STUDY OF NON-TIMBER FOREST PRODUCTS: PHENOLOGICAL AND PHYTOCHEMICAL ANALYSIS OF SELECTED PLANT SPECIES WITHIN LUNGLEI DISTRICT, MIZORAM

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This is to certify that this study "**Study of Non-Timber Forest Products: Phenological and Phytochemical analysis of Selected Plant Species within Lunglei District Mizoram**" submitted by P.C. Lalbiaknii (MZU/Ph.D/1115 of 03.05.2018) in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Botany is a record of bonafide work carried out by her under my supervision and guidance.

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DECLARATION

MIZORAM UNIVERSITY

MAY, 2024

I **P.C. Lalbiaknii**, 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.

This is being submitted to the Mizoram University for the **Degree of Doctor of Philosophy in Botany.**

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(P.C. LALBIAKNII)

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LISTS OF ABBREVIATIONS AND SYMBOLS

NTFPs	Non- Timber Forest Products
IPR	Intellectual Property Right
FAO	Food and Agriculture Organization
ROS	Reactive Oxygen Species
DNA	Deoxyribonucleic acid
HIV/AIDS	Human immunodeficiency virus infection/ Acquired immune deficiency syndrome
AMR	Antimicrobial Resistance
IUCN	International Union for Conservation of Nature
EX	Extinct
EW	Extinct in the Wild
CR	Critically Endangered
EN	Endangered
VU	Vulnerable
NT	Near Threatened
LC	Least Concern
DD	Data Deficient
RET	Rare Endangered and Threatened
WHO	World Health Organization
ICF	Informant Consensus Factor
FL	Fidelity Level
FC	Frequency Citation
RFC	Relative Frequency Citation
UV	Use Value
UR	Use Report
L	Leaf
R	Root
WP	Whole Plant
Fr	Fruit
B	Bark

Rh	Rhizome
ML	Milky Latex
S	Steam
J	Juice
De	Decoction
Co	Cooked
Pa	Paste
R	Raw
Po	Powder
WONCA	The World Organization of Family Doctors
WICC	World International Classification Committee
ICPC-2	International Classification of Primary Care-2
WHO-FIC	World Health Organization's Family of International Classifications
%	Percentage
<	Less than
±	Plus or minus
µg	Microgram
ml	Millilitre
H_2SO_4	Sulfuric Acid
HNO_3	Nitric Acid
HCl	Hydrochloric acid
mg GAE/g	Milligram Gallic Acid Equivalents Per Gram
$NaNO_2$	Sodium Nitrite
mg QE/g	Milligrams Quercetin Equivalent Per Gram
DPPH	2,2'-Diphenyl-2-Picrylhydrazyl
BHT	Butylated Hydroxytoluene
ABTS	2,2'-azinobis(3-Ethylbenzothiazoline-6-Sulfonic Acid)
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
IMTECH	Institute of Microbial Technology
mm	Millimetre

°C	Degree Celsius
w/v	Weight by Volume
nm	Nanometre
mg	Milligram
mM	Millimolar
ml	Millilitre
SD	Standard deviation
Ic	Inhibitory concentration
Kms	Kilometres
Aq	Aqueous
Met	Methanol
ANOVA	Analysis of Variance
BSI	Botanical Survey of India

CHAPTER I

1. Introduction

1.1. Background

Non-timber forest products (NTFPs) encompass all natural resources derived from forests, excluding timber, that serve various human purposes. Notably, this category excludes materials such as sand, stones, water, and ecotourism services (De Beer and McDermott, 1989; Chandrasekharan, 1995). A universally accepted and precise definition of NTFPs remains elusive. Consequently, the understanding and categorization depends on their intended utilization or their source of origin (Ahenkan and Boon, 2011). It encompasses a wide variety of plant and animal products that have both ecological and economic significance and are often associated with traditional and indigenous knowledge systems and play a vital role in the livelihoods of many forest-dependent communities worldwide (Cocksedge 2006; Edamana 2016).

It includes diverse range of biotic components: forested and wooded ecosystems, excluding timber, and include a diverse array of resources derived from wild flora and fauna. These resources, ranging from fruits, nuts, and vegetables, medicinal plants, resins, bark, fibres, palms, grasses, as well as minor wood products and firewood, are typically acquired through extraction activities conducted by local households and communities (Shackleton and Charlie, 2017). These activities primarily occur in the vicinity of residential areas, agricultural fields, grazing lands, and relatively undisturbed vegetation. The purpose of gathering NTFPs is typically for local consumption or trade. The harvesting, processing, and commercialization of NTFPs frequently constitute the sole source of employment opportunities for residents inhabiting isolated rural regions (Dau and Elisha, 2014).

NTFPs can be categorized in various ways, which can encompass purposes such as food, fuel, medicine, household utensils, and farm implements. These categories can further be refined based on the level of utilization, differentiating between those primarily used for personal sustenance and those harvested for commercial purposes.

Additionally, NTFPs can also be classified based on the specific plant parts collected, such as leaves, fruits, stems, or roots. Moreover, it is essential to consider that NTFPs can extend beyond plant-based resources and encompass products derived from wild animals, with considerations for the ecological impact of such extraction (Rijsoort, 2000).

The idea that the sustainable management of forests could be achieved through the extraction and utilization of NTFPs was initially grounded on the belief that the commercial harvesting of NTFPs from natural forests could concurrently contribute to both the preservation of global biodiversity and the generation of income for rural communities in need (Ros-Tonen, 2000; Perez et al., 1996). Employing a structured and methodical strategy to enhance the NTFPs sector can frequently align with the objectives of rural development and the preservation of invaluable natural resources.

NTFPs also offer a buffer against the negative impacts of climate change. Climate-related challenges, when coupled with non-climatic stress factors such as resource depletion and market fluctuations, frequently lead to increased insecurity and heightened vulnerability within agricultural systems (Sumukwo et al., 2013; Heubes et al., 2012; Niang et al., 2017). While it is true that the impoverished are disproportionately impacted by such pressures, others are not immune to the unpredictability of extreme climatic events such as floods and droughts. In these situations, NTFPs can step in to offer additional sustenance to rural communities. Moreover, beyond helping to alleviate the impacts of climate-related stress, NTFPs also play a role in conserving soil and landscapes, while creating habitats for various wildlife species (Nkem et al., 2013; Rowhani et al., 2011; Chidumayo, 2011; Sarmah, 2012). Simultaneously, NTFPs have frequently been observed to preserve a stable microclimate, which serves as a vital habitat for microorganisms. Their significant contribution to forest ecosystems has earned them "minor forest," highlighting their substantial role in maintaining ecological balance (Shankar et al., 1996).

In India, over 95% of the medicinal plants essential for the production of medicines by various industries are sourced from the wild (Saha and Sundriyal, 2012). Hence, comprehensive scientific records detailing the diversity, geographical prevalence,

utilization trends, and economic significance of these plant species can play a crucial role in the preservation and responsible utilization of such resources within specific states and regions. Furthermore, quantitative insights into the interplay between biological and cultural diversity, along with the relative significance of natural resources to local communities, can significantly contribute to the sustainable management and conservation of numerous NTFPs (Kufer et al., 2005).

In numerous instances, it holds greater significance for the communities than the sporadic income they receive from commercial logging activities. The conservation and protection of NTFPs play a pivotal role in sustaining and carrying forward various traditional ways of life that has been passed down through generations. However, it is crucial to acknowledge that these invaluable NTFP resources are facing escalating threats due to the encroachment of deforestation and the expansion of land development activities. These environmental and land use changes pose a growing challenge to the preservation of both the natural resources and the cultural heritage intricately intertwined with these products (Rocky and Sahoo, 2002).

Recognizing and upholding Intellectual Property Rights (IPR) is of significant consequence for many Non-Timber Forest Products (NTFPs), particularly in the swiftly advancing realms of herbal medicines and biomedical research. Often, these plants, along with their traditional uses and harvesting methods, have been honed over generations by the indigenous communities who have relied on them. As these innovations turn into profitable endeavours, it is imperative to ensure a fair distribution of benefits to the very communities, people, and nations from which this knowledge and these resources originate, honouring their invaluable contributions and protecting against unjust exploitation of their cultural heritage and traditional wisdom (Tiwari, 2000).

For generations, NTFPs have served as a vital resource for indigenous communities residing in forested regions, supplying them with sustenance, medicinal remedies, and materials for crafting. As we delve into scientific research aimed at recognizing and enhancing the significance of NTFPs, it holds the promise of paving the way for the creation of innovative nutraceutical products in the future. With the exception of a few studies on plant diversity, there has been a notable absence of comprehensive

research focusing on the true value of significant ethnomedicinal NTFPs within the study area. This research initiative aims to explore the utilization of NTFPs by the indigenous Mizo community, assess various ethnobotanical indicators, and investigate the level of reliance of forest-dwelling populations on these resources within the Thorangtlang Wildlife Sanctuary, situated in the Lunglei District of Mizoram.

CHAPTER II

2. Review of literature

2.1. NTFPs in ethnobotanical medicine.

The term 'ethnobotany' was initially coined by J.W. Harsh Berger in 1895, during the late 19th century (Cotton, 1996). In recent years, ethnobotany has gained considerable importance in the realms of healthcare advancement and specific conservation initiatives. It plays a pivotal role in the discovery and safeguarding of traditional knowledge (Kunwar and Bussmann, 2008). It primarily encompasses the examination of indigenous insights into plants and their interplay with culture and tradition. This exploration aims to uncover how local communities harnessed forest resources to fulfil their fundamental requirements and integrated plant resources into their cultural and religious practices (Balick and Cox, 2020).

The scientific field of ethnobotany focuses on investigating the intricate relationships between human societies and plants. This discipline holds significant relevance and applications in several contemporary global issues, including but not limited to food security, climate change, biodiversity conservation, and human health. Ethnobotanical research offers valuable insights into how various societies interact with their surrounding natural resources at the local level. Moreover, it has the potential to facilitate the convergence and synthesis of both indigenous and scientific knowledge to promote the overarching goal of biocultural conservation (Gaoue et al., 2017). It also demonstrates how ethnobotany can play a pivotal role in biodiversity conservation, particularly through the documentation and preservation of indigenous and local botanical knowledge (Prance, 2007).

Native populations have engaged in the collection and utilization of medicinal plants to address diverse ailments throughout history. The utilization of medicinal plants by distinct ethno-linguistic communities has garnered significant attention from both the scientific community and the general public, making it a prominent subject within the field of ethnobotany. Indigenous healers and traditional healthcare providers worldwide have accumulated extensive reservoirs of knowledge pertaining to the

acquisition and application of medicinal plants in their service to communities (Xiong et al., 2020). Indigenous communities residing in areas abundant in biodiversity rely on NTFPs to meet a wide array of their requirements. They hold extensive expertise regarding these resources and their sustainable management (Martin, 2010). The wisdom concerning these plants is traditionally passed down from one generation to the next through oral traditions. However, there is growing apprehension in recent times about the rapid erosion of this knowledge (Hedge et al., 1966).

According to the Food and Agriculture Organization (FAO), about 80% of the population in developing countries rely on NTFPs to meet their nutritional and health needs. Moreover, over 1.2 billion people in rural areas depend on NTFPs to supplement their essential requirements (FAO, 2003; Adhikari et al., 2004). Also, it plays a crucial role in creating employment opportunities for millions of individuals annually. They make a substantial contribution to the rural economy, with over half of these products being consumed by tribal communities residing in and around forested regions to fulfil their essential requirements. Consequently, the economic well-being of rural populations is closely tied to the availability and utilization of a variety of NTFPs within their vicinity (Kennedy, 2006).

The northeastern region of India, situated within the boundaries of two significant biodiversity hotspots, namely the Himalayas and the Indo-Burma region, is widely acknowledged as one of the most culturally, ethnically, and biologically diverse areas on the planet (Paul et al., 2005). India boasts a substantial array of NTFPs, which include various plant species totalling around 3,000 in number (Pradhan and Badola, 2008; Singh and Pradhan, 2019). As primarily residing in forested regions, tribal communities possess extensive knowledge and have a significant reliance on forests and their resources (Dattagupta et al., 2010). Agricultural output from tribal lands is typically insufficient, merely sustaining households at subsistence levels. Consequently, communities rely on the collection of NTFPs as a vital source of sustenance (Ives, 2002). Additionally, these resources hold considerable cultural importance and are highly cherished by diverse ethnic communities worldwide.

Numerous studies have demonstrated that NTFPs serve as a vital safety net for the livelihoods of tribal communities (Borah et al., 2020) in which findings have unveiled a substantial reliance on and a diverse array of NTFPs in the humid tropical regions of Northeast India. Among these products, the highest proportion was employed for medicinal purposes, with fruits, wild vegetables, fodder, dye-yielding materials, mushrooms, firewood, house construction materials, and agricultural tools following suit in terms of utilization (Saha and Sundriyal, 2012). These investigations have shed light on the diverse ways in which these resources are harnessed by local populations.

Furthermore, several researchers have contributed valuable reports regarding the ethnomedicinal applications of NTFPs, providing insights into the medicinal uses of these plant resources within traditional healthcare systems (Shackleton and Shackleton, 2004; Dattagupta and Gupta, 2016; Babalola, 2009; Melese, 2016). NTFPs not only cater to domestic necessities but also generate income for these communities while contributing revenue to the State government. Additionally, the economic assessment underscores the requirement for specific guidelines in livelihood and policy development within tribal regions. Strengthening livelihood strategies based on NTFPs will play a significant role in supporting the management and conservation of forest resources (Dattagupta et al., 2010, 2014).

2.2. Phenology of NTFPs

Phenology involves the examination of recurring stages or events in the life cycles of organisms and how these are influenced by various climatic factors (Sakai, 2001; Cleland et al., 2007). The patterns of plant phenology are revealed through the timing, frequency, and quantity of events like leaf emergence, leaf shedding, flowering, and fruit production in their life cycles (Morellato et al., 2010). A "phase" or "phenophase" could represent events such as the initial flowering, budburst, leaf unfolding, or the onset of bird migration.

Phenological observations offer a valuable means to investigate the consequences of climate changes since climatic factors frequently play an important role in influencing the timing of these phenophases. Comprehending this pattern is essential

for gaining insight into a plant growth and reproductive processes. Additionally, it also plays a crucial role in serving as a source of food for animals within a particular region, especially at specific times (Van Schaik et al., 1993). Also, it is valuable for comprehending the restoration process, aiding in the choice of tree species, and determining the optimal timing for seed harvesting to populate a seed bank (Ehrenfeld and Toth, 1997). Phenological processes are inherent characteristics of species and ecosystem dynamics, and they have a considerable impact on biodiversity across various levels, ranging from genetic diversity to entire ecosystems. Moreover, these processes influence the interactions between different species within these ecosystems (Van Vliet, 2008).

Climate-related elements, including temperature, rainfall, and extreme weather occurrences have the potential to impact phenological patterns and forest productivity by influencing the timing of flowering and fruiting seasons, as well as the quantity of flowers and fruits produced. Such shifts are notably influencing the timing for harvesting both timber and non-timber forest products (Winarni et al., 2016). In the realm of plants, the timing of reproductive phenology and related events is influenced by a combination of both biotic and non-living abiotic factors, along with their intricate interactions (Ramírez and Briceno, 2011).

While we acknowledge the well-established impact of climate-related factors on the reproductive phases of plants, it is important to note that there are additional elements that can also shape the patterns of flowering and fruit production. This includes the concentration of solutes in the soil (Mantovani and Martins, 1988; Williams et al., 1997), soil type (Ramírez and Briceno, 2011; Cardoso et al., 2012), life-form (Golluscio et al., 2005), phylogenetic history (Silva et al., 2011), water stress (Idso et al., 1978; Maes et al., 2009; Brachi et al., 2012), light availability (Kilkenny and Galloway, 2008; Galloway and Burgess, 2012), and interactions with pollinators (Brody, 1997; Aizen, 2003), seed predators (Brody, 1997; Collin and Shykoff, 2010), and herbivores (Osada and Sugiura, 2006; Kawagoe and Kudoh, 2010).

In addition to these ecological factors, human-induced pressures (anthropogenic factors) like land degradation and extensive harvesting can likewise impact the

phenological patterns and the overall reproductive success of plants (Bisigato et al., 2013; Peres et al., 2003; Ticktin, 2004; Brachi et al., 2012) delved into the literature concerning the harvesting of non-timber forest products. Their findings indicated that 67 percent of the studies examining the specific impacts on the reproductive rates of harvested species revealed adverse effects, while the remaining studies did not report significant impacts.

Given the limited existing literature on the subject and the significant relevance of phenological observations, this study was conducted to investigate the phenological events of selected ethnomedicinal Non-Timber Forest Products (NTFPs) within the Thorangtlang Wildlife Sanctuary, situated in the Lunglei district of Mizoram. The primary aim was to gain insights into how plant species respond to climatic influences and the seasonal patterns specific to the district of Lunglei, Mizoram.

2.3. Phytochemical activity and free radical scavenging activity of NTFPs

Naturally occurring compounds exhibit antioxidant properties, while extracts obtained from a wide array of plant species, notably berries, fruits, vegetables, medicinal herbs, aromatic plants, spices and other botanical sources have been thoroughly documented for their bioactive qualities and manifold applications (Biapa et al., 2011; Choumessi et al., 2012; Dimo et al., 2001). Polyphenols, a class of bioactive compounds extensively distributed throughout plant life, also represent integral components within the human diet (Pauline et al., 2013). Plants stand as primary sources of antioxidants, boasting a vast array of compounds encompassing diverse categories like flavonoids (anthocyanins, flavanols, flavones) and various nonflavonoids (phenolic acids, lignins, stilbenes, terpenoids, among others). These compounds exhibit structural disparities, differing in the configuration, quantity, and positioning of phenolic hydroxyl groups. Consequently, these structural variations contribute to the diversity observed in their antioxidative and biological capabilities (Erkan et al., 2011). Antioxidants are compounds that counteract the effects of free radicals or their activities by neutralizing them. Regenerative antioxidants endeavour to restore or replenish cellular components affected by oxidative damage caused by reactive oxygen species (ROS) (Sies, 1996).

Reactive oxygen species (ROS) represent a group of exceptionally reactive molecules originating from oxygen metabolism (Cerutti, 1991). These ROS variants, encompassing superoxide radicals, hydroxyl radicals, and hydrogen peroxide, typically arise as byproducts of biological reactions or from external factors. Certain ROS serve beneficial roles in cell physiology (Harman, 1994). Nevertheless, they can also inflict substantial harm to cell components such as membranes and DNA, triggering oxidative reactions that result in lipid peroxidation, reduced membrane flexibility, and DNA mutations, thereby contributing to diseases like cancer, degenerative conditions, and various other ailments (Ames, 1998; Finkel and Holbrook, 2000).

Medicinal plants harbour biologically active compounds known for their therapeutic potential (Herborne, 1973; Njoku et al., 2011). These intricate chemical compounds characterized by diverse compositions, serve as secondary metabolites within one or more of these plant species. Their efficacy has been evidenced in treating various ailments like HIV/AIDS, malaria, diabetes, sickle-cell anaemia (Elujoba et al., 2005), mental disorders and microbial infections (Iwu et al., 1999; Okigbo et al., 2005). The documented effects of plant extract rich in flavonoids include both diuretic and antibacterial activities (Delle Monache et al., 1996; Rao et al., 1996). Plant-derived alkaloids serve as crucial components in medicinal applications, particularly as anaesthetic agents (Hérouart et al., 1988). Additionally, the presence of saponins within plants has been noted for their role in the tonic and stimulating properties observed in traditional Chinese and Japanese medicinal herbs (Alinnor, 2008).

Plants containing glycosides have been documented for their ability to exert an antihypertensive impact on both blood pressure and the serum composition of hypertensive individuals (Enwerem et al., 2001). This effect is potentially linked to the existence of a steroidal nucleus and deoxy-sugar, both integral components within glycosides. For instance, thymol contains a phenolic group and has been recognized as constituting up to 75% of certain steam volatile oils (Sofowara, 1993) and is credited to the existence of tannins, possessing styptic properties and the ability to precipitate proteins, thereby conferring resistance against degradation by proteolytic enzymes.

Over the past three decades, there has been a notable emergence of phytochemical analysis and antioxidant-based medications and formulations aimed at preventing and treating intricate conditions such as atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer (Devasagayam et al., 2004). This advancement has sparked significant research attention toward natural antioxidants. Following an era of extensive research into oxidants and antioxidants, it is imperative to undertake a critical re-evaluation of these domains. The theory indicating a possible connection between phytochemicals and different illnesses has to be supported by more evidence (Dahiru et al., 2006). Likewise, a thorough re-examinations of the possibility of antioxidants preventing or treating many degenerative disorders is necessary. It is suggested that the customary medical applications for these plants be followed, along with further investigation into the identification, isolation, and purification of the particular active ingredients responsible for the medicinal qualities displayed by these plants (Shalaby and Shanab, 2013).

Further investigation involving phytochemical analysis and assessment of free radical scavenging activities is necessary to ascertain the presence of bioactive compounds within the ethnomedicinal NTFPs chosen for the treatment of various ailments. The identified phytochemical compounds, coupled with their demonstrated antioxidant activity might constitute the active constituents responsible for the observed efficacy in the studied plants. This comprehensive exploration aims to elucidate the specific bioactive elements contributing to the therapeutic properties of these plant-based remedies.

2.4. Antimicrobial activity of NTFPs

Numerous plant species harbour secondary metabolites within their tissues that exhibit promise in combating pathogenic microorganisms. Among these compounds are glycosides, saponins, flavonoids, steroids, tannins, alkaloids, and terpenes (Kamali and Amir, 2010). Extracts derived from various plant organs, such as roots, leaves, bark, flowers, fruits, and seeds, often bears unique phytochemical compositions exhibiting activity against bacterial or fungal pathogens (Tiwari et al., 2011). In regions with costly medicinal options, exploration into the antimicrobial potential of ethnomedicinal plants remains crucial. Researchers persist in studying

medicinal plants to formulate optimal pharmaceuticals for therapeutic applications (Usman and Osuji, 2007).

Microbes inhabiting soil, aquatic environments, the atmosphere, animal ecosystems, and built structures harbour antimicrobial resistance (AMR) elements alongside the genetic machinery required for their transfer. The extensive variety and prevalence of resistance within these environments align with the existence of ancient reservoirs of antibiotics. Research findings corroborate a lengthy natural lineage of interconnected resistance mechanisms in these settings (Jacoby, 2017). In the early 1970s, physicians confronted the reality that not all bacterial infections were readily treatable despite the availability of a wide range of effective antimicrobial agents (Cohen, 1992). This realization dawned upon them as resistance to multiple antibiotics surfaced among prominent pathogens like *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Mycobacterium tuberculosis* (Tomasz, 1994). The evolution of increasingly resistant bacterial species to antimicrobials results from various factors, notably the widespread and sometimes improper use of these agents, their extensive application as growth promoters, and the ease with which antimicrobial-resistant bacteria traverse geographical boundaries (Swartz, 1997). The emergence of drug resistance among human pathogens to conventional antibiotics has prompted the exploration for novel antimicrobial compounds sourced from alternative reservoirs, such as plants (Erdogrul, 2002).

The richness and prevalence of resistance found in various environments align with the presence of ancient reservoirs of antibiotics. Scientific studies provide substantial evidence supporting a longstanding natural history intertwined with resistance mechanisms (Donadio et al., 2010). Plants are an excellent source of beneficial compounds with an extensive spectrum of benefits in the pharmaceutical industry. The expansion and development of drug-resistant pathogenic organisms, which have developed novel resistance structures, pose an ongoing threat by compounding antimicrobial resistance, thus hindering our capacity to effectively combat recurrent infections (Iskandar et al., 2022).

Herbal medicine has gained traction in developing nations as a viable alternative for addressing health issues and mitigating the expenses associated with pharmaceutical

products. There has been a scarcity of literature focusing on the exploration of the antimicrobial properties of ethnomedicinal Non-Timber Forest Products (NTFPs) in Mizoram. Existing studies predominantly revolved around establishing the sustainable livelihood linkages between indigenous communities and forest products. Consequently, the current study endeavours to examine the in-vitro antimicrobial activity of methanol and aqueous extract of six ethnomedicinal NTFPs against bacterial pathogens. This investigation aims to contribute to the development of formulations utilizing the potential plants for effective management of both plant and animal pathogens.

2.5. Screening of RET species

Herbal medicines are gaining popularity due to their affordability and lack of side effects attributed to natural ingredients (Ekor, 2014). Increased interest in ethnobotanical and ethnomedicinal studies has propelled research into herbal products. As a result, there is mounting pressure on medicinal plants due to their heightened demand. Studies highlight a concerning decline in various high-value medicinal plant species, largely attributed to ongoing exploitation (Sajem et al., 2008). Loss of these medicinal plant species can be attributed to several potential factors, such as habitat specificity and a limited distribution range. Factors like habitat alteration, impact of climate change, land use disturbance, introduction of non-native species, further exacerbate the situation. Additionally, heavy livestock grazing, the expanding human population, population fragmentation and degradation, as well as genetic drift and population bottlenecks contribute significantly to this decline (Kala et al., 2006). The success of protected areas in conserving biodiversity greatly hinges on their design, management, maintenance, and protection measures. It is crucial to conduct a comprehensive species inventory within these areas to pinpoint and protect the species needing special attention and care (Heywood, 2017).

It is critical to prioritise species, evaluate their threat level, and determine the best conservation techniques due to the enormous number of species and the limited resources accessible for conservation efforts. These become fundamental prerequisites for effective conservation programs. In pursuit of this goal, the International Union for Conservation of Nature (IUCN) was formed. It comprises

government and civil society organizations, working collectively as a membership union (Collen et al., 2016). The development of the IUCN's 'Red List of Threatened Species' offers information on extinction risk and species distribution (Pollock et al., 2003; Brooks et al., 2004). Extinct (EX), extinct in the wild (EW), critically endangered (CR), endangered (EN), vulnerable (VU), near threatened (NT), least concern (LC), and data deficient (DD) are some of the threat categories that are ascribed to species according to the IUCN classification system (Hammer and Khoshbakht, 2005). The threat classification on this list continues to be a fundamental component of the global conservation effort prioritisation process, and it is widely recognised as the accepted way to assess the risk of extinction for a given species (Collar, 1996). These criteria are being used by nations all around the world to prioritise species for conservation, develop conservation plans, and influence choices and regulations under multilateral agreements for conservation (Maxted et al., 1997; Rodrigues et al., 2006).

Numerous species under consideration for inclusion on the Red List play pivotal roles in human health and sustenance. Assessments concentrated on the wild progenitors of cultivated plants and medicinal herbs illuminate species with potential for novel pharmaceutical discovery and the preservation of agricultural resilience amidst shifting climates. Shedding light on the status of these species and undertaking conservation efforts are paramount for safeguarding our own well-being. Data sourced from The IUCN Red List significantly influences human health and livelihoods. Researchers in the health sector often rely on Red List information when analysing the geographic ranges of species known or suspected to serve as disease vectors for humans and domestic animals. This data facilitates the development of effective treatments by aiding in the creation of predictive models for the future emergence of diseases.

Traditional medicines in terms of utilizing natural products hold significant importance in healthcare and have been globally practiced for centuries, evolving into well-structured medical systems. Despite potential limitations, these systems represent invaluable repositories of human wisdom and knowledge, underscored by their extensive historical use and continued relevance in contemporary healthcare

paradigms. The research aims to delineate the interconnections between ethnomedicinal NTFPs as cited by indigenous informants. Through a methodical investigation of the usage patterns and cultural significance associated with ethnomedicinal NTFPs, this research endeavours to offer insights into their potential as nutraceuticals. Utilizing rigorous analysis and synthesis of empirical data, the study aims to furnish a thorough comprehension of the complex interplay between traditional knowledge systems, which have hitherto remained unexplored in the research domain. Thus, the current research entitled "**Study of Non-Timber Forest Products: Phenological and Phytochemical analysis of Selected Plant Species within Lunglei District, Mizoram**" consisting the following five objectives:

1. Documentation and quantitative ethnobotanical study of important Ethnomedicinal NTFPs within the study area.

2. Phenological observation of selected Ethnomedical NTFPs.

3. Qualitative and quantitative phytochemical analysis and free- radical scavenging activity of selected Ethnomedicinal NTFPs.

4. Anti-bacterial activity of selected Ethnomedicinal NTFPs.

5. Documentation of RET species within the study area.

CHAPTER III

3. Documentation and quantitative ethnobotanical study of important Ethnomedicinal NTFPs within the study area.

3.1. Introduction

Non-Timber Forest Products (NTFPs) are a diverse range of natural resources obtained from forests and other wooded ecosystems, excluding timber and other wood-based products. It encompasses a wide variety of plant and animal products that have both ecological and economic significance and are often associated with traditional and indigenous knowledge systems and play a vital role in the livelihoods of many forest-dependent communities worldwide (Cocksedge, 2006; Endamana, 2016). It includes the biotic components of forested and wooded ecosystems, excluding timber, and includes a diverse array of resources derived from wild flora and fauna. These resources, ranging from fruits, nuts, and vegetables, medicinal plants, resins, bark, fibres, palms, grasses, as well as minor wood products and firewood, are typically acquired through extraction activities conducted by local households and communities (Gosling et al., 2017). These activities primarily occur in the vicinity of residential areas, agricultural fields, grazing lands, and relatively undisturbed vegetation. The purpose of gathering NTFPs is typically for local consumption or trade. The harvesting, processing, and commercialization of NTFPs frequently constitute the sole source of employment opportunities for residents inhabiting isolated rural regions (Andel, 2006).

Nonetheless, the significance of NTFPs often goes unrecognized, primarily due to their absence from formalized trading platforms and their omission from national economic datasets. This oversight persists despite the fact that numerous forest inhabitants engage in substantial NTFPs harvesting activities, for sustenance as well as commercial purposes, be it on a routine basis or in response to unforeseen circumstances (Shaanker at al., 2004). The international community has recognized that numerous ethnic communities rely on natural resources, which encompass the utilization of medicinal plants. The utilization of plants as traditional therapeutics

offers a tangible alternative within the healthcare system of developing nations, particularly for rural communities (Mahomoodally et al., 2018).

Recently, the World Health Organization (WHO) conducted an assessment revealing that approximately 80% of the global population incorporates herbal medicines into their primary healthcare regimens. WHO also identified a potential pool of approximately 21,000 plant species with medicinal attributes. Extensive data further demonstrates that over three-quarters of the global population primarily rely on botanical resources and plant-derived compounds for their healthcare requirements. Surprisingly, more than 30% of the entirety of plant species worldwide has been historically employed for medicinal purposes. Remarkably, in developed nations such as the United States, plant-based pharmaceuticals constitute a substantial proportion, approximately 25%, of the overall pharmacopoeia. Conversely, in rapidly emerging economies like India and China, this contribution soars to an impressive 80%. Consequently, the economic significance of medicinal plants is considerably greater in countries such as India in comparison to the rest of the world (World Health Organization's 2019 global report).

Over the past few years, ethnobotanical knowledge has consistently served as a valuable foundation for numerous effective drug screening initiatives (Heinrich and Bremner, 2006). The influence of traditionally crafted remedies from diverse ancient healing systems has significantly enhanced the overall well-being of the community in India, and this connection is deeply rooted in cultural traditions (Ravishankar and Shukla, 2007). Utilizing quantitative indices to analyse ethnobotanical data is believed to reveal the utilization and significance of medicinal plants within ethnic knowledge systems. These methodologies gauge the extent of consensus regarding their usage through various hypotheses, playing a crucial role in the subsequent selection of medicinal plants for biomedical research aimed at treating specific ailments, guided by survey findings enriched with valuable information (Mutheeswaran et al., 2011). The main objective of the study was to gather knowledge of ethnomedicinal NTFPs from the local traditional healers in order to create a complete database of medicinal plants and their customary uses. This

endeavour fits with our continuous documentation of ethnomedicinal practices spanning numerous years in various indigenous groups (Rahman et al., 2016).

3.2. Materials and methods

3.2.1. Description of the study area

Mizoram, an Indian state, comprises two National Parks and eight Wildlife Sanctuaries, collectively spanning 1728.75 square kilometres, constituting 8% of the state's total land area. Among these, Thorangtlang Wildlife Sanctuary is situated approximately 245 kilometres to the south of Aizawl, the state's capital. It is geographically positioned between 23°17'20"N - 23°11'30"N latitude and 92°30'35"E - 92°37'12"E longitude, with its highest point reaching an elevation of 1396 meters. Located in the Lunglei District **(Figure 3.1)**, Thorangtlang Wildlife Sanctuary received its official designation as a Wildlife Sanctuary from the Government in 2002, as per Notification No.B. 12012/171/2001-FST dated 23.04.2002, covering an area of 180 square kilometres.

The Sanctuary is surrounded by nine neighbouring villages, namely Thenhlum, Laisawral, Sesawm, West Bunghmun, Tleu, West Phulpui, Hruiduk, Dengsur, and Changpui. It boasts a diverse landscape featuring both evergreen and semi-evergreen forests, setting it apart from other Wildlife Sanctuaries in the Mizoram Forest. The region primarily comprises rural communities, with a significant portion of the population lacking access to modern education and healthcare services. Consequently, the local inhabitants heavily rely on traditional herbal remedies native to the area for their healthcare needs and additional sources of income. This reliance on traditional remedies persists even in the modern era, reflecting the enduring importance of these practices in the community.

