralte et al, 2022). In the present investigation, molecular docking analysis of LC-MS-identified phytocompounds targeting bacterial FtsZ proteins and human AKT1 kinase provides *in silico* validation and mechanistic understanding of the antibacterial and anticancer activities observed through *in vitro* assays.

Chapter 3

Materials and Methods

3.1. Collection of Samples and Preparation of Extracts

Pods of *P. timoriana*, *L. leucocephala*, *A. odoratissima*, *E. phaseoloides*, and *S. siamea* were collected from Aizawl, Mizoram (23.7°N, 92.7°E, WGS84). Herbarium specimens of each species were prepared and deposited at the Department of Botany, Mizoram University, for future reference.

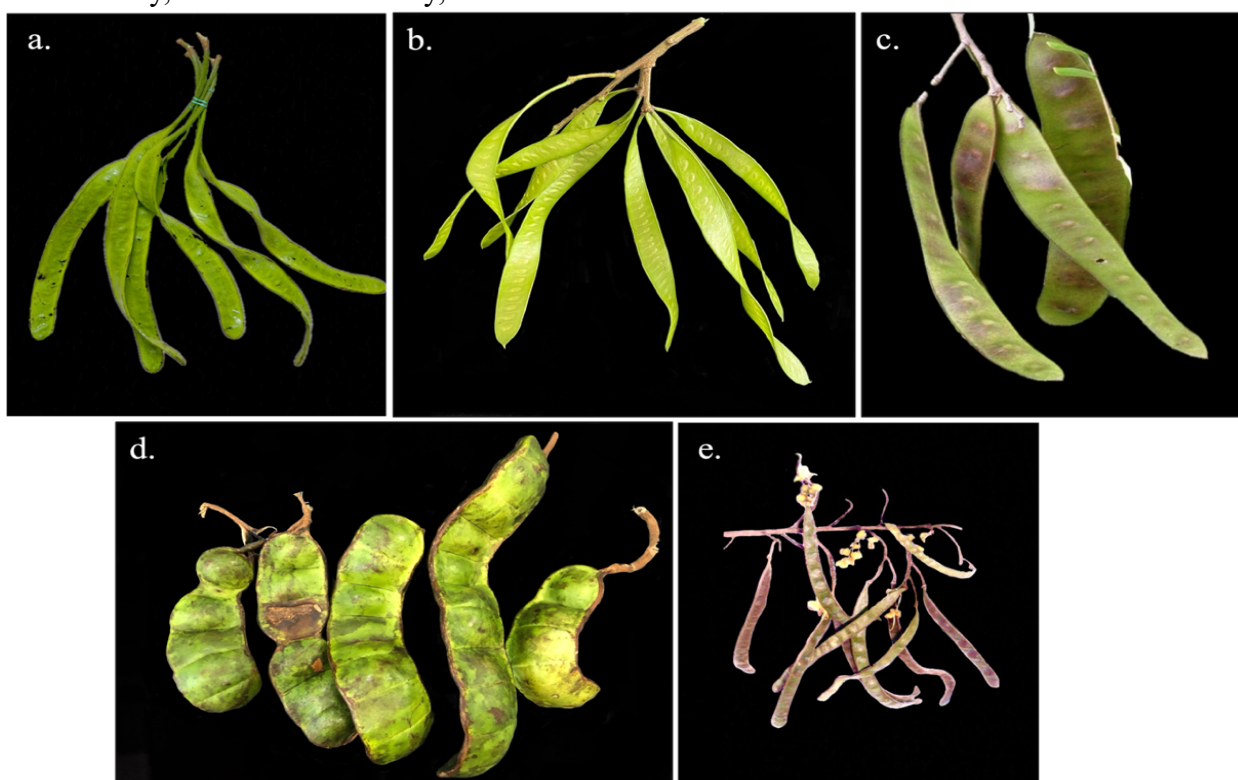


Fig 1. Collected Pods of (a) *P. timoriana*, (b) *L. leucocephala*, (c) *A. odoratissima*, (d) *E. phaseoloides*, and (e) *S. siamea*.

The collected pod samples were dried at room temperature and subsequently pulverized using a mechanical grinder. Methanolic extraction was performed using a Soxhlet apparatus, with dried ground pod material subjected to solvent extraction. Following extraction, the liquid extracts were filtered and concentrated using a rotary evaporator maintained at 40°C until complete solvent evaporation occurred, yielding

a paste-like residue. The concentrated extracts were collected and stored at appropriate conditions for subsequent phytochemical and pharmacological analyses.

3.1.1. *Parkia timoriana* (DC.) Merr.

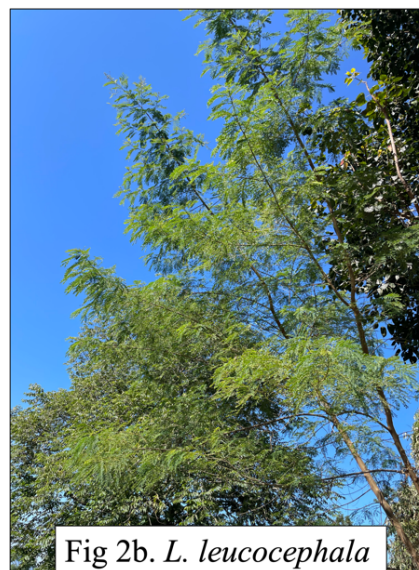
Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Eudicots
Order	:	Fabales
Family	:	Fabaceae
Subfamily	:	Mimosoideae
Genus	:	<i>Parkia</i>
Species	:	<i>P. timoriana</i>



A deciduous tree with bipinnate compound leaves featuring 20-30 pinnae, each bearing 50-60 pairs of narrow leaflets (6-12 mm long) displaying feathery, finely divided foliage. Small, pale yellow to cream-colored flowers are arranged in distinctive ball-like spherical inflorescences (glomerate heads). Elongated, pendulous, dark brown legume pods (30-60 cm long) hang characteristically in clusters. Flowering usually occurs from September to October, followed by fruiting from November to March.

3.1.2. *Leucaena leucocephala* (Lam.) de Wit.

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Eudicots
Order	:	Fabales
Family	:	Fabaceae
Subfamily	:	Mimosoideae
Genus	:	<i>Leucaena</i>
Species	:	<i>L. leucocephala</i>



A fast-growing deciduous shrub to small tree with fine, lacy bipinnate compound leaves displaying multiple leaflet pairs arranged symmetrically, creating

delicate, feathery foliage. Small white flowers with prominent stamens are arranged in dense spherical heads (1.5-2 cm diameter). Flat, elongated legume pods (15-20 cm long) are light brown to tan when mature, arranged in clusters. Flowering predominantly occurs from April to July with peak flowering during May-June, followed by fruiting from August to October.

3.1.3. *Albizia odoratissima* (L.f.) Benth.

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Eudicots
Order	:	Fabales
Family	:	Fabaceae
Subfamily	:	Mimosoideae
Genus	:	<i>Albizia</i>
Species	:	<i>A. odoratissima</i>



Fig 2c. *A. odoratissima*

A deciduous to semi-deciduous tree with bipinnate compound leaves bearing 6-16 pairs of oblong leaflets (1-3.5 cm length) of dark green coloration, creating an umbrella-like crown with massive branching architecture. Fragrant white to pale orange/cream-colored flowers with prominent red-tipped stamens are arranged in dense spherical heads (2-3 cm diameter). Flat, elongated legume pods (12-18 cm long) are brown to dark brown when mature, containing 4-8 seeds. Flowering occurs from March to June, with fruiting from August to October.

3.1.4. *Entada phaseoloides* (L.) Merr.

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Eudicots
Order	:	Fabales
Family	:	Fabaceae
Subfamily	:	Mimosoideae
Genus	:	<i>Entada</i>
Species	:	<i>E. phaseoloides</i>



Fig 2d. *E. phaseoloides*

A woody liana or perennial woody vine with bipinnate compound leaves showing reduced pinna pairs (typically 1-2 pairs) and 1-2(-3) pairs of large, leathery, asymmetrical leaflets (4-10 cm long), exhibiting a distinctive climbing growth habit within forest understories. Small, greenish-white to pale yellow flowers are arranged in characteristic cylindrical spike inflorescences. Massive, woody legume pods (up to 1+ meter long) are flattened, containing large disc-shaped seeds (2-3 cm diameter), representing among the largest pods in the Fabaceae family. Flowering occurs seasonally during monsoon to post-monsoon periods, between the months of March and June, with fruiting extending through monsoon and post-monsoon periods in highly variable patterns.

3.1.5. *Senna siamea* (Lam.) H.S. Irwin & Barneby.

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Eudicots
Order	:	Fabales
Family	:	Fabaceae
Subfamily	:	Caesalpinioideae
Genus	:	<i>Senna</i>
Species	:	<i>S. siamea</i>



Fig 2e. *S. siamea*

An evergreen to semi-deciduous shrub to small tree with pinnate compound leaves bearing 6-12 pairs of oblong leaflets (3-7 cm length) of bright to dark green coloration, maintaining evergreen foliage throughout the year. Bright yellow flowers with red stamens are arranged in large pyramidal panicles (20-40 cm length), highly conspicuous and fragrant. Elongated, flattened, dark brown to black legume pods (15-25 cm long) contain 8-15 seeds arranged linearly. Flowering predominantly occurs from September to December with peak flowering during October-November, followed by fruiting from December to March with maturation extending into early summer.

3.2. Molecular Study: Isolation and Amplification of DNA

3.2.1. Isolation of DNA

Genomic DNA was isolated from the plant samples following the protocol described by Doyle and Doyle (1987). In brief, 100 mg of tender leaf tissue from each sample was placed in a 1.5 mL centrifuge tube and ground thoroughly using a micro-pestle with the addition of 500 μ L of CTAB extraction buffer. The homogenized samples were incubated at 60°C for 30 minutes in a water bath. After incubation, the tubes were centrifuged at 14,000 rpm, and the supernatant was transferred to a new tube. RNaseA (20 μ L) was added, followed by incubation at 34°C for 20 minutes. To precipitate DNA, 0.7 volumes of ice-cold isopropanol were added and the tubes were stored at –20°C for 30 minutes. The samples were centrifuged again at 14,000 rpm for 10 minutes, the supernatant was discarded, and the resulting pellet was washed with 70% ethanol. The DNA pellet was air-dried to remove the remaining ethanol and then, 20 μ L of TE buffer was added to dissolve the DNA.

3.2.2. Amplification of DNA using Polymerase Chain Reaction (PCR)

PCR amplification of isolated DNA was conducted in a 25 μ L reaction volume containing MgCl₂, dNTPs, Taq DNA polymerase, and specific primers. Each reaction underwent 45 cycles, with denaturation at 94°C, primer annealing at 55°C, and elongation at 72°C. Five standard plant DNA barcoding primers, ITS2, rbcL, matK, trnH-psbA, and rpoC1 were utilised for amplification. Amplified products were quantified and verified by electrophoresis on 1.5% agarose gel. The PCR products were subsequently sent off to Eurofins Genomics India Pvt Ltd., Bengaluru, India for sequencing.

Table 1. DNA Barcoding Primers Utilised for Polymerase Chain Reaction (PCR) Amplification.

Sl. No.	Gene	Forward 5'-3'	Reverse 5'-3'	Reference
1	<i>ITS2</i>	ATGCGATACTTGGTGT GAAT	GACGCTTCTCCAGACTACA AT	Chen et al., (2010)
2	<i>rbcL</i>	ATGTCACCACAAACA GAGACTAAAGC	GAAACGGTCTCTCCAACGC AT	CBOL Plant Working Group (2009)
3	<i>makK</i>	CGTACAGTACTTTTGT GTTTACGAG	ACCCAGTCCATCTGGAAAT CTTGTTTC	CBOL Plant Working Group (2009)
4	<i>trnH-psbA</i>	CGCGCATGGTGGATTC ACAATCC	GTTATGCATGAACGTAATG CTC	Kress et al., (2005)
5	<i>rpoC1</i>	GGCAAAGAGGGAAGA TTTCG	CCATAAGCATATCTTGAGT TGG	Sass et al., (2007)

3.2.3. Sequence Analysis and Construction of Phylogenetic Trees

The obtained DNA barcode sequences were analysed using NCBI BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), with each sequence compared against the NCBI nucleotide database and assigned identity based on the top-scoring hit, considering both sequence similarity and stringent E-value thresholds. Phylogenetic trees for each marker region (*ITS2*, *rbcL*, *matK*, *trnH-psbA*, and *rpoC1*) were constructed in MEGA 12 using the Maximum Likelihood (ML) method. The optimal nucleotide substitution model was selected for each dataset, and the General Time Reversible model with gamma distribution and a proportion of invariant sites (GTR+G+I) was employed where appropriate. Statistical support for phylogenetic relationships was evaluated by performing 1,000 bootstrap replications.

3.3. Qualitative Phytochemical Screening

Preliminary phytochemical screening of the plant samples was performed using standard qualitative chemical tests to detect the presence of major classes of secondary metabolites including alkaloids, flavonoids, phenols, steroids, glycosides, terpenoids, saponins, and tannins (Harborne, 1998).

3.3.1. Alkaloids (Wagner's Test)

Extract (1 mL) was treated with Wagner's reagent (1.27 g iodine and 2 g potassium iodide in 100 mL distilled water). Formation of reddish-brown precipitate within 5 minutes indicated alkaloid presence.

3.3.2. Flavonoids (Ammonia/H₂SO₄ Test)

Extract (2 mL) was mixed with concentrated H₂SO₄ (5 mL). Ammonia solution (28%) was then added dropwise. Development of yellow or orange coloration indicated positive flavonoid presence.

3.3.3. Phenols (Lead Acetate Test)

Extract (2 mL) was treated with lead acetate solution (10%, w/v, 2 mL). White or yellowish precipitate formation within 2 minutes confirmed phenolic compound presence.

3.3.4. Steroids (Liebermann-Burchard Test)

Extract (1 mL) was treated with acetic anhydride (1 mL) followed by concentrated H₂SO₄ (1 mL). Formation of brown ring at the junction of two layers indicated steroid presence.

3.3.5. Glycosides (Salkowski Test)

Extract (2 mL) was treated with chloroform (2 mL) and concentrated H₂SO₄ (3 mL) was carefully layered below. Reddish-brown coloration at the interface indicated glycoside presence.

3.3.6. Terpenoids (Chloroform Test)

Extract (2 mL) was mixed with chloroform (2 mL) and concentrated H₂SO₄ (3 mL). Reddish-brown coloration at the interface within 2 minutes indicated terpenoid presence.

3.3.7. Saponins (Froth Test)

Extract (5 mL) was diluted with distilled water (5 mL) and shaken vigorously for 1 minute. Persistent froth or foam layer (≥ 1 cm) lasting for at least 15 minutes indicated saponin presence.

3.3.8. Tannins (Ferric Chloride Test)

Extract (2 mL) was treated with ferric chloride solution (5%, w/v, 1 mL). Green or blue-black coloration development within 5 minutes indicated tannin presence.

3.4. Quantification of Total Phenolic and Flavonoid Content

3.4.1. Estimation of Total Phenolic Content (TPC)

The total phenolic content was determined using the Folin-Ciocalteu reagent method (Phuyal et al., 2020). Briefly, 100 μ l of plant extract was mixed with 100 μ l of Folin-Ciocalteu reagent and incubated for 15 minutes. Subsequently, 1 mL of 8% sodium carbonate solution was added, and the mixture was incubated in darkness for 30 minutes. Absorbance was measured at 760 nm using a UV-Vis spectrophotometer. Gallic acid served as the standard reference compound, and total phenolic content was expressed as gallic acid equivalents (GAE) in mg/g of dry extract.

3.4.2. Estimation of Total Flavonoid Content (TFC)

The total flavonoid content of the extracts was determined using the standard aluminum chloride colorimetric method (Phuyal et al., 2020). In brief, 1 mL of extract was mixed with 1 mL of methanol, followed by incubation for 5 minutes. Aluminum chloride (0.5 mL of 10% w/v) and sodium hydroxide (0.5 mL of 1 M NaOH) were subsequently added to the mixture. The final volume was adjusted to 2.5 mL with distilled water, and absorbance was measured at 510 nm using a UV-Vis

spectrophotometer. Quercetin was employed as the standard reference compound, and total flavonoid content was expressed as quercetin equivalents (QE) in mg/g of dry extract.

3.5. Fourier Transform Infrared Spectroscopy (FTIR)

The functional groups present in the pod extracts were detected using Fourier transform infrared spectroscopy (Shimadzu IRAffinity-1S, Japan) with spectral frequency ranging from 400-4000 cm^{-1} .

3.6. Liquid Chromatography Mass Spectrometry (LC-MS)

LC-MS analysis of the extracts was performed using an AB SCIEX Triple TOF 5600plus mass spectrometer (AB SCIEX, Framingham, MA, USA) coupled to a Waters UPLC system (Waters Corp., Milford, MA, USA) equipped with a UV-VIS detector. Mass spectrometry was conducted in negative ionization mode with the following parameters: scan range of m/z 100-2000, source voltage of -4.5 kV, source temperature of 500°C , nebulizer gas (N_2) pressure of 50 psi, and curtain gas pressure of 30 psi. For MS/MS analysis, collision energy was set to -35 V, collision energy spread to 10 V, and declustering potential to -100 V. The injection volume was 10 μL , and the eluent flow rate to the mass detector was approximately 0.7 mL/min. Data acquisition and processing were performed using Analyst® TF 1.6 software (AB SCIEX) integrated with ChemSpider database for compound identification.

3.7. Determination of Free Radical Scavenging Potential

3.7.1. DPPH Radical Scavenging Assay

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of the extracts was determined according to the method of Gaber et al., (2023), with minor modifications. In brief, 2 mL of DPPH solution was mixed with 50 μL of extract (10-100 $\mu\text{g/mL}$) and incubated for 30 minutes in darkness. Absorbance was measured at 517 nm using ascorbic acid as the reference standard and a mixture of 1 mL

methanol and 2 mL DPPH as the negative control. The percentage radical scavenging activity (%RSA) was calculated using the following equation:

$$\%RSA = \left[\frac{(A_{control} - A_{treatment})}{A_{control}} \right] \times 100$$

Where, $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the extracts.

3.7.2. ABTS Radical Scavenging Assay

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity was determined according to the method of Gaber et al., (2023). A stock solution containing 7 mM ABTS and 2.45 mM potassium persulfate was prepared and incubated for 12 hours. A working solution was subsequently prepared by diluting the stock solution with 50% methanol to achieve an absorbance of 0.70 (± 0.02) at 745 nm. Fractions of the extract (10-100 $\mu\text{g/mL}$, 150 μl) were mixed with 1.5 mL of ABTS working solution and absorbance was measured at 745 nm using ascorbic acid as the reference standard. The negative control consisted of the ABTS working solution without extract. Percentage radical scavenging activity (%RSA) was calculated using the following equation:

$$\%RSA = \left[\frac{(A_{control} - A_{treatment})}{A_{control}} \right] \times 100$$

Where, $A_{control}$ is absorbance of the control (mixture of reagents without extracts) and A_{sample} is the absorbance of the extracts.

3.7.3. Ferric Reducing Power Assay

The ferric reducing power of each extract was determined according to the method of Adjimani and Asare (2015). Extracts (1-100 $\mu\text{g/mL}$) were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide solution. Following incubation at 50°C for 20 minutes, 2.5 mL of 10% trichloroacetic acid

(TCA) was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. The supernatant (0.5 mL) was mixed with an equal volume of distilled water and 0.5 mL of 1% ferric chloride solution. Absorbance was measured at 700 nm, with increasing absorbance indicating enhanced reducing power of the extracts.

3.8. Cytotoxicity Assay (MTT Assay)

Cytotoxicity of the pod extracts was evaluated using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] reduction assay (Mosmann, 1983). HeLa cells were cultured in Minimum Essential Medium (MEM) supplemented with fetal bovine serum (FBS) and 1% L-glutamine at 37°C with 5% CO₂. Cells were seeded in 96-well plates at a density of 1×10^4 cells per well (100 µL medium), allowed to adhere for 24 hours, then treated with various concentrations of extracts (5-100 µg/mL) for 24 hours. After treatment, the media were removed and cells were washed with FBS-free medium. Then, 10 µL of MTT solution (5 mg/mL) was added to each well, and they were incubated for a further 2 hours at 37°C in a CO₂ incubator. The formed formazan crystals were dissolved in 100 µL DMSO, and after 30 minutes at room temperature, absorbance was measured at 570 nm on a microplate reader. Cytotoxicity was calculated as percent inhibition using the formula:

$$\%RSA = \left[\frac{(A_{control} - A_{treatment})}{A_{control}} \right] \times 100$$

Where, $A_{control}$ is absorbance of the control (mixture of reagents without extracts) and $A_{treatment}$ is the absorbance of the treatments.

3.9. Determination of Antibacterial Potential

3.9.1. Agar-well Diffusion Method

The antibacterial activity of each extract was evaluated using the agar-well diffusion method (Manandhar et al., 2019). Five bacterial strains, *Bacillus pumilus* (ATCC14884), *Bacillus subtilis* (ATCC11774), *Escherichia coli* (ATCC11229), *Pseudomonas aeruginosa* (ATCC9027), and *Staphylococcus aureus* (ATCC6908)

were used for this assay and, Ampicillin was used as a standard control. Then, the zone of inhibition for each extract was measured using the Antibiotic Inhibition Scale (HiMedia, India).

3.9.2. Minimum Inhibitory Concentration (MIC) using Resazurin Assay

Minimum inhibitory concentration (MIC) was determined following the method of Rakhmawatie et al., (2019). Serial dilutions of the plant extracts were prepared in the range of 20 to 0.1 mg/mL. In a 96-well plate, each well was loaded with 75 µl Mueller-Hinton broth (MHB), 75 µl bacterial suspension (10^5 CFU/mL), and 75 µl of the test extract. Columns 1-11 received varying extract concentrations, while column 12 served as a control with bacterial suspension only. Plates were incubated at 37°C for 24 hours. After incubation, 10 µl of 0.5 mg/mL resazurin solution was added to each well and incubated for a further 3 hours. A colour change from blue (oxidized) to pink (reduced) signified bacterial growth. The MIC was identified as the lowest extract concentration that prevented this colour change.

3.10. *In Silico* Molecular Docking

Molecular docking was conducted to assess the potential antibacterial and anticancer activities of compounds identified by LC-MS. Three-dimensional structures of the phytochemicals were retrieved from the NCBI PubChem database (<http://pubchem.nlm.nih.gov/>). The selected target proteins included *E. coli* FtsZ(L178E) GTP-complex (PDB ID: 6UNX, representative of Gram-negative bacteria), *S. aureus* FtsZ GDP-form (PDB ID: 3VO8, representative of Gram-positive bacteria), and human AKT1 kinase (PDB ID: 3O96) for evaluation of anticancer potential. Molecular docking was performed using AutoDock Vina to calculate binding affinities and predict optimal ligand-protein binding conformations. Docking results were analysed and visualized using Discovery Studio Visualizer.