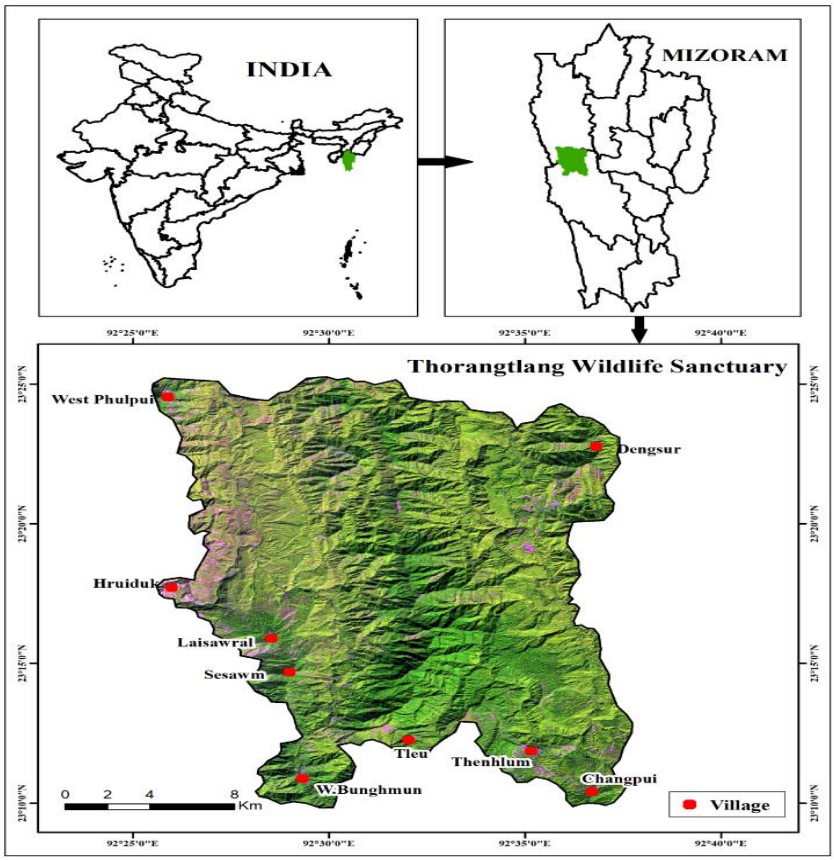


Figure 3.1: Geographical map of Thorangtlang Wildlife Sanctuary, showing the study area.

3.2.2. Data collection

In the research area, a field survey was conducted, during which traditional herbalists were randomly selected for interviews. They were asked about their usage of ethnobotanical plants. The utilization of ethnobotanical plants by these herbalists encompasses a diverse range of medicinal formulations crafted from natural ingredients. The traditional herbalists possess extensive knowledge about the medicinal and nutritional applications of various plants. Within the Mizo community residing in the research area, traditional healing practices involve the utilization of botanicals in different forms, such as freshly prepared juices, decoctions, oral powders, and topical pastes for treating skin conditions and wounds, and for meeting their health care remedies. Through semi-structured interviews, a comprehensive list of 63 ethnomedicinal NTFPs, each associated with specific ailments was documented

by gathering insights from 91 informants. The Mizo language served as the medium for recording their rich knowledge of medicinal plants.

Figure 3.2: Photoplates of informants participated and field visit of the study area.

3.2.3. Herbarium specimen and identification

The ethnomedicinal NTFPs associated with specific ailments were identified and documented in accordance with the data provided in the 'Flora of Mizoram' (Singh et al., 2002). Subsequently, herbarium specimens were collected and deposited at the Department of Botany, Mizoram University, each accompanied by unique accession numbers for the purpose of authentication. The scientific nomenclature was verified through the utilization of an online resource known as 'Plants of the World Online' https://powo.science.kew.org/.

3.2.4. Data interpretation of ethnomedicinal NTFPs

Investigation of ethnomedicinal practices and their associated methodologies employed by traditional healers and herbalists was conducted utilizing semi-structured interviews. The ethnobotanical data acquired in this study was presented as primary data, adhering to the guidelines and methodologies outlined by Heinrich et al. (2009); Weckerle et al. (2018); Leonti, 2022. The resultant dataset was meticulously organized and analysed using Microsoft Excel, leading to the derivation of quantitative ethnomedicinal metrics.

Information procured from traditional healers and herbalists was employed to document patterns of consumption and routes of administration, while ailment categories were categorized into fourteen distinct groupings. Quantitative ethnomedicinal indices, including the Informant Consensus Factor (ICF), Fidelity Level (FL), Frequency Citation (FC), Relative Frequency Citation (RFC), Use Report (UR) and Use Value (UV), were subsequently employed to assess and evaluate the information gathered from the informants. Quantitative ethnobotanical analysis was conducted using R Studio version 4.1.2, employing the 'ethnobotanyR' package and the 'Circlize' package (Whitney, 2022) for data processing and visualization. Descriptive statistics and Pearsons's correlation was calculated using Graphpad Prism 8.0.1.

3.2.5. Quantitative ethnobotanical analysis

The ethnomedicinal NTFPs gathered were subjected to analysis employing six distinct ethnobotanical indices viz., Use Report (UR), Use Value (UV), Frequency Citation (FC), Fidelity Level (FL) and Informant Consensus Factor (ICF).

3.2.5.1. Use Report (UR)

Each instance of a plant being referenced for a specific utilization was regarded as a single Use Report (UR) (Amiquet et al., 2005).

3.2.5.2. Use Value (UV)

The assessment of relative importance was conducted through the application of the Use Value (UV), a quantitative metric employed to gauge the relative significance of a species. The UV calculation takes into account both the frequency of usage and the number of individuals within a population who cited a specific plant, thereby identifying species deemed of paramount importance within that particular community (Silva et al., 2006). Use-Value was calculated using the formula

$$UV = \sum Ui/n$$

where: Ui = the number of uses mentioned by each informant for a given species, n = the total number of informants.

The Use Value (UV) assumes higher values when a particular species is associated with a greater number of Use Reports (URs), indicative of its significance within the context of ethnobotanical knowledge. Conversely, a lower UV is observed when there are a limited number of reports documenting its utilization, suggesting a lower level of importance.

3.2.5.3. Fidelity Level (FL)

This analysis aims to discern the species that exhibit a higher degree of preference among the informants when employed for the treatment of specific ailments (Friedman et al., 1986). In simpler terms, FL was employed to ascertain the proportion of the most favoured and esteemed medicinal plant specifically designated for the treatment of a particular disease or utilization category. It was calculated by the formula

$$FL = (np/N) \times 100$$

where 'np' represents the number of use-reports associated with a particular plant-use category for a given plant species, and 'N' denotes the total number of use-reports recorded for that specific plant species.

3.2.5.4. Frequency Citation (FC)

FC metric quantifies the collective focus of informants on the utilization of a specific plant for a particular ailment. FC was computed as the ratio of the number of instances a particular species was mentioned relative to the total number of times all species were referenced in the study (Tardío and Pardo-de-Santayana, 2008). FC was calculated by the formula

FC = (The frequency of a particular species was mentioned) / (The frequency of all species was mentioned) × 100

3.2.5.5. Informant Consensus Factor (ICF)

It indicates the level of homogeneity among informants for the plants to be used in each ailment category. The index is calculated as follows,

$$ICF = n_{ur} - n_t / n_{ur} - 1$$

where n_{ur} is the number of use reports in each category and n_t is the number of species (taxa) in each category.

The ICF scale ranges from 0 to 1, with a value of "1" signifying the utmost consensus among informants regarding the utilization of plant species for a particular ailment category. Conversely, lower values trending towards zero, indicate a lack of consensus or disagreement among informants concerning the application of plant species for a specific ailment category (Heinrich et al., 1998). ICF values were computed for a set of 14 ailment categories that were organized in accordance with the International Classification of Primary Care (ICPC-2) system (https://www.who.int/standrds/classifications/other-classifications/international-classification-of-primary-care).

The World Organization of Family Doctors (WONCA) and the International Classification Committee (WICC) are responsible for creating and continually updating this classification system. It serves as the predominant global reference for the systematic documentation and organization of clinical information within the realm of primary care. ICPC-2 categorization is primarily informed by patients perspectives, which lends it a lesser degree of reliance on clinical categorization, as

noted in studies by Staub et al., (2015); Panmei et al., (2019). The establishment of this classification system was driven by the escalating demand for trustworthy primary healthcare data, spurred by heightened global consciousness surrounding primary health care objectives, exemplified by the World Health Organization's overarching aim of achieving 'health for all'.

3.3. Results

3.3.1. Demographic profile of informants

A total of 91 informants were surveyed, comprising 69 (78.80%) males and 22 (24.17%) females. These informants were stratified into four age cohorts, as delineated in **Table 3.1.** Among the surveyed informants, 23 (25.27%) fell within the 20-40 age range, 17 (18.70%) within the 41-50, 44 (48.35%) within the 51-60 and 7 (7.69%) were aged above 60. The educational profiles of the informants were systematically recorded. Among the total of 91 informants, 31 (34.06%) were found to be illiterate, 34 (37.36%) had attained education up to the primary level, 19 (20.80%) had completed their secondary education, and 7 (7.69%) had received education at the university level.

Table 3.1: Demographic profile of Informants in the study area.

Gender	**No of informants**	**%**
Male	69	75.80%
Female	22	24.17%
Age		
20-40	23	25.27%
41-50	17	18.70%
51-60	44	48.35%
>60	7	7.69%
Education		
Illiterate	31	34.06%
Primary	34	37.36%
Secondary	19	20.80%
University	7	7.69%

Table 3.2: Lists of ethnomedicinal NTFPs documented from 91 informants.

SlNo.	Botanical name	Vernacular name	Family	Part used	Life form	Mode of preparation	Accession no	UV	FC	RFC
1	*Acmella calva* (DC.) R.K. Jansen	Ankasa	Asteraceae	L	Herb	Ju	MZUH000900	0.362	36.2	0.39
2	*Adiantum philippense* L.	Lungpui sam	Pteridaceae	R	Herb	De, Ju	MZUH000901	0.296	30	0.37
3	*Aeginetia indica* L.	Sanghar vaibel	Orobanchaceae	Wp	Herb	Co, Ju, De	MZUH000904	0.12	12	0.13
4	*Aganope thyrsiflora* (Benth.) Polhill	Hulhu	Fabaceae	Fr, B	Shrub	De	MZUH000905	0.12	12	0.13
5	*Ageratum houstonianum* Mill.	Vailen -hlo	Asteraceae	L, R	Herb	Co, Ju	MZUH000906	0.241	24.1	0.26
6	*Alpinia malaccensis* (Burm.f.) Roscoe	Ai chal	Zingiberaceae	Rh	Herb	De, Ju, Co	MZUH000907	0.153	15.3	0.16
7	*Alstonia scholaris* (L.) R.Br.	Thuamriat	Apocynaceae	B, Ml	Tree	Co, Ju	MZUH000913	0.329	33	0.36
8	*Aporosa octandra* (Buch.-Ham. ex D.Don) Vickery	Chhawntual	Phyllanthaceae	B, L	Tree	De, Ju	MZUH000908	0.549	55	0.6
9	*Artocarpus lacucha* Roxb. ex Buch.-Ham.	Theitat	Moraceae	B	Tree	Pa	MZUH000911	0.274	27.4	0.3
10	*Bacopa monnieri* (L.) Wettst.	Unnamed	Plantaginaceae	Wp	Herb	Pa, Ju	MZUH000910	0.472	47.2	0.52
11	*Bauhinia glauca* (Benth.) Wall. ex Benth.	Hrui vaube	Fabaceae	B	Shrub	Ju, De	MZUH000926	0.428	43	0.47
12	*Begonia roxburghii* (Miq.) A.DC.	Sekhupthur	Begoniaceae	Wp	Herb	Ju, De	MZUH000909	0.736	74	0.8
13	*Bidens pilosa* L.	Cha-bet	Asteraceae	Wp	Herb	Po, De	MZUH000912	0.384	38.4	0.42
14	*Bischofia javanica* Blume	Khuang-thli	Phyllanthaceae	L	Tree	Ju	MZUH000914	0.263	26.3	0.28
15	*Blumea lanceolaria* Druce	Buarze	Asteraceae	L	Herb	Ju, De	MZUH000915	0.527	53	0.57
16	*Bombax insigne* Wall.	Pang	Malvaceae	L	Tree	Co, De	MZUH000917	0.274	27.4	0.3
17	*Bonnaya ruellioides*	Thasuih	Linderniaceae	Wp	Herb	Co, Ju, De	MZUH000902	0.351	35.1	0.38

	(Colsm.) Spreng.									
18	*Callicarpa arborea* Roxb.	Hnahkiah	Lamiaceae	B, L	Tree	Ju, Po, Pa	MZUH000922	0.417	42	0.45
19	*Canavalia ensiformis* (L.) DC.	Fangra	Fabaceae	Fr	Climber	Co, Ju	MZUH000923	0.373	37.3	0.41
20	*Ceiba pentandra* (L.) Gaertn.	Japan pang	Malvaceae	R	Tree	Ju, De	MZUH000924	0.208	21	0.22
21	*Centella asiatica* (L.) Urb.	Lambak	Apiaceae	Wp	Herb	Ra, Ju, Co	MZUH000925	0.571	57.1	0.62
22	*Chromolaena odorata* (L.) R.M.King & H.Rob.	Tlangsam	Asteraceae	L	Shrub	Pa, Ju, De	MZUH000933	0.549	55	0.6
23	*Cissampelos pareira* L.	Hnahbial-hrui	Menispermaceae	R, S	Climber	Ju	MZUH000934	0.516	52	0.56
24	*Cissus repens* Lam.	Hruipawl	Vitaceae	R, S, L	Climber	Ju, Pa	MZUH000927	0.296	30	0.32
25	*Clerodendrum glandulosum* Lindl.	Phuihnam	Lamiaceae	L	Tree	Co, Ju	MZUH000940	0.318	32	0.35
26	*Clerodendrum infortunatum* L.	Phuihnam chhia	Lamiaceae	R, L	Shrub	Pa, Co, Ju, De	MZUH000941	0.384	38.4	0.42
27	*Colocasia esculenta* (L.) Schott	Dawl	Araceae	Wp	Herb	Ju	MZUH000935	0.252	25.2	0.27
28	*Crassocephalum crepidiodes* (Benth.) S. Moore	Buar thau	Asteraceae	L	Shrub	Ju, Co, De	MZUH000916	0.175	17.5	0.19
29	*Croton caudatus* Geiseler	Ranlung damdawi	Euphorbiaceae	L, R	Shrub	Ju, De	MZUH000936	0.758	76	0.83
30	*Curcuma caesia* Roxb.	Ailai dum	Zingiberaceae	Rh	Herb	Pa, Ra, Co	MZUH000937	0.483	48.3	0.53
31	*Curcuma longa* L.	Aieng	Zingiberaceae	Rh	Herb	Ra, Po, Ju, De	MZUH000938	0.604	60.4	0.66
32	*Drymaria cordata* Willd. ex Schult.	Chang-kal-rit	Caryophyllaceae	L	Herb	Pa, Ju, De	MZUH000939	0.472	47.2	0.52
33	*Drynaria coronans* J.Sm.	Awmvel	Polypodiaceae	L	Epiphytic fern	Ju, De	MZUH000942	0.439	44	0.48
34	*Embelia ribes* Burm.f.	Naufa dawn tuai	Primulaceae	Wp	Climber	De, Ju	MZUH000943	0.307	31	0.33
35	*Euphorbia heterophylla* L.		Euphorbiaceae	L	Herb	De, Ju	MZUH000945	0.461	46.1	0.51
36	*Euphorbia hirta* L.	Zawhte hlo	Euphorbiaceae	Wp	Herb	De, Ju	MZUH000946	0.307	30.7	0.34
37	*Flueggea virosa* (Roxb. ex Willd.) Royle	Saisiak	Phyllanthaceae	L	Shrub	De	MZUH000947	0.769	77	0.84

38	*Gelsemium elegans* (Gardner & Champ.) Benth.	Hnam-tur	Gelsemiaceae	R	Climber	Ju, Pa	MZUH000950	0.241	24.1	0.26
39	*Hedyotis scandens* Roxb.	Kel hnam tur/ Laiking tuibur	Rubiaceae	R, L	Climber	De	MZUH000951	0.175	17.5	0.19
40	*Hellenia speciosa* (J.Koenig) Govaerts	Sumbul	Costaceae	Rh	Herb	Ju, Co, De	MZUH000949	0.406	41	0.47
41	*Homalomena aromatica* Schott	An-chiri	Araceae	R	Herb	Ju, Co	MZUH000962	0.274	27.4	0.3
42	*Justicia adhatoda* L.	Kawldai	Acanthaceae	L	Shrub	De	MZUH000948	0.461	46.1	0.51
43	*Leucaena leucocephala* (Lam.) de Wit	Japan zawngtah	Fabaceae	R, B	Shrub	Ju, Co, De	MZUH000952	0.263	26.3	0.28
44	*Linostoma decandrum* (Roxb.) Steud.	Ngaihhih	Thymelaeaceae	R	Shrub	Pa	MZUH000953	0.362	36.2	0.4
45	*Lobelia nummularia* Lam.	Choaka thi	Campanulaceae	L	Herb	Ju, Co, De	MZUH000954	0.417	42	0.46
46	*Melastoma malabathricum* L.	Builukham	Melastomaceae	Wp	Shrub	Ju, De, Pa	MZUH000955	0.813	81.3	0.89
47	*Mikania micrantha* (L.) Willd.	Japanhlo	Asteraceae	L	Climber	Ra, Pa, Ju	MZUH000956	0.439	44	0.48
48	*Mimosa pudica* L.	Hlo zak	Fabaceae	Wp	Shrub	De	MZUH000957	0.373	37.3	0.41
49	*Mirabilis jalapa* L.	Artuk khuan	Rutaceae	L, R	Root	De	MZUH000960	0.142	14.2	0.16
50	*Mussaenda glabra* Vahl	Vakep	Rubiaceae	L, R	Shrub	Ju, Ra, Pa	MZUH000959	0.373	37.3	0.41
51	*Oroxylum indicum* (L.) Benth. ex Kurz	Ar changkawm	Bignoniaceae	R, B	Tree	De	MZUH000958	0.659	66	0.72
52	*Pandanus odorifer* (Forssk.) Kuntze	Ram lakhuih	Pandanaceae	L, R	Shrub	De, Co, Ju	MZUH000961	0.285	28.5	0.31
53	*Phyllanthus emblica* L.	Sunhlu	Phyllanthaceae	Fr, S	Tree	Ra, Ju, Co	MZUH000944	0.505	50.5	0.55
54	*Plantago major* L.	Kel-ba-an	Plantaginaceae	Wp	Herb	De, Co, Ju	MZUH000928	0.472	47.2	0.52
55	*Rhus chinensis* Mill.	Khawmhma	Anacardiaceae	L, Fr	Tree	De	MZUH000929	0.395	39.5	0.43
56	*Scoparia dulcis* L.	Perh-pawng chaw	Plantaginaceae	L, S, R	Shrub	Ju, Dec	MZUH000930	0.340	34	0.37

57	*Senegalia pennata* (L.) Maslin	Khanghu	Fabaceae	L, B	Shrub	De	MZUH000931	0.241	24.1	0.26
58	*Terminalia phillyreifolia* (Van Heurck & Mull. Arg.) Gere & Boatwr.	Zairum	Combretaceae	B, L	Tree	De	MZUH000903	0.395	39.5	0.43
59	*Thunbergia grandiflora* Roxb.	Vako	Acanthaceae	L	Climber	Ju	MZUH000932	0.736	74	0.8
60	*Toddalia asiatica* (L.) Lam.	Nghar dai	Rutaceae	R	Shrub	Ju, Co	MZUH000920	0.263	26.3	0.28
61	*Triumfetta rhomboidea* Jacq.	Sehnap	Malvaceae	Wp	Shrub	Ju, Co	MZUH000919	0.241	24.1	0.26
62	*Vitex peduncularis* Wall.	Thingkhawilu	Lamiaceae	L	Tree	Co, Ju, De	MZUH000921	0.769	77	0.84
63	*Zingiber officinale* Roscoe	Sawhthing	Zingiberaceae	Rh	Herb	Ju, Co	MZUH000918	0.439	44	0.48

UV- Use Value, FC- Frequency Citation, RFC- Relative Frequency Citation, L- Leaf, R- Root, Wp- Whole plant, Fr- Fruit, B- Bark, Rh- Rhizome, ML- Milky latex, S- Stem, J- Juice, De- Decoction, Co- Cooked, Pa-Paste, R- Raw, Po- Powder.

3.3.2. Floristic composition and formulation of documented ethnomedicinal NTFPs

A comprehensive documentation of ethnomedicinal Non-Timber Forest Products (NTFPs) revealed the presence of 63 plant species, distributed across 34 families and 59 genera (**Table 3.2 & Figure 3.9**). The predominant taxonomic group within the ethnomedicinal flora consisted of the Asteraceae family, encompassing 6 genera and 7 species, including *Acmella calva* (DC.) R.K. Jansen, *Ageratum haustonianum* Mill., *Bidens pilosa* L., *Crassocephalum crepidiodes* (Benth.) S. Moore, *Blumea lanceolaria* Druce, *Chromolaena odorata* (L.) R. M. King & H. Rob., and *Mikania micrantha* (L.) Willd. This taxonomic cluster accounted for 11.11% of the total ethnomedicinal plant specimens amassed. The Fabaceae family emerged as the second most prominent taxonomic group, comprising 6 genera and 6 species, including *Aganope thyrsiflora* (Benth.) Polhill, *Canavalia ensiformis* (L.) DC., *Bauhinia glauca* (Benth.) Wall. ex Benth., *Leucaena leucocephala* (Lam.) de Wit, *Mimosa pudica* L., and *Senegalia pennata* (L.) Maslin. This taxonomic assemblage constituted 9.5% of the aggregate ethnomedicinal plant species documented. Three families, namely Zingiberaceae, Phyllanthaceae, and Lamiaceae, collectively accounted for 6.3% of the entire spectrum of plant species investigated. Additionally, another trio of families viz., Plantaginaceae, Malvaceae, and Euphorbiaceae contributed to 4.7% of the recorded species. Furthermore, 3.17% of the total species were attributed to the families Araceae, Rubiaceae, and Acanthaceae. The remaining taxonomic families were each represented by a solitary plant species, collectively constituting 1.58% of the dataset. The majority of documented ethnomedicinal NTFPs were categorized as herbaceous plants, representing 36% of the total, followed by shrubs (28%), trees (21%), climbers (13%), and epiphytic ferns (2%), as visually depicted in **Figure 3.3**.

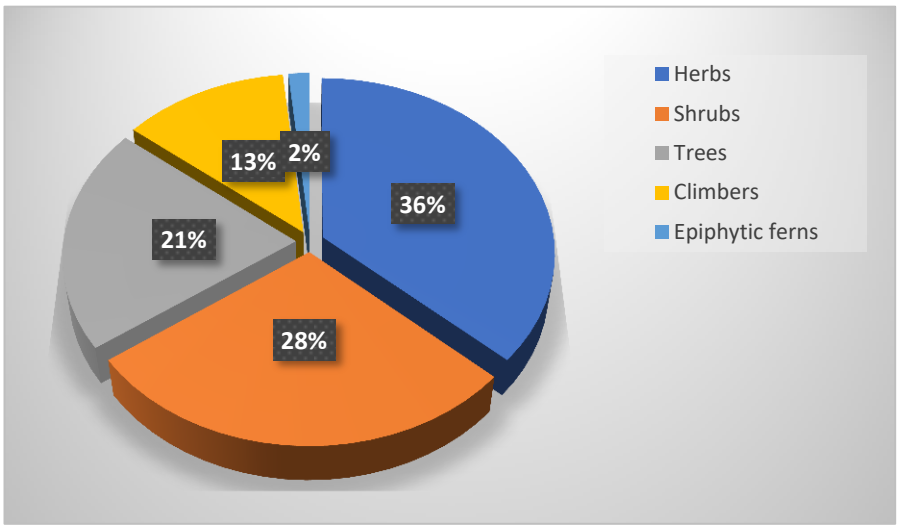


Figure 3.3: Life forms of ethnomedicinal NTFPs documented

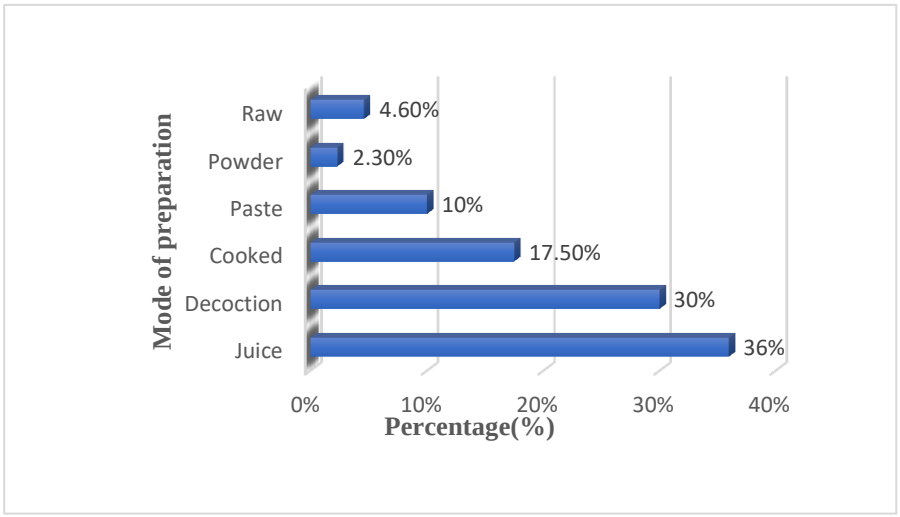


Figure 3.4: Mode of preparation.

Diverse modes of administration, including raw consumption, powder formulation, paste application, cooking, decoction preparation, and juice extraction, were employed by local informants to implement herbal remedies. The highest utilization rate was associated with juice extraction, accounting for 36% of cases, followed by decoction (30%), cooking (17.50%), paste application (10%), and raw consumption

(4.60%). The lowest utilization rate was observed in powder ingestion, constituting only 2.30% of cases (**Figure 3.4)**.

Local people use different plant parts for curing distinct ailments. Majority of ethnomedicinal NTFPs were prepared mostly from the leaves (896 URs/39.33%), whole plant (519 URs/22.7%), root (340 URs/14.9%), rhizome (213 URs/9.3%), bark (158 URs/6.9%), stem (72 URs/3.16%), fruit (70 URs/3.07%) and latex (12 URs/0.53%) (**Figure 3.6**).

3.3.3. Quantitative assessment of botanical indices

3.3.3.1. Use Report (UR)

In the current investigation, six ethnobotanical indices were utilized as analytical parameters. In the realm of quantitative ethnobotanical research, UR serves as a tool for the aggregation and quantification of ethnobotanical utilization reports associated with diverse plant species within a defined dataset. *Melastoma malabathricum* L. garnered the highest UR, with 74 citations, indicating its prominence in local traditional medicine. Following closely were *Vitex peduncularis* Wall. and *Flueggea virosa* (Roxb. ex Willd.) Royle, each with 70 citations, along with *Croton caudatus* Geiseler, which received 69 citations, and *Thunbergia grandiflora* Roxb. and *Begonia roxburghii* (Miq.) A.DC., both with 67 citations. Conversely, *Aeginetia indica* L. and *Aganope thyrsiflora* (Benth.) Polhill received the lowest UR, with only 11 citations, signifying their comparatively limited use among the 91 informants interviewed during semi-structured interviews **(Figure 3.5)**.

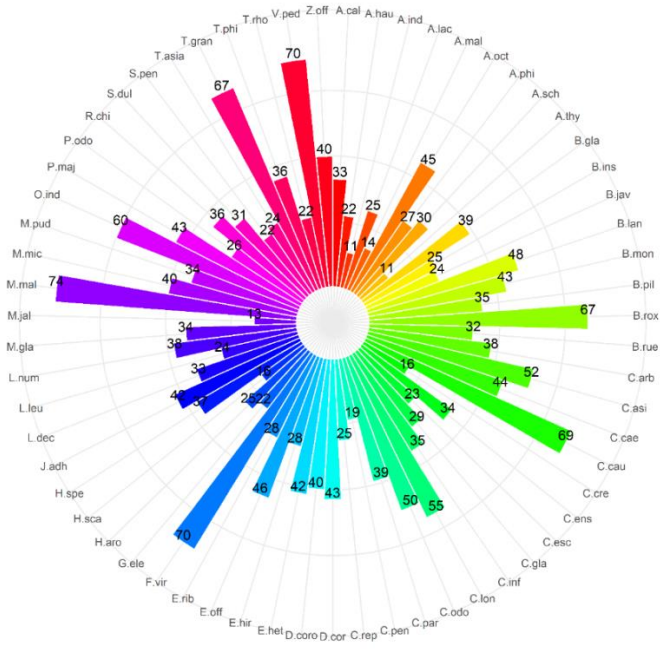


Figure 3.5: UR of reported ethnomedicinal NTFPs

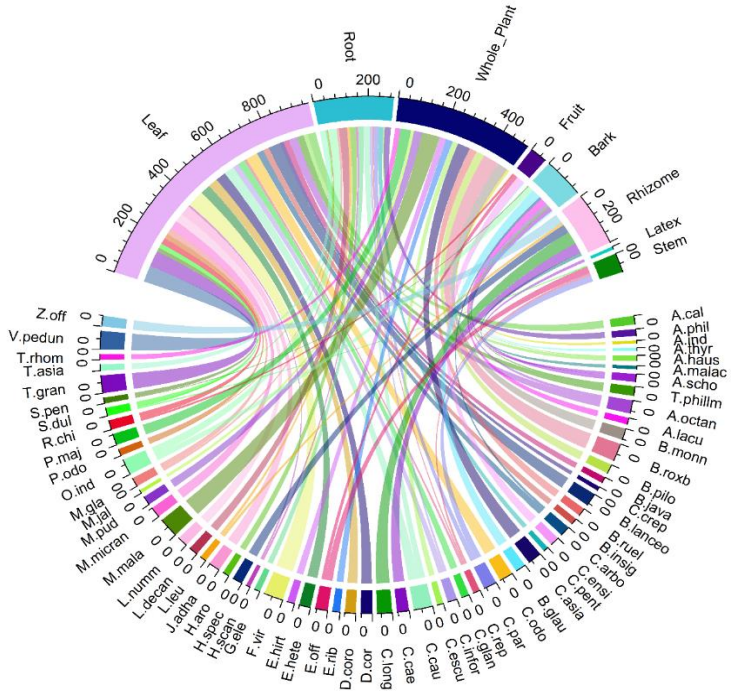


Figure 3.6: Chord diagram of UR for 63 plant taxa with part uses.

3.3.3.2. Use Value (UV)

In the present study, utilizing the ethnobotanical use-reports gathered from ethnic communities, we computed Use Value (UV). This index was employed to elucidate the prioritization of usage, significance, endorsement, and dissemination of medicinal knowledge pertaining to specific plant species within the informant group. UV value ranges from 0.12 to 0.813. *M. malabathricum* displayed the highest UV values at 0.813, followed by *F. virosa* and *V. peduncularis* (0.769), *C. caudatus* (0.758), and *T. grandiflora* and *B. roxburghii* (0.736) (**Figure 3.7 & Table 3.2**). This phenomenon can be attributed to the presence of these species in abundance within the study area. The prevalence of these species facilitates their accessibility to local traditional healers and herbalists, which in turn aids in treating associated ailments.

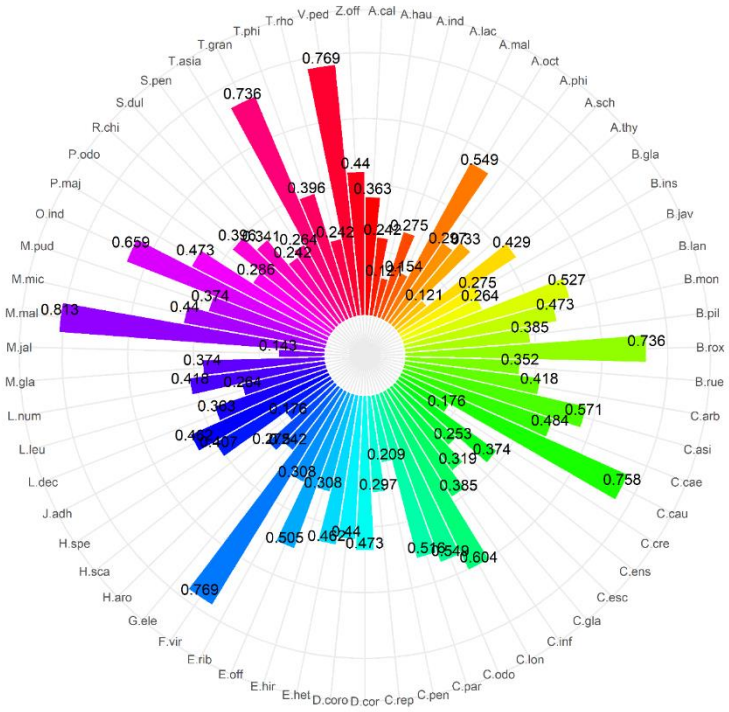


Figure 3.7: UV of reported ethnomedicinal NTFPs.

3.3.3.3. Frequency Citation (FC)

The Frequency Citation (FC) values observed for the studied ethnomedicinal plants exhibited a range between 12 and 81.3, indicative of varying degrees of citation frequency within the dataset. Notably, the highest FC was recorded for *M. malabathricum* at 81.3, followed by *V. peduncularis* and *F. virosa*, both with an FC of 77, and *C. caudatus* at 76. *B. roxburghii and T. grandiflora* also featured prominently with an FC of 74 (**Table 3.2**). The elevated FC values for these medicinal plants signify their prominence and prevalence in the study area, reflecting their popularity and common usage among local communities.

3.3.3.4. Fidelity Level (FL)

FL analysis serves as a valuable tool for the identification of the plant species most preferred by informants in the treatment of specific ailments. Within the dataset of reported ethnomedicinal NTFPs, four species exhibited the highest fidelity level, attaining a perfect score of 100%. Notably, these plant species were predominantly employed in the context of single ailment categories, often with the consensus of multiple informants. Specifically, these plant species were *Acmella calva* (DC.) R.K. Jansen, employed for addressing throat-related symptoms or complaints; *Aganope thyrsiflora* (Benth.) Polhill, prescribed for the treatment of fractured bones; *Artocarpus lacucha* Roxb. ex Buch.-Ham, used for lacerations; and *Homalomena aromatica* Schott, sought after for alleviating ear pain (**Table 3.3**).

Table 3.3: Fidelity Level (FL %) of reported ailment categories.

No.	Plants	Ailments	No of citation	No. of informants	FL %
1	*Acmella calva* (DC.) R.K. Jansen	Throat symptoms or complaint (R21)	33	33	100
2	*Adiantum philippense* L.	Mumps (D71)	16	27	59
		Lump or swelling localized (S04)	11	27	41
3	*Aeginetia indica* L.	Diabetes (T89)	8	11	73
		Arthritis(L88)	3	11	27
4	*Aganope thyrsiflora* (Benth.) Polhill	Fractured bones (L74)	11	11	100
5	*Ageratum houstonianum* Mill.	Peptic ulcer (D86)	10	22	45
		Toothache (D19)	5	22	23
		Cuts and wounds (S18)	7	22	32
6	*Alpinia malaccensis* (Burm.f.) Roscoe	Loss of appetite (T03)	6	14	43
		Bronchitis (R78)	8	14	57
7	*Alstonia scholaris* (L.) R.Br.	Hypertension (K87)	7	30	23
		Cuts and wounds (S18)	11	30	37
		Asthma (R96)	12	30	40
8	*Terminalia phillyreifolia* (Van Heurck & Mull. Arg.) Gere & Boatwr.	Contusion (S16)	9	36	25
		Stomach function disorder (D87)	21	36	58
		Hypertension (K87)	6	36	17
9	*Aporosa octandra* (Buch.-Ham. ex D.Don) Vickery	Duodenal ulcer (D85)	16	50	32
		Diarrhoea (D11)	34	50	68
10	*Artocarpus lacucha* Roxb. ex Buch.-Ham.	Laceration (S18)	25	25	100
11	*Bacopa monnieri* (L.) Wettst.	Lump or swelling localized (S04)	16	43	37
		Skin injury other (S19)	27	43	63
12	*Begonia roxburghii* (Miq.) A.DC.	Skin texture symptoms (S21)	21	67	31
		Diarrhoea (D11)	26	67	39
		Abdominal pain epigastric (D02)	20	67	30

13	*Bidens pilosa* L.	Digestive symptom (D29)	15	35	43
		Laceration (S18)	7	35	20
		Hypertension (K87)	6	35	17
		Diabetes insulin dependent (T89)	7	35	20
14	*Bischofia javanica* Blume	Throat symptoms or complaint (R21)	16	24	67
		Tonsilitis (R76)	8	24	33
15	*Crassocephalum crepidiodes* (Benth.) S. Moore	Urethral discharge (Y03)	5	16	31
		Hypertension (K87)	11	16	69
16	*Blumea lanceolaria* Druce	Asthma (R96)	38	48	80
		Tuberculosis (A70)	5	48	10
		Hypertension (K87)	5	48	10
17	*Bonnaya ruellioides* (Colsm.) Spreng.	Muscle pain (L18)	13	32	40
		Laceration (S18)	7	32	22
		Eye pain (F01)	7	32	22
		Urethral discharge (Y03)	5	32	16
18	*Bombax insigne* Wall.	Throat symptoms or complaint (R21)	19	25	76
		Hypertension (K87)	6	25	24
19	*Callicarpa arborea* Roxb.	Risk factor for Malignancy (A21)	25	38	66
		Abdominal distension (D25)	10	38	26
		Fractured bones (L74)	3	38	8
20	*Canavalia ensiformis* (L.) DC.	Peptic ulcers (D86)	25	34	74
		Burns (S14)	9	34	26
21	*Ceiba pentandra* (L.) Gaertn.	Diabetes insulin dependent (T89)	14	19	74
		Hypertension (K87)	3	19	16
		Fractured bones (L74)	2	19	10
22	*Centella asiatica* (L.) Urb.	Peptic ulcers (D86)	30	52	58
		Diarrhoea (D11)	22	52	42
23	*Bauhinia glauca* (Benth.) Wall. ex Benth.	Diarrhoea (D11)	33	39	85
		Hypertension (K87)	4	39	10
		Arthritis(L88)	2	39	5

24	*Chromolaena odorata* (L.) R.M.King & H.Rob.	Cuts (S18)	30	50	60
		Hypertension (K87)	20	50	40
25	*Cissampelos pareira* L.	Urethral discharge (Y03)	9	47	19
		Diarrhoea (D11)	31	47	66
		Fever (A03)	4	47	8
		Hypertension (K87)	3	47	7
26	*Cissus repens* Lam.	Teeth complaint (D19)	25	27	92
		Eye pain (F01)	2	27	8
27	*Clerodendrum glandulosum* Lindl.	Hypertension (K87)	11	29	38
		Jaundice (D13)	18	29	62
28	*Clerodendrum infortunatum* L.	Scabies (S72)	18	35	52
		Skin texture symptoms (S21)	17	35	48
29	*Colocasia esculenta* (L.) Schott	Laceration (S18)	20	23	87
		Headache (N01)	3	23	13
30	*Croton caudatus* Geiseler	Stomach function disorder (D87)	67	69	97
		Urethral discharge (Y03)	2	69	3
31	*Curcuma caesia* Roxb.	Risk factor for Malignancy (A21)	26	44	59
		Diarrhoea (D11)	8	44	18
		Asthma(R96)	10	44	23
32	*Curcuma longa* L.	Asthma (R96)	51	55	93
		Menstruation irregular (X07)	1	55	2
		Risk factor for Malignancy (A21)	3	55	5
33	*Drymaria cordata* Willd. ex Schult.	Fractured bones (L74)	16	43	37
		Menstruation irregular (X07)	5	43	12
		Asthma (R96)	19	43	44
		Ear pain (H01)	2	43	5
		Eye pain (F01)	1	43	2
34	*Drynaria coronans* J.Sm.	Herpes zoster (S70)	36	40	90
		Eye pain (F01)	1	40	3
		Kidney symptoms (U14)	3	40	7
35	*Embelia ribes* Burm.f.	Jaudice (D13)	14	28	50

		Epilepsy (N88)	5	28	18
		Haemorrhage (A10)	7	28	25
		Ear pain (H01)	2	28	7
36	*Emblica officinalis* Gaertn.	Weight loss (T08)	18	46	39
		Respiratory injury (R88)	21	46	46
		Haemorrhoids (K96)	7	46	15
37	*Euphorbia heterophylla* L.	Bronchitis (R78)	19	42	45
		Asthma (R96)	20	42	48
		Kidney symptoms (U14)	3	42	7
38	*Euphorbia hirta* L.	Kidney symptoms (U14)	7	28	25
		Asthma (R96)	12	28	43
		Urethral discharge (Y03)	4	28	14
		Menstruation irregular (X07)	5	28	18
39	*Flueggea virosa* (Roxb. ex Willd.) Royle	Chicken-pox (A72)	62	70	88
		Scabies (S72)	8	70	12
40	*Gelsemium elegans* (Gardner & Champ.) Benth.	Skin infections other (S76)	15	22	68
		Ear pain (H01)	4	22	19
		Post-partum symptoms or complaint (W18)	1	22	4
		Kidney symptoms (U14)	2	22	9
41	*Hedyotis scandens* Roxb.	Urinary complaint (U29)	10	16	63
		Complicate labour (W92)	6	16	37
42	*Hellenia speciosa* (J.Koenig) Govaerts	Kidney symptoms (U14)	16	37	43
		Urinary disease (U99)	13	37	35
		Haemorrhoids (K96)	8	37	22
43	*Homalomena aromatica* Schott	Ear pain (H01)	25	25	100
44	*Justicia adhatoda* L.	Fever (A03)	19	42	45
		Asthma (R96)	17	42	41
		Ear pain (H01)	6	42	14
45	*Leucaena leucocephala* (Lam.) de Wit	Peptic Ulcer (D86)	16	24	67
		Diabetes insulin dependent (T89)	8	24	33
46	*Linostoma decandrum* (Roxb.) Steud.	Scabies (S72)	11	33	33
		Fever (A03)	7	33	21

		Respiratory compaint (R29)	15	33	46
47	*Lobelia nummularia* Lam.	Diarrhoea (D11)	14	38	37
		Peptic ulcer (D86)	16	38	42
		Teeth complaint (D19)	5	38	13
		Ear pain (H01)	3	38	8
48	*Melastoma malabathricum* L.	Cuts and wounds (S18)	11	74	15
		hypertension (K87)	61	74	82
		Tuberculosis (A70)	2	74	3
49	*Mikania micrantha* (L.) Willd.	Laceration (S18)	15	40	38
		Fever (A03)	7	40	17
		Eye pain (F01)	3	40	7
		Ear pain (H01)	15	40	38
50	*Mimosa pudica* L.	Infertility (W15)	7	34	21
		Insect bite (S12)	18	34	53
		Sinus complaint (R09)	9	34	26
51	*Mirabilis jalapa* L.	Diabetes insulin dependent (T89)	7	13	54
		Fever (A03)	6	13	46
52	*Mussaenda glabra* Vahl	Cough (R05)	20	34	59
		Snake bite (S12)	9	34	26
		Risk factor for Malignancy (A21)	5	34	15
53	*Oroxylum indicum* (L.) Benth. ex Kurz	Asthma (R96)	25	60	42
		Indigestion (D07)	17	60	28
		contusion (S16)	11	60	18
		Tuberculosis (A70)	7	60	12
54	*Pandanus odorifer* (Forssk.) Kuntze	Scabies (S72)	6	26	23
		Heart burn (D03)	16	26	62
		Kidney symptoms (U14)	4	26	15
55	*Plantago major* L.	Skin symptom (S29)	9	43	21
		Fever (A03)	11	43	26
		Digestive symptom (D29)	23	43	53
56	*Rhus chinensis* Mill.	Diarrhoea (D11)	24	36	67
		Fever (A03)	7	36	19
		Endocrine infection	5	36	14

		(T70)			
57	*Scoparia dulcis* L.	Diarrhoea (D11)	17	31	55
		Toothache (D19)	7	31	23
		Ear pain (H01)	7	31	22
58	*Senegalia pennata* (L.) Maslin	Cholecystitis (D98)	7	22	32
		Bronchiolitis (R78)	5	22	23
		Endocrine disorder (T99)	3	22	13
		Asthma (R96)	7	22	32
59	*Thunbergia grandiflora* Roxb.	Throat symptoms or complaint (R21)	37	67	55
		Laceration (S18)	7	67	11
		Asthma (R96)	23	67	34
60	*Toddalia asiatica* (L.) Lam.	Fever (A03)	7	24	29
		Indigestion (D07)	14	24	58
		Endocrine disorder (T99)	3	24	13
61	*Triumfetta rhomboidea* Jacq.	Diabetes insulin dependent (T89)	17	22	77
		Fever (A03)	5	22	23
62	*Vitex peduncularis* Wall.	Malaria (A73)	27	70	39
		Jaudice (D13)	15	70	21
		Peptic ulcer (D86)	28	70	40
63	*Zingiber officinale* Roscoe	Bronchitis (R78)	14	40	35
		Ear pain (H01)	8	40	20
		Throat symptoms or compaint (R21)	18	40	45

FL- Fidelity Level.

3.3.3.5. Informant Consensus Factor (ICF)

The categorization of ailments in this study adhered to a comprehensive system known as the International Classification of Primary Care-2 (ICPC-2), which is endorsed by the World Health Organization's Family of International Classifications (WHO-FIC). Each ailment, or the corresponding disease conditions, was assigned specific codes for reference (**Table 3.4**). ICF was employed to evaluate the significance of culturally important medicinal plants utilized by multiple informants within the same use or disease category. ICF values, falling within the range of 0 to

1, reflect the extent of consensus among informants regarding the utility of particular plants for specific health concerns. Notably, the highest ICF values were observed in the D-Digestive category, registering at 0.96, derived from 665 citations spanning 26 distinct plant species and R-Respiratory category, also with an ICF of 0.96, supported by 476 citations encompassing 20 plant species, and the S-Skin category, recording an ICF of 0.93, with 391 citations involving 25 different plant species. In contrast, the F-Eye category displayed the lowest ICF value, standing at 0.69, based on 14 citations referencing 5 plant species (**Table 3.5**).

Table 3.4: International Code of Primary Care (ICPC) code of reported ailments.

No.	Botanical name	ICPC code
1	*Acmella calva* (DC.) R.K. Jansen	RES-R:21
2	*Adiantum philippense* L.	GAS-D:71, DER-S:04
3	*Aeginetia indica* L.	MET-T:90, SKE-L:88
4	*Aganope thyrsiflora* (Benth.) Polhill	SKE-L:74
5	*Ageratum houstonianum* Mill.	GAS-D:86, DER-S:18, GAS-D:19
6	*Alpinia malaccensis* (Burm.f.) Roscoe	EMN-T:03, RES-R:78
7	*Alstonia scholaris* (L.) R.Br.	CAR-K:87, RES-R:96, DER-S:18
8	*Aporosa octandra* (Buch.-Ham. ex D.Don) Vickery	GAS-D:85, GAS-D:11, Nil
9	*Artocarpus lacucha* Roxb. ex Buch.-Ham.	DER-S:18
10	*Bacopa monnieri* (L.) Wettst.	DER-S:04, DER-S:19
11	*Bauhinia glauca* (Benth.) Wall. ex Benth.	GAS-D:11, Car-K:87, SKE-L:88
12	*Begonia roxburghii* (Miq.) A.DC.	DER-S:21, GAS-D:11, GAS-D:02
13	*Bidens pilosa* L.	GAS-D:29, DER-S:18, CAR-K:87, MET-T:89
14	*Bischofia javanica* Blume	RES-R:21, RES-R:76
15	*Blumea lanceolaria* Druce	RES-R:96, Nil, OTH-A:70, Car-K:87
16	*Bombax insigne* Wall.	RES-R:21, Car-K:87
17	*Bonnaya ruellioides* (Colsm.) Spreng.	SKE-L:18, DER-S:18, EYE-F:01, MGD-Y:03
18	*Callicarpa arborea* Roxb.	OTH-A:21, GAS-D:25, SKE-L:74
19	*Canavalia ensiformis* (L.) DC.	GAS-D:86, DER-S:14
20	*Ceiba pentandra* (L.) Gaertn.	MET-T:89, Car-K:87, SKE-L:74
21	*Centella asiatica* (L.) Urb.	Nil, GAS-D:86, GAS-D:11
22	*Chromolaena odorata* (L.) R.M.King & H.Rob.	DER-S:18, CAR-K:87
23	*Cissampelos pareira* L.	MGD-Y:03, GAS-D:11, Nil, OTH-A:03, Car-

		K:87
24	*Cissus repens* Lam.	GAS-D:19, F-E:01
25	*Clerodendrum glandulosum* Lindl.	CAR-K:87, GAS-D:13
26	*Clerodendrum infortunatum* L.	DER-S:72, DER-S:21
27	*Colocasia esculenta* (L.) Schott	DER-S:18, NER-N:88
28	*Crassocephalum crepidiodes* (Benth.) S. Moore	MGD-Y:03, CAR-K:87
29	*Croton caudatus* Geiseler	GAS-D:87, MGD-Y:03
30	*Curcuma caesia* Roxb.	OTH-A:21, GAS-D:11, RES-R:96
31	*Curcuma longa* L.	RES-R:96, OTH-A:21, FG-X:07
32	*Drymaria cordata* Willd. ex Schult.	SKE-L:74, FG-X:07, RES-R:96, E-F:01, H-E:01
33	*Drynaria coronans* J.Sm.	DER-S:70, URO-U:14, E-F:01
34	*Embelia ribes* Burm.f.	GAS-D:13, NER-N:88, OTH-A:10, H-E:01
35	*Euphorbia heterophylla* L.	RES-R:78, RES-R:96, URO-U:14
36	*Euphorbia hirta* L.	URO-U:14, RES-R:96, Nil, FG-X:07, MGD-Y:03
37	*Flueggea virosa* (Roxb. ex Willd.) Royle	OTH-A:72, DER-S:72
38	*Gelsemium elegans* (Gardner & Champ.) Benth.	DER-S:76, H-E:01, URO-U:14, PRE-W:18
39	*Hedyotis scandens* Roxb.	URO-U:29, PRE-W:92
40	*Hellenia speciosa* (J.Koenig) Govaerts	URO-U:14, URO-U:99, Car-K:96
41	*Homalomena aromatica* Schott	E-H:01
42	*Justicia adhatoda* L.	OTH-A:03, RES-R:96, H-E:01
43	*Leucaena leucocephala* (Lam.) de Wit	GAS-D:86, MET-T:89
44	*Linostoma decandrum* (Roxb.) Steud.	DER-S:72, OTH-A:03, RES-R:29
45	*Lobelia nummularia* Lam.	GAS-D:11, GAS-D:86, GAS-D:19, H-E:01
46	*Melastoma malabathricum* L.	DER-S:18, Car-K:87, OTH-A:70
47	*Mikania micrantha* (L.) Willd.	DER-S:18, OTH-A:03, E-H:01, E-F:01
48	*Mimosa pudica* L.	PRE-W:15, DER-S:12, RES-R:09
49	*Mirabilis jalapa* L.	MET-T:89, OTH-A:03
50	*Mussaenda glabra* Vahl	RES-R:05, DER-S:12, OTH-A:21
51	*Oroxylum indicum* (L.) Benth. ex Kurz	RES-R:96, GAS-D:07, Nil, DER-S:16. OTH-A:70
52	*Pandanus odorifer* (Forssk.) Kuntze	DER-S:72, GAS-D:03, URO-U:14
53	*Phyllanthus emblica* L.	MET-T:08, RES-R:88, Car-K:96
54	*Plantago major* L.	DER-S:29, OTH-A-03, GAS-D:29
55	*Rhus chinensis* Mill.	GAS-D:11, OTH-A:03, MET-T:70
56	*Scoparia dulcis* L.	GAS-D:11, Nil, GAS-D:19, H-E:01

57	*Senegalia pennata* (L.) Maslin	GAS-D:98, RES-R:78, RES-R:96, MET-T:99
58	*Terminalia phillyreifolia* (Van Heurck & Mull. Arg.) Gere & Boatwr.	DER-S:16, GAS-D:87, CAR-K:87
59	*Thunbergia grandiflora* Roxb.	RES-R:21, DER-S:18, RES-R:96
60	*Toddalia asiatica* (L.) Lam.	OTH-A:03, GAS-D:07, MET-T:99
61	*Triumfetta rhomboidea* Jacq.	MET-T:89, OTH-A:03
62	*Vitex peduncularis* Wall.	OTH-A:73, GAS-D:13, GAS-D:86
63	*Zingiber officinale* Roscoe	RES-R:78, E-H:01, RES-R:21

Table 3.5: Informant Consensus Factor (ICF) of reported ailments.

No.	Ailment category	Nur	Nt	ICF
1	A- General and Unspecified	242	19	0.92
2	D- Digestive	665	26	0.96
3	F- Eye	14	5	0.69
4	H- Ear	72	9	0.88
5	K- Cardiovascular	158	14	0.91
6	L- Musculoskeletal	50	7	0.87
7	N- Neurological	8	2	0.85
8	R- Respiratory	476	20	0.96
9	S- Skin	391	25	0.93
10	T- Endocrine/ Metabolic and Nutritional	96	11	0.89
11	U- Urological	58	7	0.89
12	W- Pregnancy, Childbearing, Family planning	14	3	0.84
13	X- Female genital	11	4	0.7
14	Y- Male genital	25	5	0.83

Table 3.6: Summary statistics for Relative Frequency Citation (RFC) and Use Value (UV).

Mean	Standard deviation	Maximum	Minimum
RFC	0.1860	0.8936	0.1328
UV	0.1693	0.8132	0.1209

Association between RFC and UV by using Pearson's correlation method

r 1.000

r^2 1.000

Alpha=0.005

P (two tailed) <0.0001****

** Figure in parenthesis is P-values for significance of correlation coefficient.

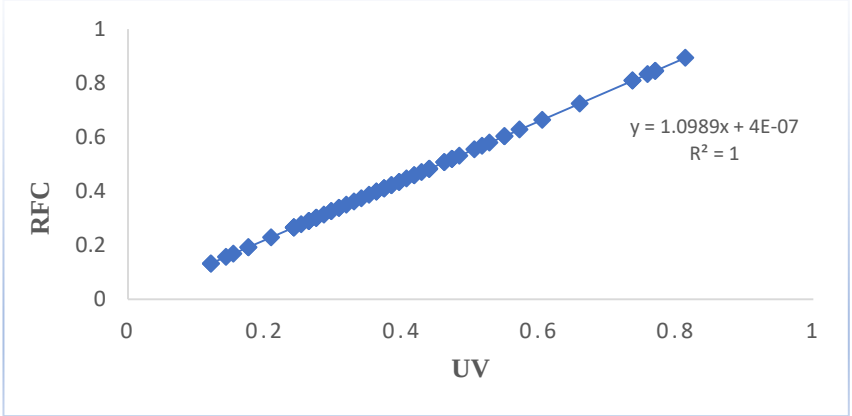


Figure 3.8: Association between Relative Frequency Citation (RFC) and Use Value (UV).

3.3.3.6. Pearson's correlation coefficient

Relative Frequency Citation (RFC) and Use Value (UV) showed a Pearson's correlation coefficient of 1.000 and a P-value of less than 1%. The statistical analysis offers compelling evidence in favour of a robust, affirmative, and statistically significant correlation between the local relevance of each species under investigation and the relative importance of plant consumption. The observation suggests that a higher frequency of useful medicinal plants is correlated with an increase in the utilisation of species by informants. There is consistency in the

patterns of RFC and UV across species, as seen by the significant and positive correlation between them. Nevertheless, it's crucial to remember that there may be situations in which some species show higher RFC and UV values than others. Consequently, the results indicate a substantial empirical consistency between these two variables (**Table 3.6**). These findings receive additional support from a scatter plot, which visually represents a pronounced strongly positive association between RFC and UV (**Figure 3.8**).

3.4. Discussion

In the contemporary landscape of rapid technological and innovative progress, researchers and scientists are diligently pursuing significant advancements within their respective fields. This study within the broader context, focuses on the utilization of ethnomedicinal NTFPs by traditional practitioners for the treatment of various ailments. These anecdotes underscore the enduring value ascribed to traditional healing practices, often placing them in high regard when compared to modern medical approaches. Despite the efficacy of established medicines that have undergone rigorous scientific evaluation by experts, traditional medicinal practices should not be pushed aside; instead, there should be an effort to seek integration and harmonization. The quantification of ethnobotanical data has identified specific plant species of considerable significance, indicating their extensive utilization within the study area.

The findings align with similar observations reported in parallel studies conducted in diverse geographical regions (Kayani et al., 2015; Malla et al., 2015; Faruque et al., 2018) where herbaceous group represents majority of the recorded plants. The prevalence of herbaceous plant utilization can be attributed to the location of the study area within dense forest zones, where herbs are abundant. Traditional healers exhibit a preference for herbs due to their relatively facile accessibility in remote forest environments and their ease of preparation for ethnomedicinal use. Due to its potential for bioactive compounds and the relative convenience they provide for phytochemical and pharmacological investigations compared to other plant parts, leaves are commonly used in the creation of herbal remedies. The prevalence of plant

parts employed in ethnobotanical investigations conducted by previous researchers has exhibited diversity. Similar findings were observed by other researchers (Boesi, 2014; Haq et al., 2022; Uzun and Koca, 2020). Also, research reported that leaves exhibit active engagement in both food synthesis and metabolite generation (Ghorbani, 2005). Higher UR values frequently correlate with more culturally and economically valuable species, thus rendering desirable targets for further investigation, conservation initiatives, or sustainable management strategies (Phillips and Gentry, 1993). UV metric signifies the relative significance of plant species based on the species (Uzun and Koca, 2020). The UV of reported uses for each respective outcomes exhibit a concordance, as evidenced by findings derived from studies conducted by Silva et al. (2006), Tardio and Pardo-de-Santayana (2008). The substantial FL values observed in this context suggest that the informants exhibit a clear preference for relying on specific plant species in the treatment of distinct diseases, indicating a degree of specialization in traditional medicinal knowledge (Hossain and Rahman, 2018). The outcomes of ICF calculated aligned consistently with prior investigations conducted by Padhan and Panda (2016), Chinnasamy et al. (2019), Panmei et al. (2019) and Faruque et al. (2018). It is worth noting that these studies have also observed a prevalent focus on the treatment of digestive system disorders. This emphasis on digestive ailments also corresponds with the findings reported in the works of Rahman et al. (2016), Lee et al. (2008), Suleiman (2015) and Sadat-Hosseini et al. (2017) suggesting a recurrent emphasis on this category in the ethnobotanical literature. Consistency in the patterns of RFC and UV is evident, as indicated by a notable and positive correlation between them. This observation is further substantiated by previous studies conducted by Bano et al. (2014) and Vijayakumar et al. (2015). This study has the potential to chart new avenues for future pharmacological research, offering valuable reference points, particularly in the context of quantitative ethnobotanical inquiries conducted among heterogeneous ethnolinguistic indigenous populations. The findings of this research hold the promise of advancing our understanding of traditional plant-based medicines and their utility in contemporary pharmacological studies, fostering collaboration between indigenous knowledge and modern science (Ong and Kim, 2014).


Acmella calva
Adiantum philippense
Aeginetia indica
Aganope thyrsiflora
Ageratum haustonianum
Alpinia malaccensis
Alstonia scholaris
Terminalia phillyreifolia
Aporosa octandra
Artocarpus lacucha
Begonia roxburghii
Bidens pilosa
Bischofia javanica
Crassocephalum crepidiodes
Bonnaya ruellioides
Bombax insigne
Callicarpa arborea
Centella asiatica
Bauhinia glauca
Chromolaena odorata
Cissampelos pareira
Cissus repens
Clerodendrum glandulosum
Clerodendrum infortunatum
Embelia ribes
Phyllanthus emblica
Euphorbia hirta
Euphorbia heterophylla
Flueggea virosa
Gelsemium elegans
Colocasia esculenta
Croton caudatus
Curcuma caesia
Curcuma longa
Drymaria cordata
Drynaria coronans
Hedyotis scandens
Hellenia speciosa
Homalomena aromatica
Justicia adhatoda

Figure 3.9: List of 63 ethnomedicinal NTFPs documented.

CHAPTER IV

4. Phenological observations of selected ethnomedicinal NTFPs

4.1. Introduction

Plant phenology encompasses the study of recurring life cycle events in plants, including the timing of growth, reproduction, and senescence, and how these events are influenced by seasonal and interannual variations in climate, as well as other factors such as competition and predation (Cleland et al., 2012). Phenological events include leafing, flowering, fruiting, and senescence, which are influenced by various factors such as temperature, photoperiod, precipitation, and biotic interactions. It refers to the study of the timing of periodic biological events in plants, such as flowering, fruiting, leafing, and senescence, in relation to climate and environmental changes, especially seasonal and interannual variations (Schwartz et al., 2003).

Research in plant phenology has gained significant attention in recent years due to the need to comprehend the consequences of shifting climate patterns on plant communities (Menzel et al., 2006). Observations and studies on plant phenology provide valuable insights into the responses of plants to climate change, such as alterations in growing seasons, species distribution patterns, and ecological interactions (Piao et al., 2019). It plays a crucial role in the study of plant ecology and environmental science, particularly in relation to temperature and rainfall patterns. Understanding the importance of plant phenology in response to temperature and rainfall helps us comprehend ecosystem dynamics, climate change impacts, and agricultural practices.

Plant phenology serves as an important indicator of climate change impacts on ecosystems. Changes in temperature and rainfall patterns can directly influence the timing of phenological events in plants. For instance, warmer temperatures can advance the onset of flowering, while alterations in rainfall patterns can affect the timing of leaf senescence. These shifts in phenological events provide valuable information on the ecological responses to climate change (Parmesan and Hanley, 2015). Many animal species rely on specific plant phenological stages for feeding,

reproduction, and migration. Also, changes in temperature and rainfall patterns can disrupt the synchrony between plants and their associated animal species, leading to potential mismatches in resource availability. Monitoring plant phenology helps assess the vulnerability of species interactions to climate change, enabling targeted conservation strategies (Thackeray et al., 2010).

Understanding the phenological responses of plants to these climatic variables is crucial for optimizing agricultural practices. Accurate knowledge of phenological stages enables farmers to time sowing, irrigation, and harvest activities effectively. By aligning crop phenology with temperature and rainfall patterns, farmers can improve crop yields and minimize the risk of yield losses due to climate variability (Farinon et al., 2022). Also, plant phenology is closely linked to ecosystem functioning. The timing of plant growth and reproductive events influences nutrient cycling, pollination dynamics and interactions with other organisms. Changes in temperature and rainfall regimes can disrupt these critical interactions, affecting ecosystem productivity and stability. Monitoring plant phenology helps assess the health and resilience of ecosystems in response to environmental changes (Panchen et al., 2014).

While much of the research in plant phenology has focused on ecological and climatic implications, there is a growing recognition of the importance of studying phenological observations specifically in relation to ethnomedicinal plants. Ethnomedicinal plants are those traditionally used by indigenous communities for medicinal purposes. By examining the phenology of these plants, we can gain a deeper understanding of their medicinal properties, sustainable harvesting practices, and potential impacts of climate change on traditional medicine systems (Vandebroek et al., 2004; Tripathi et al., 2017; Li et al., 2021; Christmann et al., 2023).

Phenological observations of ethnomedicinal plants help in identifying the optimal time for harvesting plant parts that possess the highest concentration of bioactive compounds. By studying phenological patterns, researchers can determine the stages at which plants exhibit maximum medicinal potency (Vandebroek et al., 2004). Knowledge of phenological patterns aids in developing sustainable harvesting

practices. It allows communities and practitioners to gather plant material at the right time, ensuring the survival and regeneration of the plants (Bussmann et al., 2016). This approach helps to avoid overexploitation and depletion of valuable medicinal plant resources. Also, studies of ethnomedicinal plants contribute to understanding the impacts of climate change on traditional medicine systems. Changes in phenological events, such as flowering or fruiting time, can provide insights into climate change effects on plant populations and associated medicinal practices (Byg and Salick, 2009). Phenological research helps in the conservation and management of ethnomedicinal plant species. By documenting and analysing phenological data, it becomes possible to develop conservation strategies and protected area management plans that consider the specific needs and ecological requirements of ethnomedicinal plants (Negi et al., 2022).

A comprehensive study on the phenology of ethnomedicinal plants has never been conducted in Mizoram, a state located in northeastern India. Many indigenous communities in Mizoram rely on ethnomedicinal plants for their healthcare needs. Exploring the phenology of these plants would enhance our understanding of their growth and reproductive patterns, helping to identify optimal periods for collection, propagation, and sustainable utilization. This knowledge can contribute to the conservation of ethnomedicinal plant resources and the preservation of traditional healing practices in Mizoram.

4.2. Methodology

4.2.1. Phenological observations

The current investigation cantered on the phenological observations of six selected ethnomedicinal plant species, chosen from a pool of previously examined ethnomedical indices namely *Flueggea virosa*, *Melastoma malabathricum*, *Vitex peduncularis*, *Thunbergia grandiflora*, *Begonia roxburghii* and *Croton caudatus*, which holds significant value in traditional medicine and are commonly utilized by traditional healers and herbalists within the study area. The observation process involved random selection and aimed to investigate the phenological patterns of these plants in relation to temperature and rainfall. One healthy branch was carefully

chosen and marked with tags from each of the five individuals per plant. Weekly observations were conducted to monitor various phenological events, including new leaf formation, leaf maturation, flower bud formation, flowering, completion of flower development, fruiting and complete leaf fall (Newstorm et al., 1994). Observations was made to record various phenophases of each plant from 2018, 2019 and 2021 from the month of January to December. Phenological calendar was made for each plant species studied. Simultaneously, data on rainfall and temperature were collected during the same period. These data were then presented as average monthly values.

4.2.2. Ecological signals and environmental indicators

Lunglei district experiences a subtropical highland climate. The region receives moderate to heavy rainfall, especially during the monsoon season, which typically lasts from May to September. The temperature remains relatively cool throughout the year, with mild summers and chilly winters. It is characterized by hilly terrain and valleys and the landscape consists of rolling hills, steep slopes, and deep gorges. The topography plays a crucial role in shaping the microclimates of the region, vegetation patterns, and water drainage. It is characterized by dense forests, including both evergreen and semi-evergreen forests. These lush green forests are home to a wide variety of plant and animal species contributing to the rich biodiversity. Like many regions, Lunglei district faces environmental challenges, including deforestation, soil erosion, and loss of biodiversity. Human activities such as shifting cultivation and unsustainable logging practices, contribute to these challenges.

4.2.3. Statistical analysis

The phenological events of five individuals within each species were assembled by documenting the monthly activities of the plants. The percentage of plant species exhibiting various phenophases was evaluated for each month. To analyse any correlations between phenological events and monthly mean temperature and rainfall, Spearman correlation coefficient was calculated using R Studio version 4.1.2.