3.11. Statistical Analysis

All experiments were performed in triplicate, and results are reported as mean $\pm$ standard error of the mean (SEM). To identify statistically significant differences in antioxidant, antibacterial, and cytotoxic activities among different extracts, one-way analysis of variance (ANOVA) was conducted, followed by Tukey's multiple range test for post hoc multiple comparisons. The IC₅₀ values for free radical scavenging and cytotoxicity assays were determined by plotting the percentage inhibition against the logarithm of concentrations and fitting the data with nonlinear regression using GraphPad Prism version 8. A significance threshold of $p < 0.05$ was applied for all statistical assessments.

Chapter 4

Results and Discussion

4.1. Collection of Samples, Extraction of DNA, PCR Amplification and Phylogenetic Tree Analysis:

Flowers, leaves, and pods of *P. timoriana*, *L. leucocephala*, *A. odoratissima*, *E. phaseoloides*, and *S. siamea* were collected between the months of September 2021 and March 2022, from Aizawl, Mizoram (23.7°N, 92.7°E, WGS84), and processed as voucher specimens. Herbarium sheets were then deposited to the Department of Botany, Mizoram University (MZU), with accession numbers assigned for future reference (Table 2).

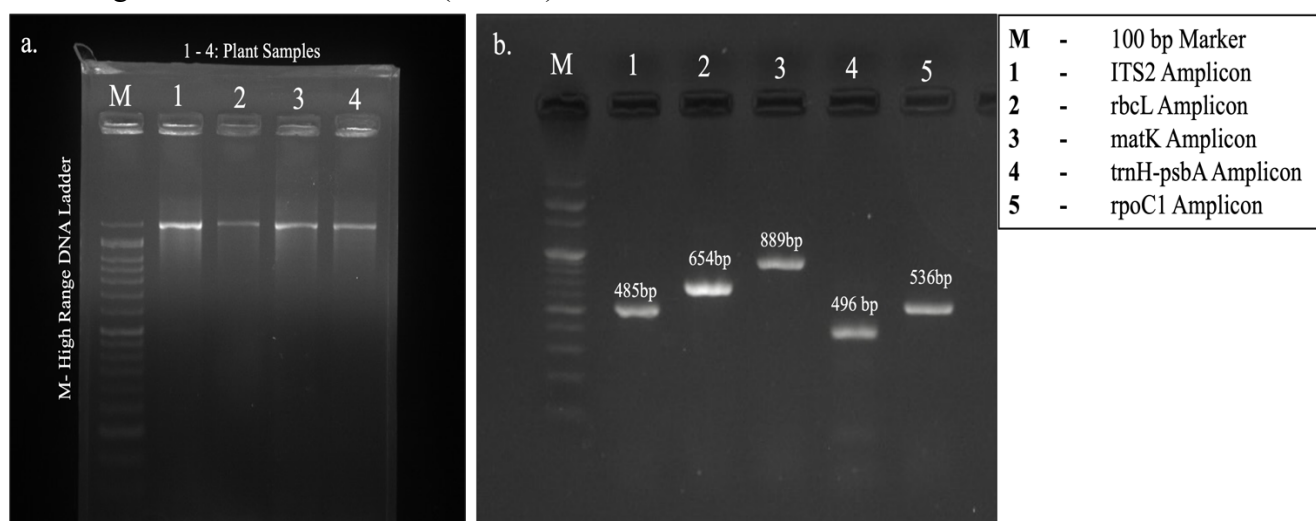


Fig 3. (a) 0.8% Agarose Gel Image of Extracted DNA products.
(b) 1.5% Agarose Gel Image of Amplified DNA products.

Extraction of DNA from the leaves of *P. timoriana*, *L. leucocephala*, *A. odoratissima*, *E. phaseoloides* and *S. siamea* was successfully carried out using CTAB plant DNA extraction. PCR amplification of extracted DNA from each sample was done using five plant DNA barcoding primers - ITS2, rbcL, matK, trnH-psbA and rpoC1 (Fig 3). The amplified products were sequenced, and all sequences were deposited in the GenBank database (NCBI) and assigned accession numbers (Table 2). Homologous sequences were retrieved from GenBank based on BLAST search results and used for phylogenetic analyses.

Table 2. Herbarium References and GenBank Accession Numbers of Voucher Specimen.

Sl. No.	Plant Sample	Herbarium Accession	Genbank Accession				
			ITS2	rbcL	matK	trnH-psbA	rpoC1
1	<i>P. timoriana</i>	MZU000201	OQ581823	PP453728	PP474206	PQ431963	PP474201
2	<i>L. leucocephala</i>	MZU000202	OP526359	PP453729	PP458217	PQ431964	PP474202
3	<i>A. odoratissima</i>	MZU000203	OQ581824	PP453730	PP458218	PQ431965	PP474203
4	<i>E. phaseoloides</i>	MZU000203	OP526360	PP453731	PP474207	PQ431966	PP474204
5	<i>S. siamea</i>	MZU000205	OP526361	PP453732	PP474208	PQ431967	PP474205

Maximum Likelihood phylogenetic analysis across all five barcode regions (ITS2, rbcL, matK, trnH-psbA, and rpoC1) demonstrated that each of the five Fabaceae specimens formed well-supported monophyletic clades with their respective reference sequences from GenBank (Fig 4). The phylogenetic trees obtained from the analysis showed that the sequences consistently grouped with their closest relatives, forming well-supported monophyletic clades with high bootstrap values, thereby confirming the reliability of each grouping. Moreover, the consistent clustering patterns observed at both species and genus levels across all five independent barcode analyses, together with strong phylogenetic support (>70%

bootstrap values), unequivocally validated the species identification and clarified the distinct evolutionary relationships within the Fabaceae family.

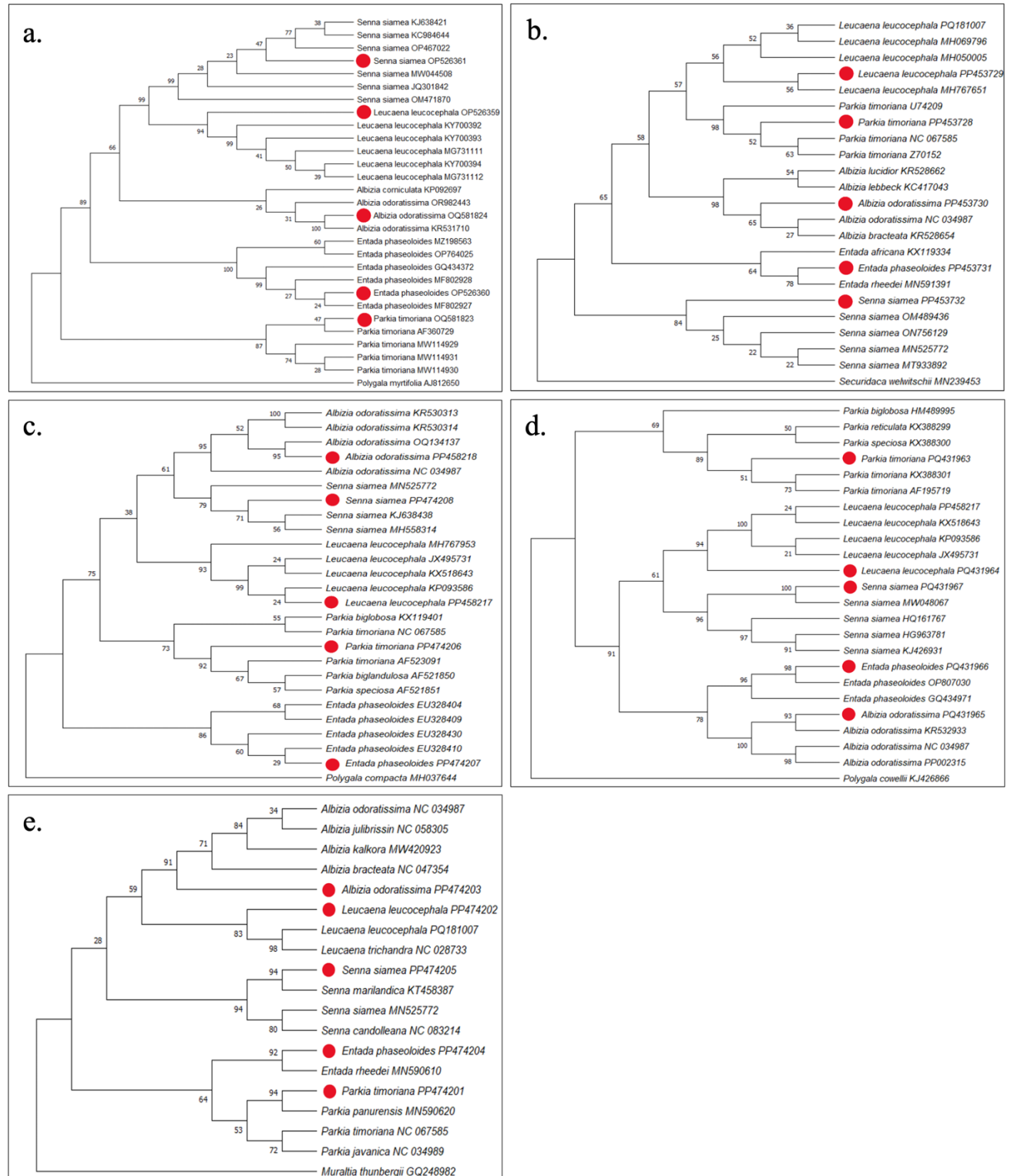


Fig 4. Maximum Likelihood (ML) Phylogenetic Tree with 1000 Bootstrap Replicates using (a) ITS2, (b) rbcL, (c) matK, (d) trnH-psbA and (e) rpoC1.

DNA barcoding is a molecular technique used for species identification and classification based on short, standardized genetic regions. It provides a rapid and reliable method for distinguishing plant species, particularly when morphological identification is challenging due to phenotypic plasticity, cryptic species, or incomplete specimens (Hebert et al., 2003). Furthermore, barcoding has been instrumental in medicinal plant authentication, preventing adulteration and ensuring the quality of herbal products (Newmaster et al., 2013).

In plants, several DNA regions have been proposed as potential barcodes, with the most widely accepted being the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) and the internal transcribed spacer 2 (ITS2) (Kress et al., 2005; Hollingsworth et al., 2009). The *rbcL* gene, located in the chloroplast genome, is highly conserved and provides reliable species-level resolution, while ITS2, a nuclear ribosomal region, offers higher discriminatory power due to its greater sequence variation (Chen et al., 2010). The *matK* gene is considered one of the core molecular markers in plant DNA barcoding and serves as a reliable tool for species identification and phylogenetic analysis across diverse plant lineages due to its balance of conservation and variability (Ho et al., 2021). The *trnH-psbA* intergenic spacer, a non-coding region of the chloroplast genome, exhibits high sequence variability and is therefore effective in distinguishing closely related plant species. When used in combination with other barcode loci such as *matK*, *rbcL*, and ITS2, it often enhances species-level resolution (Pang et al., 2021). The *rpoC1* gene encodes a subunit of the chloroplast RNA polymerase and represents a conserved coding region commonly employed in plant DNA barcoding. It is characterised by high PCR amplification success and broad universality, particularly in taxa where *matK* or *rbcL* may show inconsistent performance. However, *rpoC1* alone often lacks sufficient variability for species-level discrimination, and it is therefore considered more effective as a supplementary barcode in combination with more variable regions such as *trnH-psbA* or *matK* (Parveen et al., 2017).

For accurate plant identification, the application of many DNA barcode loci (ITS2, *rbcL*, *matK*, *trnH-psbA*, and *rpoC1*) proved successful and efficient. Phylogenetic trees constructed also showed strongly supported monophyletic groups

that validated intimate evolutionary links, improving species-level resolution through the combined examination of conserved and variable areas. These results demonstrated that multi-locus DNA barcoding is a reliable method for accurate taxonomy identification and phylogenetic inference.

4.2. Phytochemical Screening

Preliminary phytochemical screening of the methanol extracts was performed to detect the presence of eight major classes of secondary metabolites: alkaloids, flavonoids, phenols, steroids, glycosides, terpenoids, saponins, and tannins (Table 3). All eight metabolite classes were detected in the extracts of *P. timoriana*, *L. leucocephala*, *A. odoratissima*, *E. phaseoloides*, and *S. siamea*. The comprehensive phytochemical profile revealed that these Fabaceae species possess a diverse array of bioactive compounds across multiple structural classes. This rich secondary metabolite composition indicates substantial pharmacological potential and supports the traditional ethnomedicinal applications of these plants, warranting further investigation of their specific biological activities and therapeutic properties.

The detection of diverse secondary metabolite classes including alkaloids, flavonoids, phenols, tannins, glycosides, terpenoids, saponins, and steroids across the five Fabaceae species analysed in this study aligns well with existing literature, which consistently highlights these compounds as hallmark features of the Fabaceae family. Previous reports show that *P. timoriana* contains alkaloids, tannins, terpenoids, phenols, and flavonoids in its seeds and edible pods when extracted with methanol (Suryanti et al., 2022; Fakhrah et al., 2025). *L. leucocephala* is well recognized for the presence of flavonoids, saponins, alkaloids, tannins, and cardiac glycosides in both leaf and pod extracts (Prakash et al., 2020; Deivasigamani et al., 2018). In addition, methanolic extracts of *A. odoratissima* have been confirmed to contain steroids, tannins, phenolics, and saponins through qualitative phytochemical screening (22 et al., 2015; Banothu et al., 2017). These observations reinforce that Fabaceae species consistently contain structurally diverse secondary metabolites, and the metabolite classes detected in the present investigation are consistent with the phytochemical profiles characteristic of this family.

Table 3. Preliminary Phytochemical Screening of Secondary Metabolites in the Extracts.

Sl. No.	Compounds	Test	Expected Outcome	Results				
				<i>P. timoriana</i>	<i>L. leucocephala</i>	<i>A. odoratissima</i>	<i>E. phaseoloides</i>	<i>S. Siamea</i>
1	Alkaloids	Wagner's test	Reddish-brown precipitate	+	+	+	+	+
2	Flavonoids	Ammonia/ H ₂ SO ₄ test	Yellow or orange colouration	+	+	+	+	+
3	Phenols	Lead acetate test	White or yellowish precipitate	+	+	+	+	+
4	Steroids	Liebermann-Burchard test	Brown ring at junction	+	+	+	+	+
5	Glycosides	Salkowski test	Reddish-brown colour at the interface	+	+	+	+	+
6	Terpenoids	Chloroform test	Reddish-brown coloration at the interface	+	+	+	+	+
7	Saponins	Froth Test	Persistent froth or foam formation	+	+	+	+	+
8	Tannins	Ferric Chloride Test	Green or blue-black coloration	+	+	+	+	+

4.3. Total Phenolic Content and Total Flavonoid Content

The total phenolic content (TPC) and total flavonoid content (TFC) of each plant extract were determined using standardized assays (Fig 5). Among the five Fabaceae species examined, *P. timoriana* exhibited the highest TPC value (58.88 ± 0.34 GAE/g), followed by *L. leucocephala*, *E. phaseoloides*, *A. odoratissima*, with *S. siamea* showing the lowest TPC (31.10 ± 0.08 GAE/g). Phenolic compounds are well-documented for their potent antioxidant properties and have been traditionally utilised for wound healing and treatment of skin infections (Okwu et al., 2003). Plant phenolic compounds confer protection against oxidative damage from insects, microorganisms, and herbivores (Wu et al., 2015), and possess diverse pharmacological properties including anti-inflammatory, antimutagenic, hypocholesterolemic, and antiplatelet aggregation activities (Shahidi and Yao, 2018).

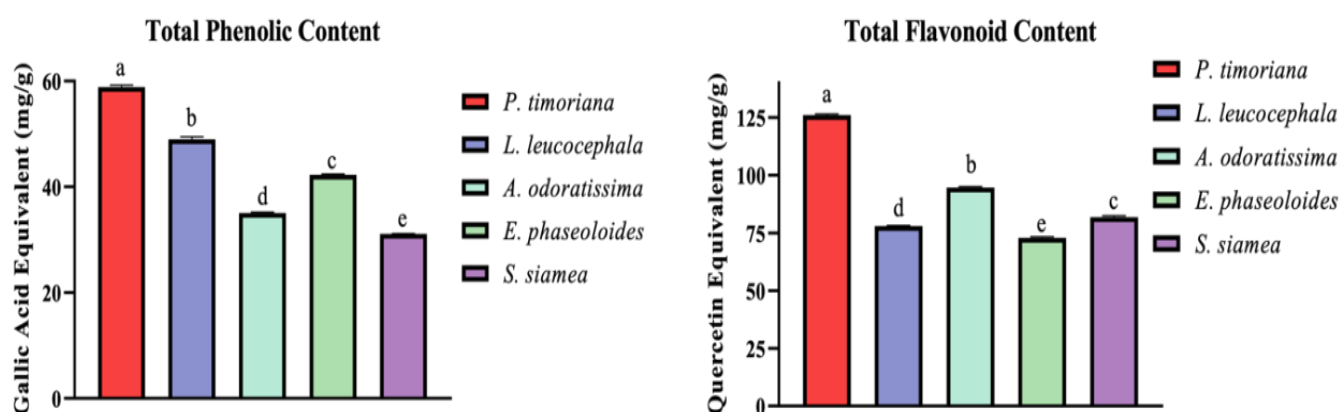


Fig 5. Estimation Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) from the extracts.

The total flavonoid content demonstrated a similar pattern to TPC, with *P. timoriana* exhibiting the highest TFC (126.05 ± 0.47 QE/g), followed by *A. odoratissima*, *S. siamea*, and *L. leucocephala*, while *E. phaseoloides* displayed the lowest TFC (72.90 ± 0.41 QE/g). Flavonoids represent another important class of polyphenolic compounds with diverse biological activities. They have demonstrated potent anticancer potential through multiple mechanisms including cell cycle arrest, apoptosis induction, and inhibition or inactivation of carcinogenic compounds (Chahar et al., 2011).

The quantification of total phenolic and flavonoid contents serves as a key indicator for assessing the phytochemical richness and potential health-promoting properties of plant extracts. The pod extracts of the five Fabaceae species demonstrated substantial levels of phenolic and flavonoid compounds, indicating their potential as valuable sources of bioactive molecules with significant pharmacological applications. The diverse phenolic and flavonoid profile of these plant extracts collectively contributes to their broad spectrum of biological activities, including antioxidant, anti-inflammatory, antimicrobial, anti-angiogenic, anticancer, and anti-allergic properties (Chahar et al., 2011; Wu et al., 2015).

The antioxidant effectiveness of plant extracts is closely linked to their total phenolic content (TPC) and total flavonoid content (TFC). Phenolic compounds and flavonoids are well-recognized bioactive metabolites that function as potent free radical scavengers, capable of neutralizing reactive oxygen species (ROS) through hydrogen atom donation from their hydroxyl groups (Hassanpour et al., 2023). In the present study, following quantification of TPC and TFC across the five Fabaceae extracts, the antioxidant activity was subsequently assessed using complementary radical scavenging assays (DPPH, ABTS) and ferric reducing power assay. The strong mechanistic relationship between phenolic and flavonoid abundance and antioxidant capacity is widely documented, with numerous studies demonstrating that higher TPC and TFC levels consistently correlate with improved free radical scavenging performance (Aryal et al., 2019; Muflihah et al., 2021).

4.4. Fourier Transform Infrared (FTIR) Analysis

Fourier Transform Infrared spectroscopy was employed to identify and characterise the functional groups present in the plant extracts of all five Fabaceae species (Fig 6). The FTIR analysis revealed distinct spectral peaks across both the functional group region ($1500\text{--}4000\text{ cm}^{-1}$) and the fingerprint region ($500\text{--}1500\text{ cm}^{-1}$), with the majority of peaks detected within the fingerprint region (Table 4). The analysis identified a diverse array of functional groups corresponding to hydroxyl groups (O-H), carboxyl groups (C=O), alkanes (C-C), alkenes (C=C),

alkynes ($\text{C}\equiv\text{C}$), aromatic primary amines (C-N), secondary amines (N-H), nitrogen-containing groups (N-O), aliphatic bromo compounds (C-Br), and aldehydes (C-H). These functional groups represent key structural components of important secondary metabolite classes, including alkaloids, flavonoids, phenols, polyphenols, tannins, and terpenoids (Lingegowda et al., 2012).

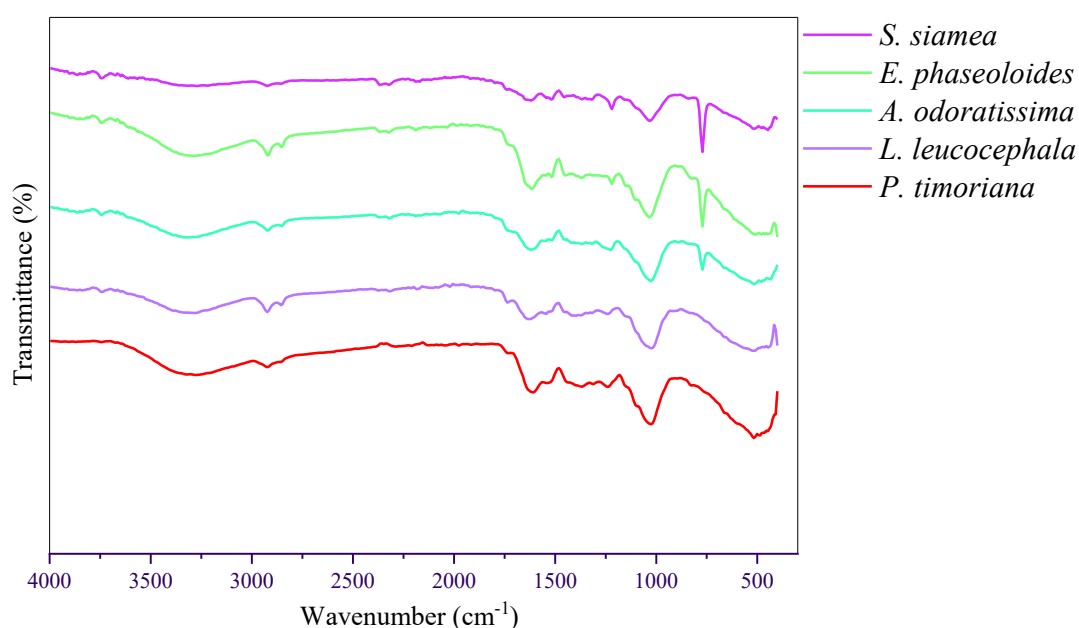


Fig 6. FTIR Spectra of the Extracts.

The spectral profiles obtained from FTIR analysis act as unique molecular fingerprints for each plant extract, facilitating accurate compound identification through comparison with reference spectral databases (Maobe et al., 2013). FTIR spectroscopy has become an established analytical technique in phytochemical analysis, offering high sensitivity and reliability in detecting and characterizing functional groups present in plant materials. The comprehensive functional group profile identified in the five Fabaceae species demonstrates the phytochemical complexity of these plant extracts and validates the utility of FTIR spectroscopy as a complementary analytical tool for phytochemical characterisation and authentication of medicinal plant materials.

Table 4. FTIR Peak Frequencies and Associated Functional Groups in the Extracts.

Frequency range (cm ⁻¹)	<i>P. timoriana</i>	<i>L. leucocephala</i>	<i>A. odoratissima</i>	<i>E. phaseoloides</i>	<i>S. siamea</i>	Functional Group
3870 - 3550	3734.88	3845.03	3863.08	3746.7	3743.59	O-H stretch alcohol (Polyphenols, flavonoids, tannins)
3500 - 3200	3324.14	3278.71	3320.4		3273.11	O-H stretch vibration presence of alcohols, phenols
3300 - 2850	2852.1	2868.63	2925.22	3286.8	2927.71	O-H stretch vibration, carboxylic acids (Alkaloids (amines), terpenoids, steroids)
2500 - 2300		2324.67	2373.21	2366.99	2326.36	C-H stretch vibration, alkenes (Organic acids, phenolic acids)
2260 - 2100		2185.89		2187.75	2188.38	C=C stretch vibration, Alkynes
1990 - 1739	1972.42	1740.29	1978.03		1742.16	Ester C=O stretch, lipid, triglycerides
1700 - 1600	1608.36	1627.03	1621.43	1615.83	1620.18	C=C stretch vibration, alkenes (Glycosides, flavonoid carbonyls, proteins)
1550 - 1475	1541.77	1546.12	1515.63	1521.23	1515.01	N-O asymmetric stretch, nitro compounds (Phenolics, Steroids, terpenoids, lipids)
1470 - 1400			1425.36	1447.17		C-C stretch vibration, aromatics
1400 - 1320	1369.38	1367.51	1371.25	1365.65	1365.02	N-O stretch vibration,

						nitro compounds (Phenolic OH, alkaloids)
1275 - 1150	1235.58	1246.79	1226.24	1220.64	1215.04	C-H wag stretch vibration, alkyl halides (Flavonoid glycosides, ethers, esters)
790 - 690		765.98	773.81	766.96	772.56	C(triple bond)C-HC-H bend stretch vibration, alkynes (Flavonoids, tannins)
680 - 510	519.27	519.27	513.67	509.94	521.14	C-Br stretch vibration, (Alkyl halides, glycogen)
490 - 400	486.91	446.46	471.97	465.89	448.32	Halogen compound

4.5. Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis

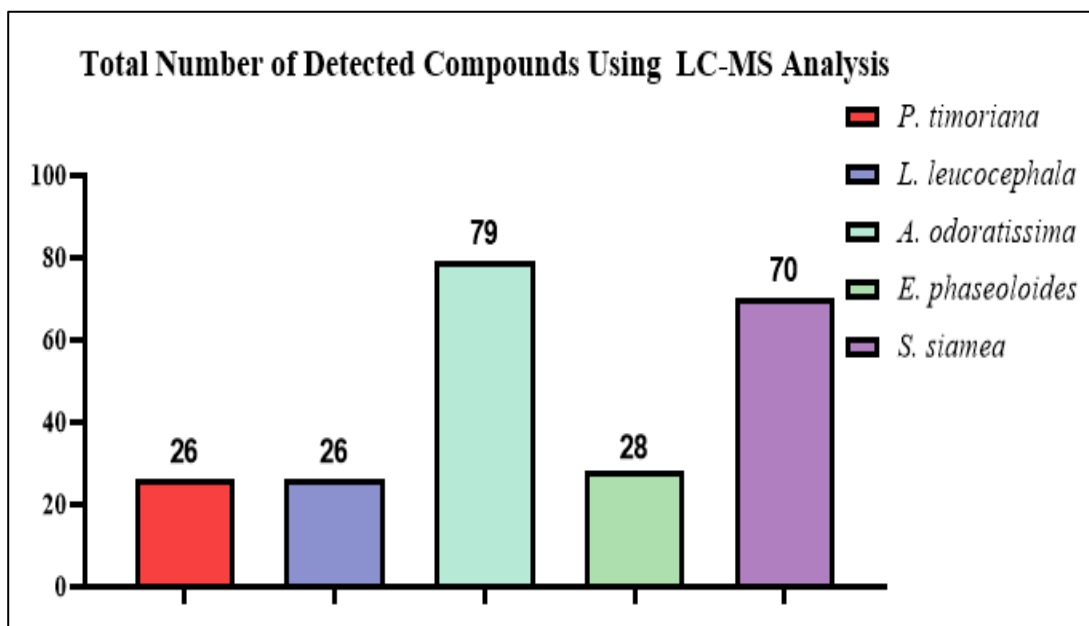


Fig 7. Total Number of Phytochemical Compounds Identified in Each Extract using LC-MS Analysis.

LC-MS analysis revealed considerable variation in the number of phytochemical constituents among the studied species. *A. odoratissima* exhibited the highest diversity with 79 compounds, followed by *S. siamea* with 70 compounds. In contrast, *E. phaseoloides* (28 compounds), *P. timoriana* (26 compounds), and *Leucaena leucocephala* (26 compounds) showed relatively fewer metabolites (Fig 7). Across these extracts, several bioactive metabolites were identified, including hodgekinsine, mycinamicin-VII, calcitriol, sophorane, atovaquone, and rutecarpin, among many others. Moreover, the compounds identified in the extracts have been reported to be associated with a wide spectrum of pharmacological properties (Table 5a-5e), such as antimicrobial, anticancer, anti-inflammatory, antifungal, antibacterial, antiparasitic, and analgesic activities, thereby underscoring their therapeutic potential (Lucci et al., 2017; Asma et al., 2022; Pereira et al., 2022; Dar et al., 2023). LC-MS has emerged as a powerful analytical technique for the identification and quantification of phytochemical constituents (Lucci et al., 2017). Plants have long been acknowledged as rich sources of bioactive compounds with significant therapeutic potential, and the analysis of their extracts is essential for the discovery

and characterisation of such metabolites. LC-MS has revolutionized this field by providing a sensitive and precise platform for the identification and quantification of a broad spectrum of phytochemical constituents (Vijayalakshmi et al., 2015).

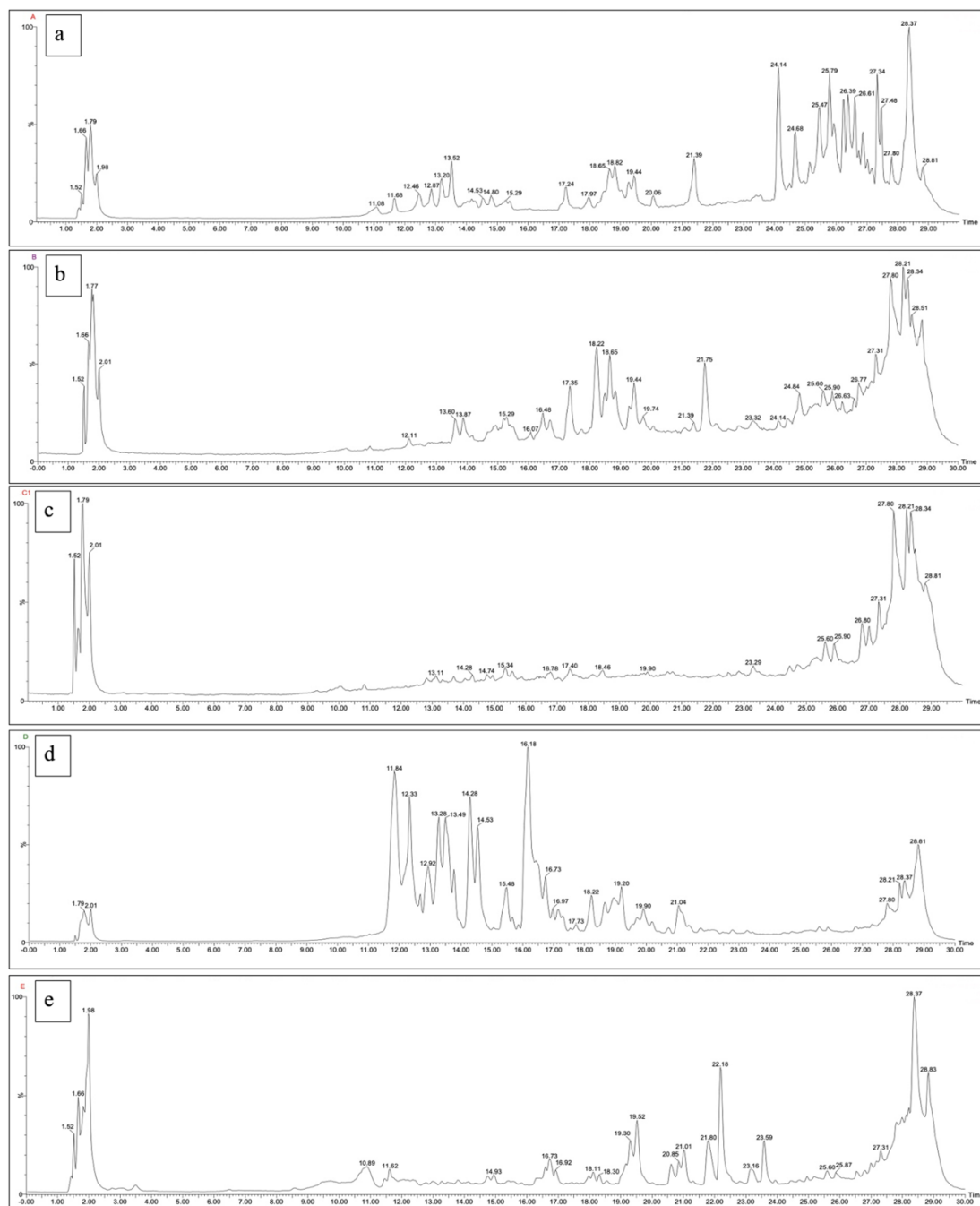


Fig 8. LC-MS Spectra of Extracts: (a) *P. timoriana*, (b) *L. leucocephala*, (c) *A. odoratissima*, (d) *E. phaseoloides*, and (e) *S. siamea*.

The diverse bioactivities observed among the five extracts including antioxidant capacity, cytotoxicity against HeLa cells, and broad-spectrum antibacterial effect may be attributed to their distinct phytochemical compositions as revealed through LC-MS profiling, confirming their broad therapeutic potential and pharmacological significance.

Table 5a. Identified Compounds from *P. timoriana* using LC-MS.

Sl. No.	m/z ratio	Compounds	Molecular Formula	Molecular Weight (g/mol)
1	28.37	6-Imino-5-oxocyclohexa-1,3-dienecarboxylate	C ₇ H ₅ NO ₃	151.12
2	1.79	O-Ureido-L-serine	C ₄ H ₉ N ₃ O ₄	163.13
3	1.66	D-Glucuronate	C ₆ H ₁₀ O ₇	194.14
4	20.06	5-(alpha-Phenylethyl)semioxamazide	C ₁₀ H ₁₃ N ₃ O ₂	207.23
5	18.79	1-(Methylnitrosoamino)-4-(3-pyridinyl)-1,4-butanediol	C ₁₀ H ₁₅ N ₃ O ₃	225.24
6	1.66	N(delta)-Hydroxy-N(omega)-methyl-N(omega)-nitroso-L-citrulline	C ₇ H ₁₄ N ₄ O ₅	234.21
7	28.51	3-[(3aS,4S,7aS)-7a-Methyl-1,5-dioxo-octahydro-1H-inden-4-yl]propanoate	C ₁₃ H ₁₇ O ₄	237.27
8	1.88	Guanidinoproclavaminic acid	C ₉ H ₁₆ N ₄ O ₄	244.25
9	1.69	Bluensidine	C ₈ H ₁₆ N ₄ O ₆	264.24
10	1.77	7-(Acetyloxy)-3-(3-pyridinyl)-2H-1-benzopyran-2-one	C ₁₆ H ₁₁ NO ₄	281.26
11	28.37	Nicofetamide	C ₂₀ H ₁₈ N ₂ O	302.4
12	28.37	Nicotianamine	C ₁₂ H ₂₁ N ₃ O ₆	303.31
13	18.82	Panamine	C ₂₀ H ₃₃ N ₃	315.5
14	27.01	(-)-Ormosanine	C ₂₀ H ₃₅ N ₃	317.5
15	1.55	N-Glycoloyl-neuraminate	C ₁₁ H ₁₈ NO ₁₀ ⁻	324.26
16	1.98	2-O-alpha-L-Rhamnopyranosyl-D-glucopyranose	C ₁₂ H ₂₂ O ₁₀	326.3
17	22.51	Deacetylcolchicine	C ₂₀ H ₂₃ NO ₅	357.4
18	27.45	17-(4-Hydroxyphenyl)heptadecanoate	C ₂₃ H ₃₇ O ₃ ⁻	361.5
19	28.37	7,8-Didemethyl-8-hydroxy-5-deazariboflavin	C ₁₆ H ₁₆ N ₃ O ₇ ⁻	362.31
20	1.77	Celecoxib	C ₁₇ H ₁₄ F ₃ N ₃ O ₂ S	381.4
21	17.97	O-Carbamoyl-deacetylcephalosporin C	C ₁₅ H ₂₀ N ₄ O ₈ S	416.4
22	27.04	1-O-Hexadecyl-lyso-sn-glycero-3-phosphocholine	C ₂₄ H ₅₂ NO ₆ P	481.6
23	28.13	Hodgkinsine	C ₃₃ H ₃₈ N ₆	518.7
24	28.83	Mycinamicin VII	C ₂₉ H ₄₇ NO ₇	521.7
25	25.38	Caracurine V	C ₃₈ H ₄₀ N ₄ O ₂	584.7
26	26.01	all-trans-Hexaprenyl diphosphate	C ₃₀ H ₅₂ O ₇ P ₂	586.7

Table 5b. Identified Compounds from *L. leucocephala* using LC-MS.

Sl. No.	m/z ratio	Compounds	Molecular Formula	Molecular Weight (g/mol)
1	156.06	N-Ethylammelide	C ₅ H ₈ N ₄ O ₂	156.14
2	188.07	2-Oxosuberate	C ₈ H ₁₂ O ₅	188.18
3	207.03	[5-(Aminomethyl)furan-3-yl]methyl phosphate	C ₆ H ₉ NO ₅ P ⁻	206.11
4	223.06	Selfotel	C ₇ H ₁₄ NO ₅ P	223.16
5	238.12	3-[(3aS,4S,7aS)-7a-Methyl-1,5-dioxo-octahydro-1H-inden-4-yl]propanoate	C ₁₃ H ₁₇ O ₄ ⁻	237.27
6	281.07	Flufenamic acid	C ₁₄ H ₁₀ F ₃ NO ₂	281.23
7	297.11	Psicofuranine	C ₁₁ H ₁₅ N ₅ O ₅	297.27
8	301.07	1-Guanidino-1-deoxy-scylo-inositol phosphate	C ₇ H ₁₆ N ₃ O ₈ P	301.19
9	302.14	Nicofetamide	C ₂₀ H ₁₈ N ₂ O	302.40
10	313.07	2-(Formamido)-N1-(5'-phosphoribosyl)acetamidine	C ₈ H ₁₆ N ₃ O ₈ P	313.20
11	315.27	Panamine	C ₂₀ H ₃₃ N ₃	315.50
12	323.05	Pirinixic acid	C ₁₄ H ₁₄ ClN ₃ O ₂ S	323.80
13	325.1	N-Glycoloyl-neuraminate	C ₁₁ H ₁₈ NO ₁₀ ⁻	324.26
14	351.09	Sulpyrine	C ₁₃ H ₁₆ N ₃ NaO ₄ S	333.34
15	359.11	Quinoline-3-carboxamides	C ₂₄ H ₂₈ N ₂ O ₃	392.50
16	361.12	Pyribencarb	C ₁₈ H ₂₀ ClN ₃ O ₃	361.80
17	366.1	Atovaquone	C ₂₂ H ₁₉ ClO ₃	366.80
18	381.07	Celecoxib	C ₁₇ H ₁₄ F ₃ N ₃ O ₂ S	381.40
19	399.14	Oxmetidine	C ₁₉ H ₂₁ N ₅ O ₃ S	399.50
20	410.21	Vismione D	C ₂₅ H ₃₀ O ₅	410.50
21	416.33	Calcitriol	C ₂₇ H ₄₄ O ₃	416.60
22	437.19	Azocyclotin	C ₂₀ H ₃₅ N ₃ Sn	436.20
23	444.36	3beta-Hydroxy-4beta-methyl-5alpha-cholest-7-ene-4alpha-carboxylate	C ₂₉ H ₄₇ O ₃ ⁻	443.70
24	460.26	Sophoranone	C ₃₀ H ₃₆ O ₄	460.60
25	518.32	Hodgkinsine	C ₃₃ H ₃₈ N ₆	518.70
26	521.34	Mycinamicin VII	C ₂₉ H ₄₇ NO ₇	521.70

Table 5c. Identified Compounds from *A. odoratissima* using LC-MS.

Sl. No.	m/z ratio	Compounds	Molecular Formula	Molecular Weight (g/mol)
1	126.05	5-Amino-4-imidazolecarboxamide	C ₄ H ₆ N ₄ O	126.12
2	151.03	6-Imino-5-oxocyclohexa-1,3-dienecarboxylate	C ₇ H ₅ NO ₃	151.12
3	182.08	Mannitol	C ₆ H ₁₄ O ₆	182.17
4	194.04	D-Glucuronate	C ₆ H ₁₀ O ₇	194.14
5	194.08	5-O-Methyl-myo-inositol (Sequoyitol)	C ₇ H ₁₄ O ₆	194.18
6	195.07	D-Glucose oxime	C ₆ H ₁₃ NO ₆	195.17
7	225.09	2-Chloro-N-(2,6-diethylphenyl)acetamide	C ₁₂ H ₁₆ ClNO	225.71
8	226.08	Cyclopenta[cd]pyrene	C ₁₈ H ₁₀	226.3
9	238.07	3-Deoxy-D-manno-octulosonate (KDO)	C ₈ H ₁₄ O ₈	238.19
10	246.09	Naphazoline hydrochloride	C ₁₄ H ₁₅ ClN ₂	246.73
11	256.13	Decarbamoylsaxitoxin	C ₉ H ₁₆ N ₆ O ₃	256.26
12	258.11	Fluenetil	C ₁₆ H ₁₅ FO ₂	258.29
13	261.11	Imazapyr	C ₁₃ H ₁₅ N ₃ O ₃	261.28
14	264.1	2-(6'-Methylthio)hexylmalic acid	C ₁₁ H ₂₀ O ₅ S	264.34
15	265.15	Esprocarb	C ₁₅ H ₂₃ NOS	265.4
16	266.12	Vasconine	C ₁₇ H ₁₆ NO ₂	266.31
17	269.11	Fenothiocarb sulfoxide	C ₁₃ H ₁₉ NO ₃ S	269.36
18	274.1	2,3-Dihydrothienamycin	C ₁₁ H ₁₈ N ₂ O ₄ S	274.34
19	275.17	Cyclobenzaprine	C ₂₀ H ₂₁ N	275.4
20	277.1	Metazachlor	C ₁₄ H ₁₆ ClN ₃ O	277.75
21	278.12	2-(7'-Methylthio)heptylmalic acid	C ₁₂ H ₂₂ O ₅ S	278.37
22	283.13	Brevianamide F	C ₁₆ H ₁₇ N ₃ O ₂	283.32
23	284.13	Promazine	C ₁₇ H ₂₀ N ₂ S	284.4
24	288.13	Karwinaphthol B	C ₁₇ H ₂₀ O ₄	288.34
25	290.12	Carbinoxamine	C ₁₆ H ₁₉ ClN ₂ O	290.79
26	292.11	Coumatetralyl	C ₁₉ H ₁₆ O ₃	292.3
27	300.1	Farrerol	C ₁₇ H ₁₆ O ₅	300.3
28	301.14	Febrifugine	C ₁₆ H ₁₉ N ₃ O ₃	301.34
29	303.14	Nicotianamine	C ₁₂ H ₂₁ N ₃ O ₆	303.31
30	307.19	Benztropine	C ₂₁ H ₂₅ NO	307.4
31	314.08	Ventinone A	C ₁₇ H ₁₄ O ₆	314.29
32	316.07	Cysteinyldopa	C ₁₂ H ₁₆ N ₂ O ₆ S	316.33
33	317.28	(-)-Ormosanine	C ₂₀ H ₃₅ N ₃	317.5
34	318.11	Paeoniflorigenone	C ₁₇ H ₁₈ O ₆	318.32
35	319.15	Metconazole	C ₁₇ H ₂₂ ClN ₃ O	319.8
36	327.11	Terrestriamide	C ₁₈ H ₁₇ NO ₅	327.3
37	333.16	Tebufenpyrad	C ₁₈ H ₂₄ ClN ₃ O	333.9
38	334.08	19-Hydroxy-8-O-methyltetrangulol	C ₂₀ H ₁₄ O ₅	334.3
39	336.12	Fenbuconazole	C ₁₉ H ₁₇ ClN ₄	336.8
40	338.08	L-Ascorbic acid-2-glucoside	C ₁₂ H ₁₈ O ₁₁	338.26
41	338.26	Clofilium	C ₂₁ H ₃₇ ClN	339
42	347.16	N-Methyl-cyclo(L-Trp-L-Phe)	C ₂₁ H ₂₁ N ₃ O ₂	347.4
43	349.3	8,11,14-Eicosatrienoylethanolamide	C ₂₂ H ₃₉ NO ₂	349.5
44	352.1	Hexythiazox	C ₁₇ H ₂₁ ClN ₂ O ₂ S	352.9
45	352.09	Savinin	C ₂₀ H ₁₆ O ₆	352.3
46	361.19	Phalaenopsine T	C ₂₀ H ₂₇ NO ₅	361.4
47	363.11	Rufloxacin	C ₁₇ H ₁₈ FN ₃ O ₃ S	363.4

48	366.1	Atovaquone	C ₂₂ H ₁₉ ClO ₃	366.8
49	371.02	Tetraconazole	C ₁₃ H ₁₁ Cl ₂ F ₄ N ₃ O	372.14
50	372.34	A-Nor-5alpha-cholestan-2-one	C ₂₆ H ₄₄ O	372.6
51	381.07	Metsulfuron-methyl	C ₁₄ H ₁₅ N ₅ O ₆ S	381.37
52	394.32	Ergosta-5,7,22,24(28)-tetraen-3beta-ol	C ₂₈ H ₄₂ O	394.6
53	395.09	Tribenuron methyl	C ₁₅ H ₁₇ N ₅ O ₆ S	395.4
54	400.05	Bis(glycerophospho)-glycerol	C ₉ H ₂₂ O ₁₃ P ₂	400.21
55	411.12	Mandipropamid	C ₂₃ H ₂₂ ClNO ₄	411.9
56	416.33	Calcitriol	C ₂₇ H ₄₄ O ₃	416.6
57	421.28	Talatizamine	C ₂₄ H ₃₉ NO ₅	421.6
58	426.19	Abscisic acid glucose ester	C ₂₁ H ₃₀ O ₉	426.5
59	437.19	Azocyclotin	C ₂₀ H ₃₅ N ₃ Sn	436.2
60	437.2	FMLP (N-Formylmethionyl-leucyl-phenylalanine)	C ₂₁ H ₃₁ N ₃ O ₅ S	437.6
61	444.36	3beta-Hydroxy-4beta-methyl-5alpha-cholest-7-ene-4alpha-carboxylate	C ₂₉ H ₄₇ O ₃	443.7
62	452.23	Hoechst 33342	C ₂₇ H ₂₈ N ₆ O	452.6
63	460.26	Sophoranone	C ₃₀ H ₃₆ O ₄	460.6
64	465.16	Bacampicillin	C ₂₁ H ₂₇ N ₃ O ₇ S	465.5
65	468.35	Scymnol	C ₂₇ H ₄₈ O ₆	468.7
66	469.12	Kalbreclasine	C ₂₀ H ₂₃ NO ₁₂	469.4
67	469.16	Indol-3-ylacetyl-myo-inositol L-arabinoside	C ₂₁ H ₂₇ NO ₁₁	469.4
68	476.27	Emetamine	C ₂₉ H ₃₆ N ₂ O ₄	476.6
69	478.28	Dehydroemetine	C ₂₉ H ₃₈ N ₂ O ₄	478.6
70	479.29	Delcorine	C ₂₆ H ₄₁ NO ₇	479.6
71	506.33	Iriomoteolide 1a	C ₂₉ H ₄₆ O ₇	506.7
72	508.34	Contignasterol	C ₂₉ H ₄₈ O ₇	508.7
73	518.31	Hodgkinsine	C ₃₃ H ₃₈ N ₆	518.7
74	521.33	Mycinamicin VII	C ₂₉ H ₄₇ NO ₇	521.7
75	542.31	Belladonnine	C ₃₄ H ₄₂ N ₂ O ₄	542.7
76	576.33	Cerberin	C ₃₂ H ₄₈ O ₉	576.7
77	650.33	Thapsigargin	C ₃₄ H ₅₀ O ₁₂	650.8
78	692.34	Scillaren A	C ₃₆ H ₅₂ O ₁₃	692.8
79	699.34	Adouetine Z	C ₄₂ H ₄₅ N ₅ O ₅	699.8

Table 5d. Identified Compounds from *E. phaseoloides* using LC-MS.