4.3. Results

4.3.1. Meteorological phenomena of the study area

In Figure 4.1, a monthly record of temperature and rainfall data from 2018, 2019 and 2021 was presented. The data was collected from the Department of Statistics in Lunglei. The average temperature and monthly rainfall values were used to represent the data. The total annual rainfall varied slightly among the three years with the highest recorded in 2018 at 3094.3 mm, followed by 2021 at 2985.8 mm and 2019 at 2514.8 mm. During the first year of the study (2018), January (14.7 mm), March (16.5 mm), and April (92.3 mm) experienced relatively low rainfall during the pre-monsoon period. From May to October, consistent rainfall was observed, with the monsoon season lasting from June to October. In the second year (2019), the pre-monsoon rain period occurred from February to April. From May to October, there was continuous rainfall and the monsoon season lasted from June to October. In 2021, pre-monsoon rain was observed in January and April. The monsoon season started in May and lasted until October, with continuous rainfall occurring from April to November. In each of the years 2018, 2019 and 2021, the highest recorded rainfall varied across different months. In 2018, the highest rainfall occurred in June with a measurement of 1158.2 mm. In 2019, July had the highest rainfall with a recorded value of 686.1 mm. Lastly, in 2021, the month of October received the highest amount of rainfall with a measurement of 661.8 mm. Regarding the maximum temperature, different months stood out for each year. In 2018, the highest temperature of 29.7°C was reached in April. For the year 2019, both April and May had notable high temperatures of 31.43°C and 31.42°C, respectively. Lastly, in 2021, the highest temperature of 31.03°C was observed in April.

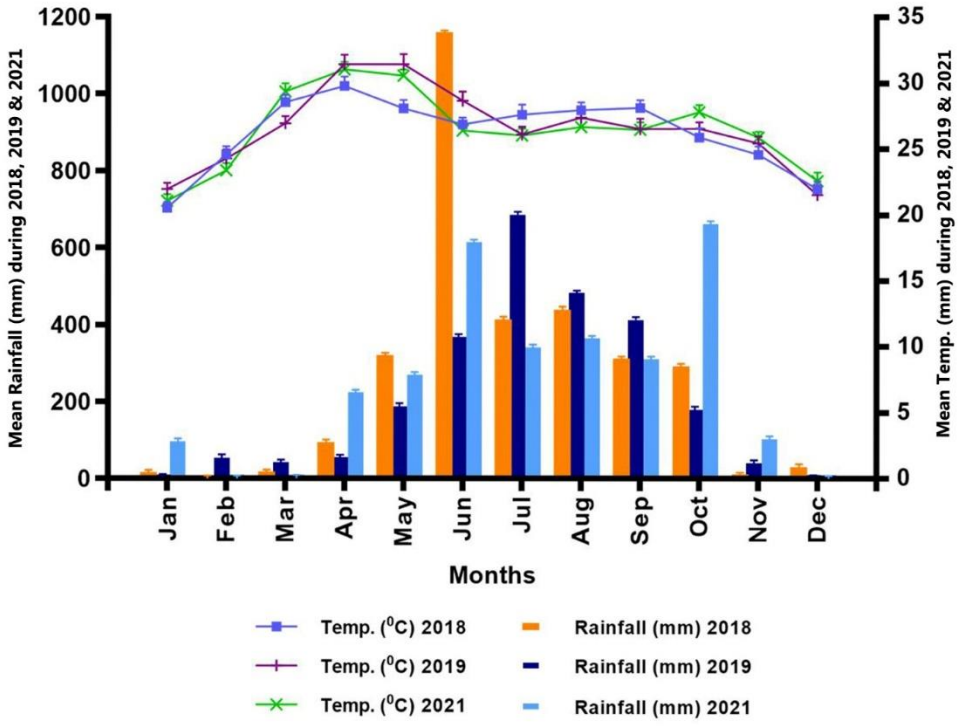


Figure 4.1: Meteorological data of the study area during 2018, 2019 and 2021.

4.3.2. Phenological observations

Based on our research findings, the six scrutinized plant species exhibited the highest utilization reports and use values among those commonly employed by the Mizo people. Additionally, we documented the medicinal applications of each species with ailment codes sourced from the Internal Classification of Primary Care (ICPC2) (**Table 4.1**) which was briefly discussed in the preceding chapter. Phenological observations were conducted revealing that the ethnomedicinal plant species are categorized within the families Melastomaceae, Acanthaceae, Lamiaceae, Begoniaceae, Euphorbiaceae and Phyllanthaceae. The selection of these plant species was guided by the preferences and dependencies of the local population within the study area.

Phenological observations were conducted on five individuals of each plant species, spanning from January 2018 to December 2021. The selection of this three-year period was necessitated by the circumstances surrounding the COVID-19 pandemic which led to a complete lockdown in 2020, prohibiting data collection and human activities within the study area. Consequently, only data from 2018, 2019, and 2021 were available for assessment. This timeframe allowed for a comprehensive examination of the phenological patterns of the studied plant species, despite the interruption caused by the pandemic. The six ethnomedicinal NTFPs each exhibited unique timings for diverse phenological phases, encompassing new leaf emergence duration, flower bud development, flowering onset, fruiting, maturation of fruits, and leaf shedding periods (**Figure 4.2**). Notably, within individuals of the same species, data for flowering and fruiting phases were represented, acknowledging the variability observed in these critical stages of plant development (**Table 4.2** and **Table 4.3**).

Table 4.1: List of plant species observed with their Family, Local names, Life-forms, Leaf habit, Fruit type, Mature fruit colour and Flower colour with their respective ethnomedicinal use and UV and FC score.

Sl no.	Plant Species	Ethnomedicinal uses	UV	FC	Life forms	Leaf habitat	Fruit type	Mature fruit colour	Flower colour
1	*Melastoma malabathricum* LC: Builukham Family: Melastomaceae	Cut (S18), hypertension (K87), tuberculosis (A70)	0.813	81.3	Shrub	Evergreen	Berries	Green	Purplish black
2	*Flueggea virosa* LC: Saisiak Family: Phyllanthaceae	Chicken-pox (A72), scabies (S72)	0.769	77	Shrub	Deciduous	Capsule	Green	Brown
3	*Vitex peduncularis* LC: Thing-	Malaria (A73), Jaudice (D13), Peptic ulcer	0.769	77	Tree	Evergreen	Drupe	Green	Dark purple

	khawilu Family: Lamiaceae	(D86)							
4	*Croton caudatus* LC: Ranlung-damdawi Family: Euphorbiaceae	Stomach function disorder (D87), urethral discharge (Y03)	0.758	76	Herb	Deciduous	Capsule	Red	Red
5	*Thunbergia grandiflora* LC: Vako Family: Acanthaceae	Throat symptoms or complaint (R12), laceration (S18), asthma (R96)	0.736	74	Shrub	Evergreen	Globose	Green	Brown
6	*Begonia roxburghii* LC: Sekhupthur Family: Begoniaceae	Skin texture symptoms (S21), Diarrhoea (D11), abdominal pain epigastric (D02)	0.736	74	Shrub	Deciduous	Berries	Yellowish green	Pale white

4.3.2.1. Leaf initiation Phenology

Over the course of a three-year investigation, an analysis of six ethnomedicinal plant species revealed noteworthy patterns in leaf initiation peaks. Specifically, the highest peak amounting to 66.67% was recorded in January 2018. This was succeeded by a peak of 63.3% in March 2019 and a peak of 60% in January 2021, as illustrated in the accompanying **Figure 4.3.A**. These peak percentages endured until the onset of the monsoon season in May each year indicating the continuation of favourable conditions for plant growth although there may be some variability influenced by fluctuations in temperature and rainfall. The percentages decrease significantly which possibly might be due to cooling temperatures and reduced rainfall, signalling the onset of winter conditions and decreased metabolic activity in plants. Among the six species under scrutiny, *M. malabathricum*, *V. peduncularis*, and *C. caudautus* displayed a consistent similar leaf initiation period throughout the three successive

years. *T. grandiflora* and *F. virosa*, however, initiated leaf growth between March and May coinciding with the onset of monsoon. Interestingly, the investigation also uncovered that *V. peduncularis* exhibited the most protracted leaf flushing duration, spanning 14 to 16 weeks across all three years (**Figure 4.2**). This extended duration persisted until the point of attaining mature leaf production. The developmental trajectory regarding leaf maturation encompasses a duration ranging from 6 to 9 months across a spectrum of studied species. However, an exception to this pattern is observed in *B. roxburghii* where the retention of mature leaves persists throughout the year barring the months of June and July in both 2018 and 2019.

4.3.2.2. Flowering Phenology

Floral bud initiation patterns were observed across various plant species occurring typically between February and May. This timeframe aligns with the transition from the pre-monsoon to summer seasons just before the onset of the monsoon, which is the period conducive to flowering. Among the species studied, *B. roxburgii* stood out for its notably prolonged duration of floral bud development spanning approximately 6-7 weeks. In contrast, other plant species exhibited a shorter period of 3-4 weeks for floral bud maturation followed by the subsequent flowering phase (**Figure 4.2**). This phenomenon can be elucidated by the intricate interplay of environmental cues and plant physiology. The transition from pre-monsoon to summer marks a critical period of environmental change, characterized by alterations in temperature, humidity, and photoperiod, which influence plant development. During this time plants undergo physiological changes, including the initiation and maturation of floral buds, in preparation for the forthcoming flowering season.

The extended duration of floral bud development observed in *B. roxburgii* may be attributed to species-specific genetic factors and adaptive mechanisms. Certain plant species may require more time to complete the complex processes involved in bud development, such as organogenesis and floral differentiation. Additionally, environmental factors such as light intensity, temperature fluctuations, and nutrient availability can modulate the pace of floral bud maturation. Consequently, *B. roxburgii* with its distinct genetic makeup and physiological traits might exhibits a

prolonged duration of floral bud development compared to other species **(Table 4.2** and **Figure 4.2).**

The study revealed varying flowering percentages across different months and years, with the highest recorded at 96.6% in June 2018, followed by 83% in July 2019, and again at 77% in June 2021(**Figure 4.3.B**). These fluctuations in flowering percentages corresponded to the progression of average flowering phenophases, which were aligned with distinct seasonal transitions, starting from the pre-monsoon period and gradually increasing during the rainy seasons before declining towards winter for all species under investigation. Notably, all studied species exhibited a single annual blooming cycle.

The average duration of the flowering period, calculated from the onset of flower formation, ranged from 54 days (*V. peduncularis*) to 215 days (*B. roxburghii*) across all observed species. *B. roxburghii* consistently displayed the longest mean blooming duration, with records of 212 days in 2018, 217 days in 2019, and 215 days in 2021. Conversely, *V. peduncularis* showcased the shortest average blooming period, with durations of 56 days in 2018, 54 days in 2019, and 58 days in 2021 (**Table 4.2**). These patterns can be explained by a combination of genetic predispositions and environmental influences. The synchronization of flowering phenophases with distinct seasons reflects the adaptation of plant species to prevailing environmental conditions. The variation in blooming duration among species may stem from inherent genetic traits governing flower development and reproductive strategies. Factors such as pollination mechanisms, resource allocation, and sensitivity to environmental cues contribute to the observed differences in blooming duration. Overall, the study highlights the interplay between genetic factors and environmental cues in shaping the flowering patterns and durations of plant species, providing insights into their reproductive strategies and adaptive capabilities in response to seasonal fluctuations.

Table 4.2: Date of first floral bud initiation and average days of blooming of selected NTFPs (range of 5 individuals).

Species	Year					
	2019		2020		2021	
	Date of first floral bud initiation	**Average days of blooming**	**Date of first floral bud initiation**	**Average days of blooming**	**Date of first floral bud initiation**	**Average days of blooming**
M. malabathricum	15th to 19th March	137 days	25th to 29th March	129 days	19th to 23rd March	125 days
T. grandiflora	3rd to 7th May	211 days	5th to 8th May	202 days	6th to 10th May	209 days
V. peduncularis	4th to 8th April	56 days	6th to 9th April	54 days	5th to 8th April	58 days
B. roxburghii	26th February to 1st March	212 days	27th February to 1st March	217 days	28th February to 3rd March	215 days
C. caudatus	18th to 21st April	99 days	16th to 19th April	96 days	17th to 20th April	98 days
F. virosa	23rd to 26th April	96 days	24th to 26th April	92 days	26th to 28th April	89 days

4.3.2.3. Fruiting and mature fruit formation Phenology

Across three consecutive years, the majority of observed plant species exhibited a consistent pattern of fruiting and mature fruit development, predominantly occurring from the rainy season through the winter months, spanning from June to January. The pinnacle of fruiting and mature fruit formation was noted in October 2018 with a prevalence of 66.6%, succeeded by 65% in 2021 and 60% in 2019 (**Figure 4.3.C**). Remarkably, all species displayed their peak fruiting activity and mature fruit formation following the conclusion of the rainy seasons. The scientific rationale behind this phenomenon lies in the relations between environmental cues and biological processes inherent to these plant species. Rainfall patterns typically serve as a primary trigger for the initiation of reproductive phases in many plant species. The onset of rains marks the beginning of favourable conditions providing ample moisture and nutrients essential for robust vegetative growth and subsequent reproductive development.

The duration of the fruiting phase exhibited variability among different plant species, contingent upon the peak flowering time of each species. On average, the duration of fruiting and mature fruit formation spanned approximately 3 to 5 months across the studied plant species. *M. malabathricum* exhibited the lengthiest period of fruit production, lasting 113 days in 2018, 108 days in 2019, and 109 days in 2021. Conversely, *F. virosa* displayed the shortest duration, with fruiting periods of 47 days in 2018, 50 days in 2019, and 52 days in 2021 (**Table 4.3**).

As the rainy season progresses, plants accumulate resources and energy directing them towards reproductive process such as flower production and fruit maturation. The transition from the rainy season to the drier winter months prompts plants to optimize their reproductive output culminating in peak fruiting activity. This strategic timing ensures the dissemination of seeds during periods conducive to germination and establishment maximizing the chances of offspring survival and dispersal. Moreover, factors such as temperature fluctuations and day length variations also play crucial roles in regulating physiological processes associated with fruiting and mature fruit formation. The synchronization of these environmental cues with internal hormonal signalling mechanisms governs the timing and duration

of reproductive phases in plants, contributing to the observed seasonal patterns of fruiting across successive years.

Table 4.3: Initial date of fruit formation and average days of fruiting and mature fruit formation of selected NTFPs (range of 5 individuals).

Species	Year					
	2019		2020		2021	
	Initial date of fruiting	Average days of fruiting	Initial date of fruiting	Average days of fruiting	Initial date of fruiting	Average days of fruiting
M. malabathricum	15th to 17th July	113 days	16th to 19th July	108 days	17th to 19th July	109 days
T. grandiflora	3rd to 6th November	64 days	4th to 8th November	66 days	5th to 8th November	67 days
V. peduncularis	22nd to 25th July	59 days	24th to 27th July	61 days	23rd to 28th July	57 days
B. roxburghii	9th to 12th July	88 days	11th to 14th July	85 days	13th to 16th July	80 days
C. caudatus	8th to 12th July	65 days	7th to 10th July	67 days	9th to 13th July	63 days
F. virosa	5th to 7th October	47 days	6th to 9th October	50 days	3rd to 6th October	52 days

4.3.2.4. Leaf fall Phenology

Among all species under scrutiny the peak occurrence of leaf abscission commonly known as leaf fall was notably observed during the months of November and December. This phenomenon reached its highest proportion recording a value of 47% in 2018, followed by rates of 43% in 2019 and 40% in 2021 (**Figure 4.3.D**). Furthermore, a slight increase in leaf fall was consistently noted during January and February across all observed years. The period of leaf fall observed in all

investigated plant species consistently spanned a duration of 2 to 3 months throughout the years under study (**Figure 4.2**).

Notably, these instances aligned with the arid season subsequently followed by neo-foliar emergence and the initiation of floral bud formation. The pronounced leaf abscission during November and December as well as the minor surge in January and February can be attributed to a combination of environmental cues and physiological responses in plants. During autumn, as days become shorter and temperatures decrease plants sense these changes and initiate processes to prepare for winter dormancy. This includes the breakdown of chlorophyll in leaves, resulting in colour changes and eventual leaf drop. Cooler temperatures and reduced water availability further contribute to leaf senescence and detachment. Additionally, plants redistribute nutrients from senescing leaves to other parts of the plant, aiding in winter survival and supporting future growth. Leaf abscission during this period serves as an adaptive strategy to conserve water and energy and protect against potential damage from harsh environmental conditions.

Species	Year	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec
Melastoma malabathricum	2018	●	●ɗ	●ɗ•	ɗf	ɗf	ɗf	ɗf❁	ɗf❁	ɗ❁	ɗ❁	❁Ļ	❁Ļ
	2019	●	●	●ɗ•	ɗf	ɗf	ɗf	ɗf❁	ɗf❁	ɗ❁	ɗ❁	❁Ļ	❁Ļ
	2021	●	●ɗ	●ɗ•	ɗf	ɗf	ɗf	ɗf❁	ɗf❁	ɗ❁	ɗ❁	❁Ļ	❁Ļ
Thunbergia grandiflora	2018	❁Ļ	Ļ	●	●	●ɗ•	ɗf	ɗf	ɗf	ɗf	ɗf	ɗf❁	f❁
	2019	❁Ļ	Ļ	●	●	●ɗ•	ɗf	ɗf	ɗf	ɗf	ɗf	ɗf❁	f❁
	2021	❁Ļ	Ļ	●	●	●ɗ•	ɗf	ɗf	ɗf	ɗf	ɗf	ɗf❁	f❁
Vitex peduncularis	2018	●	●	●	●ɗ•	ɗf	ɗf	ɗ❁	ɗ❁	ɗ❁	ɗ❁	Ļ	Ļ
	2019	●	●	●	●ɗ•	ɗf	ɗf	ɗ❁	ɗ❁	ɗ❁	ɗ❁	Ļ	Ļ
	2021	●	●	●	●ɗ•	ɗf	ɗf	ɗ❁	ɗ❁	ɗ❁	ɗ❁	Ļ	Ļ
Begonia roxburghii	2018	●ɗ	ɗ•	ɗ•f	ɗf	ɗf	ɗfĻ	ɗf❁Ļ	ɗf❁	ɗf❁	ɗf❁	ɗ❁	●ɗ
	2019	●ɗ	ɗ•	ɗ•f	ɗf	ɗf	ɗfĻ	ɗf❁Ļ	ɗf❁	ɗf❁	ɗf❁	ɗ❁	●ɗ
	2021	●ɗ	ɗ•	ɗ•f	ɗf	ɗf	ɗfĻ	ɗf❁Ļ	ɗf❁	ɗf❁	ɗf❁	ɗ❁	●ɗ
Croton caudatus	2018	●	●	●	●•	ɗf	ɗf	ɗf❁	ɗf❁	ɗ❁	ɗ❁	Ļ	Ļ
	2019	●	●	●	●•	ɗf	ɗf	ɗf❁	ɗf❁	ɗ❁	ɗ❁	Ļ	Ļ
	2021	●	●	●	●•	ɗf	ɗf	ɗf❁	ɗf❁	ɗ❁	ɗ❁	Ļ	Ļ
Flueggea virosa	2018	Ļ	Ļ	●	●•	●ɗf	ɗf	ɗf	ɗf	ɗ	ɗ❁	❁	❁Ļ
	2019	Ļ	Ļ	●	●•	●ɗf	ɗf	ɗf	ɗf	ɗ	ɗ❁	❁	❁Ļ
	2021	Ļ	Ļ	●	●•	●ɗf	ɗf	ɗf	ɗf	ɗ	ɗ❁	❁	❁Ļ

●- New leaf, ɗ- Mature leaf, •- Floral bud, f- Flower, ❁- Fruiting and mature fruit formation, Ļ- Leaf fall.

Figure 4.2. Phenological calendar of selected ethnomedicinal NTFPs.

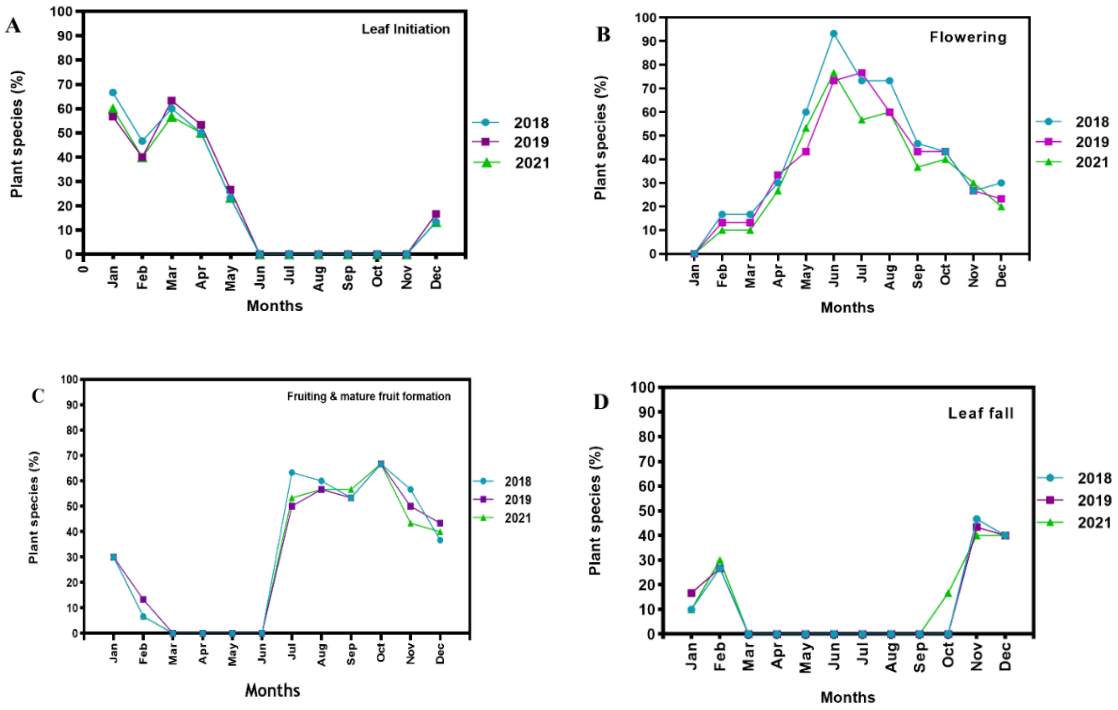


Figure 4.3: Percentage of plant species (%) under study exhibiting various phenological events: A- Leaf initiation, B- Flowering, C- Fruiting & mature fruit formation and D- Leaf fall.

Figure 4.4: Phenological phases of *M. malabathricum* (A) Leaf initiation, (B) Floral bud, (C) Flower and (D) Fruiting and mature fruit formation.

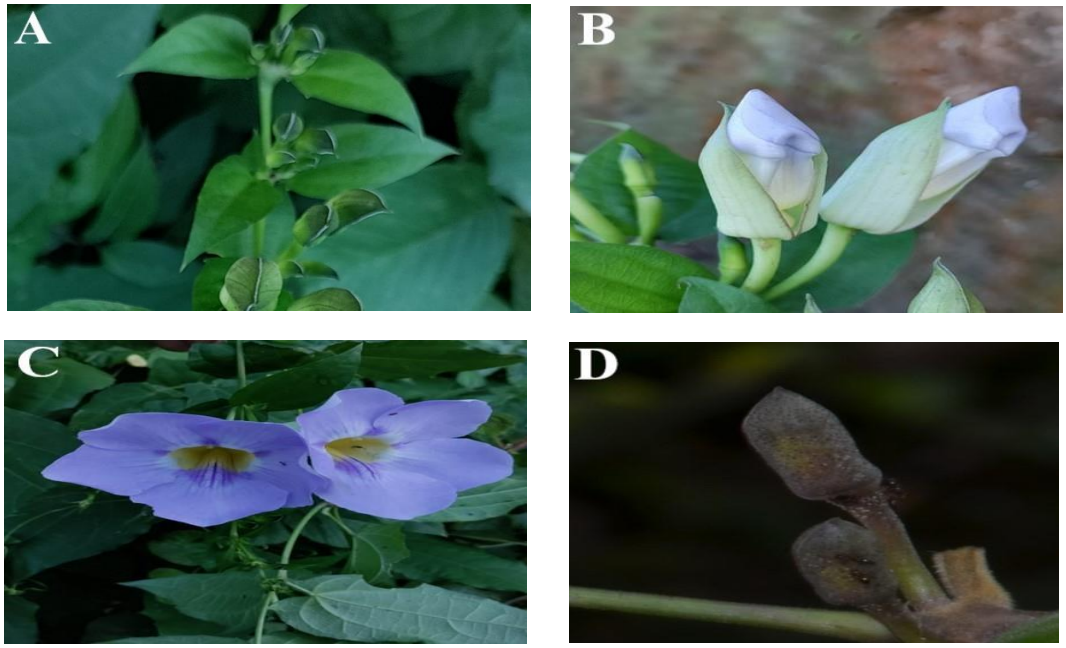


Figure 4.5: Phenological phases of *T. grandiflora* (A) Leaf initiation, (B) Flower bud, (C) Flower and (D) Fruiting and mature fruit formation

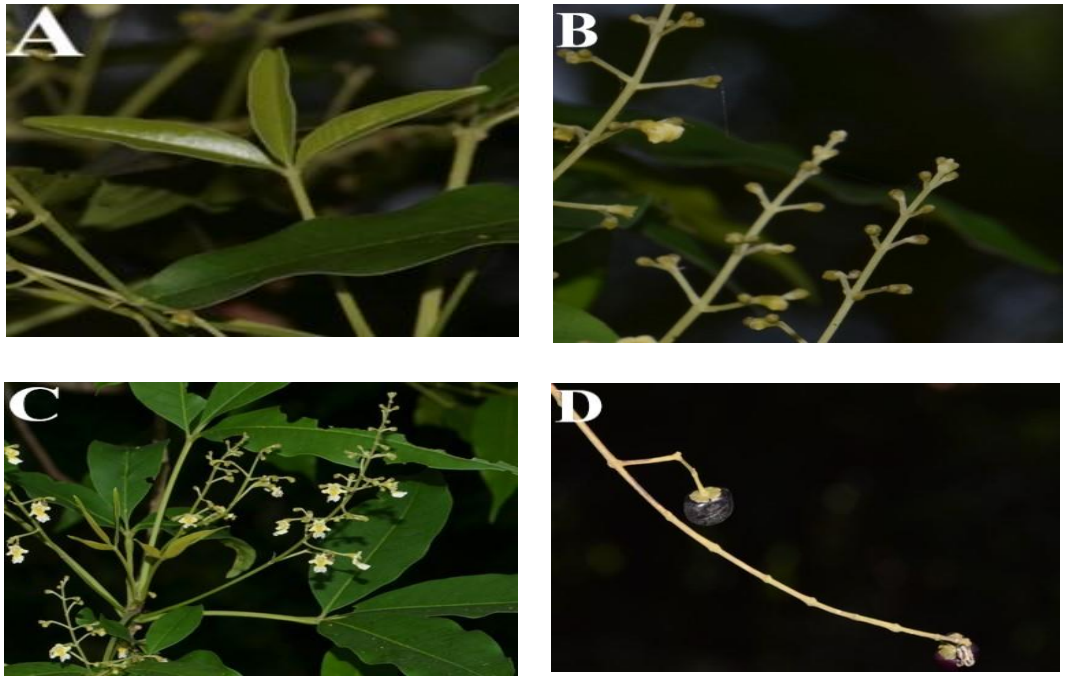


Figure 4.6: Phenological phases of *V. peduncularis* (A) Leaf initiation, (B) Flower bud, (C) Flower and (D) Fruiting and mature fruit formation.

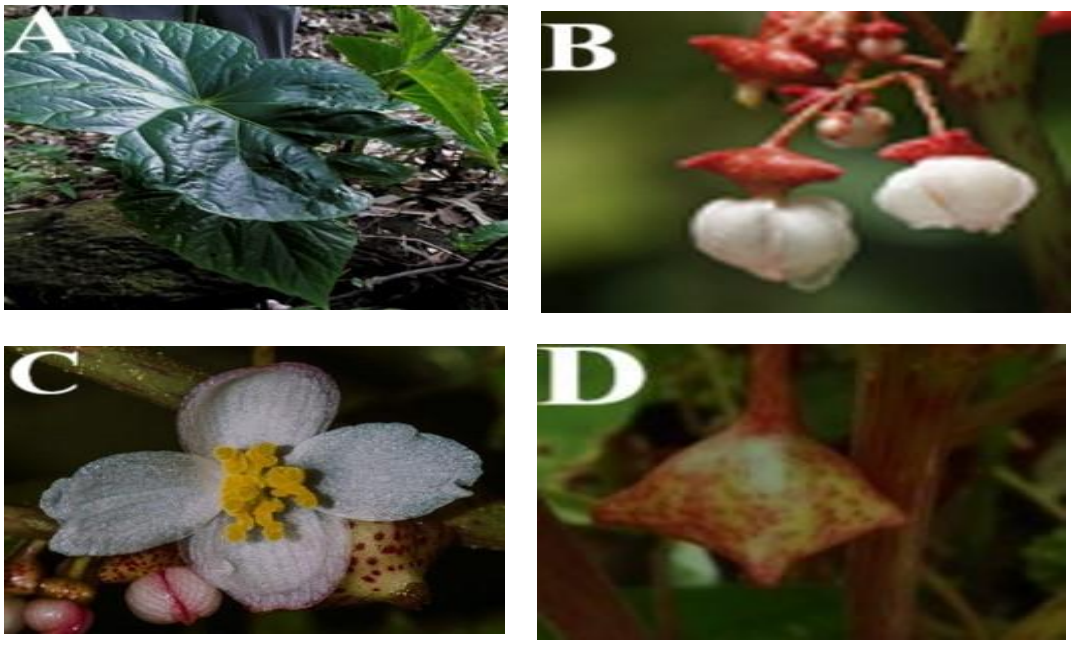


Figure 4.7: Phenological phases of *B. roxburghii* (A) Leaf initiation, (B) Flower bud, (C) Flower and (D) Fruiting and mature fruit formation.

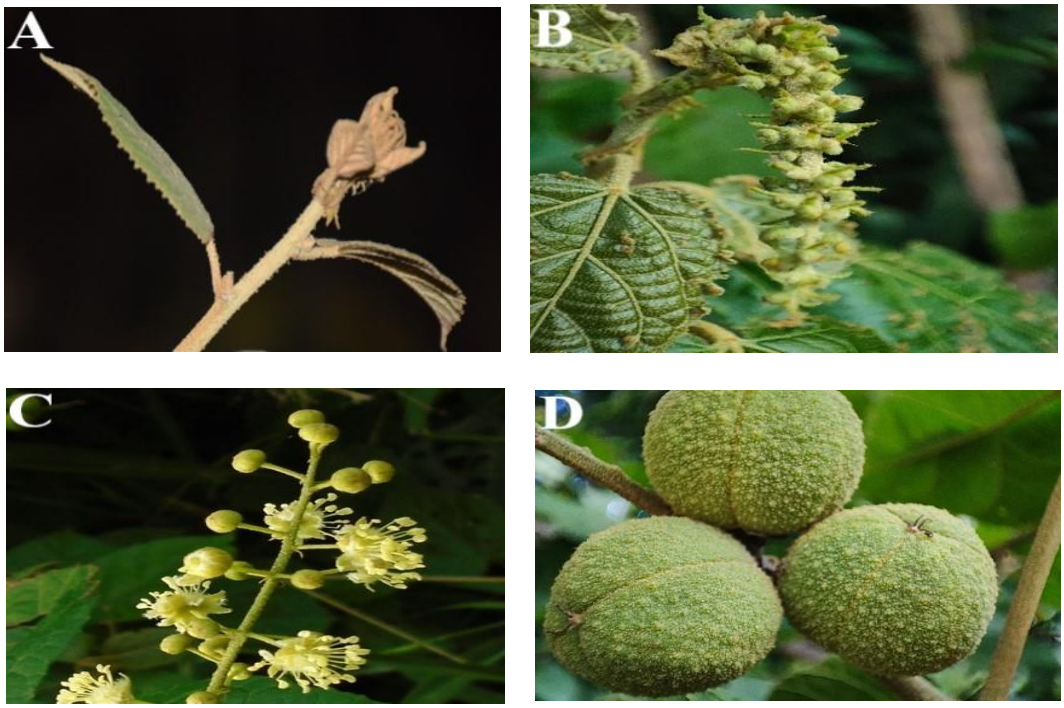


Figure 4.8: Phenological phases of *C. caudatus* (A) Leaf initiation, (B) Flower bud, (C) Flower and (D) Fruiting and mature fruit formation.

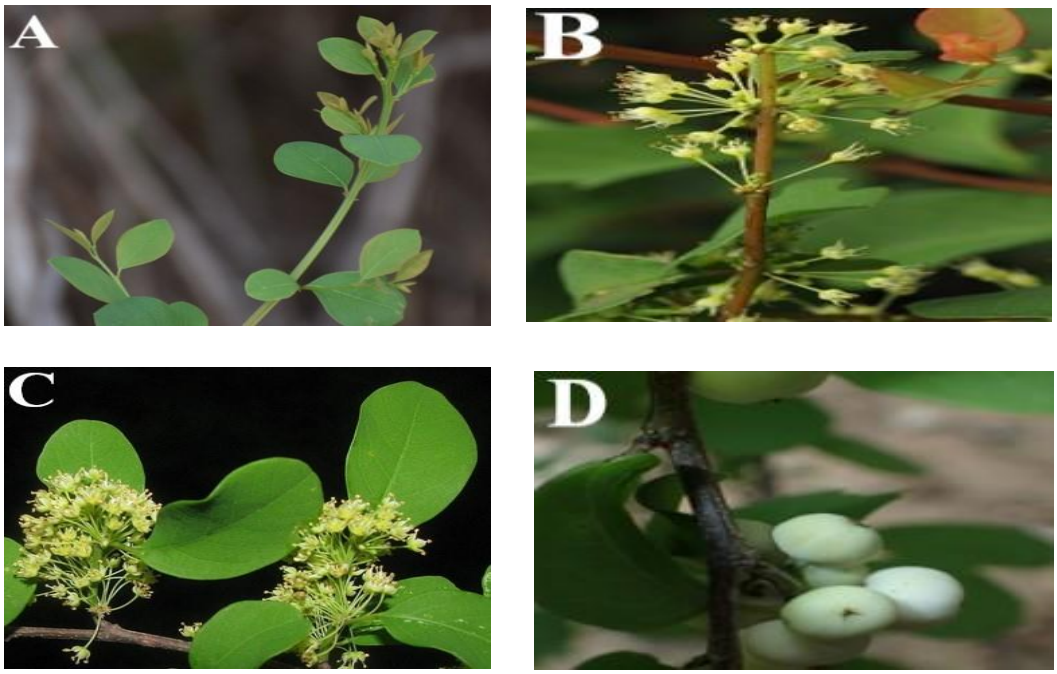


Figure 4.9: Phenological phases of *F. virosa* (A) Leaf initiation, (B) Flower bud, (C) Flower and (D) Fruiting and mature fruit formation.

4.3.3. Correlation between climatic factors and phenological phases

Pearsons correlation analysis was utilized to evaluate the correlation between various phenophases and the variables of rainfall and temperature during the three years of the study. The correlation was expressed using the "r" value, which provides a measure of the strength and direction of the relationship between the variables studied. Also, its value typically falls within a range of -1 to 1, with -1 indicating a perfect negative correlation, 1 indicating a perfect positive correlation, and 0 indicating no correlation between the variables. Variability in the correlation between rainfall and different phenophases was noted across the three years under investigation. There is a strong and negative correlation (r=-0.82, p=0.046*) between rainfall and fruiting and mature fruit formation in 2018 (**Figure 4.10**). When rainfall decreases it often signifies drier conditions which can stimulate fruiting and mature fruit formation in certain plant species. This is because reduced water availability can trigger physiological responses in plants such as stress-induced flowering and fruiting, as a survival mechanism to ensure reproduction. Also, a negative correlation between rainfall and leaf fall in 2019 (r=-0.59, p=0.05*) and 2021 (r=-0.77, p=0.039*). These statements indicate that lower rainfall levels coincide with increased leaf shedding. This relationship is often observed because reduced moisture availability can trigger responses in plants such as leaf abscission as a mechanism to conserve water and adapt to drier conditions. Rainfall had a strong positive and statistically significant correlation with flowering in 2021 (r=0.87, p=0.034*). This relationship is often observed because ample moisture availability provided by rainfall can promote plant growth and reproductive processes including flowering.

In 2018 and 2019 (**Figure 4.10**), there is a strong positive (r=0.71, p=0.043*) and negative (r=-0.78, p=0.043*) correlation between temperature and leaf initiation. The variation in the correlation between temperature and leaf initiation across the same plants over different years can occur due to fluctuations in environmental conditions, genetic diversity within the plant population, plant acclimation and adaptation mechanisms, and interactions with other environmental factors. These factors collectively influence how plants respond to temperature cues resulting in differing

outcomes in leaf initiation from year to year. In 2018, a robust negative correlation (r=-0.91, p=0.002**) was observed between temperature and flowering indicating that as temperature decreased there was a significant increase in flowering. Conversely, in 2019 (r=0.71, p=0.043*) and 2021 (r=0.69, p=0.046*), positive correlations were found between temperature and flowering, indicating that higher temperatures were associated with increased flowering during these years. This variability in the relationship between temperature and flowering across different years may be attributed to the nuanced interplay of various environmental factors, genetic influences, and plant physiology. Furthermore, there are positive (r=0.77, p=0.039*) and negative (r=-0.94, p=0.002**) correlations identified between temperature and leaf fall. The positive correlation suggests that higher temperatures can accelerate leaf senescence and promote leaf shedding possibly due to increased metabolic activity. Conversely, the negative correlation implies that cooler temperatures may delay leaf senescence resulting in reduced leaf fall. In addition to temperature and rainfall several factors may include light intensity, soil moisture levels, nutrient availability, and biotic interactions such as pollination and herbivory. The interplay of these environmental factors along with genetic factors inherent to the plant species collectively influences the timing of key phenological events such as flowering, leaf initiation, and fruiting. Therefore, while temperature and rainfall are important drivers, it also interacts with other environmental factors such as photoperiod (day length), moisture, nutrient availability and hormonal signalling. Depending on the balance on these factors, it is entirely plausible for the same species in the same area to display different correlations throughout the three years under investigation. This variability emphasizes the need for long term observations to understand the nuances of how plants respond to their environment and the challenges needed for making generalized predictions.

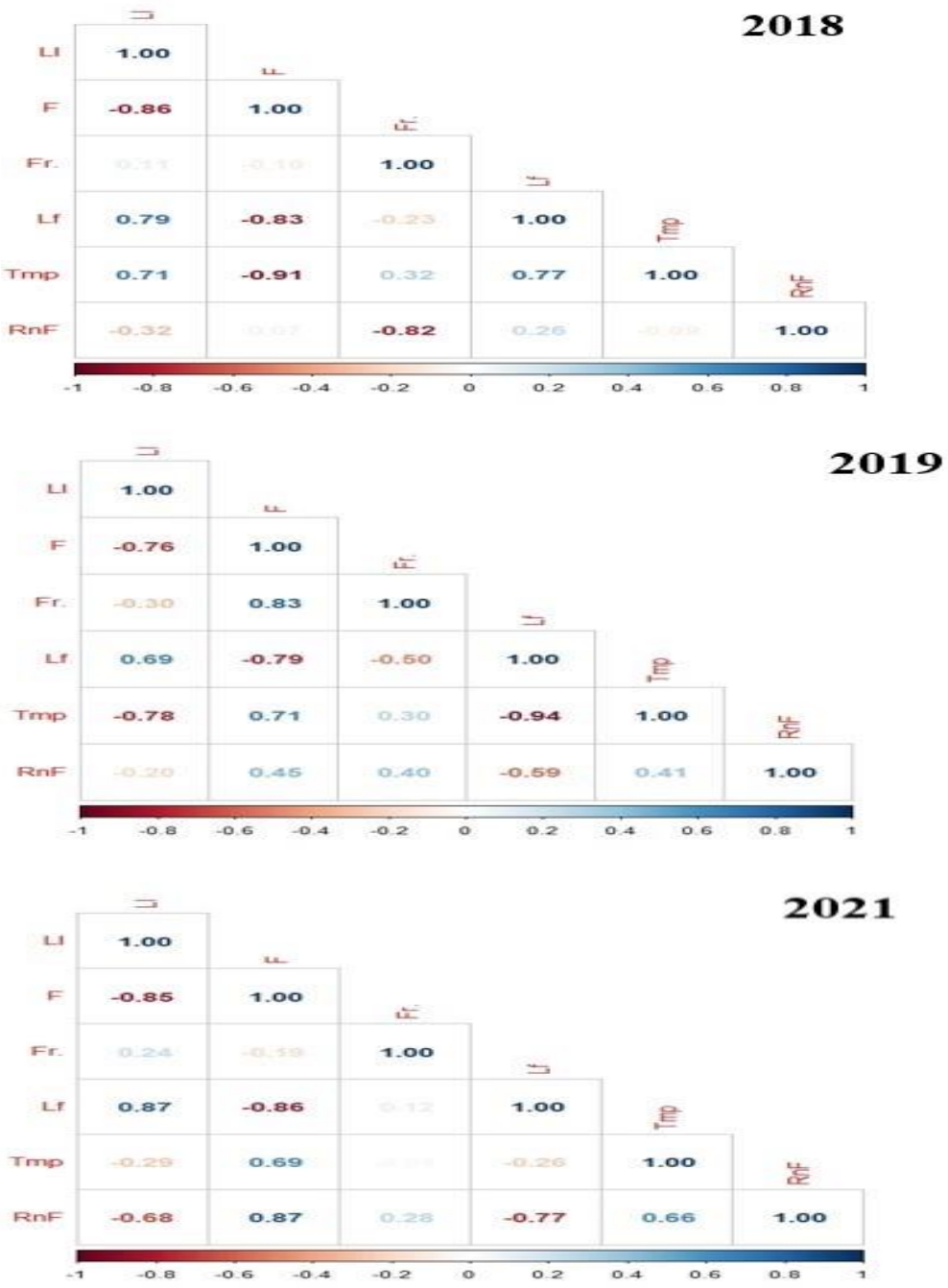


Figure 4.10: Pearson's correlation between climatic variables and phenological phases during three years investigation (2018, 2019 and 2021).

*Correlation is significant at the 0.05 level.

** Correlation is significant at 0.01 level.

4.3.4. Discussions

There has been a significant surge in interest surrounding phenological patterns and their underlying processes. This rise in interest can be attributed at least in part to a growing interest about the impact of a changing climate on the timing of plant life stages (Price and Waser, 1998; Corlett and LaFrankie, 1998; Mac Mynoski and Root, 2007). Rising temperatures have affected the dynamics of populations and enhanced a number of processes including leaf unfolding, emergence, flowering and breeding. On the other hand, the timing of leaf fall has been delayed resulting in a prolonged growing season (Menzel and Fabian, 1999). Colder and temperate forests have evolved to withstand the seasonal cycle; they undergo dormancy in the winter mainly because of changes in light and temperature. These seasonal stages can also be influenced by biological variables including genetic characteristics, soil conditions, and water availability (Cotton, 2003). Thus, plants can serve as biological markers of shifting climatic scenarios with springtime stages of the plant being especially temperature-sensitive.

This study examines the phenological variations of six ethnomedicinal NTFPs across three years. Most species exhibit leaf initiation between January and March, except for *B. roxbughii,* which begins in December. Additionally, *F. virosa* shows leaf initiation from March, extending until May. The deviations in leaf initiation timings among these species might be attributed to specific genetic traits, or adaptations to their respective habitats. Factors such as variation in light exposure or even soil moisture could influence these differing phenological patterns. Variations in microclimates within their habitats might influence when these plants initiate their leaves. Microclimates can differ due to factors like elevation, aspect, or local environmental conditions. Different species may have evolved to thrive in specific ecological niches. Their phenological patterns could be an adaptation to optimize growth and survival in these niches. Also, their relationships with other organisms such as pollinators, pests, or symbiotic partners could influence the timing of leaf initiation as a response to these interactions. Human activities including land-use changes or disturbances might impact the phenology of these species by altering their habitats or disrupting their natural cycles. The exact reasons behind the varying leaf

initiation timings are likely influenced by a combination of these and possibly other factors, showcasing the complexity of plant responses to their environment.

In this study, the process of new leaf formation spans a timeframe of 14 to 16 weeks, indicating a gradual and prolonged emergence of foliage. Notably, the pinnacle of this emergence consistently aligns with the pre-monsoon period and endures until the onset of the monsoon season in each observed year. This observation resonates with several documented findings by various researchers establishing a recurring pattern where leaf initiation tends to coincide with the commencement of the monsoon. The synchronization of this leaf emergence with the pre-monsoon and monsoon phases might be attributed to the influence of environmental stimuli. Fluctuations in temperature, humidity, and potentially other ecological triggers characteristic of the pre-monsoon and monsoon periods could serve as critical cues triggering this surge in leaf growth. Plants often exhibit such synchronized patterns in response to environmental changes, optimizing their growth and development to make the most of available resources during favourable conditions. Leaf initiation and the peak of leaf flushing are linked to the hotter months preceding the rainfall. Peaks in all phenophases were discernible as a result of distinct wet and dry seasons. This phenomenon could be attributed to the stimulative impact of escalating temperatures and the extended duration of daylight exposure known as photoperiods (Yadav and Yadav, 2008). This study aligns with prior research conducted by other workers where exhibits a noteworthy adverse impact signifying that leaf initiation predominantly occurs during the dry season (Kikim and Yadava, 2001). Additionally, the production of leaves has been observed towards the end of the dry season and preceding the onset of the rainy season in tropical tree species (Singh and Kushwaha, 2005). The findings are also consistent with the observations documented by (Bullock and Solis-Magallenus, 1990; Sundriyal, 1990). This leaf initiation and leaf flushing phase which correlates with increase in temperature could favour to enhance the photosynthesis and vegetative growth (Bhat, 1992; Bhat and Murali, 2001; Rivera et al., 2002; Hamann, 2004; Dar and Malik, 2009).

The synchronicity of bud burst or flowering closely correlates with the species-specific environmental signal providing a reliable indirect method to identify the

triggering cue. The seasonal change in daylight duration prompts simultaneous bud burst or flowering annually across an entire geographic area (Rivera and Borchert, 2001). This investigation demonstrated that floral formation consistently coincided with the pre-rainfall and rainy seasons. Additionally, the duration of the fruiting phase exceeds that of the flowering phase. This pattern of peak flowering and extended fruiting periods has been observed in previous studies as well (Anderson et al., 2005; Gunter et al., 2008). It has been observed that a positive correlation between flower formation and mean monthly temperature and rainfall aligning with previous findings by Osmondi et al. (2016); Elliott et al. (1994).

CHAPTER V

5. Qualitative and quantitative phytochemical analysis and free-radical scavenging activity of selected Ethnomedicinal NTFPs.

5.1. Introduction

Plant-derived products have been integral components of phytomedicines for centuries. These sources encompass leaves, roots, seeds, flowers, barks and fruits, contributing to diverse medicinal formulations (Cragg and Newman, 2001). Medicinal plants harbour a range of organic compounds that exert distinct physiological effects on the human body. These bioactive substances encompass flavonoids, carbohydrates, tannins, terpenoids, alkaloids and steroids (Edeoga et al., 2005). These compounds are produced through either primary or secondary metabolism within living organisms. Secondary metabolites represent a chemically and taxonomically diverse group of compounds with often unclear functions. They find extensive applications in veterinary medicine, human therapy scientific research agriculture, and various other fields (Vasu et al., 2009). These chemicals collaborate with nutrients and fibres to constitute an integral component of the defence system against diverse diseases and stress conditions (Thilagavathi et al., 2015). Comprehending the connection between phytoconstituents and plant biological activity is essential for the synthesis of substances with specific activities intended to cure a range of diseases and persistent medical conditions (Pandey et al., 2013). Fundamental elements include proteins, common sugars and chlorophyll; secondary ingredients include phenolic, alkaloids and terpenoid chemicals (Krishnaiah et al., 2007). Strong natural antioxidants, phytochemicals found in plants such as carotenoids, tocopherols, ascorbates, and phenols play a vital role in the health care system. A significant class of compounds having antioxidant qualities, phenols include subclasses including phenolic acids, flavonoids, biflavonoids, anthocyanins, and isoflavonoids. They have anti-inflammatory, anti-allergenic, anti-tumour, anti-platelet aggregation, and anti-cancer effects (Bendich, 1996).

Oxidative stress results from the production of free radicals also known as reactive oxygen species (ROS), during metabolism and other activities that exceed the capacity of biological system to produce antioxidants (Zima et al., 2001). Heart conditions, malaria, neurological illnesses, AIDS, cancer, and the ageing process are all influenced by oxidative stress (Astley, 2003). The growing body of evidence substantiates the notion that oxidative damage is implicated in the pathogenesis of chronic, age-related degenerative diseases. Concurrently, dietary antioxidants are recognized for their ability to counteract this process thereby reducing the risk of disease. Hence, there is a pressing need to extract these antioxidants from plant matrices (Atoui et al., 2005; Alasalvar et al., 2005). The total phenolic content, antioxidant and free radical scavenging properties of extracts from various plant sections were investigated in an effort to identify possible natural sources of antioxidants.

Antioxidants including ascorbates, polyphenols, limonoids, tocopherols and carotenoids presently draws an increasing amount of interest in the biological benefits of phenols because research indicates that they can protect heart disease and cancer (West, 2003). In a recent investigation diverse extraction methodology including Soxhlet, microwave-assisted extraction, dispersed-solids, percolation and supercritical fluid extraction, were employed to isolate antioxidants from plant sources (Grigonis et al., 2005). However, nearly all organisms possess defence mechanisms against free radical attacks. These mechanisms include a preventive antioxidant system which decreases the rate of free radical formation as well as a system that generates chain-breaking antioxidants capable of scavenging and stabilizing free radicals. Nevertheless, when the production rate of free radicals surpasses the capacity of these antioxidant defence mechanisms significant tissue damage occurs (Rahman and Moon, 2007). Hence, antioxidants exhibiting free radical scavenging properties hold significant promise in both the prevention and treatment of diseases mediated by free radicals (Hasan et al., 2009). Phytochemical research informed by ethno-pharmacological knowledge is widely recognized as an effective strategy for identifying novel anti-infective agents derived from higher plants. Qualitative and quantitative analyses play an important role in identifying and

quantifying active compounds within medicinal plants, crucial for understanding their medicinal properties and facilitating drug formulation (Bhumi and Savithramma, 2014).

5.2. Methodology

5.2.1. Collection of plant materials

Fresh parts of six ethnomedicinal NTFPs viz., *M. malabathricum* (leaf), *V. peduncularis* (leaf), *F. virosa* (leaf), *C. caudatus* (leaf), *T. grandiflora* (leaf) and *B. roxburghii* (whole plant) were collected from the study area. The plant materials undergo cleaning with running water followed by shade drying until complete evaporation of water molecules ensuring thorough drying for subsequent grinding. Subsequently, the dried plant materials are finely ground using a mechanical blender and then transferred into airtight containers with appropriate labelling for future utilization.

5.2.2. Preparation of plant samples for extraction

A crude plant extract was prepared via the Soxhlet extraction method. Initially, 50-100g of powdered plant material was packed uniformly into a thimble. This material was then subjected to extraction with 250ml volumes of two distinct solvents: methanol and an aqueous solution. The extraction process was allowed to continue for a duration of 72 hours or until the solvent within the siphon tube of the extractor became colourless. The resulting extract was transferred into a beaker and placed on a hot plate where it was heated at a temperature range of 30-40°C until complete evaporation of the solvent occurred. The dried extract was then stored in a refrigerator at a temperature of 4°C to ensure its preservation for future analysis.

5.2.3. Qualitative phytochemical analysis

The chemical analysis of the plant extracts was conducted according to established protocols outlined by Harborne (1998) employing standard procedures. This involved systematic testing to identify the various constituents present in the extracts.

5.2.3.1 Test for Alkaloids

Mayer's test

A volume of 2ml of the plant extract was mixed with 2N hydrochloric acid (HCl). Following this, one or more drops of Mayer's reagent were introduced into the mixture for further analysis. The observation of a white creamy precipitate within the solution signified the potential presence of alkaloids.

Wagner's test

A total of 1ml of the plant extract was mixed with an equal volume of Wagner's reagent which is a dilute iodine solution. Upon thorough mixing, the appearance of reddish-brown precipitates within the mixture served as an indication of the potential presence of alkaloids.

5.2.3.2. Test for Carbohydrates

Fehling's test

A volume of 2ml of the test solution was combined with Fehling solution A and B, the mixture was then heated. The formation of a brick-red precipitate within the solution indicated the potential presence of carbohydrates.

Benedict's test

2ml of plant extract was mixed with 2ml of Benedict's reagent and subsequently boiled. Formation of a reddish-brown precipitate occurred indicating the presence of carbohydrates within the extract.

Molisch's test

2ml of the plant extract was combined with 2ml of Molisch's reagent and thoroughly shaken to ensure proper mixing. Following this, 2ml of concentrated sulfuric acid (H_2SO_4) was added carefully along the side of the test tube. The observation of a violet ring formation at the interphase of the solution indicated the potential presence of carbohydrates.

5.2.3.3. Test for Phytosterols

Liebermann-Burchard's test

50 mg of the extract was dissolved in 2 ml of acetic anhydride. Following this, 1 or 2 drops of concentrated sulfuric acid were carefully added slowly along the sides of the test tube. The emergence of a spectrum of colour changes within the solution indicated the potential presence of phytosterols.

5.2.3.4. Test for Saponins

Foam test

2ml portion of the test solution was mixed with water followed by vigorous agitation. After allowing the mixture to settle for 15 minutes without disturbance, the appearance of a foam-like substance reminiscent of leather indicates the presence of saponins.

5.2.3.5. Test for Phenols and Tannins

$FeCl_3$ Test

The crude extract was mixed with 2ml of a 2% solution of $FeCl_3$. The emergence of a blue-green or black coloration upon this reaction served as an indicator for the presence of phenols and tannins within the extract.

5.2.3.6. Test for Flavonoid

H_2SO_4 test

The plant extract underwent treatment with a few drops of H_2SO_4. The appearance of an orange coloration following this reaction suggested the presence of flavonoids within the plant extract.

5.2.3.7. Test for terpenoid

Salkowski test

5mg aliquot of the extract was combined with 2ml of chloroform and 3ml of sulfuric acid. Observation of a reddish-brown coloration layer forming on the inner surface indicated the presence of terpenoid.

5.2.3.8. Test for proteins and amino acids

Xanthoproteic test

1 ml of concentrated nitric acid (HNO_3) was added, followed by heating and subsequent cooling of the mixture. Sodium hydroxide solution (40% w/v in water) was then gradually introduced until the mixture achieved alkalinity and a noticeable colour change was observed. The transition from yellow to orange hue indicated the potential presence of an aromatic amino acid within the sample.

5.2.4. Quantitative Phytochemical analysis and free- radical scavenging activity

5.2.4.1. Determination of total phenolic content

With a few modifications, the Folin ciocalteu technique (Kujala et al., 2000) was used to determine the total phenolic content. In a nutshell, a methanolic extract stock solution (1 mg/ml) was prepared. Additionally, a 100µg/ml of gallic acid standard solution was prepared by mixing 10 mg of gallic acid with 100 ml of water. Various concentrations of (20 µg/ml - 100 µg/ml) were generated using this stock solution. 1 ml of the sample was added with 0.5 ml of Folin Ciocalteu's reagent (1:10). Following an incubation period of 5 minutes, 1.5ml of 7% Na_2CO_3 was added, and the final volume was adjusted to 10ml using Millipore water. After an additional 2 hours of incubation, optical density measurements were taken at 760 nm. The standard curve preparation followed the same procedure. The determination of total phenolic content was derived from the standard curve and the outcomes were expressed as milligrams of Gallic acid equivalents per gram (mg GAE/g) of dry weight.

5.2.4.2. Determination of total flavonoid content

The total flavonoid content was assessed using Aluminium Chloride (Zhishen et al., 1999) outlined with slight modifications. In test tubes, 1 ml aliquots and 1 ml of Standard Quercetin (20–100 µg/mL) were prepared. Subsequently, 1 ml of Quercetin/plant extract was combined with 3 ml of 5% Sodium Nitrite ($NaNO_2$) followed by the addition of 0.3 ml of 10% aluminium chloride after 5 minutes. Following a 6-minute incubation period at room temperature, 1 ml of 1M Sodium hydroxide was introduced into the reaction mixture and the final volume was

adjusted to 10 ml with distilled water. Absorbance measurements of the samples were conducted at 510 nm against a blank. The blank was prepared identically to the samples excluding the plant extract. Consequently, the total flavonoid content was determined using the standard curve of Quercetin with results expressed as milligrams of Quercetin equivalent per gram (mg QE/g) of dry weight.

5.2.4.3. DPPH radical scavenging assay

The DPPH (2,2'-diphenyl-2-picrylhydrazyl) free radical scavenging activity was evaluated following the methodology described by Braca et al., (2001) with slight modifications. Initially, various concentrations of the plant extract (10 µg/ml - 100 µg/ml) were prepared from the stock solution (1mg/ml). 1ml of the plant extract was combined with 2ml of DPPH solution (0.004% w/v) and incubated in darkness at room temperature for 60 minutes. Optical density readings were then taken at 517nm. A negative control was established by mixing 1ml of methanol with 2ml of DPPH solution. Butylated hydroxytoluene (BHT) was used as standard. The percentage of inhibition was determined by comparing the absorbance values of the test samples with those of the controls. The inhibition percentage (I) was evaluated using the following formula:

$$\% \text{ Inhibition} = (\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{control}} \times 100$$

5.2.4.4. ABTS radical scavenging assay

The 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity was assessed following the protocol outlined by Re et al. (1999). Initially, an ABTS solution was prepared by dissolving 7mM of ABTS in distilled water. The ABTS radical was then prepared by adding a 2.45mM potassium persulfate solution to the mixture. The resulting mixture was then incubated in a dark room for 12 hours to ensure completion of the reaction. A working standard for the ABTS radical was prepared by extracting 1ml from the stock solution and diluting it with 50% methanol to achieve an absorbance of 0.700 ± 0.001 at 745nm. Subsequently, the free radical scavenging activity was evaluated by combining 500µl of various fractions of plant extracts (10 - 100 µg/ml, dissolved in distilled water) with 1 ml of the ABTS working solution. The reduction in absorbance was

monitored for up to 3 minutes following the mixing of the solutions. BHT at concentrations ranging from 10 µg/ml to 100 µg/ml was utilized as a standard for comparison with the analysed data and were recorded in triplicate. The scavenging activity was determined using the following formula:

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

5.2.5. Statistical analyses

The results were expressed in means of triplicate (Mean ± SD). IC_{50} values and Pearsons correlation coefficient of antioxidant activity with TPC and TFC (heat map) was generated using R studio version 4.1.2.

5.3. Results

5.3.1 Qualitative phytochemical analysis of ethnomedicinal NTFPs

Preliminary phytochemical analysis was conducted on both methanol and aqueous extracts of the plants. Overall, the methanolic extracts exhibited more promising results compared to the aqueous extracts. Eight phytoconstituents were examined, revealing the presence of alkaloids, carbohydrates, saponins, phenols, tannins and flavonoids in the methanolic extracts of six ethnomedicinal NTFPs. Additionally, both the methanolic and aqueous extracts tested negative for phytosterols and amino acids. While terpenoids were absent in the methanol and aqueous extracts of *T. grandiflora* and *B. roxburghii*, they were found in the remaining methanolic plant extracts (**Table 5.1**).

Table 5.1: Qualitative phytochemical analysis of six ethnomedicinal NTFPs.

Sl. no	**Compounds**	**Phytochemical testing**	***F. virosa***		***M. malabathricum***		***B. roxburghii***		***V. peduncularis***		***C. caudatus***		***T. grandiflora***	
			Met	Aq	Met	Aq	Met	Aq	Met	Aq	Met	Aq	Met	Aq
1	Alkaloids	**Mayer's test**	+	+	+	-	+	-	+	-	+	+	+	+
		Wagner's test	+	+	+	-	+	-	+	-	+	+	+	+
2	Carbohydrates	**Molisch's test**	+	+	+	+	+	+	+	+	+	+	+	+
		Benedict's test	+	+	+	+	+	+	+	+	+	+	+	+
		Fehling's test	+	+	+	+	+	+	+	+	+	+	+	+
3	Phytosterols	**Liebermann Burchard's test**	-	-	-	-	-	-	-	-	-	-	-	-
4	Saponins	**Foam or froth test**	+	+	+	+	+	+	+	+	+	+	+	+
5	Phenols and Tannins	**FeCl3**	+	+	+	+	+	+	+	+	+	+	+	+
6	Proteins and amino acids	**Xanthoproteic test**	-	-	-	-	-	-	-	-	-	-	-	-
7	Flavonoids	**H2SO4**	+	+	+	+	+	+	+	+	+	+	+	+
8	Terpenoids	**Salkowski**	+	-	+	+	-	-	+	+	+	-	-	-

5.3.2. Evaluation of quantitative phytochemical analysis and free radical scavenging activity

5.3.2.1. Total phenolic content (TPC)

The total phenol content of various methanol and aqueous plant extracts was determined by extrapolating from the linear regression curve derived from the standard Gallic acid ($y=0.0062x - 0.0194$, $R^2= 0.99$) (**Figure 5.1**). It was quantified and expressed as milligrams of Gallic acid equivalents per gram of dry extract. Both methanolic and aqueous plant extracts exhibited remarkably elevated levels of phenolic content. This phenomenon can be attributed to the presence of a diverse array of phenolic compounds within the extracts which may include flavonoids, phenolic acids, and other related compounds known for their antioxidant properties and potential health benefits. Among the studied samples, the methanolic extract of *C. caudatus* (155 ± 0.5mg GAE/g) exhibited the highest phenolic content. Following closely were *F. virosa* (153.3 ± 1mg GAE/g), *V. peduncularis* (148.8 ± 0.1mg GAE/g), *M. malabathricum* (147 ± 1mg GAE/g), and *B. roxburghii* (142.6 ± 0.2mg GAE/g). *T. grandiflora* (122.8 ± 1.5mg GAE/g) displayed the lowest phenolic content. Similarly, among the aqueous plant extracts, *V. peduncularis* (139 ± 2.5mg GAE/g) exhibited the highest total phenolic content followed by *T. grandiflora* (137.7 ± 1.5mg GAE/g), *C. caudatus* (135 ± 1mg GAE/g), *F. virosa* (133. 6 ± 1mg GAE/g), *M. malabathricum* (123.3 ± 0.1mg GAE/g), and *B. roxburghii* (122.2 ± 1.1mg GAE/g), in descending order of phenolic concentration (**Table 5.2**).

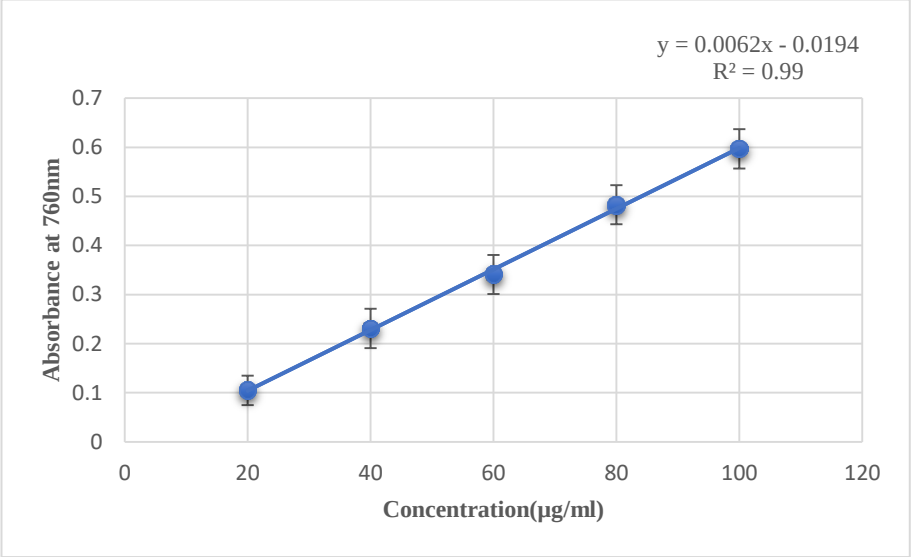


Figure 5.1: Standard curve of Gallic acid.

5.3.2.2. Total Flavonoid Content (TFC)

The total flavonoid content of the selected plants was assessed using a standard curve of quercetin derived from a linear regression analysis (y= 0.0064x + 0.0272, r^2= 0.9966) (**Figure 5.2**). Results were expressed as milligrams of quercetin equivalent (QE) per gram of dry extract. The highest flavonoid content was observed in both methanol and aqueous extracts of *M. malabathricum* (139.6 ± 1.8 mg QE/g and 126 ± 2 mg QE/g) respectively. Conversely, *T. grandiflora* exhibited the lowest flavonoid content (108.6 ± 1.09 mg QE/g in methanol and 92.4 ± 2 mg QE/g in aqueous extract) (**Table 5.2**).

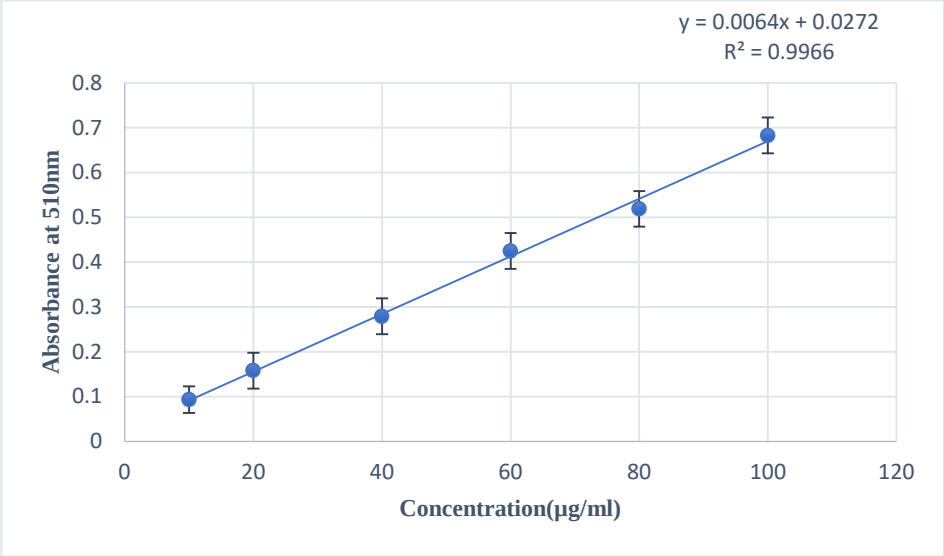


Figure 5.2: Standard curve of Quercetin.

Table 5.2: Evaluation of TPC and TFC in methanol and aqueous extracts of tested ethnomedicinal NTFPs.