Sl. No.	m/z ratio	Compounds	Molecular Formula	Molecular Weight (g/mol)
1	146.06	2-Aceto-2-hydroxybutanoate	C ₆ H ₁₀ O ₄	Molecular Weight
2	151.03	6-Imino-5-oxocyclohexa-1,3-dienecarboxylate	C ₇ H ₅ NO ₃	146.14
3	163.06	O-Ureido-L-serine	C ₄ H ₉ N ₃ O ₄	151.12
4	182.08	Mannitol	C ₆ H ₁₄ O ₆	163.13
5	188.07	2-Oxosuberate	C ₈ H ₁₂ O ₅	182.17
6	194.08	5-O-Methyl-myo-inositol	C ₇ H ₁₄ O ₆	188.18
7	207.03	[5-(Aminomethyl)furan-3-yl]methyl phosphate	C ₆ H ₉ NO ₅ P ⁻	194.18
8	223.06	Selfotel	C ₇ H ₁₄ NO ₅ P	206.11
9	278.12	2-(7'-Methylthio)heptylmalic acid	C ₁₂ H ₂₂ O ₅ S	223.16
10	281.07	Flufenamic acid	C ₁₄ H ₁₀ F ₃ NO ₂	278.37
11	283.05	N2-Acetyl-L-aminoadipyl-delta-phosphate	C ₈ H ₁₄ NO ₈ P	281.23
12	302.22	Abietate	C ₂₀ H ₃₀ O ₂	283.17
13	303.14	Nicotianamine	C ₁₂ H ₂₁ N ₃ O ₆	302.50
14	317.28	(-)-Ormosanine	C ₂₀ H ₃₅ N ₃	303.31
15	325.10	N-Glycoloyl-neuraminate	C ₁₁ H ₁₈ NO ₁₀ ⁻	317.50
16	363.11	Rufloxacin	C ₁₇ H ₁₈ FN ₃ O ₃ S	324.26
17	366.10	Atovaquone	C ₂₂ H ₁₉ ClO ₃	363.40
18	381.07	Metsulfuron-methyl	C ₁₄ H ₁₅ N ₅ O ₆ S	366.80
19	383.07	Pantoprazole	C ₁₆ H ₁₅ F ₂ N ₃ O ₄ S	381.37
20	383.14	Tolfenpyrad	C ₂₁ H ₂₂ ClN ₃ O ₂	383.40
21	399.14	Oxmetidine	C ₁₉ H ₂₁ N ₅ O ₃ S	383.90
22	416.33	Calcitriol	C ₂₇ H ₄₄ O ₃	399.50
23	437.19	Azocyclotin	C ₂₀ H ₃₅ N ₃ Sn	416.60
24	438.21	Kurarione	C ₂₆ H ₃₀ O ₆	436.20
25	444.36	3beta-Hydroxy-4beta-methyl-5alpha-cholest-7-ene-4alpha-carboxylate	C ₂₉ H ₄₇ O ₃ ⁻	438.50
26	460.26	Sophoranone	C ₃₀ H ₃₆ O ₄	443.70
27	518.32	Hodgkinsine	C ₃₃ H ₃₈ N ₆	460.60
28	521.34	Mycinamicin VII	C ₂₉ H ₄₇ NO ₇	518.70

Table 5e. Identified Compounds from *S. siamea* using LC-MS.

Sl. No.	m/z ratio	Compounds	Molecular Formula	Molecular Weight (g/mol)
1	127.04	5-Amino-4-imidazole carboxylate	C ₄ H ₅ N ₃ O ₂	127.1
2	190.06	N-Carbamyl-L-glutamate	C ₆ H ₁₀ N ₂ O ₅	190.15
3	207.03	[5-(Aminomethyl)furan-3-yl]methyl phosphate	C ₆ H ₉ NO ₅ P ⁻	206.11
4	213.08	N-Acetyl-6-diazo-5-oxo-L-norleucine	C ₈ H ₁₁ N ₃ O ₄	213.19
5	217.08	Captopril	C ₉ H ₁₅ NO ₃ S	217.29
6	218.05	p-Chlorobenzhydrol	C ₁₃ H ₁₁ ClO	218.68
7	223.06	Selfotel	C ₇ H ₁₄ NO ₃ P	223.16
8	233.08	Bis(1-aziridinyl)morpholinophosphine sulfide	C ₈ H ₁₆ N ₃ OPS	233.27
9	238.12	3-[(3aS,4S,7aS)-7a-Methyl-1,5-dioxo-octahydro-1H-inden-4-yl]propanoate	C ₁₃ H ₁₇ O ₄ ⁻	237.27
10	248.04	(E)-2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide	C ₁₁ H ₈ N ₂ O ₅	248.19
11	256.06	Norbaeocystin	C ₁₀ H ₁₃ N ₂ O ₄ P	256.19
12	256.13	Decarbamoylsaxitoxin	C ₉ H ₁₆ N ₆ O ₃	256.26
13	257.07	Orbencarb	C ₁₂ H ₁₆ ClNOS	257.78
14	257.07	2-Amino-6-(benzylthio)purine	C ₁₂ H ₁₁ N ₅ S	257.32
15	260.12	Maculosin	C ₁₄ H ₁₆ N ₂ O ₃	260.29
16	271.05	Clorgyline	C ₁₃ H ₁₅ Cl ₂ NO	272.17
17	281.07	Flufenamic acid	C ₁₄ H ₁₀ F ₃ NO ₂	281.23
18	283.04	N2-Acetyl-L-aminoadipyl-delta-phosphate	C ₈ H ₁₄ NO ₈ P	283.17
19	287.11	Rutaecarpine	C ₁₈ H ₁₃ N ₃ O	287.3
20	293.08	Panfuran S	C ₁₁ H ₁₁ N ₅ O ₅	293.24
21	297.08	Albendazole sulfone	C ₁₂ H ₁₅ N ₃ O ₄ S	297.33
22	298.1	Thalassemine	C ₈ H ₁₉ N ₄ O ₆ P	298.23
23	299.13	Saxitoxin	C ₁₀ H ₁₇ N ₇ O ₄	299.29
24	302.21	Emedastine	C ₁₇ H ₂₆ N ₄ O	302.4
25	303.08	Chlorambucil	C ₁₄ H ₁₉ Cl ₂ NO ₂	304.2
26	303.14	Nicotianamine	C ₁₂ H ₂₁ N ₃ O ₆	303.31
27	304.11	Imicyafos	C ₁₁ H ₂₁ N ₄ O ₂ PS	304.35
28	306.07	Phenazine methosulfate	C ₁₄ H ₁₄ N ₂ O ₄ S	306.34
29	316.14	N(beta)-Epoxysuccinamoyl-DAP-Val	C ₁₂ H ₂₀ N ₄ O ₆	316.31
30	318.06	Kinamycin	C ₂₂ H ₁₈ N ₂ O ₉	454.4
31	319.13	Norfloxacin	C ₁₆ H ₁₈ FN ₃ O ₃	319.33
32	320.13	2-[(L-Alanin-3-ylcarbamoyl)methyl]-3-(2-aminoethylcarbamoyl)-2-hydroxypropanoate	C ₁₁ H ₂₀ N ₄ O ₇	320.3
33	320.12	Mugineic acid	C ₁₂ H ₂₀ N ₂ O ₈	320.3
34	321.14	Acronycine	C ₂₀ H ₁₉ NO ₃	321.4
35	325.1	N-Glycoloyl-neuraminate	C ₁₁ H ₁₈ NO ₁₀ ⁻	324.26
36	332.11	Tos-Arg-CH ₂ Cl	C ₁₃ H ₂₁ ClN ₄ O ₂ S	332.85
37	332.14	1-Dehydro-9-fluoro-11-oxotestololactone	C ₁₉ H ₂₁ FO ₄	332.4
38	333.15	Cyclo(L-Trp-L-Phe)	C ₂₀ H ₁₉ N ₃ O ₂	333.4
39	344.12	Calaxin	C ₁₉ H ₂₀ O ₆	344.4
40	344.11	Clotrimazole	C ₂₂ H ₁₇ ClN ₂	344.8
41	350.13	Safranin	C ₂₀ H ₁₉ ClN ₄	350.8
42	357.14	9-Thiocyanato-androst-4-ene-3,11,17-trione	C ₂₀ H ₂₃ NO ₃ S	357.5
43	364.14	Pyridaben	C ₁₉ H ₂₅ ClN ₂ OS	364.9

44	366.1	Atovaquone	C ₂₂ H ₁₉ ClO ₃	366.8
45	373.09	Deacetylcephalosporin C	C ₁₄ H ₁₉ N ₃ O ₇ S	373.38
46	378.13	N2-Citryl-N6-acetyl-N6-hydroxy-L-lysine	C ₁₄ H ₂₂ N ₂ O ₁₀	378.33
47	379.13	Fluotrimazole	C ₂₂ H ₁₆ F ₃ N ₃	379.4
48	380.09	Diphyllin	C ₂₁ H ₁₆ O ₇	380.3
49	381.07	Metsulfuron-methyl	C ₁₄ H ₁₅ N ₅ O ₆ S	381.37
50	394.14	Rotenone	C ₂₃ H ₂₂ O ₆	394.4
51	399.12	7-Deoxycylindrospermopsin	C ₁₅ H ₂₂ N ₅ O ₃ ⁺	320.37
52	399.13	Oxmetidine	C ₁₉ H ₂₁ N ₅ O ₃ S	399.5
53	400.06	Bis(glycerophospho)-glycerol	C ₉ H ₂₂ O ₁₃ P ₂	400.21
54	400.11	Flavonol 3-O-D-galactoside	C ₂₁ H ₂₀ O ₈	400.4
55	411.12	Mandipropamid	C ₂₃ H ₂₂ ClNO ₄	411.9
56	412.13	4-Methylamino-4-de(dimethylamino)anhydrotetracycline	C ₂₁ H ₂₀ N ₂ O ₇	412.4
57	416.33	Calcitriol	C ₂₇ H ₄₄ O ₃	416.6
58	437.19	Azocyclotin	C ₂₀ H ₃₅ N ₃ Sn	436.2
59	444.36	3beta-Hydroxy-4beta-methyl-5alpha-cholest-7-ene-4alpha-carboxylate	C ₂₉ H ₄₇ O ₃ ⁻	443.7
60	449.1	Cyhalothrin	C ₂₃ H ₁₉ ClF ₃ NO ₃	449.8
61	460.26	Sophoranone	C ₃₀ H ₃₆ O ₄	460.6
62	463.12	Peonidin 3-O-glucoside	C ₂₂ H ₂₃ ClO ₁₁	498.9
63	488.13	Phrymarolin I	C ₂₄ H ₂₄ O ₁₁	488.4
64	493.14	Glyburide	C ₂₃ H ₂₈ ClN ₃ O ₅ S	494
65	496.16	Mycophenolic acid O-acyl-glucuronide	C ₂₃ H ₂₈ O ₁₂	496.5
66	518.32	Hodgkinsine	C ₃₃ H ₃₈ N ₆	518.7
67	521.33	Mycinamicin VII	C ₂₉ H ₄₇ NO ₇	521.7
68	542.31	Belladonnine	C ₃₄ H ₄₂ N ₂ O ₄	542.7
69	546.2	Erastin	C ₃₀ H ₃₁ ClN ₄ O ₄	547
70	548.15	Puerarin xyloside	C ₂₆ H ₂₈ O ₁₃	548.5

4.6. Determination of Antioxidant Activity

4.6.1. DPPH and ABTS Radical Scavenging Activity

Antioxidant activity of the five Fabaceae plant extracts was evaluated using both DPPH and ABTS radical scavenging assays, which represent standardized and complementary methodologies for quantifying free radical scavenging capacity. DPPH radical scavenging assay revealed that *P. timoriana* demonstrated the strongest antioxidant potential with the lowest IC₅₀ value of 13.88 ± 0.27 µg/mL, followed by *L. leucocephala*, *A. odoratissima*, *E. phaseoloides*, and *S. siamea* with an IC₅₀ value of 79.60 ± 0.26 µg/mL (Fig 9).

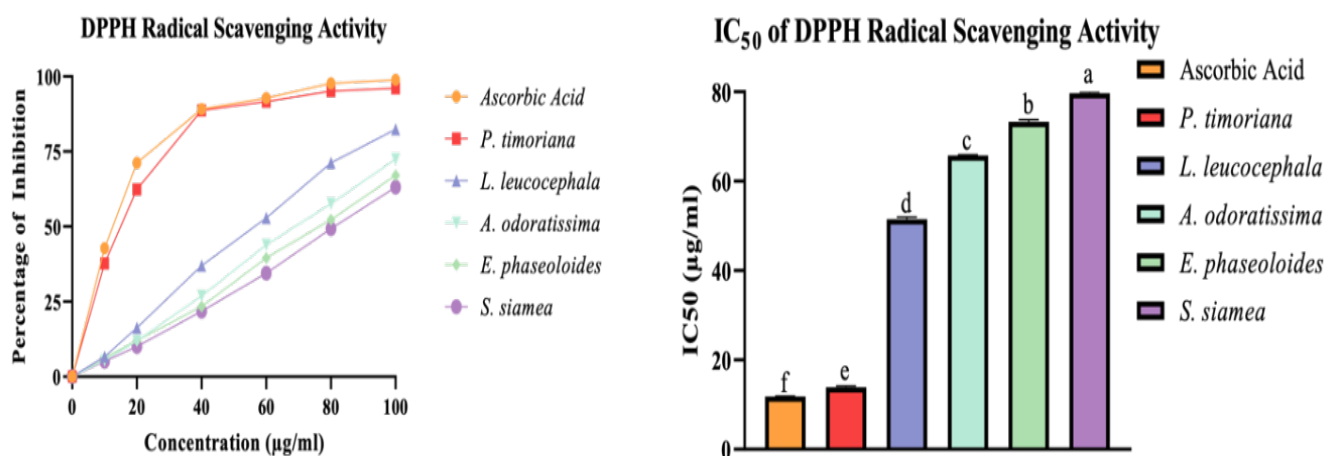


Fig 9. Comparison of DPPH Radical Scavenging Activity of Each Plant Extract.

Values are expressed as mean $\pm$ SE, $n = 3$. Different letters indicate significant variation ($p < 0.05$).

Similarly, ABTS radical scavenging activity showed consistent results (Fig 10), with *P. timoriana* exhibiting the lowest IC₅₀ value of 35.41 ± 0.31 µg/mL, indicative of superior antioxidant potential, followed by *L. leucocephala*, *A. odoratissima*, *E. phaseoloides*, and *S. siamea* (71.56 ± 0.46 µg/mL). Notably, the antioxidant potential observed in the present study was substantially higher compared to previously reported values from methanol bark extracts of *P. timoriana* (DPPH IC₅₀ = 106.85 ± 1.24 µg/mL; ABTS IC₅₀ = 127.33 ± 0.74 µg/mL) (Sariwati et al., 2024), suggesting enhanced bioactivity in the pod extracts examined in this study.

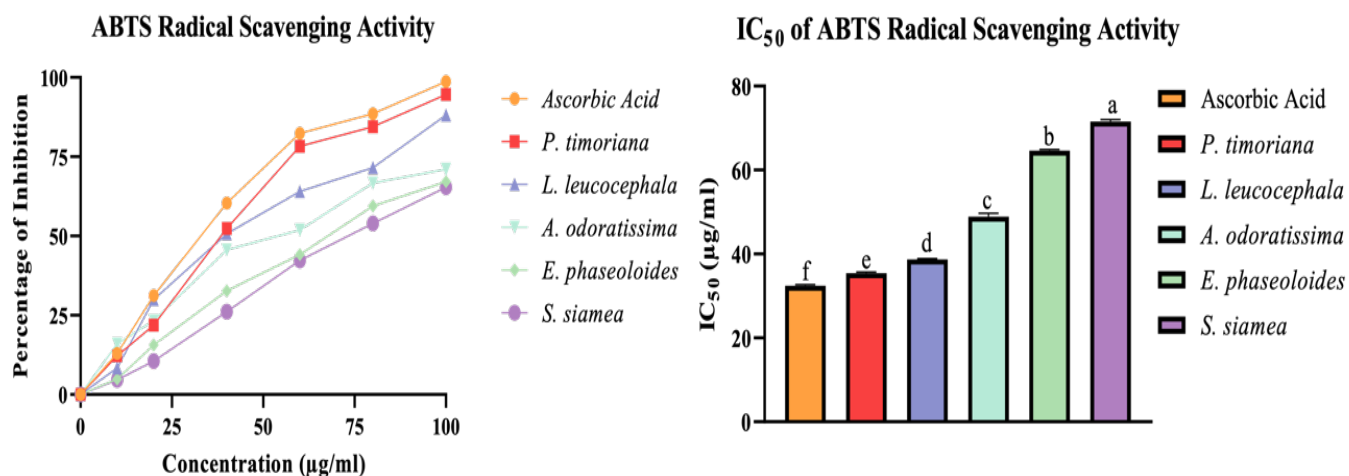


Fig 10. Comparison of ABTS Radical Scavenging Activity of Each Plant Extract.

Values are expressed as mean $\pm$ SE, $n = 3$. Different letters indicate significant variation ($p < 0.05$).

The DPPH and ABTS assays represent standardised spectrophotometric methodologies for quantifying free radical scavenging capacity through distinct mechanistic pathways. DPPH employs mixed-mode hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms, measuring the reduction of stable nitrogen-centered radicals at 515-518 nm. On the other hand, ABTS employs cationic radical scavenging detected at 734 nm with enhanced sensitivity and broader pH tolerance (3-9 versus 5-9 for DPPH), enabling comprehensive assessment of both hydrophilic and lipophilic antioxidant constituents in plant extracts for phytochemical characterisation and bioactive compound screening (Baliyan et al., 2022).

The IC₅₀ values (concentration required to inhibit 50% of radical activity) serve as critical quantitative parameters for antioxidant potency comparison, where lower IC₅₀ values indicate stronger antioxidant activity, extracts with IC₅₀ values less than 50 µg/mL are considered highly potent, 50-100 µg/mL show strong activity, and IC₅₀ values higher than 100 µg/mL demonstrate moderate to weak antioxidant capacity (Lee et al. 2015).

The DPPH and ABTS assays are widely recognized as reliable, practical, and standardized methodologies for determining antioxidant activity in plant extracts.

Their simplicity, rapid execution, minimal instrumentation requirements, and dependence on straightforward spectrophotometric measurements render them particularly effective for preliminary phytochemical screening and bioactivity evaluation (Mishra et al., 2012). The consistent antioxidant potency demonstrated across both assay platforms in the present study provides strong evidence for the bioactive potential of these Fabaceae species and supports their traditional use in ethnomedicinal applications.