Plant Species	Total Phenols (Met extracts) (mg GAE/g± SD)	Total Phenols (Aq extracts) (mg GAE/g ± SD)	Total Flavonoids (Met extracts) (mg QE/g ± SD)	Total Flavonoids (Aq extracts) (mg QE/g ± SD)
Thunbergia grandiflora **(leaf extract)**	122.8 ± 1.5	137.7 ± 1.5	108.6 ± 1.09	92.4 ± 2
Begonia roxburghii **(whole plant)**	142.6 ± 0.2	122.2 ± 1.1	119.3 ± 1.5.2	104.5 ± 2.2
Melastoma malabathricum ***(*****leaf extract)**	147 ± 1	123.3 ± 0.1	139.6 ± 1. 8	126 ± 2
Vitex peduncularis **(leaf extract)**	148.8 ± 0.1	139 ± 2.5	121.6 ± 1	113 ± 2
Flueggea virosa **(leaf extract)**	153.3 ± 1	133. 6 ± 1	134.6 ± 2.5	118 ± 2
Croton caudatus **(leaf extract)**	155 ± 0.5	135 ± 1	128 ± 2.08	109.5 ± 2.02

Each value is expressed as means ± SD (n = 3)

5.3.2.3. DPPH radical scavenging activity

In-vitro antioxidant assay of six methanolic and aqueous ethnomedicinal plants demonstrated notable antioxidant potential. The extract exhibited a concentration-dependent enhancement in scavenging DPPH radicals as evidenced by the progressive discoloration of DPPH solution. BHT served as the positive control

across concentrations ranging from 10-100µg/ml. The scavenging activity varied between 30.56% (10µg/ml) and 89.25% (100µg/ml) in methanol extracts and between 19.82% (10µg/ml) and 75.57% (100µg/ml) in aqueous extracts. Notably, at the highest concentration (100µg/ml), *F. virosa* exhibited the highest scavenging activity in methanol extracts (89.25%), followed by *T. grandiflora* (88.1%), *B. roxburghii* (86.57%), *V. peduncularis* (82.73%) and *M. malabathricum* (81.58%) with the lowest observed in *C. caudatus* (80.69%) (**Figure 5.3**). In aqueous extracts, the highest scavenging activity was observed in *C. caudatus* (75.57%), followed by *V. peduncularis* (72.89%), *F. virosa* (70.2%), *T. grandiflora* (68.67%) and the lowest activity was recorded in *B. roxburghii* and *M. malabathricum* both at 64.58% (**Figure 5.4**). Additionally, the scavenging activity of the positive control BHT at the highest concentration recorded was 96.67%. Regarding IC_{50} values, the highest values were observed in methanolic extracts of *B. roxburghii* (19.5 ± 0.14µg/ml) and *V. peduncularis* (50 ± 0.13µg/ml) in aqueous extracts. Conversely, the lowest IC_{50} value was noted in methanolic extract of *F. virosa* (37.47 ± 0.1614µg/ml) (**Figure 5.5A**), and in aqueous extract of *B. roxburghii* (75.33 ± 0.12 µg/ml) (**Figure 5.5B**).

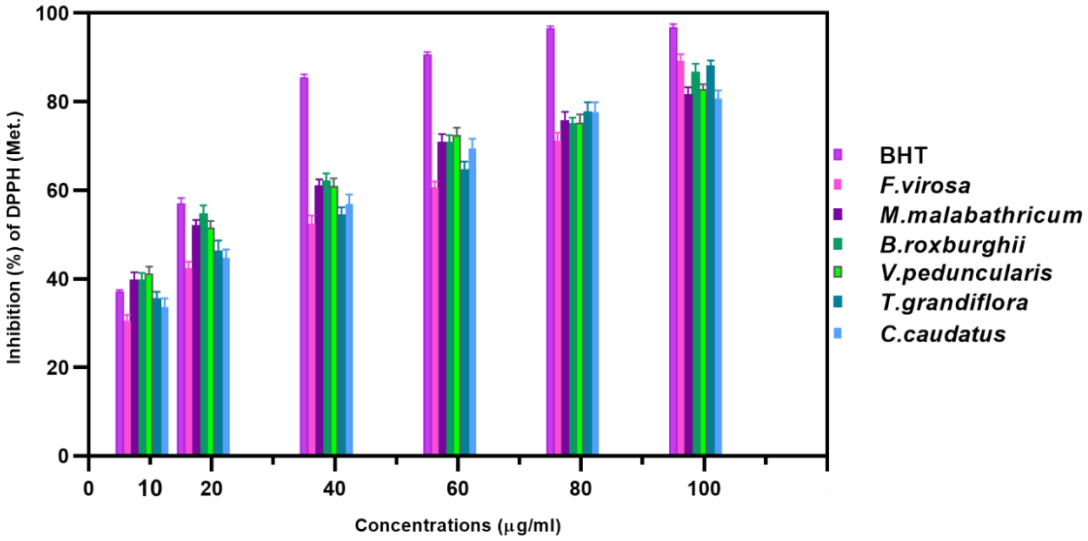


Figure 5.3: Percentages of DPPH scavenging activity of methanolic plant extract with BHT as positive control. Each value was represented as mean ± SD (n=3).

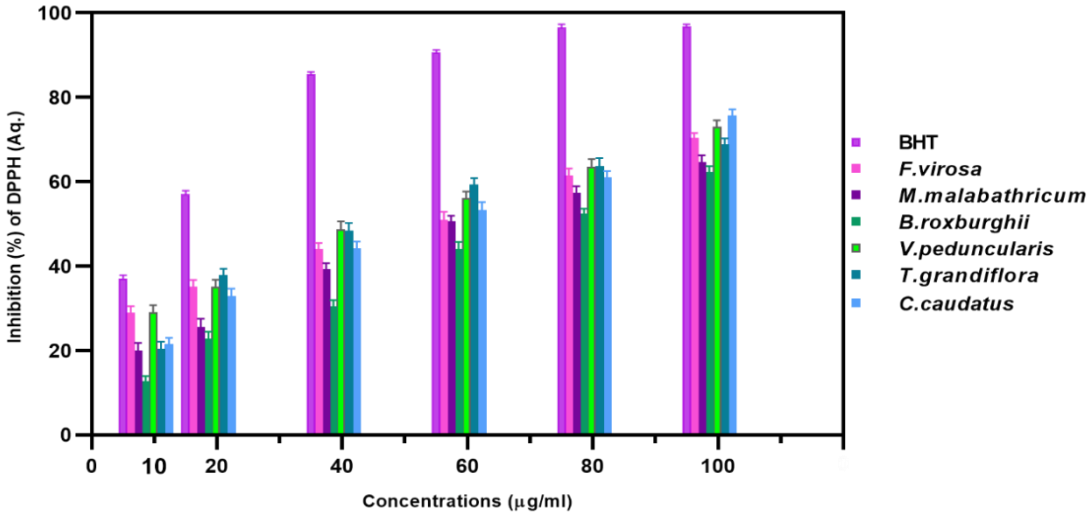


Figure 5.4: Percentages of DPPH scavenging activity of aqueous plant extract with BHT as positive control. Each value was represented as mean ± SD (n=3).

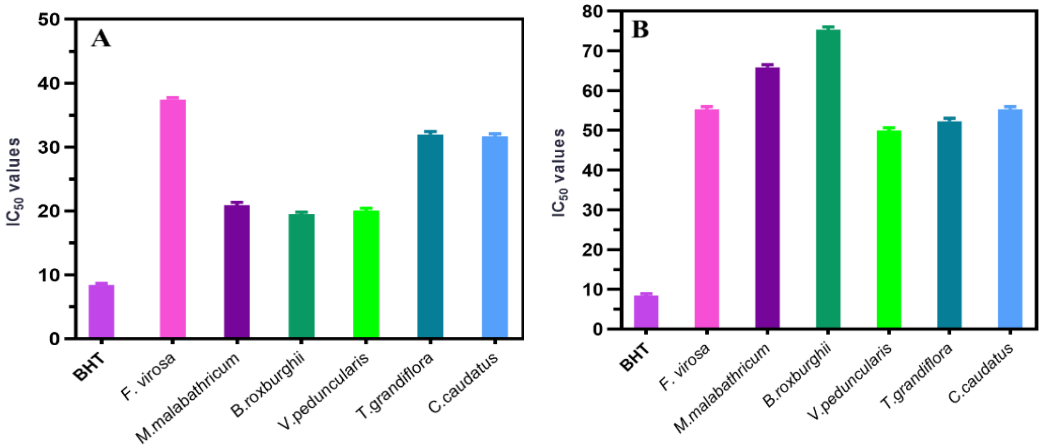


Figure 5.5. IC_{50} values of DPPH assay in methanol (A) and aqueous extract (B) of six ethnomedicinal NTFPs.

5.3.2.4. ABTS radical scavenging activity

The ABTS scavenging activity exhibited a range of values across different concentrations and solvents. Specifically, in methanol extracts the scavenging activity varied from 19.52% at a concentration of 10µg/ml to 80.32% at 100µg/ml.

Similarly, in aqueous extracts, the scavenging activity ranged from 10.08% at 10µg/ml to 76.48% at 100µg/ml. In the methanolic extract, *B. roxburghii* exhibited the highest scavenging activity at the highest concentration (100 µg/ml), reaching 80.32%. It was closely followed by *C. caudatus* (80.16%), *M. malabathricum* (79.68%), *T. grandiflora* (79.52%) and *V. penduncularis* (78.08%), while *F. virosa* demonstrated the lowest activity at 73.4% (**Figure 5.6**). Similarly, in aqueous extracts, the highest scavenging activity was observed in *C. caudatus* (76.48%) followed by *M. malabathricum* (71.84%), *T. grandiflora* (69.92%) and *V. penduncularis* (68.48%). *B. roxburghii* exhibited a scavenging activity of 67.04%, whereas F. vorosa showed the lowest activity at 59.2% (**Figure 5.7**). In both methanolic and aqueous extracts, *V. peduncularis* displayed the highest IC_{50} values at 32.47 ± 0.11µg/ml and *M. malabathricum* followed with 59 ± 0.11µg/ml, respectively (**Figure 5.8A**). Conversely, the lowest IC_{50} values were observed

in *F. virosa* (55 ± 0.03µg/ml) in methanolic extracts and *T. grandiflora* (70 ± 0.10µg/ml) in aqueous extracts (**Figure 5.8B**).

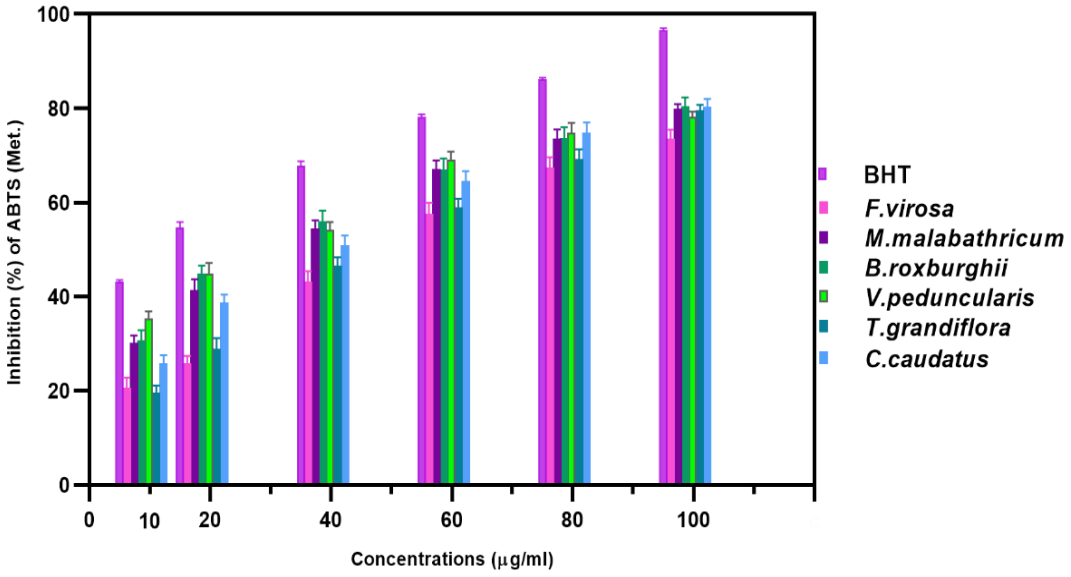


Figure 5.6: Percentages of ABTS scavenging activity of methanolic plant extract with BHT as positive control. Each value was represented as mean ± SD (n=3).

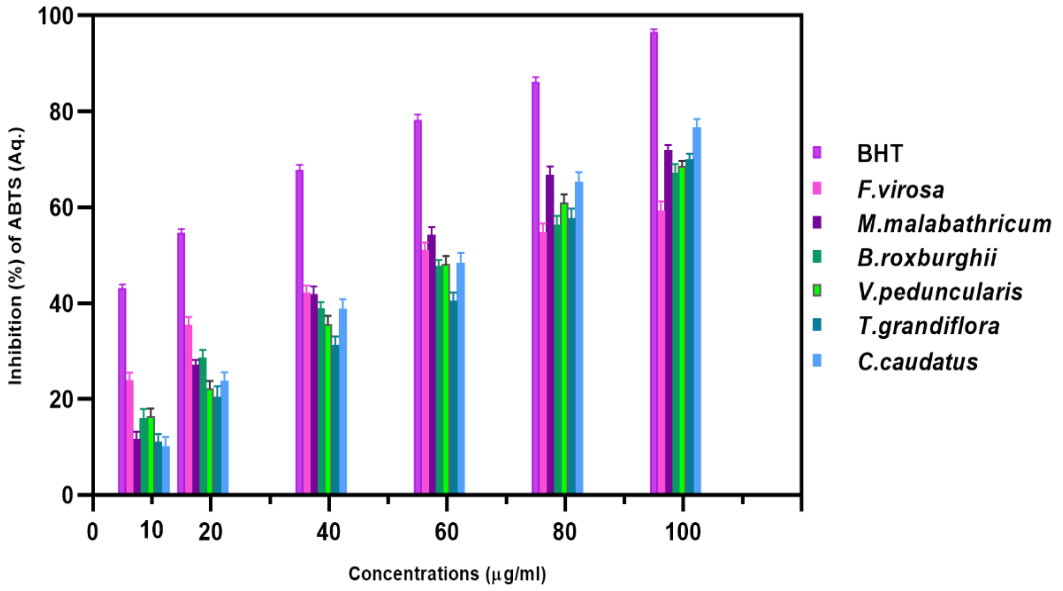


Figure 5.7: Percentages of ABTS scavenging activity of aqueous plant extract with BHT as positive control. Each value was represented as mean ± SD (n=3).

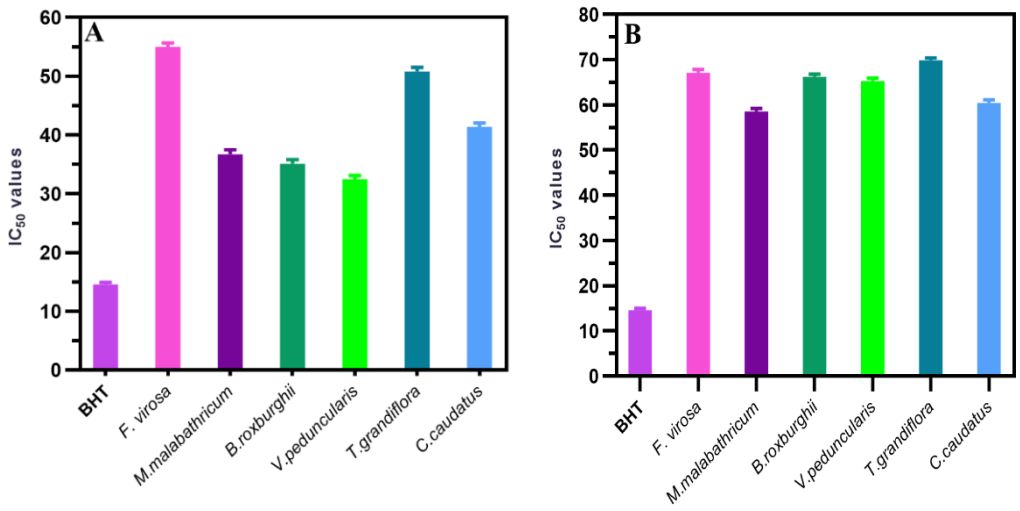


Figure 5.8: IC_{50} values of ABTS assay in methanol (A) and aqueous extract (B) of six ethnomedicinal NTFPs.

5.3.3. Pearson's Correlation coefficient between antioxidant activity, total phenol and flavonoid content

In the methanolic extract, a robust positive correlation of statistical significance was observed between Total Phenolic Content (TPC) and DPPH activity (R^2 = 0.97, p = 0.001**). Similarly, a significant correlation was found between Total Flavonoid Content (TFC) and ABTS activity (R^2 = 0.83, p = 0.039*) (**Table 5.4** and **Figure 5.9A**). Moreover, in the aqueous extract, a similar significant correlation was identified between TPC and DPPH activity (R^2 = 0.82, p = 0.022*) as well as between TFC and DPPH activity (R^2 = 0.75, p = 0.043*) (**Table 5.5** and **Figure 5.9B**).

The strong positive correlations between antioxidant activity and phenolic and flavonoid content are well-supported by scientific literatures. Phenolic compounds, including flavonoids are known for their antioxidant properties due to their ability to donate hydrogen atoms or electrons thus neutralizing free radicals and reducing oxidative stress. Consequently, higher concentrations of phenolic and flavonoid compounds in plant extracts often correspond to increased antioxidant activity as observed in these findings.

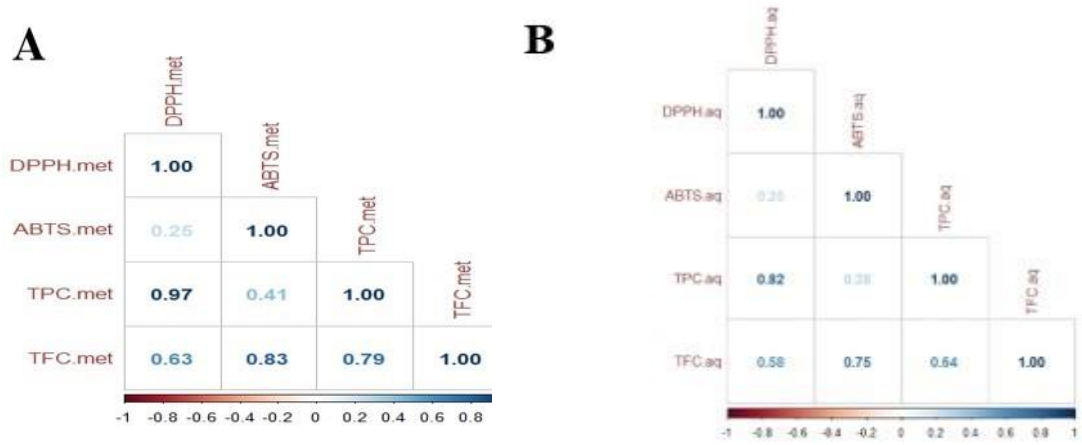


Figure 5.9: Pearson's correlation coefficient between antioxidant activity, TPC and TFC in methanol (A) and aqueous extracts(B).

Table 5.3: Pearson's correlation table of antioxidant activity with TPC and TFC in methanol extracts.

	DPPH.met	ABTS.met	TPC.met	TFC.met
DPPH.met	1			
ABTS.met	0.634			
TPC.met	0.001**	0.415		
TFC.met	0.176	0.039*	0.063	1

Table 5.4: Pearson's correlation table of antioxidant activity with TPC and TFC in aqueous extracts.

	DPPH.aq	ABTS.aq	TPC.aq	TFC.aq
DPPH.aq	1			
ABTS.aq	0.317			
TPC.aq	0.022*	0.294		
TFC.aq	0.114	0.043*	0.086	1

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

5.4. Discussions

The findings indicate the presence of pharmaceutically relevant constituents within the investigated plants. The investigation of two solvent extracts derived from the six ethnomedicinal NTFPs revealed the presence of numerous essential plant bioactive compounds. Alkaloids, flavonoids, carbohydrates, phenols, tannins and terpenoids were consistently identified in both methanol and aqueous extracts. These compounds represent crucial secondary metabolites that serve as key components

responsible for the medicinal properties attributed to the respective plants. Tannins were detected in all six plants studied, while terpenoids were present in all samples except for *B. roxburghii* and *T. grandiflora*. These compounds particularly terpenoids and tannins, are associated with analgesic and anti-inflammatory properties. Additionally, tannins contribute to the property of astringency, which accelerates wound healing and reduces inflammation in mucous membranes (Okwu and Josiah, 2006). Apart from saponins, alkaloids and flavonoids are among the secondary metabolites identified in all six ethnomedicinal NTFPs. Pure isolated alkaloids including their synthetic derivatives are recognized as fundamental medicinal agents esteemed for their analgesic, antispasmodic, and bactericidal properties (Stary, 1998; Okwu and Okwu, 2004). On the contrary, flavonoids exhibit robust antioxidant properties as potent water-soluble agents acting as scavengers of free radicals to inhibit oxidative damage to cells. Moreover, they demonstrate notable anticancer activity (Del-Rio et al., 1997; Salah et al., 1995). Plants produce flavonoids which are hydroxylated phenolic compounds in reaction to microbial infection. Studies conducted in vitro have shown that flavonoids are antimicrobial agents against a broad range of pathogens. Their capacity to form complexes with bacterial cell walls and extracellular soluble proteins is most likely what drives their activity (Cowan, 1999). One of the most significant and most prevalent classes of plant metabolites is the phenolic compounds. Their biological attributes include cardiovascular protection, anti-aging, anti-carcinogen, anti-inflammatory, anti-atherosclerosis and enhanced endothelial function. Additionally, they inhibit haemorrhage and cell division (Singh et al., 2007; Han et al., 2007).

The initial phytochemical group analysis conducted on *B. roxburghii* corroborates previous literature findings indicating the presence of alkaloids, flavonoids, phenols, tannins and carbohydrates while terpenoids were found to be absent (Mobarak et al., 2018). Our current research findings reveal elevated total phenolic content (TPC) at 142.6 ± 0.2 mg gallic acid equivalents per gram (GAE/g) and total flavonoid content (TFC) at 119.3 ± 1.5.2 mg QE/g closely resembling the outcomes of a study by Akter et al. (2021) in Chittagong, Bangladesh, which reported TPC at 180.40 ± 0.03 mg GAE/g and TFC at 60.43 ± 0.27 mg QE/g. Additionally, our investigations align

closely with those by Akter and Chowdhury (2021), where the reported IC_{50} values were 22.35 µg/ml, consistent with our findings of 19.5 ± 0.14 µg/ml. Flavonoids have been shown to prevent coronary heart disease and lower the risk of heart attacks (Osawa, 1994). Due of this there is an increased likelihood that the flavonoid content of *B. roxburghii*'s leaves might show an antioxidant defence against cardiac events and coronary heart disease. Also, the outcomes of phytochemical screening for *C. caudatus* are consistent with prior research indicating the presence of alkaloids, flavonoids, and phytosterols. However, while Shantabi et al. (2014) reported the absence of tannins, our investigation reveals their presence. Extracts derived from *C. caudatus* exhibited concentration-dependent scavenging of DPPH free radicals, a phenomenon well-documented in various plant species (Jagetia and Baliga, 2003; Wong et al., 2006; Aparadh et al., 2012). This scavenging effect is likely attributed to the donation of electrons to the DPPH free radical (Narayanaswamy and Balakrishnan, 2011; Jagetia et al., 2003b, 2012). The antioxidant capacity is presumably linked to the presence of a diverse array of phenolic compounds and other phytochemicals. These findings imply that *C. caudatus* leaves hold promise as a natural source of antioxidants potentially serving as therapeutic agents to ameliorate or delay the progression of degenerative conditions associated with oxidative stress (Shantabia et al., 2014).

In line with prior studies by Ajaib et al. (2021), which reports that the DPPH radical scavenging analysis had established that highest radical scavenging potential (92.23%) in the methanol extracts of *F. virosa*, also the present investigation demonstrated that the DPPH scavenging activity of *F. virosa* shows the highest percentage with 89.25%. Bokhari et al. (2013) reported similar outcomes demonstrating the stability of the findings. The research findings aligned with those of Ajaib et al. (2013); Siddiqui et al. (2016) who conducted separate studies on antioxidants in medicinal plants. According to Zengin et al. (2022), *F. virosa* had levels of TPC and TFC of 105.80 mg GAE/g and 32.59 mg RE/g respectively. The levels of content detected in the present investigation appeared to be higher with TPC and TFC measuring 153.3 ± 1 mg GAE/g and 134.6 ± 2.5 mg QE/g respectively. According to Zakaria et al. (2006); Latiff and Zakri (2000), the leaves of *M.*

malabathricum are chewed up, mashed, and put as a paste on cuts or wounds, or they are finely chopped and squeezed to apply the juice onto the wound to cease bleeding. The shoots can be consumed for relieving diabetes, high blood pressure, and puerperal infections, according to (Burkill, 1966; Koay, 2008). The present study aligns with previously published literature, which also documented ethnomedicinal uses for conditions such as cuts, hypertension and tuberculosis. Also, it produced favourable outcomes with the current research investigation for the presence of tannins, phenolic, terpenoid and flavonoid compounds according to comparable findings done by Isnaini et al., (2018); Sembiring et al., (2018); Zakaria et al., (2006). According to Wan Mohd Samsudin, (2019) the IC_{50} values were 111.90 µg/ml. However, the current research revealed IC_{50} values of 21 ± 0.12 µg/ml, which is significantly higher.

In accordance with observations made by Uddin et al., (2016), the methanolic extract of *T. grandiflora* exhibited higher levels of total phenolic content (98.53 mg GAE/g) and flavonoid content (191.76 mg QE/g). Our findings, however, show slight discrepancies, with total flavonoid content measured at 108.6 ± 1.09 mg QE/g and total phenolic content at 122.8 ± 1.5 mg GAE/g. Additionally, Uddin et al. (2016) reported a highly potent DPPH scavenging activity with an IC_{50} value of 10.50 ± 0.68 µg/ml, whereas our data revealed a lower IC_{50} value of 32 ± 0.15 µg/ml. Additionally, quantitative assessment of total phenolic content was determined as 119.8 mg GAE/g, while total flavonoid content yielded 36.8 mg QE/g as reported by Ibrahim and Sleem (2017). Our findings closely align with these results, although with higher flavonoid content observed in our study. A study conducted by Rashid Chowdhury et al. (2021) revealed that in *V. peduncularis*, the DPPH scavenging assay resulted in an IC_{50} value of 83.72 µg/ml and total phenolic content (TPC) analysis yielded a value of 97.27 ± 8.64 mg gallic acid equivalents per gram (GAE/g). In comparison, our findings for TPC were notably higher at 148.8 ± 0.1 mg GAE/g, and the IC_{50} value was significantly higher with the recorded value being 20.11 ± 0.13 µg/ml.

This comprehensive detection underscores the richness of bioactive constituents present in these plant extracts, further supporting their potential pharmacological

significance. The current research investigation aligns with previous studies, which have consistently highlighted the bioactive nature of these identified phytochemicals. Numerous investigations have underscored the medicinal and physiological contributions of these phytochemicals to the plants, showcasing their efficacy in treating various ailments. The discrepancies observed in the quantitative measurements of phenolic and flavonoid content, as well as antioxidant activity, between different studies can be attributed to variations in factors such as plant species, geographical location, extraction methods and analytical techniques employed. Additionally, differences in sample preparation and experimental conditions may contribute to variations in the observed results. Hence, the extracts derived from these plants hold promise as a valuable resource for the development of therapeutic drugs. Embracing traditional medicinal practices involving these plants is strongly advocated, while further research endeavours are recommended to isolate the active constituents responsible for their therapeutic effects. Additionally, there is a need for further exploration to elucidate the underlying mechanisms of action exhibited by these extracts. Such efforts would contribute significantly to our understanding of their pharmacological potential and pave the way for the development of novel pharmaceutical agents. It is noteworthy to emphasize the absence of published data regarding the phytochemical and antioxidant profiles of the selected six NTFPs, particularly with a focus on Mizoram. Therefore, there is a critical need to systematically document their profiles. Furthermore, it is imperative to conduct further exploratory research to fill this gap in knowledge. This will not only enhance our understanding of the medicinal potential and ecological significance of these NTFPs but also facilitate their utilization in various fields such as pharmacology, ethnobotany and conservation biology.

CHAPTER VI

6. Anti-bacterial activity of selected Ethnomedicinal NTFPs.

6.1. Introduction

Long before the discovery of microorganisms there was a widespread belief in the healing potential of certain plants some of which were thought to possess properties resembling what we now recognize as antimicrobial agents. Humans have relied on plants for their medicinal qualities since ancient times to combat infectious diseases, with many of these traditional remedies persisting to this day for their effectiveness in treating a variety of health issues (Rios and Recio, 2005). Medicinal plants serve as abundant reservoirs of antimicrobial compounds offering significant potential for drug development. Across various nations, these plants are harnessed for their medicinal properties presenting promising avenues for potent pharmaceuticals. Different parts of these plants exhibit diverse medicinal properties effective against a variety of microbes. Despite extensive testing of plant species for antimicrobial efficacy, a significant portion remains inadequately evaluated (Sivastava and Vietmeyer, 1997; Balandrin et al., 1985).

The emergence of drug resistance in human pathogens to conventional antibiotics underscores the urgency of exploring alternative antimicrobial sources such as plants. The systematic evaluation of medicinal plants for their antimicrobial properties and phytochemical composition is crucial for the discovery of novel therapeutic compounds (Erdogrul, 2002). Infectious diseases pose a significant burden on public health particularly in developing nations, contributing to high rates of illness and death. Consequently, pharmaceutical companies have been increasingly driven to develop novel antimicrobial medications spurred by the persistent emergence of microorganisms resistant to conventional treatments (Nascimento et al., 2000).

Evidently, bacterial species demonstrate genetic adaptability, enabling them to acquire and disseminate resistance to commonly prescribed antibacterial agents. This is evident in frequent reports of bacteria initially susceptible to standard therapies subsequently developing multidrug resistance against alternative medications

(Sakagami and Kajimura, 2002). Henceforth, prevalent tactics employed by pharmaceutical enterprises to meet the demand for new antimicrobial therapeutics involve modifying the molecular composition of existing medications. This alteration aims to enhance their efficacy or reinstate lost activity resulting from bacterial resistance mechanisms (Chartone-Souza, 1998).

In contemporary times, both the realms of cancer treatment and combating infectious diseases inclusive of fungal and bacterial infections have increasingly relied upon natural products. Among the 175 molecules constituting approved anticancer drugs, approximately 41% are either natural products or derivatives thereof sourced from animals (3%), medicinal plants (25%) and microorganisms (13%) (Thomford et al., 2018; Salehi-Sardoei and Khalili, 2022; Ahani and Attaran, 2022; Calixto, 2019). Numerous inherent challenges exist concerning the antimicrobial efficacy of medicinal plant extracts. The effectiveness of extraction methodologies which must be tailored to specific plant species directly impacts the quality and selectivity of the extracted compounds. Variability in results is often observed in antimicrobial susceptibility tests conducted on plant extracts. Furthermore, the development of novel antimicrobials from such extracts necessitates overcoming various obstacles. Despite efforts to enhance the antimicrobial activity of chemical compounds significant research endeavours are required to elucidate the mechanisms of action, interactions with other substances and the pharmacokinetic/pharmacodynamic profiles. Prioritizing these investigations is essential for characterizing these extracts as potential antimicrobial agents (Vaou et al., 2021).

Antimicrobial agents derived from medicinal plants possess the capability to inhibit the proliferation of bacteria, fungi, viruses and protozoa through mechanisms distinct from those exhibited by existing antimicrobial drugs. This unique mode of action holds considerable promise in addressing microbial strains that have developed resistance to current treatment modalities, thereby presenting valuable prospects for clinical intervention (Shankar et al., 2010). The antimicrobial efficacy of an agent primarily arises from two mechanisms: chemical interference with the synthesis or functionality of essential bacterial components and circumvention of conventional antibacterial resistance mechanisms. However, bacteria can inherently develop

resistance to multiple antimicrobial agents due to selective pressures or acquire resistance mechanisms from neighbouring microbes (Magiorakos et al., 2012; Velayati et al., 2009).

6.2. Methodology

6.2.1. Selection of bacterial strains

In this investigation, extracts derived from six selected ethnomedicinal NTFPs, as mentioned previously in Chapter 5, were subjected to test against five bacterial strains. These strains include two gram-positive bacteria, *Bacillus subtilis* (ATCC-11774) and *Micrococcus luteus* (ATCC-10240) and three gram-negative bacteria *Escherichia coli* (ATCC-10145), *Klebsiella pneumoniae* (ATCC-10031) and *Salmonella typhimurium* (ATCC 51812). The microorganisms were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, Punjab, India. Prior to experimentation, the microorganisms were sub-cultured in nutrient broth and incubated at 37°C for 24 hours.

6.2.2. Antimicrobial susceptibility test

The antimicrobial susceptibility of the plant extracts was evaluated using the disc diffusion method following the standard protocol outlined by Bauer et al. (1966). Bacterial cultures adjusted to a 0.5 McFarland standard were evenly spread onto the solidified Muller Hinton agar plates using a sterile swab. After drying for 15 minutes, discs impregnated with various plant extracts were placed onto the agar surface. Each disc was accompanied by a positive control containing a standard antibiotic ceftriaxone which is a broad-spectrum disc and a negative control was also included. The plates were then incubated at 37°C for 18 to 24 hours, depending on the bacterial species tested. Following incubation, the plates were examined for the presence of clear zone formed around the discs which indicates a positive antimicrobial activity and was measured and recorded. To ensure reliability, the experiment was repeated three times.

6.2.3. Minimum Inhibition Concentration Determination

The minimum inhibitory concentration (MIC) of the plant extract was determined using sterile 1.2ml 96-well plates employing resazurin as an indicator of bacterial

viability with slight modifications. Resazurin was prepared as a 10g/1 litre of sterile water stock solution and diluted at a ratio of 1:10 in sterile water as needed (Elshikh et al., 2016). Each well was filled with 0.5ml of sterilized Mueller Hinton Broth, followed by 0.5ml of the plant extract at various concentrations. Subsequently, 0.1ml of bacterial inoculum (at a concentration of 5×10^8 cfu/mL) was added to each well and thoroughly mixed. The plates were incubated at 37°C for 12 hours. Following this incubation period, 10µl of resazurin was added to each well and the plates were then further incubated for an additional 3-4 hours. Visual monitoring of colour changes in the wells was conducted thereafter. Pink coloration observed in wells after the modified resazurin assay indicated bacterial growth, whereas wells exhibiting blue coloration indicated the absence of growth (McNicholl et al., 2007).

6.2.4. Statistical analyses

In this chapter, one-way analysis of variance (ANOVA) was utilized and statistical analysis was performed using GraphPad Prism Software Version 8.

6.3. Results

6.3.1. Antimicrobial susceptibility test

The antibacterial activity of both methanolic and aqueous extracts from six plant samples was assessed against five bacterial strains. Methanolic extract of *M. malabathricum* (**Figure 6.4**) exhibited the highest inhibition zone against *Bacillus subtilis* (ATCC-11774) measuring 23 ± 0.4 mm, while *V. peduncularis* (**Figure 6.6**) showed the least inhibition measuring 15 ± 0.3 mm. Similarly, *M. malabathricum* ((**Figure 6.4**) methanolic extract demonstrated the highest inhibition against *Micrococcus luteus* (ATCC-10240) with a zone of 20 ± 0.3 mm while *B. roxburghii* (**Figure 6.1**) exhibited the least inhibition at 14 ± 0.4 mm. *C. caudatus* (**Figure 6.2**) methanolic extract displayed the highest inhibition zone against *Escherichia coli* (ATCC-10145) at 20 ± 0.3 mm, while *F. virosa* (**Figure 6.3**) showed the least inhibition at 12 ± 0.3 mm. Furthermore, *C. caudatus* (**Figure 6.2**) methanolic extract exhibited the highest inhibition against *Klebsiella pneumoniae* (ATCC-10031) at 20 ± 0.4 mm with *V. peduncularis* (**Figure 6.6**) displaying the least inhibition at 16 ± 0.3 mm. Additionally, the methanolic extract of *C. caudatus* (**Figure 6.2**)

demonstrated the highest inhibition zone against *Salmonella typhimurium* (ATCC 51812) at 19.2 ± 0.3 mm while *T. grandiflora* (**Figure 6.5**) exhibited the least inhibition at 13 ± 0.4 mm. The standard antibiotic ceftriaxone displayed an inhibition zone of 27 ± 0.3 mm.

The aqueous extract of *M. malabathricum* (**Figure 6.4**) exhibited the highest inhibition against *Bacillus subtilis* (ATCC-11774) with a zone measuring 18 ± 0.3 mm while *T. grandiflora* (**Figure 6.5**) displayed the least inhibition at 11 ± 0.4 mm. Furthermore, the aqueous extract of *B. roxburghii* (**Figure 6.1**) demonstrated the highest inhibition against *Micrococcus luteus* (ATCC-10240) with a zone of 17 ± 0.4 mm, whereas *T. grandiflora* (**Figure 6.5**) exhibited the least inhibition at 11 ± 0.3 mm. Additionally, the aqueous extract of *M. malabathricum* (**Figure 6.4**) displayed the highest inhibition against *Escherichia coli* (ATCC-10145) at 16 ± 0.3 mm, while both *T. grandiflora* (**Figure 6.5**) and *F. virosa* (**Figure 6.3**) exhibited the least inhibition at 10 ± 0.4 mm. The aqueous extract of *F. virosa* (**Figure 6.3**) achieved the best inhibition of *Klebsiella pneumoniae* (ATCC-10031) with a zone measuring 17.8 ± 0.4 mm, whereas *T. grandiflora* (**Figure 6.5**) displayed the least inhibition at 10 ± 0.3 mm. Moreover, the highest inhibition of *Salmonella typhimurium* (ATCC 51812) was observed with the aqueous extract of *B. roxburghii* (**Figure 6.1**) measuring 15 ± 0.3 mm while *T. grandiflora* (**Figure 6.5**) exhibited the least inhibition at 8.4 ± 0.4 mm. The standard antibiotic ceftriaxone displayed an inhibition zone of 25 ± 0.3 mm. Bacterial strains employed in this study are classified as opportunistic pathogens known for their ability to cause a range of diseases in susceptible hosts.

ANOVA was employed with a significance level set at $P < 0.05$. The analysis revealed that the methanolic extracts of *F. virosa* and *T. grandiflora* exhibited statistically significant inhibition against all five bacterial strains, whereas the remaining four plant species showed insignificant results. Furthermore, the aqueous extracts of *F. virosa*, *M. malabathricum*, and *B. roxburghii* displayed significant variation, while the remaining three were deemed insignificant.

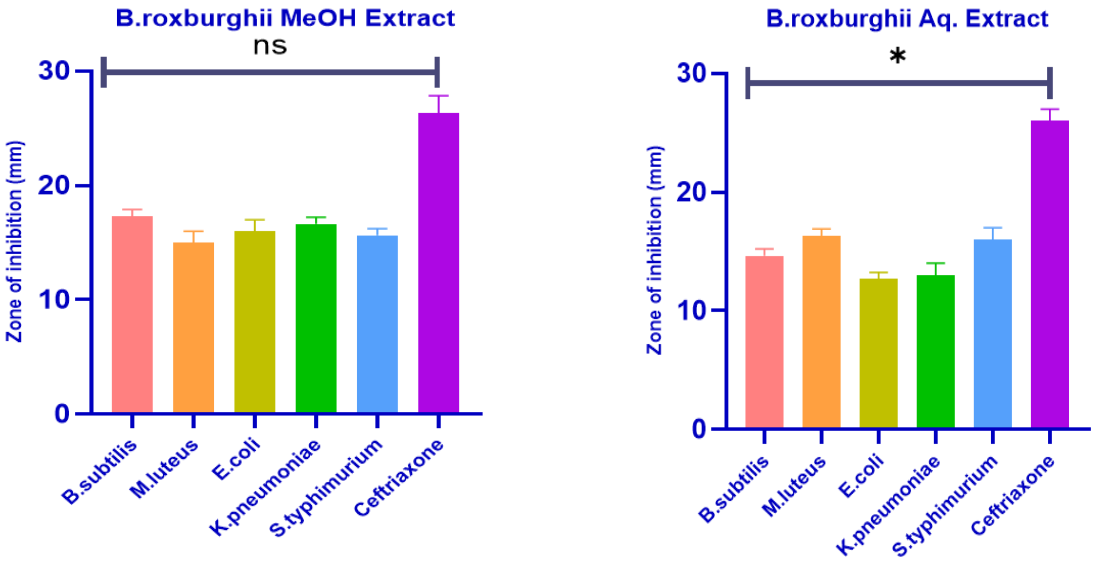


Figure 6.1: Methanol and aqueous extracts of *B. roxburghii* showing zone of inhibition (mm) against five bacterial strains at 30mg of plant extracts per 30 ml of Ceftriaxone.

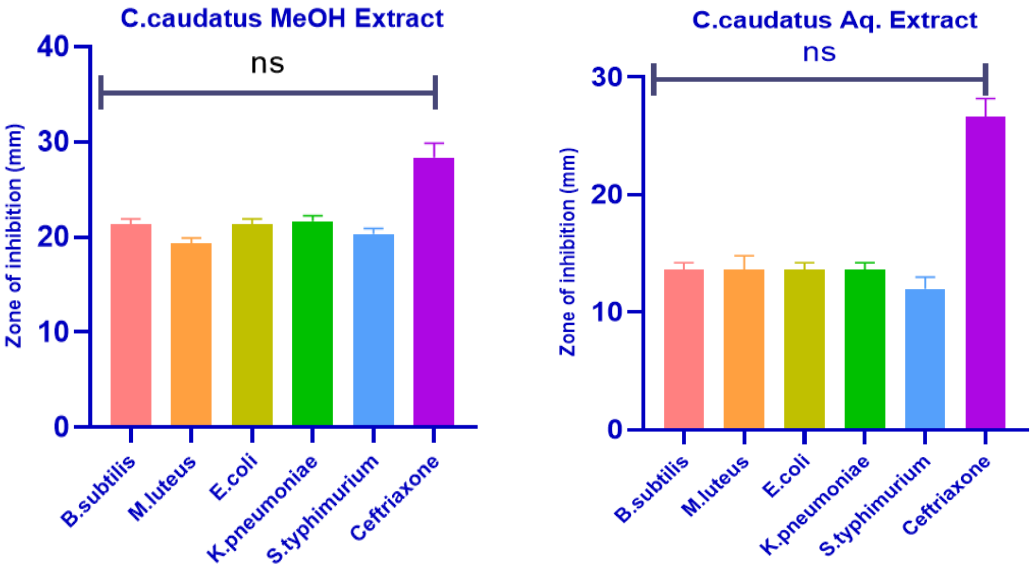


Figure 6.2: Methanol and aqueous extracts of *C. caudatus* showing zone of inhibition (mm) against five bacterial strains at 30mg of plant extracts per 30 ml of Ceftriaxone.

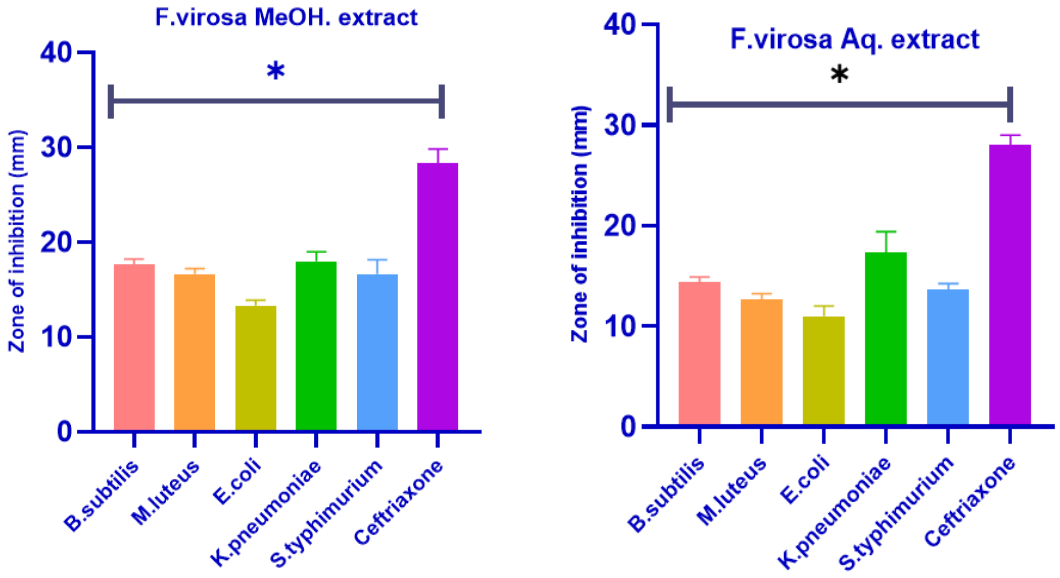


Figure 6.3: Methanol and aqueous extracts of *F. virosa* showing zone of inhibition (mm) against five bacterial strains at 30mg of plant extracts per 30 ml of Ceftriaxone

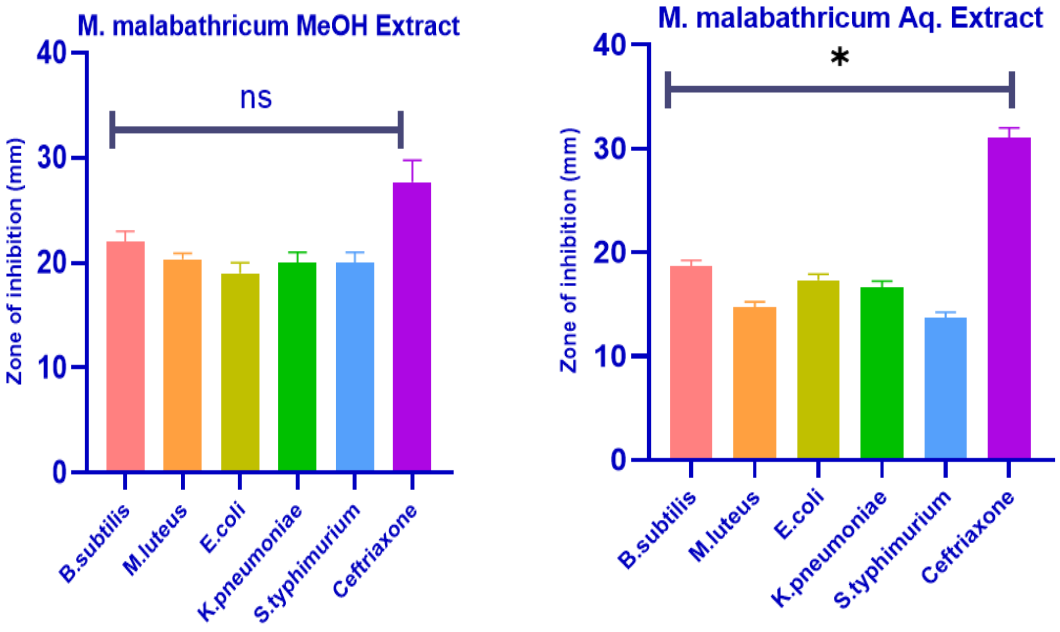


Figure 6.4: Methanol and aqueous extracts of *M. malabathricum* showing zone of inhibition (mm) against five bacterial strains at 30mg of plant extracts per 30 ml of Ceftriaxone.

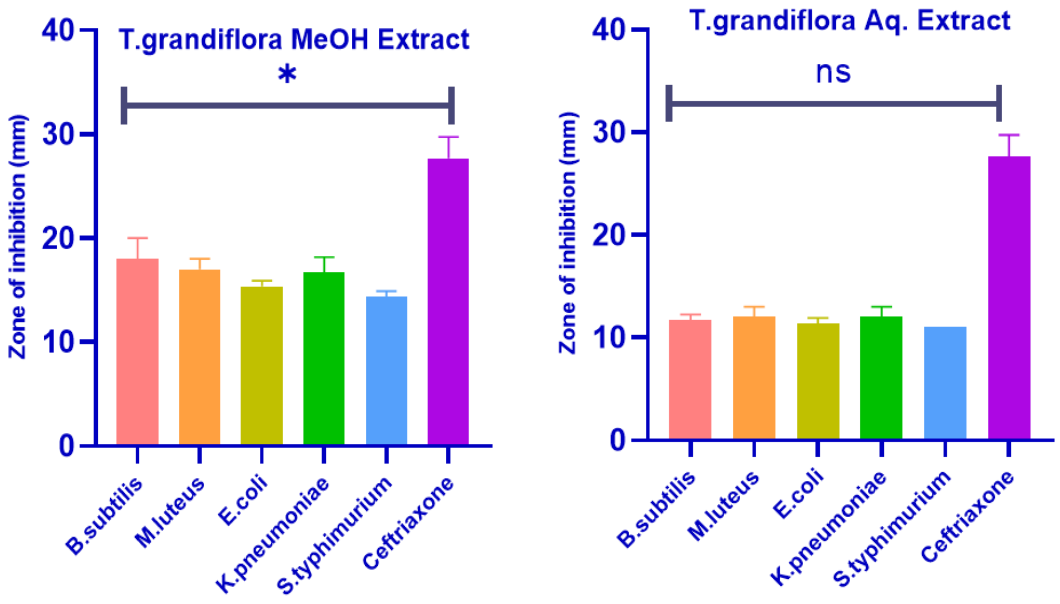


Figure 6.5: Methanol and aqueous extracts of *T. grandiflora* showing zone of inhibition (mm) against five bacterial strains at 30mg of plant extracts per 30 ml of Ceftriaxone.

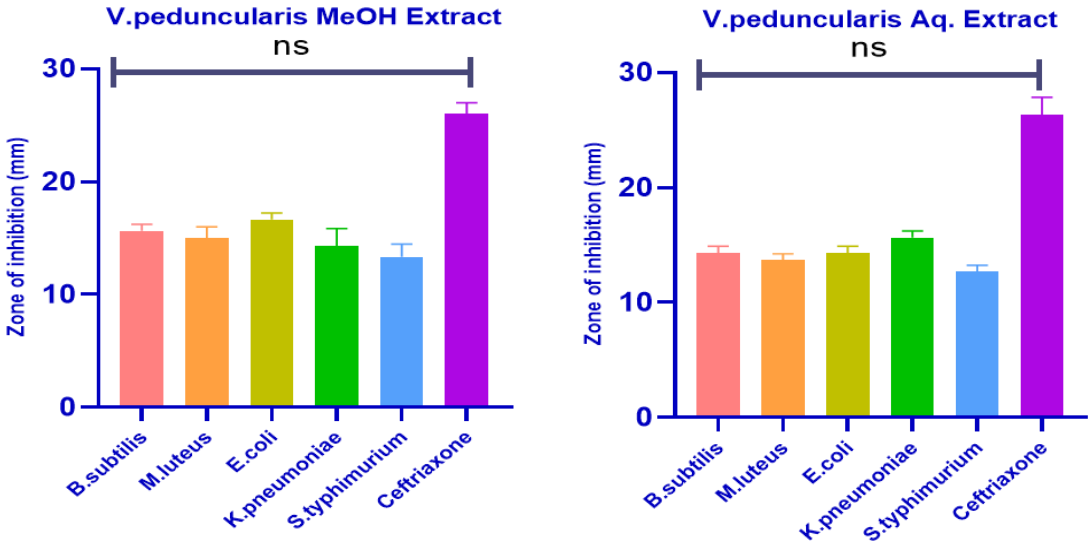


Figure 6.6: Methanol and aqueous extracts of *V. peduncularis* showing zone of inhibition (mm) against five bacterial strains at 30mg of plant extracts per 30 ml of Ceftriaxone.

*ANOVA was significant at 0.05 level.

Values are expressed as mean ± SD (n=3).

6.3.2. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was assessed for the methanolic extract derived from six ethnomedicinal NTFPs against five bacterial strains: *Bacillus subtilis* (ATCC-11774), *Micrococcus luteus* (ATCC-10240), *Escherichia coli* (ATCC-10145), *Klebsiella pneumoniae* (ATCC-10031) and *Salmonella typhimurium* (ATCC 51812). Following antimicrobial susceptibility testing, it was observed that the methanolic extracts exhibited superior efficacy compared to aqueous extracts. Consequently, methanolic extracts from the plant specimens were chosen for further investigation for MIC studies. The MIC of the plant extracts were tested in different concentrations (10, 8, 6, 4, 2, 0.5) mg/ml which were arranged from the top to bottom (highest to lowest). The determination of the minimum inhibitory concentration (MIC) involved identifying the lowest concentration of the extract at which a colour change was observed upon addition of resazurin. Visual monitoring of colour changes within the wells was conducted. Wells displaying a pink coloration following the incubation period indicated bacterial growth, whereas those exhibiting a blue hue indicated the absence of growth. In the experimental setup, Column 9 served as negative control which shows a change of resazurin natural color (blue/purple) to the reduced form (red-colourless). Column 11 functioned as positive control demonstrating the standard antibiotic Ceftriaxone's efficacy in inhibiting all five tested bacterial strains as evidenced by the blue coloration observed. The MIC values of both the highest and lowest tested against the bacterial strains were determined for each methanolic plant extract in the results (**Table 6.1**)

Table 6.1: Highest and lowest Minimum Inhibitory Concentration of methanolic plant extract against different bacteria.

Plant Species	**Highest MIC (mg/ml)**	**Lowest MIC (mg/ml)**
F. virosa	4 (*Bacillus subtilis*)	8 (*Escherichia coli*)
M. malabathricum	2 (*Bacillus subtilis*)	6 (*Escherichia coli*)
C. caudatus	4 (*Klebsiella pneumoniae*)	6 (*Micrococcus luteus*)
B. roxburghii	4 (*Bacillus subtilis*)	6 (*Micrococcus luteus*)
V. peduncularis	4 (*Escherichia coli*)	6 (*Salmonella typhimurium*)
T. grandiflora	6 (*Klebsiella pneumoniae*)	8 (*Salmonella typhimurium*)

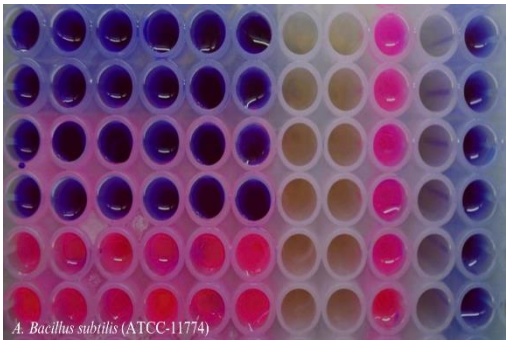
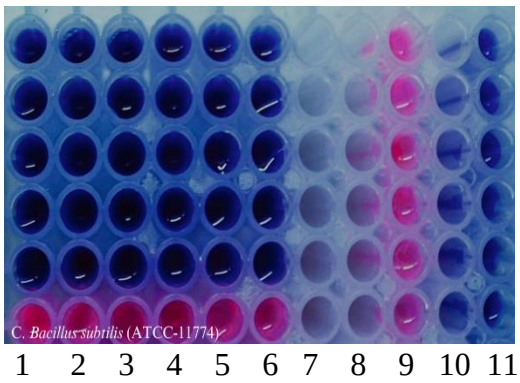


Figure 6.7 (A&B): Lowest and Highest MIC represented by *F. virosa*.



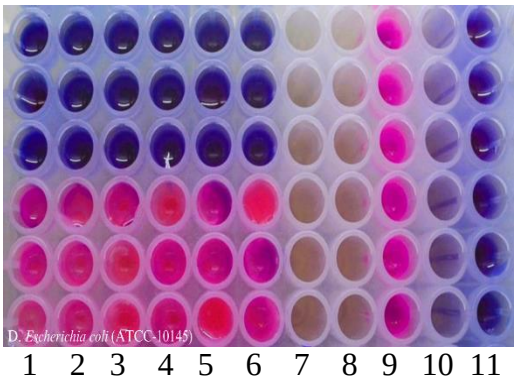
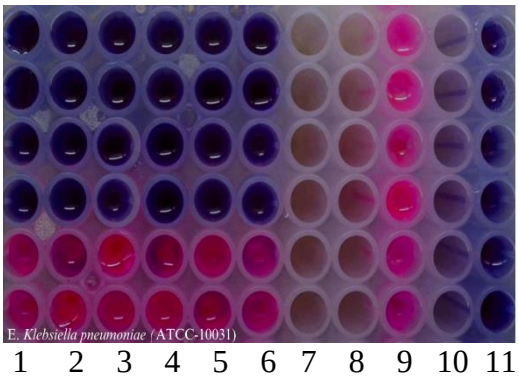


Figure 6.8 (C&D): Lowest and Highest MIC represented by *M. malabathricum*.



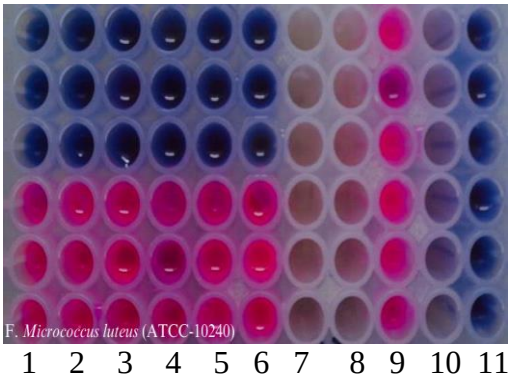
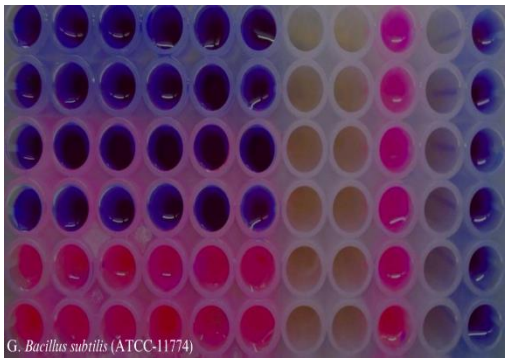


Figure 6.9 (E&F): Lowest and Highest MIC represented by *C. caudatus*.



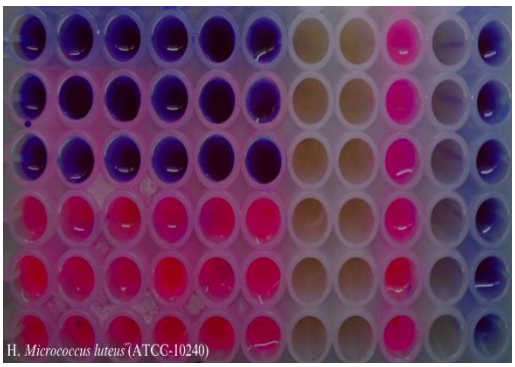
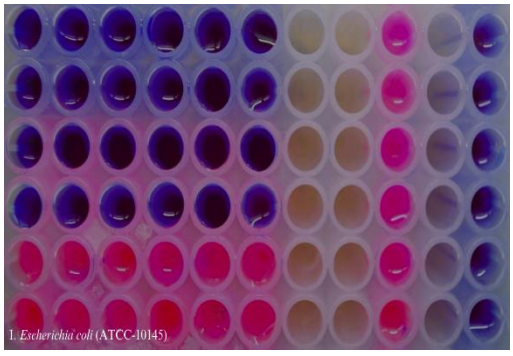


Figure 6.10 (G&H): Lowest and Highest MIC represented by *B. roxburghii*.



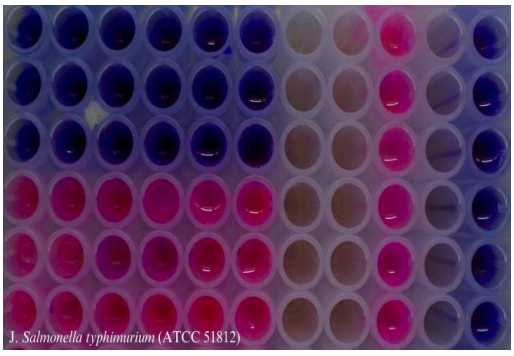
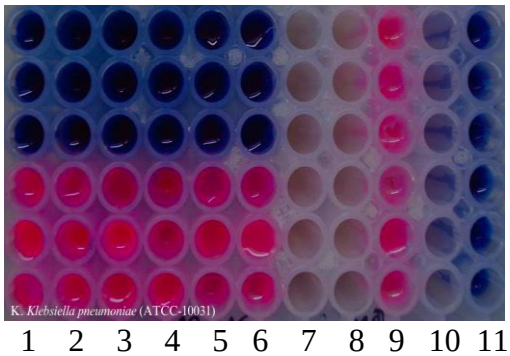


Figure 6.11 (I&J): Lowest and Highest MIC represented by *V. peduncularis*.



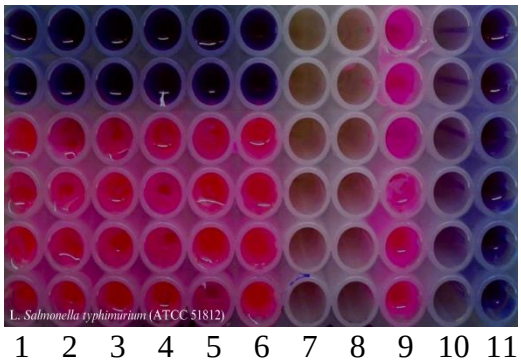


Figure 6.12 (K&L): Lowest and Highest MIC represented by *T. grandiflora*.

6.4. Discussions

The present findings provide valuable insights into the efficacy and potential of the six selected ethnomedicinal plants in inhibiting the growth of bacterial strains at a concentration of 30 mg/ml. A comparative analysis between the two types of extracts revealed that methanol extracts generally outperformed aqueous extracts. Multiple researchers have observed similar outcomes noting that methanol extracts of plants consistently yield superior results (Voravuthikunchai et al., 2004; Duraipandiyan and Ignacimuthu, 2006; Prashanth et al., 2001). This observed trend can be attributed to the differential solubility of bioactive compounds in methanol and water. Methanol being a polar solvent, has a higher capacity to extract a wider range of compounds including hydrophobic molecules, compared to water. Consequently, methanol extracts may contain a higher concentration of bioactive compounds with antimicrobial properties, leading to enhanced inhibition of bacterial growth. Additionally, certain bioactive compounds may be more readily soluble in methanol, further contributing to the superior efficacy of methanol extracts in inhibiting bacterial growth.

Overall, the antibacterial activity of the tested plant extracts exhibited greater activity against gram-positive microorganisms compared to gram-negative microorganisms. This finding is consistent with previous reports by multiple researchers (Buwa and Van Staden, 2006; Valsaraj, 1997; Srinivasan et al., 2001). The observed phenomenon of plant extracts displaying greater activity against gram-positive microorganisms compared to gram-negative ones can be attributed to differences in cell wall composition between these two types of bacteria (Samy and Raja 1999). Gram-positive bacteria possess a thicker peptidoglycan layer in their cell wall which is more susceptible to disruption by antimicrobial agents present in the plant extracts. In contrast, gram-negative bacteria have an additional outer membrane composed of lipopolysaccharides that acts as a barrier making them less permeable to antimicrobial compounds. Therefore, gram-positive bacteria may be more susceptible to the antimicrobial effects of plant extracts due to their simpler cell wall structure allowing for easier penetration and disruption of cellular functions (Parekh and Chanda, 2005). The current findings align with previous research by Amenu et

al. (2019), which reported antimicrobial activity in ethanol root extracts of *F. virosa,* ranging from 8.0 to 22.5 mm (mg/ml) for zone of inhibition and MIC values ranging from 3.13 to 25 mg/ml. Similarly, Dickson et al. (2006) documented significant antimicrobial activity in chloroform extracts of *F. virosa* exhibiting activity against 13 tested organismsnwith zone of inhibition ranging from 12 to 19 mm (5mg/ml) and MIC values ranging from 15.6 µg/mL to over 1000 µg/ml. Research conducted by Appridamayanti et al. (2021) demonstrated that ethanolic extracts of *M. malabathricum* exhibit antibacterial activity against *E. coli* and *B. subtilis* with clear zone diameters of 10.77 mm at 12.5 mg/ml and 11.08 mm at 25 mg/ml respectively. Lokendrajit et al. (2012) investigated various extracts of *C. caudatus* against both gram-positive and gram-negative human pathogenic bacteria as well as antifungal activity against human and plant pathogens. The study revealed diverse levels of antibacterial and antifungal activity across all extracts. Particularly, the ethanolic extract displayed antibacterial efficacy against all tested bacteria with zone diameters ranging from 8-12 mm at concentrations of 10 mg/ml. In their study, Kannathasan et al. (2011) reported that *V. peduncularis* exhibited the most potent activity against all screened microorganisms with zone of inhibition ranging from 11 ± 0.57 to 22.6 ± 0.66 mm and MIC values ranging between 62.5 to 1000 µg/ml. Furthermore, Islam et al. (2022) reported in their study that chloroform and methanolic crude extracts of *V. peduncularis* exhibited significant antibacterial activity against both gram-positive and gram-negative bacteria with zone of inhibition ranging from 8 to 15 mm at a concentration of 400 µg/disc. However, the available literature on the antimicrobial activity of *B. roxburghii* is limited making it challenging to draw comparisons with other studies. Ibrahim and Sleem, (2017) reported that *T. grandiflora* revealed marked antimicrobial activity showing zone of inhibition ranging from 16.3 ± 0.58 to 24.2 ± 1.2 mm (mg/ml).

This discrepancy in inhibition efficacy between different plant extracts may be attributed to variations in their chemical compositions. Factors such as the presence and concentration of bioactive compounds as well as their synergistic or antagonistic interactions can influence the antimicrobial activity of plant extracts. Additionally, variations in extraction methods and environmental factors during plant growth may

contribute to differences in extract potency. Further investigation into the specific bioactive compounds present in each extract and their mode of action against the target bacteria could provide deeper insights into these observed differences. Antibiotic side effects include nausea, pain, rashes on the skin, vomiting, headaches and nausea. Additionally, microbial pathogens are becoming resistant to drugs due to drug resistance. This has prompted research into medicinal compounds, including primary and secondary metabolites, which has resulted in the discovery of effective therapeutic compounds with minimal side effects (Ahmadi et al., 2022). The current study presents initial findings from antibacterial susceptibility testing. Further research is warranted to refine herbal compound formulations to ensure optimal bioavailability and suitability for physiological conditions.

Chapter VII

5. Documentation of RET species within the study area

5.1. Introduction

The increasing popularity of medicinal plants stems from their perceived safety and affordability compared to synthetic drugs (Ekor, 2014), thereby enhancing our comprehension of ethnobotanical and ethnomedicinal studies concerning herbal remedies. However, this heightened demand has placed considerable pressure on medicinal plant resources. Research indicates that the continued exploitation of various medicinal plant species has led to population declines in numerous high-value taxa (Sajem et al., 2008). Numerous plant species of significant medicinal importance have been identified in the Himalayan region of North-Eastern India, falling within the classification of Rare, Endangered, Threatened, Extinct, or Vulnerable plants as per the Red Data Book. This poses a substantial threat to the field of herbal medicine (Ray and Saini, 2022). Hence, there exists a pressing need to refine and strengthen existing methodologies and research programs aimed at facilitating the classification, conservation, management and utilization of plants by researchers. This necessity is especially crucial in tropical regions, characterized by the highest levels of botanical diversity. However, these areas are also the least explored and most imperilled, lacking adequate conservationists who are often insufficiently trained, and possessing limited resources for conservation efforts (Maxted et al., 1997). Due to the continuous growth of the human population and associated anthropogenic activities the rate of species extinction has escalated significantly, reaching levels hundreds or thousands of times higher than background extinction rates. This phenomenon underscores the urgent crisis of the 'sixth mass extinction' (Shivanna, 2020).

It is becoming more and more evident that despite the enormous efforts made in the last few decades to conserve plant diversity worldwide, our existing approaches have failed to stop the continuing decline in biodiversity. The World Conservation Union (IUCN; http://www.iucn.org) produces the IUCN Red List of Threatened Species

(henceforth, the "Red List") which identifies species that are most at risk of extinction and encourages their conservation by "concentrating minds on true priorities" (Collar, 1996). A resolution (RESWCC3.013) adopted by the World Conservation Congress mandates the emergence of applications for the Red List in national laws, international gatherings, conservation planning and research studies.