4.6.2. Ferric Reducing Power Assay

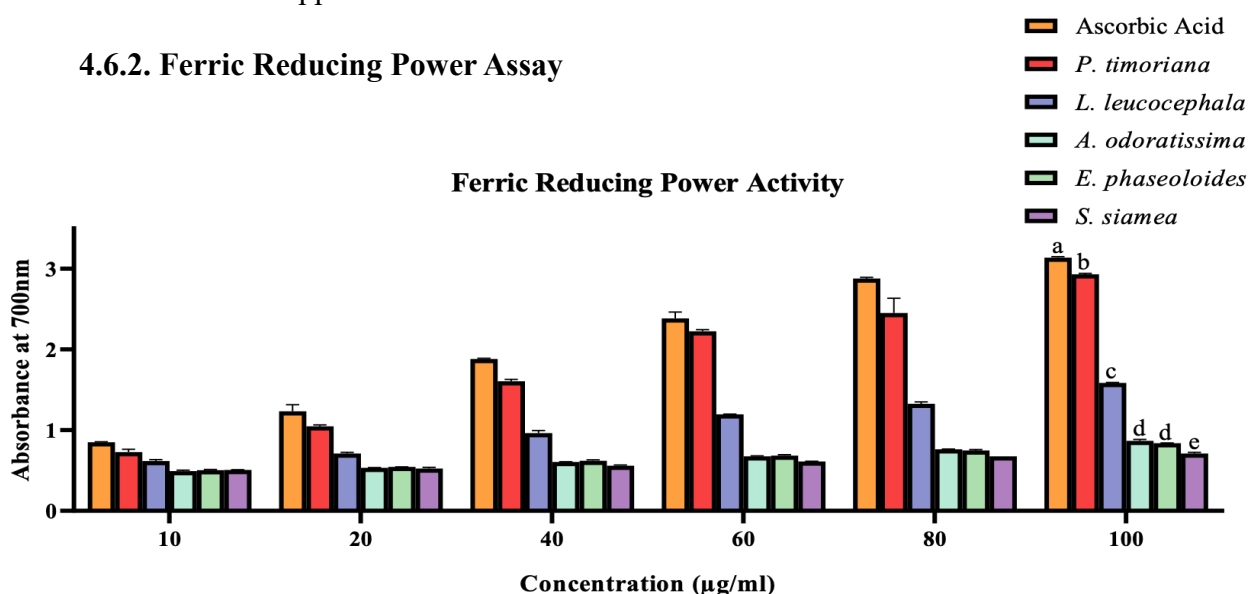


Fig 11. Zone of Inhibition Determination Using Plant Extracts Against Selected Bacterial Strains. Values are expressed as mean $\pm$ SE, $n = 3$. Different letters indicate significant variation ($p < 0.05$).

The ferric reducing power assay was employed to evaluate the electron-donating capacity and reducing potential of the plant extracts as an additional measure of antioxidant activity. The assay is based on the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}), wherein increased absorbance at 700 nm indicates enhanced reducing power and antioxidant potential (Fig 11). The five Fabaceae plant extracts exhibited pronounced dose-dependent increasing reducing activity across the tested concentration range. *P. timoriana* (2.93 ± 0.01 at 100 $\mu\text{g/mL}$) consistently demonstrated the highest reducing power among the plant extracts, approaching the antioxidant potential of the positive control ascorbic acid, while *L. leucocephala*, *A. odoratissima*, *E. phaseoloides*, and *S. siamea* showed progressively lower reducing activity. This concentration-dependent response pattern reflects the antioxidant

potential of the extracts and demonstrates that the reducing activity, and consequently the capacity for free radical inhibition, is strongly influenced by extract dosage (Adjimani and Asare, 2015). The consistent dose-response relationship observed across all five species indicates that the reducing power of these plant extracts is directly proportional to the concentration of bioactive antioxidant compounds present.

4.7. Cytotoxicity Assay

Cytotoxicity against HeLa cervical cancer cells was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay, which serves as a standardized colorimetric methodology for assessing the cytotoxic potential of plant extracts (Fig 12). The MTT assay revealed dose-dependent inhibition of HeLa cell viability by all five Fabaceae plant extracts. *P. timoriana* exhibited the strongest cytotoxic effect with the lowest IC₅₀ value of 5.31 ± 0.05 µg/mL, followed by *S. siamea* (7.86 ± 0.24 µg/mL), *L. leucocephala* (10.65 ± 0.08 µg/mL), *A. odoratissima* (12.33 ± 0.08 µg/mL), and *E. phaseoloides* (15.31 ± 0.19 µg/mL). All of the five extracts demonstrated promising cytotoxic potential, as crude extracts exhibiting *in vitro* cytotoxic activity with IC₅₀ values below 30-40 µg/mL are considered to possess significant cytotoxic potential (El-Hallouty et al., 2015).

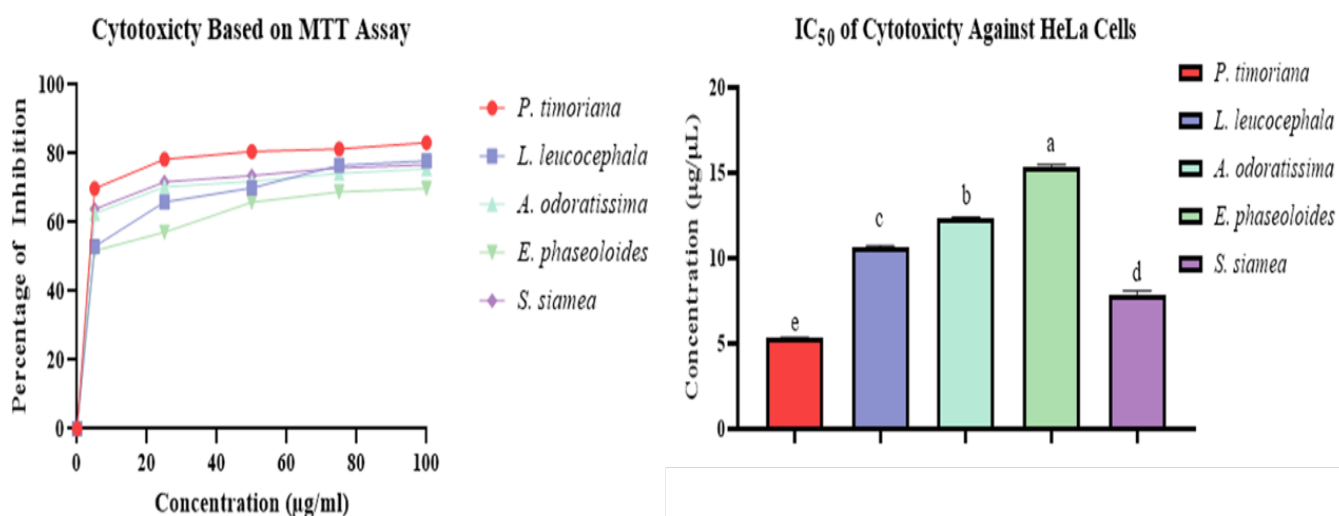


Fig 12. Cytotoxicity Against HeLa Cells of Each Plant Extract. Values are expressed as mean $\pm$ SE, n = 3. Different letters indicate significant variation (p < 0.05).

The MTT assay is a widely used colorimetric method for assessing the cytotoxicity and cell viability of various compounds, including plant extracts, owing to its simplicity, reliability, and sensitivity. This assay provides quantitative and accurate data on the cytotoxic potential of plant extracts, thereby helping to evaluate their safety for living cells. Considering the growing interest in plant-derived secondary metabolites with pharmacological potential, the MTT assay serves as a crucial tool for screening and identifying extracts with possible anticancer properties (Nair and Varalakshmi, 2011; Nemati et al., 2013; Chen et al., 2015). The pronounced cytotoxic effects observed in this study correlate closely with the elevated antioxidant potential demonstrated in DPPH, ABTS, and ferric reducing power assays, reflecting the dual bioactivity of phenolic and flavonoid compounds, which function both as free radical scavengers and as inducers of apoptotic pathways in cancer cells (Sammar et al., 2019; Akbarmehr et al., 2023). Notably, *P. timoriana*, which exhibited the highest total phenolic and flavonoid contents in quantitative analysis, also demonstrated the most pronounced cytotoxic efficacy, supporting the hypothesis that polyphenol enrichment drives both antioxidant and cytotoxic activities. This mechanistic convergence is further substantiated by growing evidence that Fabaceae-derived phytochemicals induce apoptosis, arrest cell cycle progression, and exhibit cytotoxic action against diverse cancer cell types, including breast (MCF-7), lung (A-549), colon (HCT-116), liver (HepG-2), and nasopharyngeal carcinomas (Sebastian et al., 2020; Usman et al., 2022). These results indicate that each of the studied extracts holds meaningful promise as a potential source of natural anticancer agents, and support further investigation of their mechanisms and applications in cancer therapy development.

4.8. Determination of Antibacterial Activity

4.8.1. Agar-well Diffusion Assay

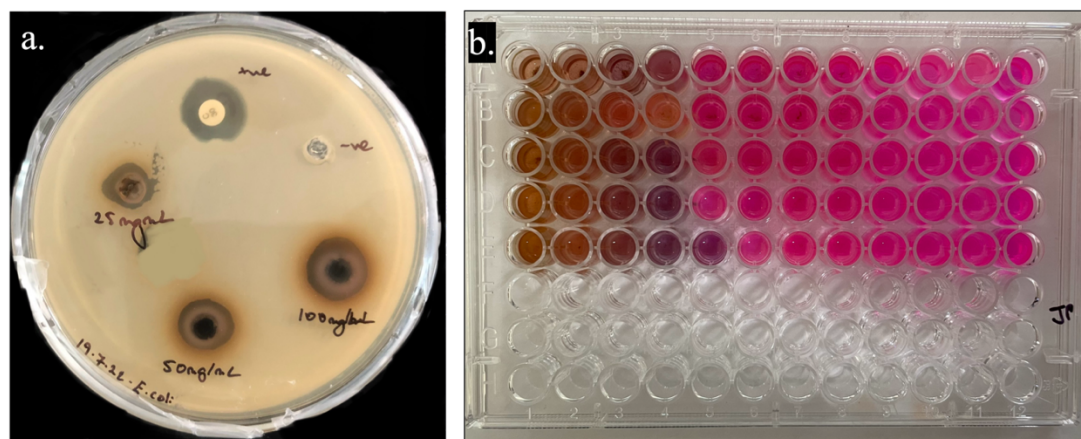


Fig 13. Determination of Antibacterial Activity using, (a) Agar well diffusion and, (b) MIC using Resazurin based assay.

Determination of antibacterial activity of the extracts using agar-well diffusion revealed that all the extracts have a strong potential of bacterial growth inhibition (Fig 14). Among the tested bacterial strains, all the extracts exhibited the strongest growth inhibitory activity against *E. coli* and the weakest activity against *B. pumilus*. When compared between gram-positive and gram-negative bacteria, the extracts were more effective against the gram-negative *E. coli* and the gram-positive *S. aureus*.

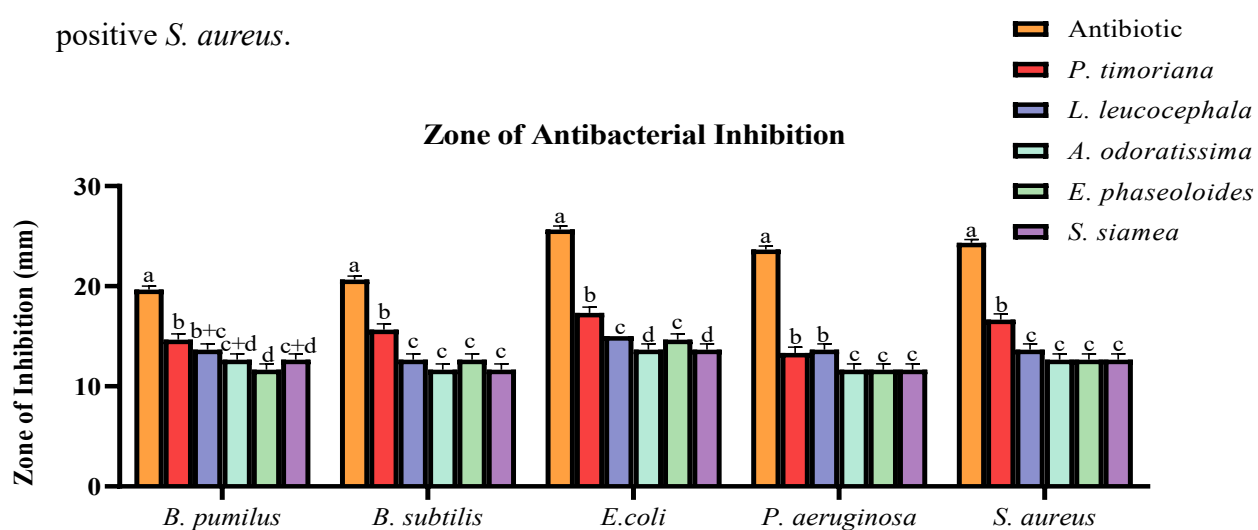


Fig 14. Zone of Inhibition Determination Using Plant Extracts Against Selected Bacterial Strains. Values are expressed as mean $\pm$ SE, $n = 3$. Different letters indicate significant variation ($p < 0.05$).

4.8.2. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) assay also revealed a pattern consistent with the growth inhibition results based on agar-well diffusion assay (Fig 15). The extracts demonstrated the lowest MIC values against *Escherichia coli*, indicating higher potency, and the highest MIC values against *B. pumilus*, indicating lower efficacy. Similarly, comparatively lower MIC values were recorded against *Staphylococcus aureus* among the Gram-positive strains. These findings further support the observed trend that the extracts were most effective against *E. coli* and *S. aureus*.

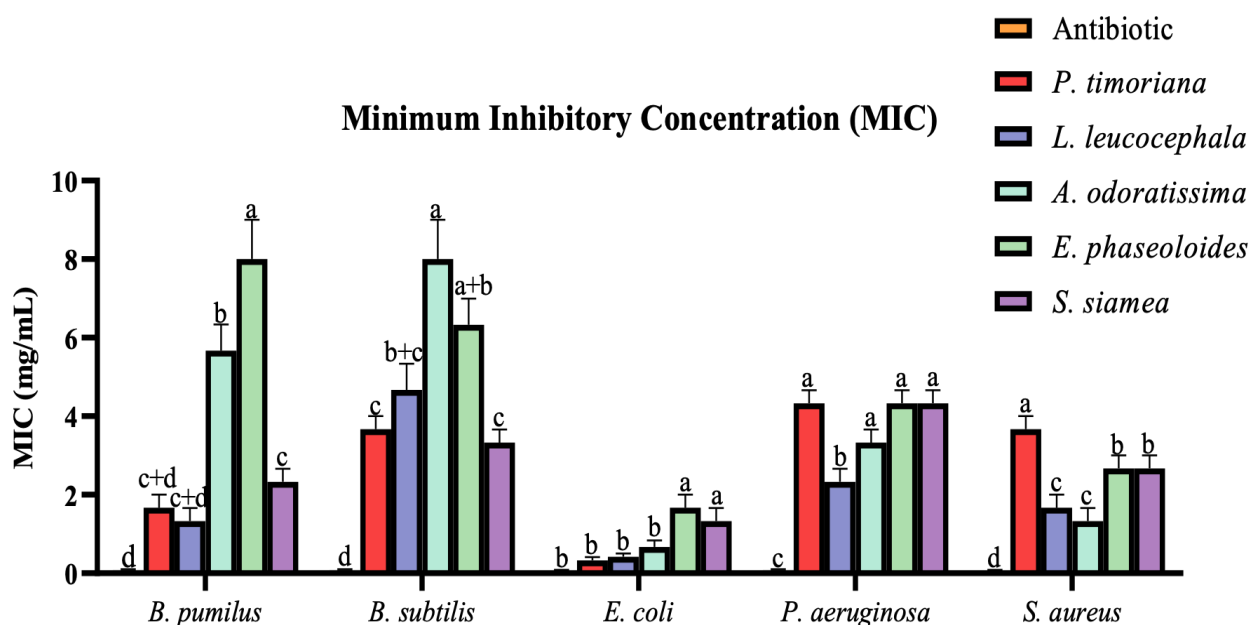


Fig 15. MIC of Plant Extracts Against Selected Bacterial Strains. Values are expressed as mean $\pm$ SE, n = 3. Different letters indicate significant variation ($p < 0.05$).

The agar well-diffusion method has been widely reported as a qualitative assay for evaluating the antimicrobial activity of natural products (Elshikh et al., 2016). The MIC assay is a rapid and straightforward technique that provides an estimate of the lowest concentration of an antimicrobial agent required to inhibit the visible growth of a microorganism under controlled conditions (Wiegand et al., 2008). Resazurin (7-hydroxy-3H-phenoxazin-3-one 10-oxide) is a blue dye that can be irreversibly reduced by oxidoreductases in metabolically active bacteria to

resorufin, a pink and highly fluorescent compound, which can be further reduced to non-fluorescent hydroresorufin. This redox conversion provides a direct and quantifiable measure of bacterial metabolic activity and is commonly employed to determine the minimum inhibitory concentration of antimicrobial substances (Chakansin et al., 2022). The antibacterial activity of the extracts may be attributed to the presence of bioactive phytochemicals such as saponins, alkaloids, and tannins, which have been reported to function as natural defense compounds protecting plants against pathogenic microorganisms. These phytochemicals have also been shown to confer protective effects in humans when plant parts are traditionally consumed as concoctions or decoctions in ethnomedicine (Bukar et al., 2009).

4.9. *In silico* Molecular Docking Studies

To complement the *in vitro* findings, select-key compounds identified from LC-MS profiling of the five Fabaceae extracts were evaluated for drug-likeness and pharmacokinetic attributes using SwissADME, a robust and internationally accepted tool for early-stage drug discovery. Comprehensive SwissADME analysis revealed that all selected ligands from *P. timoriana*, *L. leucocephala*, *A. odoratissima*, *E. phaseoloides*, and *S. siamea* met critical criteria for oral bioavailability, pharmacokinetic desirability, and Lipinski's rule compliance, supporting their feasibility as potential drug leads (Daina et al., 2017).

Table 6. Ligand selection from LC-MS data of each extract for antibacterial molecular docking studies.

Plant species	Ligand	No. of Rotatable Bonds	No. of H-bond Acceptors	No. of H-bond Donors	Gastrointestinal Absorption	Blood Brain Barrier Permeation	Drug Likelihood (Lipinski)
<i>P. timoriana</i>	Deacetylcolchicine	4	6	1	High	No	Yes
<i>L. leucocephala</i>	Sophoranone	7	4	2	Low	No	Yes
<i>A. odoratissima</i>	Paeoniflorigenone	4	6	1	High	No	Yes
<i>E. phaseoloides</i>	Kurarinone	7	6	3	High	No	Yes
<i>S. siamea</i>	Rotenone	3	6	0	High	Yes	Yes

4.9.1. *In silico* Targeting of *E. coli* FtsZ (L178E) GTP-complex (PDB ID: 6UNX) for Antibacterial Activity:

Molecular docking analysis of compounds from five plant species against *E. coli* FtsZ (L178E) GTP-complex (PDB ID: 6UNX) revealed varying binding affinities (Table 7). Kurarinone from *E. phaseoloides* exhibited the strongest binding affinity (-11.54 kcal/mol), followed by rotenone from *S. siamea* (-10.16 kcal/mol). Deacetylcolchicine from *P. timoriana* showed moderate binding affinity (-8.6 kcal/mol), while sophoranone from *L. leucocephala* (-7.2 kcal/mol) and paeoniflorigenone from *A. odoratissima* (-6.8 kcal/mol) demonstrated lower but still significant binding energies (Fig 16). All plant-derived compounds showed superior binding affinities compared to the control ampicillin (-7.01 kcal/mol), suggesting their potential as effective FtsZ inhibitors.

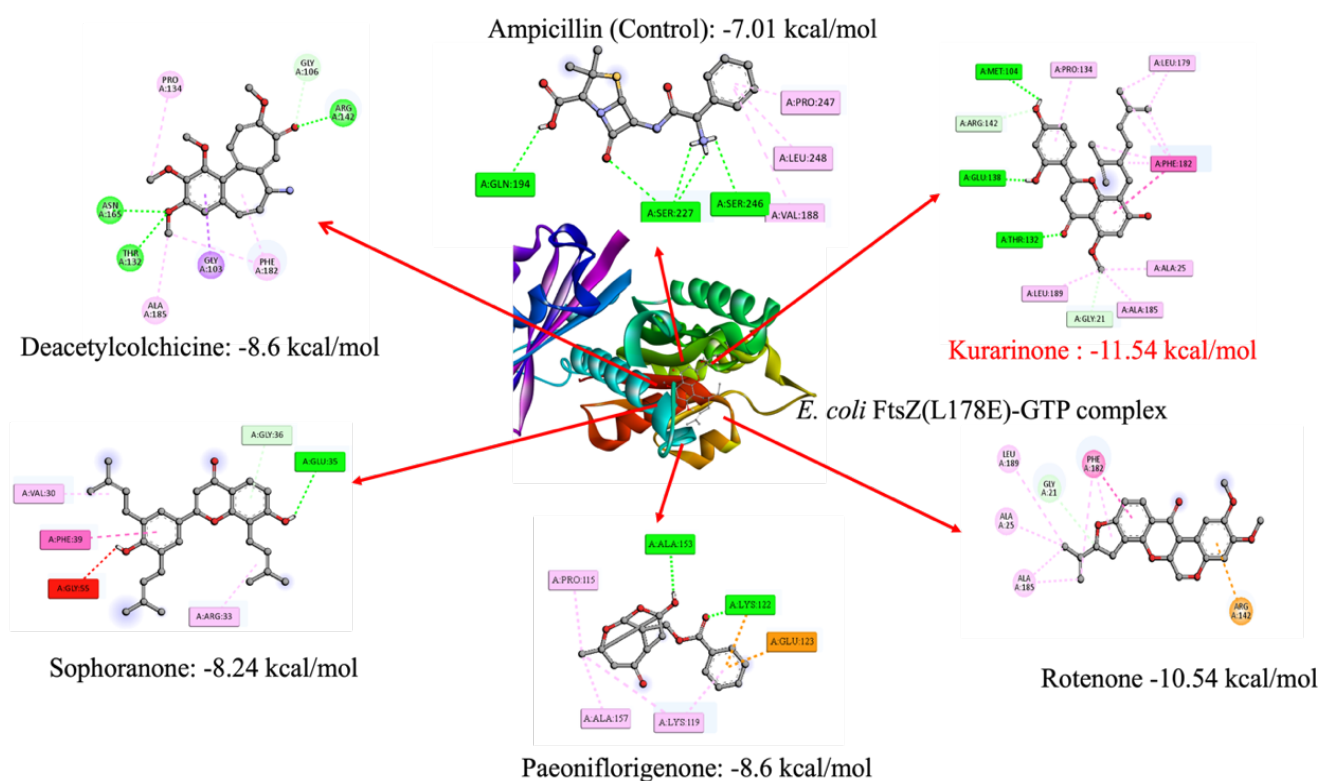


Fig 16. Molecular docking study of protein-ligand interactions of *E. coli* FtsZ (L178E) GTP-complex (PDB ID: 6UNX)

The FtsZ(L178E)-GTP crystal structure (PDB: 6UNX) is significant for molecular docking studies targeting antibacterial activity as FtsZ represents a highly attractive antimicrobial target that is essential for bacterial cell division but absent in

human cells, making it a promising candidate for developing novel antibiotics against drug-resistant bacterial strains. The availability of this high-resolution *E. coli* FtsZ structure enables computational screening of inhibitors that can disrupt bacterial cytokinesis by binding to the GTP pocket or allosteric sites, potentially leading to cell elongation and lysis, a mechanism of action that remains clinically unexploited despite the urgent need for new antibiotics against multidrug-resistant pathogens (Schumacher et al., 2020; Andreu et al., 2022; Du et al., 2022).

Table 7. Molecular docking study of protein-ligand interactions of *E. coli* FtsZ (L178E) GTP-complex (PDB ID: 6UNX)

Plant species and Control	Ligand	Binding energy (kcal/mol)	Ligand efficiency	Inhibit constant ^d (KI) (nM)
Control	Ampicillin	-7.01	-0.26	86
<i>P. timoriana</i>	Deacetylcolchicine	-8.6	-0.33	42.2
<i>L. leucocephala</i>	Sophoranone	-7.2	-0.54	12.5
<i>A. odoratissima</i>	Paeoniflorigenone	-6.8	-0.76	54
<i>E. phaseoloides</i>	Kurarinone	-11.54	-0.36	3.61
<i>S. siamea</i>	Rotenone	-10.16	-0.35	35.51

4.9.2. *In silico* Targeting of *S. aureus* FtsZ GDP-form (3VO8) for Antibacterial Activity:

In addition, molecular docking analysis of compounds against *S. aureus* FtsZ GDP-form (PDB ID: 3VO8) also demonstrated varying binding affinities (Table 8). Sophoranone from *L. leucocephala* exhibited the strongest binding affinity (-8.24 kcal/mol), followed by rotenone from *S. siamea* (-7.59 kcal/mol). Paeoniflorigenone from *A. odoratissima* showed moderate binding affinity (-7.11 kcal/mol), while deacetylcolchicine from *P. timoriana* (-7.01 kcal/mol) demonstrated comparable binding energy to the control ampicillin (-7.21 kcal/mol). Kurarinone from *E. phaseoloides* displayed the lowest binding affinity among the tested compounds (-6.27 kcal/mol). The plant-derived compounds showed competitive binding affinities compared to ampicillin, with sophoranone and rotenone demonstrating superior binding energies, suggesting their potential as effective FtsZ inhibitors against *S. aureus* (Fig 17).

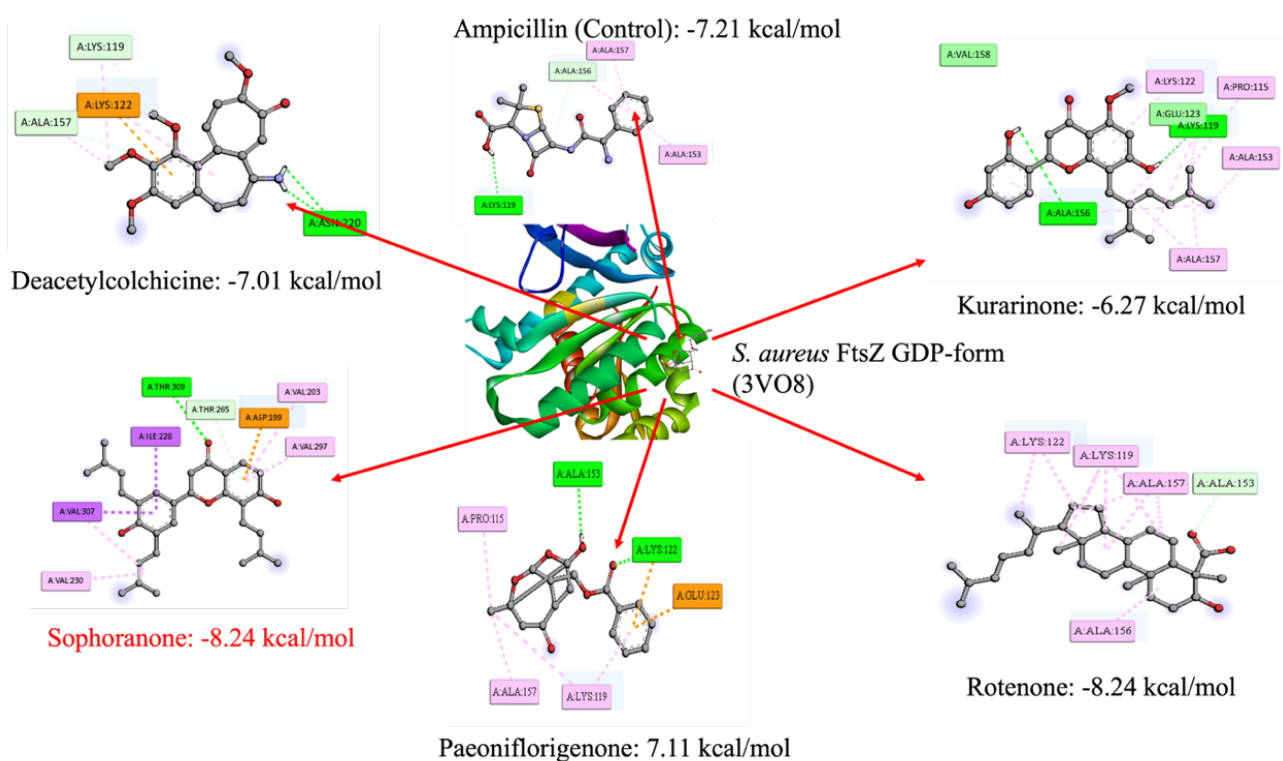


Fig 17. Molecular docking study of protein-ligand interactions of *S. aureus* FtsZ GDP-form (3VO8).

The crystal structure of *Staphylococcus aureus* FtsZ in its GDP-bound state (PDB: 3VO8) represents the first reported structure capturing the protein in a straight conformation, which is distinct from the curved conformation observed in other bacterial FtsZ structures (Matsui et al., 2012). This straight conformation is crucial for filament formation and is directly linked to its functional activity during cell division (Chan et al., 2013; Pradhan et al., 2021). Therefore, this structure was selected as a target for molecular docking studies to facilitate the identification of potential FtsZ inhibitors.

Table 8. Molecular docking study of protein-ligand interactions of *S. aureus* FtsZ GDP-form (3VO8).

Plant species and Control	Ligand	Binding energy (kcal/mol)	Ligand efficiency	Inhibit constant ^d (KI) (nM)
Control	Ampicillin	-7.21	-0.26	28.18
<i>P. timoriana</i>	Deacetylcolchicine	-7.01	-0.25	39
<i>L. leucocephala</i>	Sophoranone	-8.24	0.15	46.5
<i>A. odoratissima</i>	Paeoniflorigenone	-7.11	-0.31	6.88
<i>E. phaseoloides</i>	Kurarinone	-6.27	-0.2	25.33
<i>S. siamea</i>	Rotenone	-7.59	-0.23	14.75

4.9.3. *In silico* Targeting of AKT1 for Anticancer Activity:

Molecular docking analysis against AKT1 Serine/Threonine kinase (PDB ID: 3O96) revealed significant binding affinities (Table 10). Farrerol from *A. odoratissima* exhibited the strongest binding affinity (-11.0 kcal/mol), followed by rutecarpine from *S. siamea* (-10.9 kcal/mol). Oxmetidine, identified in both *L. leucocephala* and *E. phaseoloides*, demonstrated identical strong binding affinities (-10.2 kcal/mol). Panamine from *P. timoriana* showed the lowest binding affinity (-6.2 kcal/mol) among the tested compounds but exhibited the highest ligand efficiency (-0.77). All plant-derived compounds except panamine demonstrated superior binding affinities compared to the control fluorouracil (-7.54 kcal/mol), with farrerol, rutecarpine, and oxmetidine showing particularly strong interactions with the AKT1 kinase, suggesting their potential as effective kinase inhibitors (Fig 18).

Table 9. Ligand selection from LC-MS data of each extract for anticancer molecular docking studies targeting AKT1 Serine/Threonine kinase (3O96)

Plant species	Ligand	No. of Rotatable Bonds	No. of H-bond Acceptors	No. of H-bond Donors	Gastrointestinal Absorption	Blood Brain Barrier Permeation	Drug Likelihood (Lipinski)
<i>P. timoriana</i>	Panamine	0	3	1	High	Yes	Yes
<i>L. leucocephala</i>	Oxmetidine	8	5	3	High	No	Yes
<i>A. odoratissima</i>	Farrerol	1	5	3	High	No	Yes
<i>E. phaseoloides</i>	Oxmetidine	8	5	3	High	No	Yes
<i>S. siamea</i>	Rutecarpine	0	2	1	High	Yes	Yes

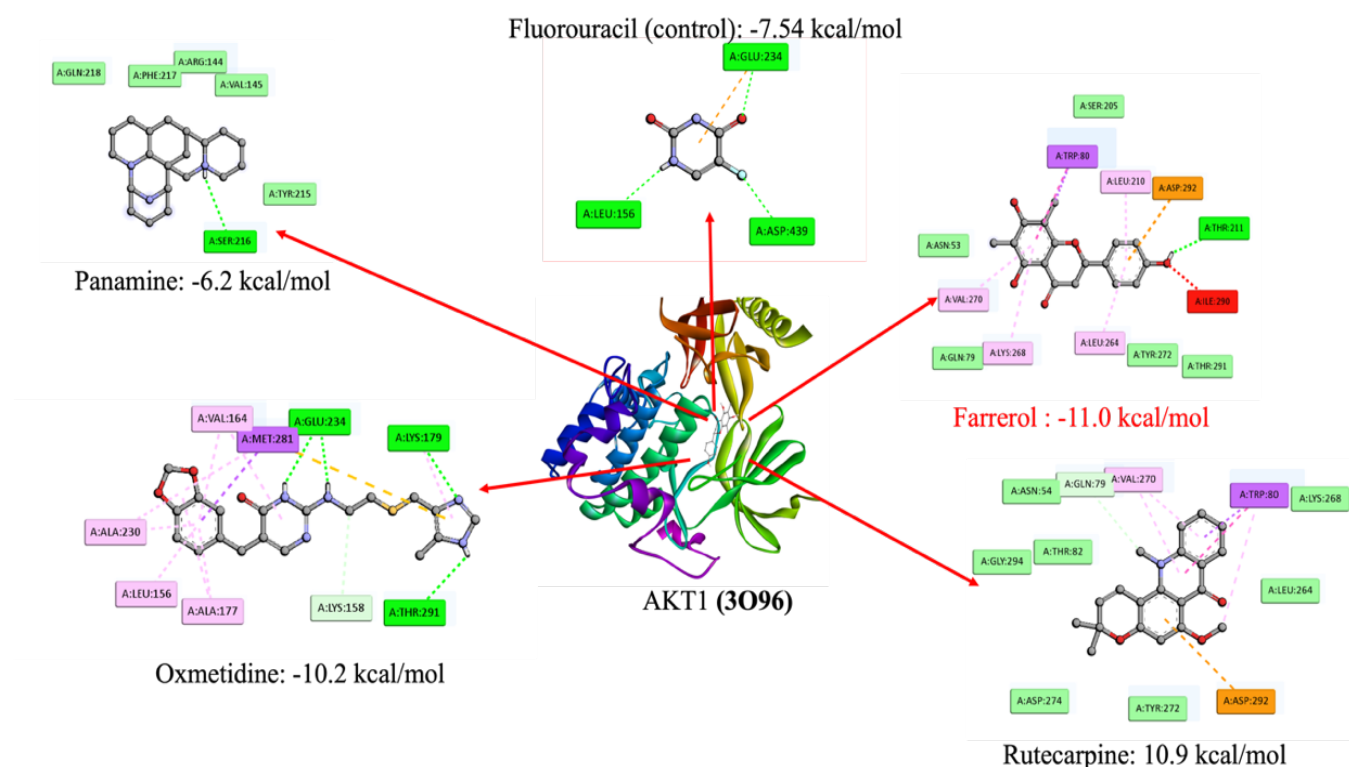


Fig 18. Molecular docking study of protein-ligand interactions of AKT1 Serine/Threonine kinase (3O96).

Autophagy and apoptosis are both regulated by the PI3K/AKT1/mTOR signalling pathway, which is one of the most frequently dysregulated pathways in malignancies, including lung cancer (Luo et al., 2003). Therefore, inhibiting survival proteins such as AKT1 and mTOR represents a promising therapeutic strategy. In cancer cells, alterations in upstream regulators, such as the activation of the epidermal growth factor receptor (EGFR) can lead to sustained activation of AKT1, thereby promoting uncontrolled cell growth and survival (Papadimitrakopoulou, 2012). Overexpression or constitutive activation of AKT has been reported in several malignancies, including ovarian, lung, and pancreatic cancers, where it is closely associated with enhanced cancer cell proliferation and survival. Therefore, targeting the AKT signaling pathway represents a crucial strategy for cancer prevention and therapy. Inhibiting AKT activity, particularly through modulation of the AKT1 gene, is one of the key mechanisms by which many compounds exert their anticancer effects (Ballou and Lin, 2008; Huang et al., 2021).

Table 10. Molecular docking study of protein-ligand interactions of AKT1 Serine/Threonine kinase (3O96).

Plant species and Control	Ligand	Binding energy (kcal/mol)	Ligand efficiency	Inhibit constant ^d (KI) (nM)
Control	Fluorouracil	-7.54	-0.52	86.34
<i>P. timoriana</i>	Panamine	-6.2	-0.77	12.5
<i>L. leucocephala</i>	Oxmetidine	-10.2	-0.42	86.3
<i>A. odoratissima</i>	Farrerol	-11.0	-0.39	39.4
<i>E. phaseoloides</i>	Oxmetidine	-10.2	-0.53	86.3
<i>S. siamea</i>	Rutaecarpine	-10.9	-0.49	25.3

The results observed from molecular studies revealed strong binding affinities with the target proteins, further suggesting their antibacterial and anticancer potential. Molecular docking has emerged as a key computational tool in drug design, enabling the systematic investigation of interactions between small molecules and target macromolecules. By predicting ligand-receptor interactions and estimating binding affinities, molecular docking accelerates the drug discovery process, making it more cost-effective and efficient (Ralte et al., 2022).

Chapter 5

Conclusion

The present study represents a comprehensive, multi-faceted evaluation of the phytochemical composition, secondary metabolite profiles, and bioactive properties of methanol pod extracts from five Fabaceae species from Mizoram- *P. timoriana*, *L. leucocephala*, *A. odoratissima*, *E. phaseoloides*, and *S. siamea*. The study involved authentication of the selected plants through rigorous morphological characterisation, complemented and validated by molecular authentication using five standardized DNA barcode regions (ITS2, rbcL, matK, trnH-psbA, and rpoC1), which provided robust species-level identification through genetic sequencing. Moreover, phylogenetic analysis of these multilocus barcode sequences generated well-supported phylogenetic trees with high bootstrap values.

Preliminary phytochemical screening using standard qualitative chemical tests detected eight major classes of secondary metabolites, such as alkaloids, flavonoids, phenols, steroids, glycosides, terpenoids, saponins, and tannins across all five species, confirming the phytochemical richness characteristic of the Fabaceae family and supporting their traditional ethnomedicinal applications in Northeast Indian folk medicine. The quantitative assessment of total phenolic content and total flavonoid content revealed substantial variation among the extracts, with *P. timoriana* demonstrating the highest phenolic and flavonoid concentration (58.88 GAE/g; 126.05 ± 0.47 QE/g). The synergistic abundance of both phenolic and flavonoid compounds in these extracts represents a significant source of bioactive metabolites, as these two secondary metabolite classes collectively contribute to the observed biological activities.

DPPH and ABTS radical scavenging assays, along with ferric reducing power assay revealed significant antioxidant potential of the extracts, with *P. timoriana* exhibiting superior antioxidant activity (DPPH IC₅₀: 13.88 ± 0.27 µg/mL; ABTS IC₅₀: 35.41 ± 0.31 µg/mL). The correlation between quantified phenolic/flavonoid levels and measured antioxidant potency supports the hypothesis that phenolic and

flavonoid metabolites are the primary bioactive constituents responsible for the radical scavenging properties observed experimentally.

FTIR analysis confirmed the presence of functional groups in the extracts including aromatic rings, hydroxyl groups, carbonyl moieties, and C-N stretches characteristic of the identified secondary metabolite classes. Comprehensive phytochemical profiling using LC-MS identified distinct phytochemical compounds, *A. odoratissima* exhibited the highest diversity with 79 compounds, followed by *S. siamea* with 70 compounds, *E. phaseoloides* (28 compounds), *P. timoriana* (26 compounds), and *Leucaena leucocephala* (26 compounds). These identified compounds possess well-documented antimicrobial, anticancer, anti-inflammatory, antifungal, antibacterial, antiparasitic, and analgesic activities, providing a chemistry-based mechanistic foundation for the observed broad-spectrum bioactivities and validating the multitarget polypharmacological potential of these plant extracts.

Cytotoxicity assessment using MTT assay demonstrated dose-dependent inhibition of HeLa cell viability across all five Fabaceae extracts, with *Parkia timoriana* exhibiting the most potent cytotoxic effect (IC_{50} : $5.31 \pm 0.05 \mu\text{g/mL}$), followed by *Senna siamea* ($7.86 \pm 0.24 \mu\text{g/mL}$), *Leucaena leucocephala* ($10.65 \pm 0.08 \mu\text{g/mL}$), *Albizia odoratissima* ($12.33 \pm 0.08 \mu\text{g/mL}$), and *Entada phaseoloides* ($15.31 \pm 0.19 \mu\text{g/mL}$). All of the five extracts demonstrated significant cytotoxic potential, with IC_{50} values substantially below the 30-40 $\mu\text{g/mL}$ threshold established for crude plant extracts to be considered cytotoxically active, therefore indicating promising anticancer potential.

Evaluation of antibacterial potential based on agar well diffusion and minimum inhibitory concentration (MIC) assays demonstrated consistent patterns of antimicrobial efficacy across all five Fabaceae extracts. The extracts exhibited superior potency against *E. coli* among the gram-negative strains, and *S. aureus* among the gram-positive strains. All five extracts showed promising broad-spectrum antibacterial potential, with *P. timoriana* consistently exhibiting the strongest inhibitory effects and lowest MIC values across all tested bacterial strains. The

preferential activity against *E. coli* and *S. aureus* with both clinically relevant multidrug-resistant pathogens, suggests that these Fabaceae species merit further investigation for potential antimicrobial therapeutic applications.

In silico molecular docking analysis targeting bacterial FtsZ proteins (*E. coli* L178E-GTP complex 6UNX and *S. aureus* GDP-bound form 3VO8) and the human AKT1 kinase (PDB 3O96) provided computational validation of the experimental antibacterial and anticancer findings. Molecular docking results demonstrated favourable binding affinities and conformational complementarity between identified phytochemical compounds and these validated therapeutic targets, thereby offering mechanistic insights into ligand-receptor interactions and supporting the biological plausibility of observed *in vitro* bioactivities through structure-based computational evidence.

Collectively, the findings of this comprehensive multi-disciplinary investigation establish the five selected Fabaceae as significant sources of bioactive phytochemicals with demonstrable antioxidant, antibacterial, and anticancer potential. The systematic integration of morphological and molecular authentication through DNA barcoding, qualitative and quantitative phytochemical characterisation through preliminary screening and quantification of TPC and TFC, advanced analytical profiling utilizing LC-MS and FTIR spectroscopy, *in vitro* bioactivity assessment through antioxidant, antibacterial, and cytotoxicity assays, and structure-based computational drug discovery employing molecular docking targeting FtsZ and AKT1 provides a rigorous, evidence-based framework for comprehensive evaluation of medicinal plant extracts and supports their scientific validation for potential therapeutic development.

Future investigations should aim to isolate and characterise the bioactive compounds identified using LC-MS analysis, accompanied by comprehensive *in vitro* mechanistic evaluations to establish structure-activity relationships. Cytotoxicity screenings must be expanded to additional cancer cell lines to systematically assess the therapeutic potential and selectivity of the most active compounds across diverse malignancies. *In vivo* efficacy assessments using

appropriate animal models are also necessary to validate pharmacological relevance. Further research should explore synergistic interactions with standard chemotherapeutic agents and integrate molecular dynamics simulations for improved prediction of binding affinity and optimization of lead compounds. Additionally, the development of advanced formulations to enhance solubility, stability, and bioavailability will be crucial to facilitate the translational progression of these promising Fabaceae-derived compounds toward clinical application.

REFERENCES

- Adjimani, J. P., & Asare, P. (2015). Antioxidant and free radical scavenging activity of iron chelators. *Toxicology Reports*, 2, 721–728.
- Agu, K. C., & Okolie, P. N. (2017). Proximate composition, phytochemical analysis, and *in vitro* antioxidant potentials of extracts of *Annona muricata* (Soursop). *Food science & nutrition*, 5(5), 1029-1036.
- Agu, P. C., Afiukwa, C. A., Orji, O. U., Ezech, E. M., Ofoke, I. H., Ogbu, C. O., ... & Aja, P. M. (2023). Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. *Scientific reports*, 13(1), 13398.
- Ahmad, F., Anwar, F., & Hira, S. (2016). Review on medicinal importance of Fabaceae family. *Pharmacologyonline*, 3, 151-157.
- Ahmad, F., Anwar, F., & Hira, S. (2016). Review on medicinal importance of Fabaceae family. *Pharmacology Online*, 3, 151–156.
- Ahmad, S. R., & Tamang, M. (2020). Study of indigenous medicinal plants of northeast states of India as potential therapies for Malaria. *Biosciences Biotechnology Research Asia*, 17(3), 567
- Akbarmehr, A., Peighambardoust, S. H., Ghanbarzadeh, B., & Sarabandi, K. (2023). Physicochemical, antioxidant, antimicrobial, and *in vitro* cytotoxic activities of corn pollen protein hydrolysates obtained by different peptidases. *Food Science & Nutrition*, 11(5), 2403–2417.
- Ali, K., Ali, A., Khan, M. N., Rahman, S., Faizi, S., Ali, M. S., & Musharraf, S. G. (2021). Rapid identification of common secondary metabolites of medicinal herbs using high-performance liquid chromatography with evaporative light scattering detector in extracts. *Metabolites*, 11(8), 489.
- Alum, E. U., Manjula, V. S., Uti, D. E., Echegu, D. A., Ugwu, O. P. C., Egba, S. I., & Agu, P. C. (2025). Metabolomics-driven standardization of herbal medicine:

- advances, applications, and sustainability considerations. *Natural Product Communications*, 20(8), 1934578X251367650.
- Andreu, J. M., Huecas, S., Araújo-Bazán, L., Vazquez-Villa, H., & Martin-Fontecha, M. (2022). The search for antibacterial inhibitors targeting cell division protein FtsZ at its nucleotide and allosteric binding sites. *Biomedicines*, 10(8), 1825.
- Angami, T., Bhagawati, R., Touthang, L., Makdoh, B., Nirmal, Lungmuana, & Ayyanar, M. (2018). Traditional uses, phytochemistry and biological activities of *Parkia timoriana* (DC.) Merr., an underutilized multipurpose tree bean: A review. *Genetic Resources and Crop Evolution*, 65(2), 679–692.
- Anuja, N., Kothakota, N. J., Bashar, S., & Vataparthy, P. (2024). Different DNA Barcoding Techniques in Forensic Botany: A Review. *Biosciences Biotechnology Research Asia*, 21(3), 935-945.
- Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants*, 8(4), 96.
- Ashokkumar, R., & Ramaswamy, M. (2014). Phytochemical screening by FTIR spectroscopic analysis of leaf extracts of selected Indian medicinal plants. *Journal of Herbal Medicine*, 4(3), 186–190.
- Asma, S. T., Acaroz, U., Imre, K., Morar, A., Shah, S. R. A., Hussain, S. Z., ... & Ince, S. (2022). Natural products/bioactive compounds as a source of anticancer drugs. *Cancers*, 14(24), 6203.
- Bahari, E. A., Zaaba, N. E., Haron, N., Dasiman, R., & Amom, Z. (2014). Antioxidant activity characterization, phytochemical screening, and proximate analysis of Cermela Hutan (*Phyllanthus gomphocarpus* Hook. f) roots and leaves. *Medical Science Monitor: Basic Research*, 20, 170–178.
- Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. M. (2022). Determination of antioxidants by DPPH radical

- scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. *Molecules*, 27(4), 1326.
- Ballou, L. M., & Lin, R. Z. (2008). Rapamycin and mTOR kinase inhibitors. *Journal of Chemical Biology*, 1(1), 27–36.
- Banothu, V., Neelagiri, C., Adepally, U., Lingam, J., & Bommareddy, K. (2017). Phytochemical screening and evaluation of *in vitro* antioxidant and antimicrobial activities of the indigenous medicinal plant *Albizia odoratissima*. *Pharmaceutical Biology*, 55(1), 1155–1161.
- Barua, C. C., Hazorika, M., & Misri, J. (2014). An overview of *Entada phaseoloides*: Current research and future prospects. *Journal of Pharmacy and Pharmacology*, 2, 1–18.
- Benjamim, J. K. F., da Costa, K. A. D., & Santos, A. S. (2020). Chemical, botanical and pharmacological aspects of the Leguminosae. *Pharmacognosy Reviews*, 14(28), 106–120.
- Bhoir, K., Varatha, A., Tamnar, L., & Jagdale, R. (2024). *Albizia odoratissima*: Review on morphology, pharmacological activity, physicochemical, phytochemical study and traditional uses. *International Journal of Research in Applied Science and Engineering Technology*, 12(10), 64701.
- Bonev, B., Hooper, J., & Parisot, J. (2008). Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. *Journal of Antimicrobial Chemotherapy*, 61(6), 1295–1301.
- Borges, A., José, H., Homem, V., & Simões, M. (2020). Comparison of Techniques and Solvents on the Antimicrobial and Antioxidant Potential of Extracts from *Acacia dealbata* and *Olea europaea*. *Antibiotics*, 9(2), 48.
- Bukar, A., Mukhtar, M., & Hassan, A. (2009). Phytochemical screening and antibacterial activity of leaf extracts of *Senna siamea* (Lam) on *Pseudomonas aeruginosa*. *Bayero Journal of Pure and Applied Sciences*, 2(1), 139–142.

- Caesar, L. K., & Cech, N. B. (2019). Synergy and antagonism in natural product extracts: When 1+ 1 does not equal 2. *Natural Product Reports*, 36(6), 869–888.
- Cavalcante, J. B., de Brito Silva, A., de Freitas Lopes, R., Rosseto, L. P., Marques, F. A., da Silva, O. V., ... & Fajemiroye, J. O. (2025). From Folk Medicine to Pharmacology: A Systematic Review of the Anti-Inflammatory Evidence for *Hymenaea* spp.(Fabaceae). *Plants*, 14(22), 3545.
- CBOL Plant Working Group. (2009). A DNA barcode for land plants. *Proceedings of the National Academy of Sciences of the United States of America*, 106(31), 12794–12797.
- Chahar, M. K., Sharma, N., Dobhal, M. P., & Joshi, Y. C. (2011). Flavonoids: A versatile source of anticancer drugs. *Pharmacognosy Reviews*, 5(9), 1–8.
- Chakansin, C., Yostaworakul, J., Warin, C., Kulthong, K., & Boonrungsiman, S. (2022). Resazurin rapid screening for antibacterial activities of organic and inorganic nanoparticles: Potential, limitations and precautions. *Analytical Biochemistry*, 637, 114449.
- Chan, F. Y., Sun, N., Neves, M. A., Lam, P. C. H., Chung, W. H., Wong, L. K., & Wong, K. Y. (2013). Identification of a new class of FtsZ inhibitors by structure-based design and *in vitro* screening. *Journal of Chemical Information and Modeling*, 53(8), 2131–2140.
- Chaudhary, P., Janmeda, P., Docea, A. O., Yeskaliyeva, B., Abdull Razis, A. F., Modu, B., ... & Sharifi-Rad, J. (2023). Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. *Frontiers in chemistry*, 11, 1158198.
- Chen, J., Duan, Y., Zhang, X., Ye, Y., Ge, B., & Chen, J. (2015). Genistein induces apoptosis by the inactivation of the IGF-1R/p-Akt signaling pathway in MCF-7 human breast cancer cells. *Food & Function*, 6(3), 995–1000.

- Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., & Leon, C. (2010). Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS ONE*, 5(1), e8613.
- Chen, S., Yin, X., Han, J., Sun, W., Yao, H., Song, J., & Li, X. (2023). DNA barcoding in herbal medicine: Retrospective and prospective. *Journal of Pharmaceutical Analysis*, 13(5), 431–441.
- Cinelli, M. A., & Wilson, A. E. (2024). Mass spectrometry does not need to be a "grind": Plant secondary metabolites in coffee and black pepper as a practical liquid chromatography-tandem mass spectrometry (LC-MS/MS) teaching tool. *Journal of Chemical Education*, 101(3), 1257–1267.
- Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific reports*, 7(1), 42717.Fpra
- Dar, R. A., Shahnawaz, M., Ahanger, M. A., & Majid, I. U. (2023). Exploring the diverse bioactive compounds from medicinal plants: a review. *J. Phytopharm*, 12(3), 189-195.
- Deivasigamani, R. (2018). Phytochemical analysis of *Leucaena leucocephala* on various extracts. *Journal of Phytopharmacology*, 7(6), 480–482.
- Del Papa, M. F., Delgado, M. J., Irisarri, P., Lattanzi, F. A., & Monza, J. (2024). Maximizing nitrogen fixation in legumes as a tool for sustainable agriculture intensification, volume II. *Frontiers in Agronomy*, 6, 1387188.
- Deniz, U. Ğ., Güneş, H., Güneş, F., & Mammadov, R. (2017). Cytotoxic activities of certain medicinal plants on different cancer cell lines. *Turkish Journal of Pharmaceutical Sciences*, 14(3), 222–228.
- Dinesha, S., Kungkho, C., Kumar, A., Gopal, R., Apshahana, K., Tiwari, D., & Shukla, G. (2025). Exploring the conservation and sustainable utilization potential of *Parkia timoriana* (DC.) Merr: An underutilized multipurpose tree bean in North-Eastern India. *The NEHU Journal*, 44, 1–15.

- Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical bulletin*.
- Du, R. L., Sun, N., Fung, Y. H., Zheng, Y. Y., Chen, Y. W., Chan, P. H., & Wong, K. Y. (2022). Discovery of FtsZ inhibitors by virtual screening as antibacterial agents and study of the inhibition mechanism. *RSC Medicinal Chemistry*, 13(1), 79–89.
- El-Hallouty, S. M., Fayad, W., Meky, N. H., El-Menshaw, B. S., Wassel, G. M., & Hasabo, A. A. (2015). *In vitro* anticancer activity of some Egyptian plant extracts against different human cancer cell lines. *International Journal of Pharmaceutical Technology Research*, 8, 267–272.
- El-Saadony, M. T., Saad, A. M., Mohammed, D. M., Korma, S. A., Alshahrani, M. Y., Ahmed, A. E., ... & Ibrahim, S. A. (2025). Medicinal plants: bioactive compounds, biological activities, combating multidrug-resistant microorganisms, and human health benefits-a comprehensive review. *Frontiers in immunology*, 16, 1491777.
- Elshikh, M., Ahmed, S., Funston, S., Dunlop, P., McGaw, M., Marchant, R., & Banat, I. M. (2016). Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. *Biotechnology Letters*, 38(6), 1015–1019.
- Enan, M. R., Palakkott, A. R., & Ksiksi, T. S. (2017). DNA barcoding of selected UAE medicinal plant species: A comparative assessment of herbarium and fresh samples. *Physiology and Molecular Biology of Plants*, 23(1), 221–227.
- Erb, M., & Kliebenstein, D. J. (2020). Plant secondary metabolites as defenses, regulators, and primary metabolites: the blurred functional trichotomy. *Plant physiology*, 184(1), 39-52.
- Fakhrah, S., Bano, N., Sarvendra, K., Lone, R. A., Nayak, S. P., Kumari, A., & Mohanty, C. S. (2025). Elucidating the secondary metabolite biosynthesis networks in underutilized tree bean (*Parkia timoriana*) through integrated

- metabolomic and transcriptomic approaches. *Applied Biochemistry and Biotechnology*, 197, 1–27.
- Fitzgerald, M., Heinrich, M., & Booker, A. (2020). Medicinal plant analysis: A historical and regional discussion of emergent complex techniques. *Frontiers in Pharmacology*, 10, 1480.
- Gaber, N. B., El-Dahy, S. I., & Shalaby, E. A. (2023). Comparison of ABTS, DPPH, permanganate, and methylene blue assays for determining antioxidant potential of successive extracts from pomegranate and guava residues. *Biomass Conversion and Biorefinery*, 13(5), 4011–4020.
- Gebrehiwot, H., Ensermu, U., Endale, M., Hunsen, M., & Dekebo, A. (2024). Exploring the medicinal potential of *Senna siamea* roots: An integrated study of antibacterial and antioxidant activities, phytochemical analysis, ADMET profiling, and molecular docking insights. *Applied Biological Chemistry*, 67, 48.
- Göger, G., Köse, Y. B., Demirci, F., & Göger, F. (2021). Phytochemical characterization of phenolic compounds by LC-MS/MS and biological activities of *Ajuga reptans* L., *Ajuga salicifolia* (L.) Schreber and *Ajuga genevensis* L. from Turkey. *Turkish Journal of Pharmaceutical Sciences*, 18(5), 616–628.
- Harborne, A. J. (1998). *Phytochemical methods a guide to modern techniques of plant analysis*. Springer science & business media.
- Hassanpour, S. H., & Doroudi, A. (2023). Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants. *Avicenna Journal of Phytomedicine*, 13(4), 354–365.
- Hebert, P. D., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, 270(1512), 313–321.

- Ho, V. T., Tran, T. K. P., Vu, T. T. T., & Widiarsih, S. (2021). Comparison of matK and rbcL DNA barcodes for genetic classification of jewel orchid accessions in Vietnam. *Journal of Genetic Engineering and Biotechnology*, 19(1), 93.
- Hollingsworth, P. M., Forrest, L. L., Spouge, J. L., et al. (2009). A DNA barcode for land plants. *Proceedings of the National Academy of Sciences of the United States of America*, 106(31), 12794–12797.
- Huang, J., Zheng, Y., Ma, J., Ma, J., Lu, M., Ma, X., & Tang, X. (2021). Exploration of the potential mechanisms of Wumei pill for the treatment of ulcerative colitis by network pharmacology. *Gastroenterology Research and Practice*, 2021(1), 4227668.
- Hyder, Z., Rizwani, G. H., Shareef, H., Azhar, I., & Zehra, M. (2024). Authentication of important medicinal herbal species through DNA-based molecular characterization. *Saudi Journal of Biological Sciences*, 31(6), 103985.
- Kasote, D. M., Katyare, S. S., Hegde, M. V., & Bae, H. (2015). Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International journal of biological sciences*, 11(8), 982.
- Kaur, J., Dhiman, V., Bhadada, S., Katare, O. P., & Ghoshal, G. (2022). LC/MS guided identification of metabolites of different extracts of *Cissus quadrangularis*. *Food Chemistry Advances*, 1, 100084.
- Khan, A., Kanwal, F., Ullah, S., Fahad, M., Tariq, L., Altaf, M. T., ... & Zhang, G. (2025). Plant secondary metabolites—Central regulators against abiotic and biotic stresses. *Metabolites*, 15(4), 276.
- Khiangte, L., Sailo, H., Ralte, L., Lalnunmawia, F., Senthilkumar, N., & Singh, Y. T. (2025). *In vitro* and *in silico* evaluation of phytochemicals from *Leucaena leucocephala* and *Entada phaseoloides* targeting DNA gyrase, topoisomerase II, AKT1. *Scientific Reports*, 15(1), 25399.
- Kokkini, M., Gazoulis, I., Danaskos, M., Kontogeorgou, V., Kanatas, P., & Travlos, I. (2025). Enhancing ecosystem services in agriculture: the special role of legume intercropping. *Frontiers in Sustainable Food Systems*, 9, 1547879.

- Kress, W. J., Wurdack, K. J., Zimmer, E. A., Weigt, L. A., & Janzen, D. H. (2005). Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences*, 102(23), 8369–8374.
- Kulkarni, N. D., Momin, S. J., Jain, L. P., More, R. B., Jadhav, S. R., & Lungase, M. B. (2025). A Comprehensive Review on Molecular Docking in Drug Discovery. *International Journal of Scientific Research and Technology*.
- Kumar, D., Jain, A., & Verma, A. (2017). Phytochemical and pharmacological investigation of *Cassia siamea* Lamk: An insight. *The Natural Products Journal*, 7(4), 255–266.
- Kumar, D., Kohli, S., Kumar, S., Gupta, J., Jain, P., & Kumar, R. (2011). Screening of methanolic bark extract of *Albizia odoratissima* for antimicrobial activity. *Pharmacognosy Communications*, 1(2), 47–49.
- 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.
- Lee, K. J., Oh, Y. C., Cho, W. K., & Ma, J. Y. (2015). Antioxidant and anti-inflammatory activity determination of one hundred kinds of pure chemical compounds using offline and online screening HPLC assay. *Evidence-Based Complementary and Alternative Medicine*, 2015(1), 165457.
- Liao, W., Mei, Z., Miao, L., Liu, P., & Gao, R. (2020). Comparative transcriptome analysis of root, stem, and leaf tissues of *Entada phaseoloides* reveals potential genes involved in triterpenoid saponin biosynthesis. *BMC Genomics*, 21(1), 1–12.
- Lingegowda, D. C., Kumar, J. K., Prasad, A. D., Zarei, M., & Gopal, S. (2012). FTIR spectroscopic studies on *Cleome gynandra*: Comparative analysis of functional group before and after extraction. *Romanian Journal of Biophysics*, 22(3–4), 137–143.

- Lucci, P., Saurina, J., & Núñez, O. (2017). Trends in LC-MS and LC-HRMS analysis and characterization of polyphenols in food. *Trends in Analytical Chemistry*, 88, 1–24.
- Luo, J., Manning, B. D., & Cantley, L. C. (2003). Targeting the PI3K-Akt pathway in human cancer: Rationale and promise. *Cancer Cell*, 4(4), 257–262.
- Manandhar, S., Luitel, S., & Dahal, R. K. (2019). *In vitro* antimicrobial activity of some medicinal plants against human pathogenic bacteria. *Journal of tropical medicine*, 2019(1), 1895340.
- Maobe, M. A., Nyarango, R. M., & Box, P. O. (2013). Fourier transformer infra-red spectrophotometer analysis of *Urtica dioica* medicinal herb used for the treatment of diabetes, malaria and pneumonia in Kisii region, Southwest Kenya. *World Applied Science Journal*, 21(8), 1128–1135.
- Maroyi, A. (2023). Medicinal uses of the Fabaceae family in Zimbabwe: A review. *Plants*, 12(6), 1255.
- Maroyi, A. (2023). Medicinal uses of the Fabaceae family in Zimbabwe: A review. *Plants*, 12(6), 1255.
- Matsui, T., Yamane, J., Mogi, N., Yamaguchi, H., Takemoto, H., Yao, M., & Tanaka, I. (2012). Structural reorganization of the bacterial cell-division protein FtsZ from *Staphylococcus aureus*. *Acta Crystallographica Section D: Biological Crystallography*, 68(9), 1175–1188.
- Mir, W. R., Bhat, B. A., Rather, M. A., Muzamil, S., Almilaibary, A., Alkhanani, M., & Mir, M. A. (2022). Molecular docking analysis and evaluation of the antimicrobial properties of the constituents of *Geranium wallichianum* D. Don ex Sweet from Kashmir Himalaya. *Scientific Reports*, 12(1), 12547.
- Mishra, K., Ojha, H., & Chaudhury, N. K. (2012). Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. *Food Chemistry*, 130(4), 1036–1043.

- Mittraphab, Y., Amen, Y., Nagata, M., Matsumoto, M., Wang, D., & Shimizu, K. (2022). Anti-phototoxicity effect of phenolic compounds from acetone extract of *Entada phaseoloides* leaves via activation of COX-2 and iNOS in human epidermal keratinocytes. *Molecules*, 27(2), 440.
- Morante-Carriel, J., Nájera, H., Samper-Herrero, A., Živković, S., Martínez-Esteso, M. J., Martínez-Márquez, A., & Bru-Martínez, R. (2024). Therapeutic potential of prenylated flavonoids of the Fabaceae family in medicinal chemistry: An updated review. *International Journal of Molecular Sciences*, 25(23), 13036.
- Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of immunological methods*, 65(1-2), 55-63.
- Muflihah, Y. M., Gollavelli, G., & Ling, Y. C. (2021). Correlation study of antioxidant activity with phenolic and flavonoid compounds in 12 Indonesian indigenous herbs. *Antioxidants*, 10(10), 1530.
- Nair, S., & Varalakshmi, K. N. (2011). Anticancer, cytotoxic potential of *Moringa oleifera* extracts on HeLa cell line. *Journal of Natural Pharmacy*, 2(3), 138–142.
- Ndezo Bisso, B., Njikang Epie Nkwelle, R., Tchuenguem Tchuenteu, R., & Dzoyem, J. P. (2022). Phytochemical screening, antioxidant, and antimicrobial activities of seven underinvestigated medicinal plants against microbial pathogens. *Advances in Pharmacological and Pharmaceutical Sciences*, 2022(1), 1998808.
- Nemati, F., Dehpouri, A. A., Eslami, B., Mahdavi, V., & Mirzanejad, S. (2013). Cytotoxic properties of some medicinal plant extracts from Mazandaran, Iran. *Iranian Red Crescent Medical Journal*, 15(11), e8871.
- Newmaster, S. G., Grguric, M., Shanmughanandhan, D., Ramalingam, S., & Ragupathy, S. (2013). DNA barcoding detects contamination and substitution in North American herbal products. *BMC Medicine*, 11, 222.

- Nobossé, P., Fombang, E. N., & Mbofung, C. M. (2018). Effects of age and extraction solvent on phytochemical content and antioxidant activity of fresh *Moringa oleifera* L. leaves. *Food science & nutrition*, 6(8), 2188-2198.
- Okwu, D. E., & Ekeke, O. (2003). Phytochemical screening and mineral composition of chewing sticks in South Eastern Nigeria. *Global Journal of Pure and Applied Sciences*, 9(2), 235–238.
- Oyebade, K. F., Daspan, A. J., Denkok, Y., Alemika, T. E., & Ojerinde, O. S. (2021). Antimicrobial, antioxidant and antiproliferative properties of the leaves of *Senna siamea*. *Journal of Complementary and Alternative Medical Research*, 14(1), 22–29.
- Pang, X., Liu, C., Shi, L., Liu, R., Liang, D., Li, H., & Chen, S. (2012). Utility of the trnH-psbA intergenic spacer region and its combinations as plant DNA barcodes: A meta-analysis. *PLoS ONE*, 7(11), e48833.
- Papadimitrakopoulou, V. (2012). Development of PI3K/AKT/mTOR pathway inhibitors and their application in personalized therapy for non-small-cell lung cancer. *Journal of Thoracic Oncology*, 7(8), 1315–1326.
- Parveen, I., Singh, H. K., Malik, S., Raghuvanshi, S., & Babbar, S. B. (2017). Evaluating five different loci (rbc L, rpo B, rpo C1, mat K, and ITS) for DNA barcoding of Indian orchids. *Genome*, 60(8), 665–671.
- Pereira, M. T. M., Charret, T. S., Pascoal, V. D. A. B., Machado, R. L. D., Rocha, L. M., & Pascoal, A. C. R. F. (2022). *Myrciaria* genus: bioactive compounds and biological activities. *Chemistry & Biodiversity*, 19(12), e202200864.
- Phuyal, N., Jha, P. K., Raturi, P. P., & Rajbhandary, S. (2020). Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* DC. *Science World Journal*, 2020(1), 8780704.
- Pradhan, P., Margolin, W., & Beuria, T. K. (2021). Targeting the achilles heel of FtsZ: The interdomain cleft. *Frontiers in Microbiology*, 12, 732796.

- Prakash, O., Malik, S., Rani, K. V., & Verma, V. K. (2020). Phytochemical screening and bioactive potential of pod seed extracts of *Leucaena leucocephala* Linn. *Pharmacognosy Research*, 12(4), 1–10.
- Puranik, A., & Taranath, T. C. (2023). FTIR characterization and validation of secondary metabolites in leaf and bark extracts and direct powder of *Erinocarpus nimmonii*, an endemic plant. *International Journal of Phytopharmacy*, 13(2), 1–12.
- Rakhmawatie, M. D., Wibawa, T., Lisdiyanti, P., & Pratiwi, W. R. (2019). Evaluation of crystal violet decolorization assay and resazurin microplate assay for antimycobacterial screening. *Heliyon*, 5(8).
- Ralte, L., Khiangte, L., Thangjam, N. M., Kumar, A., & Singh, Y. T. (2022). GC–MS and molecular docking analyses of phytochemicals from the underutilized plant, *Parkia timoriana* revealed candidate anti-cancerous and anti-inflammatory agents. *Scientific Reports*, 12(1), 3395.
- Raval, K., & Ganatra, T. (2022). Basics, types and applications of molecular docking: A review. *IP International Journal of Comprehensive and Advanced Pharmacology*, 7(1), 12–16.
- Riaz, M., Khalid, R., Afzal, M., Anjum, F., Fatima, H., Zia, S., & Aslam, M. A. (2023). Phytobioactive compounds as therapeutic agents for human diseases: A review. *Food Science & Nutrition*, 11(6), 2500–2529.
- Rumpf, J., Burger, R., & Schulze, M. (2023). Statistical evaluation of DPPH, ABTS, FRAP, and Folin-Ciocalteu assays to assess the antioxidant capacity of lignins. *International Journal of Biological Macromolecules*, 233, 123470.
- Safhi, F. A., Alshamrani, S. M., Bogmaza, A. F. M., & El-Moneim, D. A. (2023). DNA barcoding of wild plants with potential medicinal properties from faifa mountains in Saudi Arabia. *Genes*, 14(2), 469.
- Sahoo, M. R., & Umashankara, M. S. (2023). FTIR based metabolomics profiling and fingerprinting of some medicinal plants: An attempt to develop an

- approach for quality control and standardization of herbal materials. *Pharmacognosy Research*, 15(1), 1–10.
- Sammar, M., Abu-Farich, B., Rayan, I., Falah, M., & Rayan, A. (2019). Correlation between cytotoxicity in cancer cells and free radical-scavenging activity: *In vitro* evaluation of 57 medicinal and edible plant extracts. *Oncology Letters*, 18(6), 6563–6571.
- Santhosh Kumar, J. U., Krishna, V., Seethapathy, G. S., Ganesan, R., Ravikanth, G., & Shaanker, R. U. (2018). Assessment of adulteration in raw herbal trade of important medicinal plants of India using DNA barcoding. *3 Biotech*, 8(3), 135.
- Sariwati, A., Suryanti, V., Sari, F., Kamei, I., & Trisnawati, E. W. (2024). Phytochemical profile, antioxidant, antidiabetic, and antimicrobial activities of *Parkia timoriana* bark extracts. *Biodiversitas Journal of Biological Diversity*, 25(6).
- Sass, C., Little, D. P., Stevenson, D. W., & Specht, C. D. (2007). DNA barcoding in the cycadales: Testing the potential of proposed barcoding markers for species identification of cycads. *PLoS ONE*, 2(11), e1154.
- Schumacher, M. A., Ohashi, T., Corbin, L., & Erickson, H. P. (2020). High-resolution crystal structures of Escherichia coli FtsZ bound to GDP and GTP. *Protein Science*, 29(2), 94–102.
- Sebastian, R., Jaykar, B., & Gomathi, V. (2020). Current status of anticancer research in Fabaceae family. *Pathways*, 6(7).
- Shahidi, F., & Yeo, J. (2018). Bioactivities of phenolics by focusing on suppression of chronic diseases: A review. *International Journal of Molecular Sciences*, 19(6), 1573.
- Sharma, P., & Chaurasia, S. (2015). Evaluation of total phenolic, flavonoid contents and antioxidant activity of *Acokanthera oppositifolia* and *Leucaena leucocephala*. *International Journal of Pharmacy and Pharmaceutical Sciences*

- Siddiqui, S. (2025). Global patterns and drivers of species and genera richness of Fabaceae. *Frontiers in Plant Science*, 16, 1581814.
- Sonawane, P. A., Patel, A., & Ghawate, V. (2023). *Albizia odoratissima*: Review on morphology, therapeutic uses, phytochemical study and pharmacological activities. *Research Journal of Pharmacognosy and Phytochemistry*, 15(3), 225–229.
- Sowe, M. S., Etonihu, A. C., Tarigan, I. L., & Muadifah, A. (2025). Secondary Metabolites of The Fabaceae Plant Family A Review of Extraction Methods, Molecules and Bioactivity. *Chempublish Journal*, 9(1), 1-35.
- Sravan Kumar, S., Manoj, P., & Giridhar, P. (2015). Fourier transform infrared spectroscopy (FTIR) analysis, chlorophyll content and antioxidant properties of native and defatted foliage of green leafy vegetables. *Journal of Food Science and Technology*, 52(12), 8131–8139.
- Subramani, R., Narayanasamy, M., & Feussner, K. D. (2017). Plant-derived antimicrobials to fight against multi-drug-resistant human pathogens. *3 Biotech*, 7(3), 172.
- Sun, W., & Shahrajabian, M. H. (2023). Therapeutic potential of phenolic compounds in medicinal plants – Natural health products for human health. *Molecules*, 28(4), 1845.
- Suryanti, V., Sariwati, A., Sari, F., Handayani, D. S., & Risqi, H. D. (2022). Metabolite bioactive contents of *Parkia timoriana* (DC) Merr seed extracts in different solvent polarities. *HAYATI Journal of Biosciences*, 29(5), 681–694.
- Thalia, O. P. (2025). Medicinal plants in global health: a comparative study of practices. *Res Output J Public Health Med*, 5(1), 42-49.
- Thangjam, U., Sahoo, U. K., & Thong, P. (2019). Effect of agroclimate on seed and seedling traits of tree bean (*Parkia timoriana* (DC) Merr.) in North East India. *Indonesian Journal of Forestry Research*, 6(1), 17–26.

- Truong, D. H., Nguyen, D. H., Ta, N. T. A., Bui, A. V., Do, T. H., & Nguyen, H. C. (2019). Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and *in vitro* anti-inflammatory activities of *Severinia buxifolia*. *Journal of food quality*, 2019(1), 8178294.
- Umar, A. H., Syahrani, R., Ranteta'dung, I., & Rafi, M. (2023). FTIR-based fingerprinting combined with chemometrics method for rapid discrimination of *Jatropha* spp.(Euphorbiaceae) from different regions in South Sulawesi. *Journal of Applied Pharmaceutical Science*, 13(1), 139-149.
- Urumarudappa, S. K. J., Tungphatthong, C., Jaipaew, J., Pornputtapong, N., Pakdeesattayapong, D., Vimolmangkang, S., & Sukrong, S. (2022). Development of a DNA barcode library of plants in the Thai Herbal Pharmacopoeia and Monographs for authentication of herbal products. *Scientific Reports*, 12(1), 9624.
- Usman, M., Khan, W. R., Yousaf, N., Akram, S., Murtaza, G., Kudus, K. A., & Nazre, M. (2022). Exploring the phytochemicals and anti-cancer potential of the members of Fabaceae family: A comprehensive review. *Molecules*, 27(12), 3863.
- Vaou, N., Stavropoulou, E., Voidarou, C., Tsakris, Z., Rozos, G., Tsigalou, C., & Bezirtzoglou, E. (2022). Interactions between medical plant-derived bioactive compounds: Focus on antimicrobial combination effects. *Antibiotics*, 11(8), 1014.
- Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., & Bezirtzoglou, E. (2021). Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. *Microorganisms*, 9(10), 2041.
- Vijayalakshmi, M., Kiruthika, R., Bharathi, K., & Ruckmani, K. (2015). Phytochemical screening by LC-MS analysis and *in vitro* anti-inflammatory activity of *Marselia quadrifolia* plant extract. *International Journal of Pharmacological Research*, 8(9), 148–157.

- Waris, M., Kocak, E., Gonulalan, E. M., Demirezer, L. O., Kır, S., & Nemutlu, E. (2022). Metabolomics analysis insight into medicinal plant science. *TrAC Trends in Analytical Chemistry*, 157, 116795.
- Wei, S., Gao, F., Wang, Z., Yin, G., Wen, S., Ou, H., & Liu, Z. (2024). Transcriptome and metabolome analyses reveal the molecular mechanisms of *Albizia odoratissima*'s response to drought stress. *Plants*, 13(19), 2732.
- Wiegand, I., Hilpert, K., & Hancock, R. E. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols*, 3(2), 163–175.
- Wu, K., et al. (2015). Plant phenolics are detoxified by prophenoloxidase in the insect gut. *Scientific Reports*, 5(1), 16823.
- Zayed, M. Z., & Sallam, S. (2019). Comparative phytochemical constituents of *Leucaena leucocephala* (Lam.) leaves, fruits, stem barks, and wood branches grown in Egypt using GC-MS method coupled with multivariate statistical approaches. *BioResources*, 14(1), 1–20.
- Zayed, M. Z., & Samling, B. (2016). Phytochemical constituents of the leaves of *Leucaena leucocephala* from Malaysia. *International Journal of Pharmacy and Pharmaceutical Sciences*, 8(12), 174–179.
- Zhao, Y., Zhang, R., Jiang, K. W., Qi, J., Hu, Y., Guo, J., & Ma, H. (2021). Nuclear phylotranscriptomics and phylogenomics support numerous polyploidization events and hypotheses for the evolution of rhizobial nitrogen-fixing symbiosis in Fabaceae. *Molecular Plant*, 14(5), 748–773.
- Zhou, S., Zhou, T., Zhang, Y., Yang, B., Niu, J., Li, W., ... & Zhou, J. (2025). DNA metabarcoding unveils authenticity and adulteration in commercial Chinese polyherbal preparations: Renshen Jianpi Wan as a critical case study. *Frontiers in Pharmacology*, 16, 1584065.
- Zhu, S., Liu, Q., Qiu, S., Dai, J., & Gao, X. (2022). DNA barcoding: An efficient technology to authenticate plant species of traditional Chinese medicine and recent advances. *Chinese Medicine*, 17(1), 112.

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3	B. SC	Botany	2018	Mizoram University	First	77.25
4	M. SC	Botany	2020	Mizoram University	First	81.14

LIST OF PUBLICATIONS

1. **Laldinliana Khiangte**, Hmingremhlua Sailo, Laldinfeli Ralte, F. Lalnunmawia, Nachimuthu Senthil Kumar, & Yengkhom Tungenba Singh(2025). *In vitro* and *in silico* evaluation of phytochemicals from *Leucaena leucocephala* and *Entada phaseoloides* targeting DNA gyrase, topoisomerase II, AKT1. *Scientific Reports*, 15(1), 25399.
2. Laldinfeli Ralte, **Laldinliana Khiangte**, Thangjam, Nurpen M. Thangjam, Awadhesh Kumar, & Yengkhom Tungenba Singh (2022). GC–MS and molecular docking analyses of phytochemicals from the underutilized plant, *Parkia timoriana* revealed candidate anti-cancerous and anti-inflammatory agents. *Scientific Reports*, 12(1), 3395.
3. Sailo Vanlalawmpuia, Sandhyarani D. Khomdram, Yumkham, S. D., Renthlei Lalthantluanga, & **Laldinliana Khiangte** (2026). *Begonia mizoramensis* (Begoniaceae, section *Platycentrum*), a new dioecious species from Mizoram, Northeast India. *Taiwania*, 71(2), 213-220.
4. Hmingremhlua Sailo, **Laldinliana Khiangte**, Laldinfeli Ralte, & Yengkhom Tungenba Singh (2025). Grain quality and physicochemical evaluation coupled with untargeted metabolic identification provide new insight into the upland pigmented rice of Manipur, India. *LWT*, 216, 117236.
5. Hmingremhlua Sailo, **Laldinliana Khiangte**, Lalremliani, Laldinfeli Ralte, Sagolshem Priyokumar Singh, & Yengkhom Tungenba Singh (2025). Exploring the Nutritional and Antimicrobial Properties of Wild Fruit, *Castanopsis tribuloides*: *In Vitro* and *In Silico* Insights for Potential Antimicrobial Drug Development. *Scientifica*, 2025(1), 2106755.
6. Hmingremhlua Sailo, Lalremliani, **Laldinliana Khiangte**, Malsawmthanga, Laldinfeli Ralte, Remruattluanga Hnamte, Lalfakzuala Ralte, & Yengkhom Tungenba Singh (2025). Post-pandemic assessment: A 3-year study of heavy metal risks in Mizoram's paddy agroecosystem. *Environmental Geochemistry and Health*, 47(9), 384.
7. Lal Tlanhlui, Sandhyarani D. Khomdram, Sanatombi D. Yumkham, Margaret Lalhlupuii, **Laldinliana Khiangte**, & S. Sureshkumar Singh (2025).

- Chamaegastrodia reiekensis*: A new holomycotrophic orchid from Mizoram, Northeast India. *Webbia*, 80(2), 229-238.
8. Laldinfeli Ralte, Hmingremhlua Sailo, Rakesh Kumar, **Laldinliana Khiangte**, Nachimuthu Senthil Kumar, & Yengkhom Tungenba Singh (2024). Identification of novel AKT1 inhibitors from *Sapria himalayana* bioactive compounds using structure-based virtual screening and molecular dynamics simulations. *BMC Complementary Medicine and Therapies*, 24(1), 116.
 9. Yengkhom Tungenba Singh, **Laldinliana Khiangte**, Sagolshem Priyokumar Singh, Hmingremhlua Sailo, & Laldinfeli Ralte (2022). New distribution record and DNA barcoding of *Sapria himalayana* Griff.(Rafflesiaceae), a rare and endangered holoparasitic plant from Mizoram, India. *Journal of Threatened Taxa*, 14(12), 22215-22220.
 10. Tridib Kumar Rajkhowa, Kiran Jayappa, A. Z. Beithatlo, Charitha B. Balakrishna, **Laldinliana Khiangte**, & Guillermo R. Risatti (2026). Detection of African Swine Fever Virus (ASFV) in *Haematopinus suis*: Evidence for a Potential New Sylvatic Cycle of ASFV Involving Lice and Domestic Pigs. *Transboundary and Emerging Diseases*, 20 Sanatombi D. Yumkham 26(1), 6675117.
 11. Tridib Kumar Rajkhowa, Kiran Jayappa, Charitha B. Balakrishna, **Laldinliana Khiangte**, A. Z. Beithatlo, & Mamta Pathak (2025). Lumpy skin disease virus (LSDV) infection in free-ranging red serow (*Capricornis rubidus*), Mizoram, India. *European Journal of Wildlife Research*, 71(3), 49.

PAPERS PRESENTED

1. Presented, “**Antioxidant, Antimicrobial and Phytochemical Analysis of an Underutilized Plant, *Parkia timoriana* (DC) Merr., from Mizoram, India,**” at the International Conference on Biotechnology for Environment & Health (2021).
2. Presented, “**Evaluation of bioactive compounds, antioxidant and cytotoxic potentials of *Leucaena leucocephala* (Lam.) de Wit. from Mizoram, India,**” at the National Seminar on Emerging Trends in Plant Sciences (ETPS) (2022).
3. Presented, “**Antioxidant, Antimicrobial and Phytochemical Analysis of an Underutilized Plant, *Entada phaseoloides* (L.) Merr., from Mizoram, India,**” at the National Conference on Recent Advances in Plant Biology with Special References to Northeast India (2023).
4. Presented, “**Antioxidant, Antimicrobial and Phytochemical Analysis of an Underutilized Plant, *Senna siamea* (Lam.), from Mizoram, India,**” at the National Seminar on Value Addition and Marketing of Horticultural Crops, Medicinal and Aromatic Plants (2023).

SEMINARS AND WORKSHOPS ATTENDED

1. Participated in, “**Instrumentation and Applications of Trace Elemental Analysis and Electron Microscopy**” (24 – 26th November, 2021) held at Sophisticated Analytical Instrument Facility, North-Eastern Hill University, Shillong.
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Approval of the Research Proposal

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ABSTRACT

MOLECULAR CHARACTERISATION AND EVALUATION OF BIOACTIVE COMPOUNDS OF SELECTED MEDICINAL PLANTS FROM FABACEAE IN MIZORAM

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

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**DEPARTMENT OF BOTANY
SCHOOL OF LIFE SCIENCES**

MAY, 2026

**MOLECULAR CHARACTERISATION AND EVALUATION OF
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BY

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Submitted

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

ABSTRACT

Introduction:

Medicinal plants represent abundant reservoirs of bioactive molecules and constitute essential components of contemporary healthcare, with approximately 80-85% of the world's population relying on plant-derived medicines for primary healthcare, and nearly a quarter of modern pharmaceutical drugs originating from natural plant sources. Mizoram, located in Northeast India within the Indo-Burma biodiversity hotspot, possesses exceptional ethnobotanical heritage with over 300 documented medicinal plant species traditionally used by indigenous tribes to treat diverse ailments; however, this knowledge remains largely anecdotal and increasingly threatened by anthropogenic pressures. The Fabaceae family, the third-largest flowering plant family comprising approximately 765 genera and over 19,500 species, is distinguished by its economic and ecological significance through nitrogen fixation, agricultural importance as a protein source, and therapeutic potential across traditional medicine systems. Members of Fabaceae are characterized by secondary metabolites including flavonoids, terpenoids, tannins, alkaloids, and phenolic compounds, which exhibit anticancer, antioxidant, antimicrobial, and anti-inflammatory activities. In Mizoram, this family is well-represented and highly valued as dual-purpose resources providing nutrition and ethnomedicinal applications.

Accurate plant species identification remains a fundamental challenge in botany and global trade, as traditional morphological methods are insufficient due to phenotypic plasticity and loss of diagnostic features in processed materials. DNA barcoding utilizing standardized regions such as ITS2, *rbcL*, and *matK* has emerged as a robust molecular tool providing precise species authentication and traceability. The comprehensive evaluation of plant bioactive compounds depends on appropriate extraction methodologies, with methanol recognized as the superior solvent for phytochemical recovery due to its amphiphilic nature, yielding higher concentrations of bioactive constituents compared to alternative solvents.

Oxidative stress and multi-drug resistant bacterial pathogens represent critical global health threats. Plant-derived secondary metabolites, particularly phenolic compounds and flavonoids, function as potent free radical scavengers and antimicrobial agents through novel mechanisms distinct from synthetic antibiotics. Integrated analytical approaches combining Fourier-transform infrared (FTIR) spectroscopy for rapid functional group identification and Liquid chromatography-mass spectrometry (LC-MS) for precise molecular characterization enable comprehensive phytochemical profiling. Furthermore, *in silico* molecular docking provides mechanistic insights by predicting bioactive molecule-protein interactions, facilitating cost-effective screening of lead candidates through computational prediction of binding affinity and stability.

To the best of our knowledge, this study represents the first comprehensive systematic evaluation of methanolic pod extracts from *Parkia timoriana*, *Leucaena leucocephala*, *Albizia odoratissima*, *Entada phaseoloides*, and *Senna siamea* from Mizoram, India. The investigation integrates molecular authentication through DNA barcoding, phytochemical characterization via FTIR spectroscopy and LC-MS analysis, *in vitro* evaluation of antioxidant, antibacterial, and anticancer activities, and *in silico* molecular docking targeting bacterial FtsZ proteins and human AKT1 kinase to validate therapeutic mechanisms.

Objectives:

- i. To collect and identify the Fabaceae plants using the DNA barcoding technique.
- ii. To evaluate and characterise the bioactive compounds of the collected samples.
- iii. To study the antimicrobial and antioxidant potential of collected samples.

Methodology:

Collected plant samples were first authenticated and their phylogenetic relationships established using DNA barcoding with multiple genetic markers (ITS2, *rbcL*, *matK*, *trnH-psbA*, and *rpoC1*). This was followed by preliminary qualitative phytochemical screening, and quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) of the methanolic pod extracts. Subsequent characterisation of functional groups and putative metabolites was carried out using Fourier-transform infrared (FTIR) spectroscopy and Liquid chromatography-mass spectrometry (LC-MS). Antioxidant potential was then evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and reducing power assays, followed by assessment of anticancer activity against HeLa cervical cancer cells using the MTT assay, and antibacterial efficacy using agar well diffusion and minimum inhibitory concentration (MIC) analysis. In addition, molecular docking studies were performed to investigate the interaction of selected LC-MS identified compounds with bacterial FtsZ proteins and human AKT1 kinase, providing mechanistic support for the observed bioactivities.

Results:

The methanol pod extracts of *P. timoriana*, *L. leucocephala*, *A. odoratissima*, *E. phaseoloides*, and *S. siamea* from Mizoram, India were evaluated for molecular identity, phytochemical composition, and bioactivity. DNA barcoding of the samples using ITS2, *rbcL*, *matK*, *trnH-psbA*, and *rpoC1* resulted in successful amplifications and confirmed species-level identification. Qualitative phytochemical screening of the extracts revealed the presence of major groups secondary metabolites such as alkaloids, flavonoids, phenols, steroids, glycosides, terpenoids, saponins and tannins. *P. timoriana* exhibited the highest total phenolic content at 58.88 ± 0.34 GAE/g, while *S. siamea* showed the lowest at 31.10 ± 0.08 GAE/g. For total flavonoid content, *P. timoriana* again demonstrated the highest at 126.05 ± 0.47 QE/g, whereas *E. phaseoloides* showed the lowest at 72.90 ± 0.41 QE/g. FTIR analysis revealed major functional groups and LC-MS analysis also revealed a number of

bioactive compounds present in the extract where the highest number of identified compounds was observed in *A. odoratissima* (79 compounds). The strongest DPPH and ABTS activity was also observed in *P. timoriana*, with an IC₅₀ value of 13.88 ± 0.27 µg/mL and 35.41 ± 0.31 µg/mL, respectively with reducing power absorbance of 2.93 ± 0.01 at 100 µg/mL. MTT assay demonstrated that the plant extracts exhibited strong cytotoxic potential against HeLa cervical cancer cells. *P. timoriana* demonstrated the most potent cytotoxic activity with an IC₅₀ value of 5.31 ± 0.05 µg/mL. Antibacterial assays using agar well diffusion and resazurin-based MIC revealed that the extracts exhibited promising activity, with the strongest inhibition observed against *Escherichia coli* among Gram-negative strains and *Staphylococcus aureus* among Gram-positive strains. Molecular docking studies revealed favourable interactions between key phytochemicals identified from LC-MS and bacterial FtsZ as well as human AKT1, providing computational support for the observed antimicrobial and anticancer bioactivities.

Conclusion:

This comprehensive multi-disciplinary investigation systematically validated the therapeutic potential of five selected Fabaceae species, *P. timoriana*, *L. leucocephala*, *A. odoratissima*, *E. phaseoloides*, and *S. siamea* from Mizoram through integrated molecular authentication using DNA barcoding, phytochemical characterization utilising LC-MS and FTIR analysis, *in vitro* bioactivity evaluation, and *in silico* molecular docking studies targeting bacterial FtsZ and human AKT1 kinase, thereby establishing scientific evidence for their traditional ethnomedicinal applications and identifying promising candidates for further pharmaceutical development.

Keywords: Medicinal plants, Fabaceae, DNA barcoding, phytochemical profiling, bioactive compounds, molecular docking, Mizoram, , FTIR spectroscopy, LC-MS analysis, cytotoxicity, FtsZ protein, AKT1 kinase, molecular docking.