In India, over 90% of medicinal plants are endangered due to extensive and unsustainable harvesting practices, overexploitation, or unskilled collection methods (Kumari et al., 2011). Considering the global rates of plant species facing extinction, it is projected that approximately 1,000 medicinal plant species across various ecosystems in India could be at risk (FRLHTENVIS, 2016a). According to the IUCN Red List, among the 2,143 species listed under medicinal use for human and veterinary purposes a total of 457 species are identified. Among these, 73 species are categorized as threatened (Critically Endangered, Endangered, Vulnerable), 8 species are classified as Near Threatened (NT), 1 species is Data Deficient (DD) and 366 species are considered of Least Concern (LC). Despite numerous publications from organizations such as Conservation Assessment and Management Prioritization (CAMP), Botanical Survey of India (BSI) and IUCN listing threatened medicinal plants at various geographical scales, there lacks a consolidated compilation providing a comprehensive and accurate assessment of these species in one location (Gowthami et al., 2021).

Researchers are primarily concentrating on ethnobotanical and ethnomedicinal inquiries to meet the growing demand for herbal products. However, medicinal plants are currently experiencing significant pressure stemming from their excessive collection and exploitation. The ongoing exploitation of various medicinal plant species coupled with significant habitat loss has led to a decline in the populations of numerous high-value medicinal plant species over time (Kala and Sajwan, 2007). The level of endangerment facing natural populations of medicinal plants has intensified primarily due to the extraction of over 90% of medicinal plant raw materials for both India's herbal industries and export from their natural habitats (Dhar et al., 2002). The predominant risk to medicinal plants arises from human

activities particularly those involving the utilization of these plants which can impact biodiversity in various ways (Sajem and Nath, 2008).

5.2. Methodology

In this chapter, a structured protocol for data collection was not employed. Rather, the identification and documentation of the list of RET (Rare, Endangered, and Threatened) species were conducted primarily through fieldwork. This approach involved direct observation and recording of pertinent species, supplemented by the capture of photographic evidence. This methodology allowed for a comprehensive understanding of the RET species present within the study area facilitating accurate documentation and subsequent analysis. Also, the status of the plant species was assessed through https://www.iucnredlist.org/about/background-history.

5.3. Results

Throughout the entirety of our exhaustive research fieldwork, a total of six Rare, Endangered and Threatened (RET) species were documented within the study area. Among these, two species, *Saraca asoca* (Roxb.) W.J.de Wilde (**Figure 7.1**) and *Globba spathulata* Roxb. (**Figure 7.2**) were classified as Vulnerable. Additionally, two species, *Sophora wightii* Baker (**Figure 7.3**) and *Prunus ceylanica* (Wight) Miq. (**Figure 7.4**) were categorized as Endangered. Finally, the remaining two species, *Saurauia punduana* Wall. (**Figure 7.5**) and *Ilex khasiana* Purkay. (**Figure 7.6**) were identified as Critically Endangered. This comprehensive assessment provides valuable insights into the conservation status and biodiversity of the study area.

Figure 7.1: *Saraca asoca* (Roxb.) W.J.de Wilde (Vulnerable).

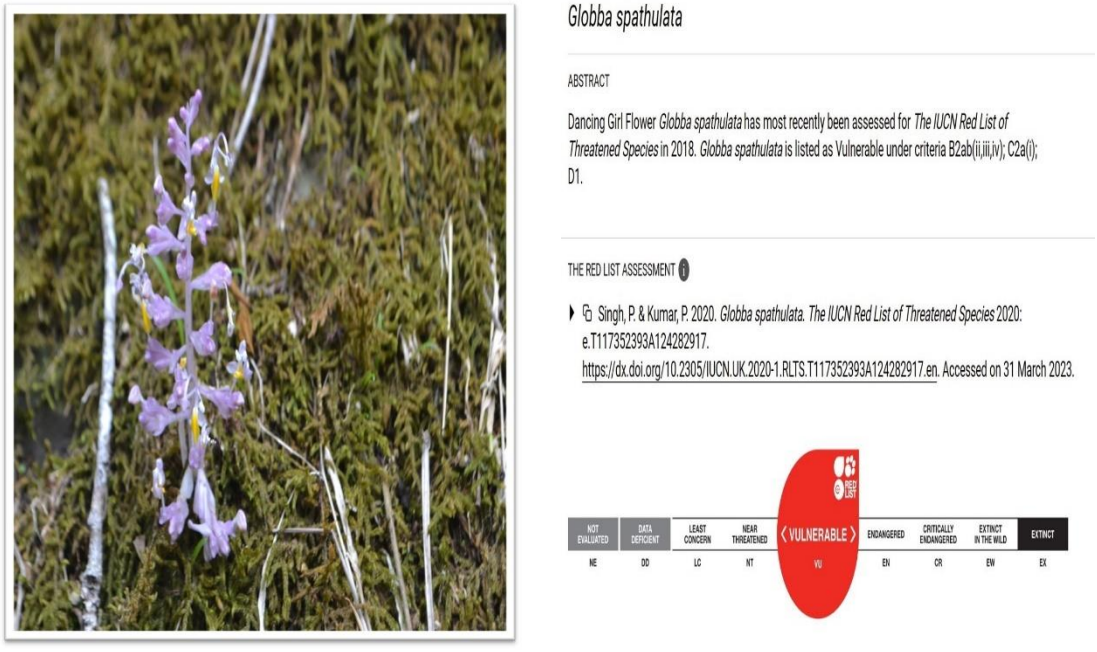


Figure 7.2: *Globba spathulata* Roxb. (Vulnerable).

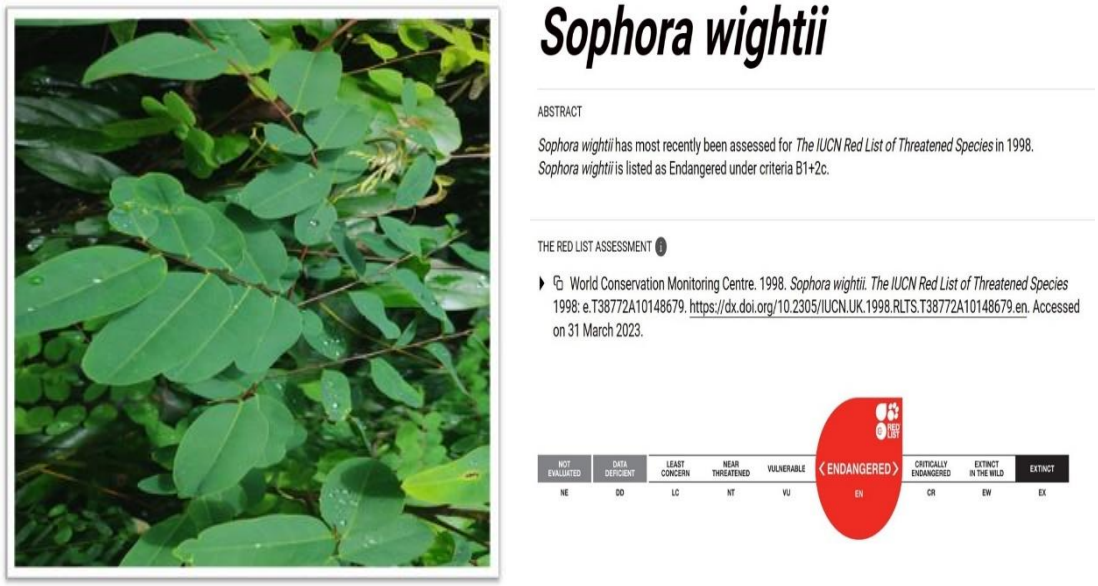


Figure 7.3: *Sophora wightii* Baker (Endangered).

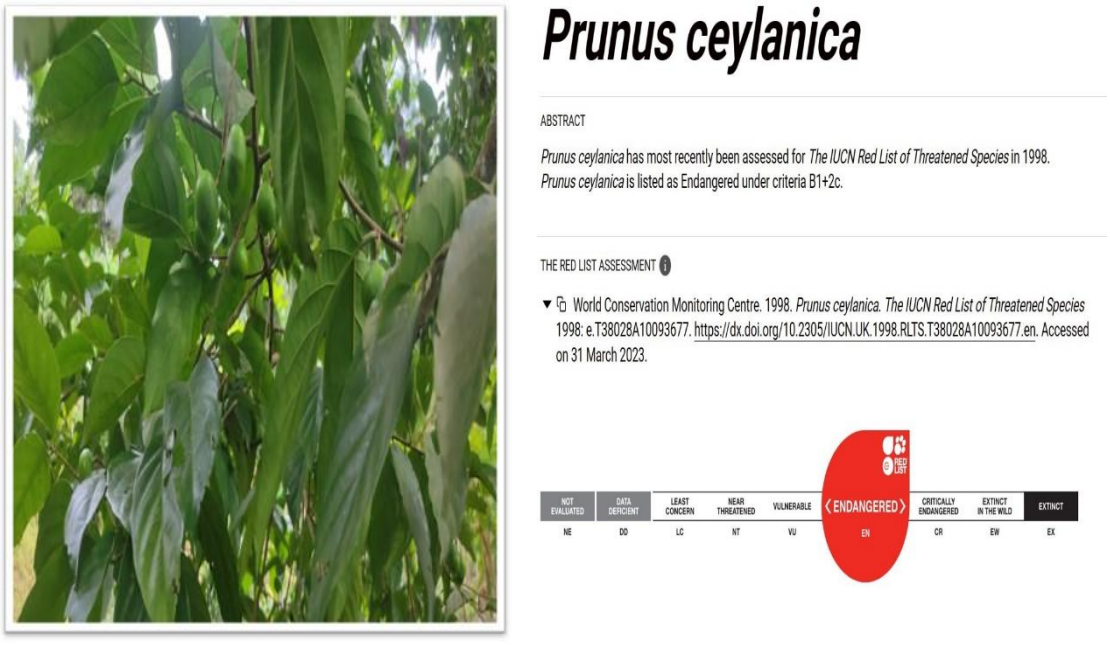


Figure 7.4: *Prunus ceylanica* (Wight) Miq. (Vulnerable).

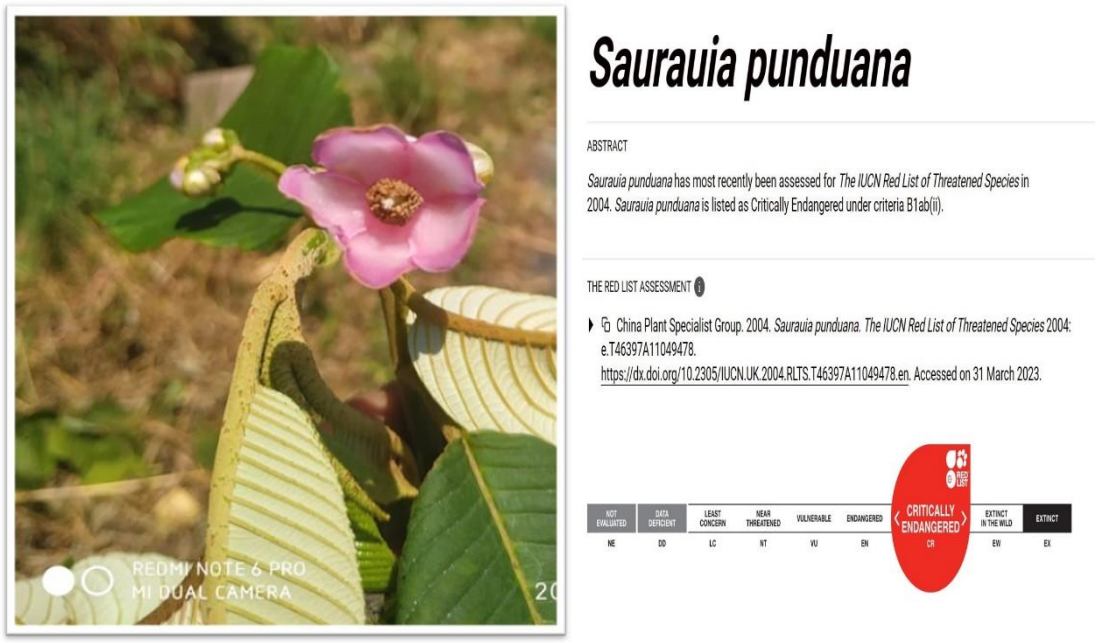


Figure 7.5: *Saurauia punduana* Wall. (Critically Endangered). *Ilex khasiana* Purkay.

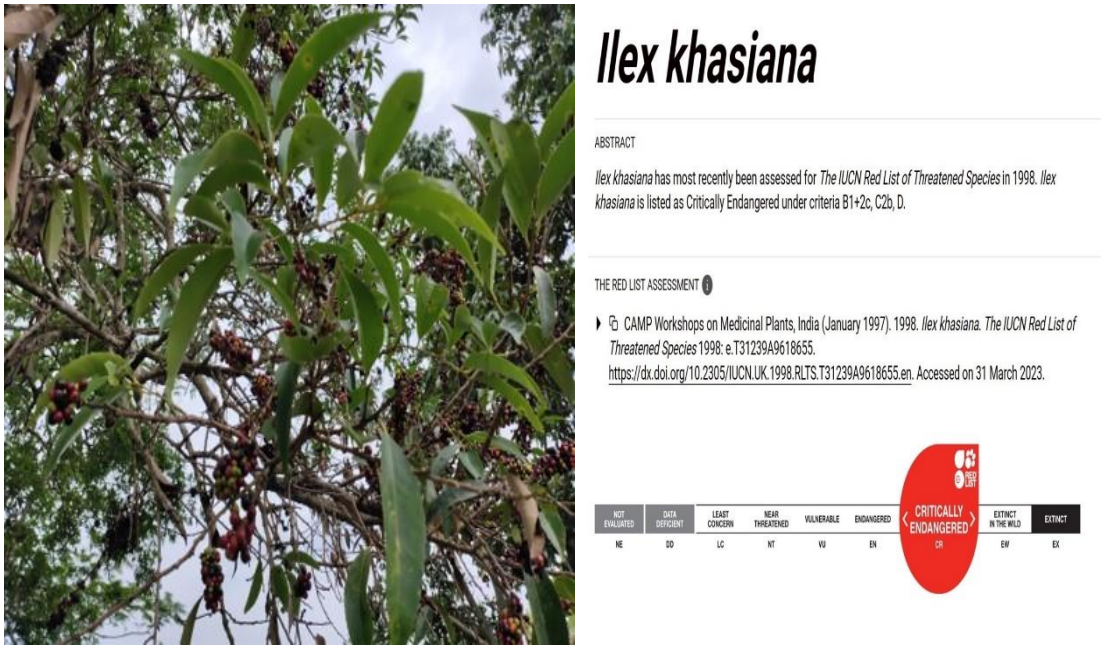


Figure 7.6: *Ilex khasiana* Purkay. (Critically Endangered).

7.4. Discussions

The present investigation indicated that Rare, Endangered and Threatened (RET) species offer valuable insights into the niches and ranges of rare, endemic, endangered and threatened species on a regional scale. This report serves as a tool for identifying areas and habitats with a high concentration of these species, thereby prioritizing critical habitats and sites for conservation efforts. Furthermore, it highlights the biodiversity richness of the study area (Thorangtlang Wildlife Sanctuary). Furthermore, these findings align with the objectives of the International Union for Conservation of Nature (IUCN), particularly regarding the conservation of plant species. The identification of Vulnerable, Endangered and Critically Endangered species within the study area underscores the urgent need for conservation measures to safeguard these plants and their habitats. By integrating these results into the broader context of IUCN conservation efforts, stakeholders can prioritize resources and interventions to mitigate threats and protect vulnerable plant species. This approach not only contributes to the preservation of biodiversity but also promotes sustainable management practices within the study area and similar ecosystems worldwide.

The absence of prior documentation regarding Rare, Endangered and Threatened (RET) plant species within the study area is noteworthy. However, it is regrettable to acknowledge that the scope of the present research was constrained by temporal limitations. Consequently, it remains plausible that additional plant species of conservation concern exist in this region, yet remain undiscovered.

Given this circumstance, it is imperative to emphasize for further exploration initiatives. Such endeavours should be accompanied by comprehensive awareness campaigns focused on RET plants. Additionally, it is crucial to implement conservation strategies that are accessible and comprehensible to local communities. By doing so, this can foster an environment of active participation and stewardship among residents, facilitating the protection of vulnerable plant species and their habitats effectively. This approach not only enriches our understanding of biodiversity but also promotes sustainable management practices essential for the long-term preservation of ecosystems within the study area.

Chapter VIII

8. Summary and Conclusions

Non-Timber Forest Products (NTFPs) encompass a diverse array of forest-derived resources, including plant and animal-based materials, whose tangible values often bypass formal economic systems. Throughout human history, these products have served myriad purposes. Additionally, they hold cultural significance intertwined with various traditional beliefs, healthcare and sustenance of indigenous communities, serving as vital resources in their livelihood practices. The current investigation was conducted within the confines of Thorangtlang Wildlife Sanctuary situated in Lunglei District, Mizoram. The current research entitled "**Study of Non-Timber Forest Products: Phenological and Phytochemical Analysis of Selected plant species within Lunglei District Mizoram**". The aim of the study was to elucidate the significance of NTFPs in the daily lives of rural communities residing in the vicinity of the sanctuary, as well as to assess the economic importance of these resources within their livelihood strategies.

The selection of Thorangtlang Wildlife Sanctuary as the study site was motivated by the absence of prior research in this region pertaining to the objectives of the present study. Comprising nine fringing villages, the communities residing within this area heavily rely on the sanctuary for their medical and healthcare requirements due to the lack of nearby medical facilities. Consequently, traditional practices deeply intertwined with local communities prevail prominently. Through a multifaceted approach encompassing direct field observation, semi-structured interviews and questionnaires, a comprehensive survey was conducted. This methodology facilitated the documentation of 63 ethnomedicinal NTFPs, gathered from insights provided by 91 informants (69 male and 22 female) with the respective ailments associated. The ethnomedicinal flora within the study area was predominantly represented by the Asteraceae family. Herbaceous plants comprised the largest proportion accounting for 36% of the total documented ethnomedicinal NTFPs in the present study followed by shrubs (28%), trees (21%), climbers (13%), and epiphytic ferns (2%).

It was observed that local informants utilized various modes of administration to implement herbal remedies, reflecting a diverse range of traditional practices. These included raw consumption, powder formulation, paste application, cooking, decoction preparation and juice extraction. Among these methods, juice extraction (36%) exhibited the highest utilization rate, representing of cases, followed by decoction (30%), cooking (17.50%), paste application (10%), raw consumption (4.60%) and powder ingestion (2.30%). This multifaceted approach underscores the rich traditional knowledge and practices surrounding ethnomedicinal plant utilization within the community residing in the study area. Majority of ethnomedicinal NTFPs were prepared mostly from the leaves (896 URs/39.33%), whole plant (519 URs/22.7%), root (340 URs/14.9%), rhizome (213 URs/9.3%), bark (158 URs/6.9%), stem (72 URs/3.16%), fruit (70 URs/3.07%) and latex (12 URs/0.53%).

Quantitative ethnobotanical indices like ICF, FL, FC, RFC, UR and UV were employed to assess and evaluate the information gathered from the informants. *M. malabathricum* emerged as the most prominently cited species, boasting 74 citations, indicative of its significant role in local traditional medicine practices. Close behind were *V. peduncularis* and *F. virosa* each garnering 70 citations. Similarly, *C. caudatus* received 69 citations, while *T. grandiflora* and *B. roxburghii* were cited 67 times each. In contrast, *A. indica* and *A. thyrsiflora* were cited the least, with only 11 citations each. This disparity in citation frequency suggests a relatively limited utilization of these species. UV values ranged from 0.12 to 0.813 across the species observed. *M. malabathricum* exhibited the highest UV values reaching 0.813, followed closely by *F. virosa* (0.78), *V. peduncularis* (0.76), *C. caudatus* (0.758), and *T. grandiflora* and *B. roxburghii* both registering at 0.736. The ICF values, ranging between 0 and 1, indicate the degree of consensus among informants regarding the efficacy of specific plants for addressing particular health concerns. Particularly noteworthy were the highest ICF values observed in the D-Digestive category, reaching 0.96. This consensus was drawn from 665 citations spanning 26 distinct plant species. Following closely was the R-Respiratory category, also displaying an ICF of 0.96, supported by 476 citations across 20 plant species. Additionally, the S-Skin category recorded an ICF of 0.93, with 391 citations

involving 25 different plant species. In contrast, the F-Eye category exhibited the lowest ICF value, standing at 0.69, based on 14 citations referencing 5 plant species. This disparity in ICF values across different health categories highlights variations in consensus among informants regarding the medicinal efficacy of plants for specific health concerns.

The phenological study serves as a crucial tool for understanding plant reproductive success and acts as a vital bio-indicator offering valuable temporal and spatial insights into climate change dynamics. The study meticulously documented the phenophases of six selected NTFPs over a span of three consecutive years. Five healthy individuals of each species were monitored to elucidate the timing and duration of various phenological events. The findings revealed that the initiation of leaf growth was most prevalent in January 2018 accounting for 66.6% of observations across all three years of investigation. Leaf fall, conversely, was predominantly observed from November to February, coinciding with the dry seasons. Notably, the species *B. roxburghii* exhibited the longest duration for flower bud formation, spanning 6-7 weeks. Furthermore, the study unveiled fluctuating flowering percentages across different months and years. The highest flowering percentage was recorded in June 2018, reaching 96.6%, followed by 83% in July 2019, and again at 77% in June 2021. These findings underscore the dynamic nature of plant phenology and its intricate relationship with seasonal variations and climatic patterns. A correlation between climatic variables and recorded phenological phases was observed although variability was noted across the three years of study.

The six selected ethnomedicinal Non-Timber Forest Products (NTFPs) underwent further research investigation, involving the preparation of methanolic and aqueous extracts for experimentation. Across the board, the methanolic extracts demonstrated more promising outcomes in comparison to aqueous extracts. Analysis of these extracts revealed the presence of eight phytoconstituents, including alkaloids, carbohydrates, saponins, phenols, tannins and flavonoids in the methanolic extracts of all six ethnomedicinal NTFPs. Furthermore, both the methanolic and aqueous extracts tested negative for phytosterols and amino acids. The quantitative analysis of methanolic plant extracts revealed remarkably high levels of phenols and flavonoids,

with the total phenolic content being particularly notable. Among the methanol extracts, *C. caudatus* exhibited the highest total phenolic content at 155 ± 0.5 mg GAE/g while *M. malabathricum* displayed the highest flavonoid content at 139.6 ± 1.8 mg QE/g. In contrast, among the aqueous extracts, *V. peduncularis* demonstrated the highest phenolic content at 139 ± 2.5 mg GAE/g while *M. malabathricum* showcased the highest flavonoid content at 126 ± 2 mg QE/g. These findings underscore the remarkable efficacy of these selected plants and their potential antioxidant properties. The methanol and aqueous extracts from various plants exhibited robust antioxidant activity demonstrating significant scavenging effects on DPPH and ABTS radicals. Furthermore, the DPPH and ABTS radical scavenging activities of the methanol extracts from all nine samples were observed to follow a dose-dependent pattern. Out of the six selected ethnomedicinal NTFPs, methanolic and aqueous extract of *B. roxburghii* and *V. peduncularis* had the strongest DPPH scavenging activity with IC_{50}= 19.5 ± 0.14 µg/ml and 50 ± 0.13 µg/ml respectively. Additionally, methanolic and aqueous extract of *V. peduncularis* and *M. malabathricum* shows the highest ABTS scavenging activity with IC50= 32.47 ± 0.11 µg/ml and 59 ± 0.11 µg/ml respectively. In the methanolic extract, a robust positive correlation of statistical significance was observed between TPC and DPPH activity (R^2 = 0.97, p = 0.001**). Similarly, a significant correlation was found between TFC and ABTS activity (R^2 = 0.83, p = 0.039*). Moreover, in the aqueous extract, a similar significant correlation was identified between TPC and DPPH activity (R^2 = 0.82, p = 0.022*) as well as between TFC and DPPH activity (R^2 = 0.75, p = 0.043*). A strong positive correlation between TPC and DPPH scavenging activity and between TFC and ABTS scavenging activity, indicates that higher levels of phenolic compounds and flavonoids in the extract are associated with greater antioxidant activity. This correlation suggests that phenolic compounds and flavonoids could be the major contributors to the antioxidant potential of the plant extract and their presence in higher concentrations enhances the ability of the extract to scavenge free radicals and protect against oxidative damage.

Antibacterial screening was conducted against five bacterial pathogens two gram positive and three gram negative bacteria: *Bacillus subtilis* (ATCC- 11774),

Micrococcus luteus (ATCC- 10240), *Escherichia coli* (ATCC- 10145) *Klebsiella pneumoniae* (ATCC- 10031) *Salmonella typhimurium* (ATCC 51812). All the methanolic and aqueous plant extracts shows their potential zone of inhibition against the bacterial strains. ANOVA was employed with a significance level set at $P < 0.05$. The analysis revealed that the methanolic extracts of *F. virosa* and *T. grandiflora* exhibited statistically significant inhibition against all five bacterial strains, whereas the remaining four plant species showed insignificant results. Furthermore, the aqueous extracts of *F. virosa, M. malabathricum,* and *B. roxburghii* displayed significant variation while the remaining three were deemed insignificant.

A total of six Rare, Endangered, and Threatened (RET) species were documented within the study area. Among these, two species, *Saraca asoca* (Roxb.) W.J.de Wilde and *Globba spathulata* Roxb. were classified as Vulnerable. Additionally, two species, *Sophora wightii* Baker and *Prunus ceylanica* (Wight) Miq. were categorized as Endangered. Finally, the remaining two species *Saurauia punduana* Wall. and *Ilex khasiana* Purkay. were identified as Critically Endangered.

The research endeavours to document ethnomedicinal Non-Timber Forest Products (NTFPs) utilized by indigenous informants residing in the peripheral villages proximate to Thorangtlang Wildlife Sanctuary. Acknowledging the expansive scope and intricacies inherent in the term NTFPs, the study deliberately focuses on ethnomedicinal NTFPs to delineate precise objectives. It elucidates the profound reliance of local communities on ethnomedicinal NTFPs to address their healthcare needs rooted deeply in traditional practices and a diverse reservoir of knowledge aimed at managing various ailments. This investigation demonstrates the integral role of ethnomedicinal NTFPs in fulfilling the medical and healthcare requirements of local populations. The dependence on these resources is intertwined with traditional practices reflecting a symbiotic relationship between human communities and their natural environment. Furthermore, the utilization of ethnomedicinal NTFPs underscores the adaptation of indigenous knowledge systems to local ecological contexts, enhancing community resilience and self-sufficiency in healthcare provision.

Analysis of the research findings indicates the presence of potential bioactive compounds within the investigated plant species. Nevertheless, a deeper phytochemical exploration is imperative to isolate bioactive molecules with diverse pharmacological activities. This study advocates for the integration of contemporary methodologies with traditional knowledge systems aiming to facilitate the utilization of NTFPs for the development of novel pharmaceutical agents. The introduction of modern techniques is closely connected with indigenous wisdom holds promise in enhancing the efficacy and sustainability of drug discovery endeavours. This approach not only underscores the importance of preserving traditional knowledge but also underscores its potential to inform and enrich modern scientific practices, thereby fostering synergistic collaborations between indigenous communities and scientific researchers.

Phenological data offers a valuable avenue for acting as a meaningful biological indicator in forecasting forthcoming climatic fluctuations and alterations in biodiversity. Such observations offer insights into the timing of natural events in plant life cycles, including flowering, leaf emergence and fruiting which are influenced by environmental cues. These cues can include temperature, precipitation and photoperiod. By analysing short-term phenological trends, researchers can glean crucial information about how plants respond to immediate environmental fluctuations, providing early warnings of ecological shifts and potential impacts on plant survival and reproductive success. However, for a comprehensive understanding of plant stability in response to environmental factors, a long-term study of phenology is indispensable. Long-term observations allow for the detection of subtle trends and patterns that may not be evident in short-term datasets. Furthermore, they enable researchers to assess the resilience of plant populations to prolonged environmental changes and to elucidate complex interactions between phenological shifts and ecosystem dynamics. Consequently, a combination of short-term and long-term phenological studies is recommended to capture both immediate responses and enduring trends, thereby facilitating informed conservation and management strategies in the face of environmental change.

Given the continued reliance of local communities on traditional medicines during the course of our interviews, informants expressed a keen interest in understanding the therapeutic properties of the plants they have been using for various ailments. Therefore, we have a profound obligation to disseminate the findings of the research, raising awareness regarding the efficacy of these plants and emphasizing the imperative for their conservation. This engagement serves not only to provide valuable insights to the local population but also to foster a sense of stewardship and sustainable utilization of these plant resources, aligning with principles of ethnomedicinal preservation and biodiversity conservation. It is highly advisable to undertake a thorough documentation and inquiry into the traditional knowledge concerning the utilization of medicinal plants. Such efforts constitute an essential initial step in the progression of pharmacological research and the quest for novel drug development. Consequently, there exists a pressing need for further scientific exploration into these medicinal plant species, encompassing investigations into their phytochemical compositions, biological attributes, and subsequent clinical assessments.

APPPENDICES

Appendix I

Questionnaires on Ethnomedicinal NTFP's Data Collection

1. Details of Informants

   Name/Hming : …….

   Gender : Male/ Female

   Age/Kum : …….

   Educational Level : …….

2. Documentation of ethnomedicinal NTFPs

   Local name of the plant/ thlai hming : …….

   Part used as medicine/ damdawi atan a hman lai : …….

   Mode of consumption/ damdawi atan a hman dan : …….

   Ailment category/ Natna bik atan a hmanna : …….

I ..... hereby give my full consent and willingly accepted to participate in this study and declare that the information provided by me during the course of interview was true and accurate to the best of my knowledge.

Appendix II

List of Publications

1. Lalbiaknii, P.C., Ngamlai E.V., Lalnunmawia F., Ralte V., Vanlalnunpuia P.C., Pachuau J.L. (2022). Morphological assessment and partial genome sequencing inferred from matK and rbcL genes of the plant Tacca chantrieri (2022). *Journal of Threatened Taxa* 18(8): 21696-21703.

2. Lalbiaknii, P.C., Ngamlai E.V., Rinmuana L., Vanlalnunpuia P.C., Lalnunmawia F., Ralte V., (2023). *In silico* validation and pharmacological activity of potent anti viral and anti inflammatory ethnomedical plants used by traditional herbalists within Thorangtlang Wildlife Sanctuary. *International Journal of Pharmaceutical Sciences and Research* 14(5): 2385-2400.

3. Vanlnunpuia P.C., Lalzarzovi S.T., Lalbiaknii P.C., Pachuau J.L (2022). An evaluation of anthropogenic impacts using remote sensing approach on forest coverage of Pualreng Wildlife Sanctuary, Mizoram, India. *Applied Ecology and Environmental Sciences* 10(1): 19-24

4. Vanlnunpuia P.C., Lalzarzovi S.T., Lalbiaknii P.C., Pachuau J.L (2022). Assessment of tree species composition and diversity of core and buffer zones in Pualreng Wildlife Sanctuary, Mizoram, India. *Indian Journal of Ecology* 48(4): 1056-1061

5. Ngamlai E.V., Lalbiaknii P.C., Vanlalpeka R., Ralte V., Lalnunmawia F (2022). Phytochemical and pharmacognostic study of *Hedyotis scandens* Roxb. from Mizoram, Northeast India. *Research Journal of Pharmacy and Technology* 15(12)

6. Ngamlai E.V., Lalthanpuii P., Lalbiaknii P.C., Ralte V., Lalnunmawia F (2022). Antioxidant property and free radical scavenging activity of *Hedyotis scandens* Roxb.Rubiaceae. *Current Trends in Biotechnology and Pharmacy* 16: 46-45.

7. Ngamlai E.V., Lalbiaknii P.C., Lalchhandama K., Ralte V., Lalnunmawia F (2022). In vitro antioxidant and phytochemical screening of *Hedyotis scandens* Roxb. Rubiaceae. *Science and Technology Journal* 10(2)

8. Ngamlai E.V., Pradhan R.B., Lalbiaknii P.C., Ralte V., Lalnunmawia F., Vanlalhluna P.C., Mehta S.K (2023). Diuretic Activity Evaluation and Chemical Composition Analysis of *Hedyotis scandens* Extract from Mizoram, India, in Rat Models. *Journal of Ethnopharmacology* 319(1)

Appendix III

List of Presentations

1. Presented paper (poster) on "*Tacca chantrieri*, a rare species of the Wild" at the International Conference on "Chemical Ecology, Environment and Human Health: Emerging Frontiers and Synthesis (ICCEEHH), 2019 organized by Department of Zoology, Sikkim University on August 9-10, 2019.

2. Presented paper (oral) on "*In silico* validation and pharmacological activity of potent anti-viral and anti-inflammatory ethnomedical plants used by traditional herbalists within Thorangtlang Wildlife Sanctuary" at the National seminar on Plant Taxonomy and Traditional knowledge in the Himalayas and Northeast India and Annual Conference of East Himalayan Society for Spermatophyte Taxonomy (EHHST). Jointly organized by Department of Botany, Rajiv Gandhi University, East Himalayan Society for Spermatophyte Taxonomy on February 21-22, 2022.

3. Presented paper (poster) on "Study on the effect of Selected Herbicides GLV-71 and Cut Off on Growth and Biochemical Activity of *Burkholderia* sp." at the 12th Annual Convention of Association of Biotechnology and Pharmacy (ABAP) & International Conference on Biodiversity, Environment and Human Health: Innovations and Emerging Trends (BEHIET 2018) organized by School of Life Sciences, Mizoram University, Aizawl and Association of Biotechnology and Pharmacy (ABAP), India on November 12-14, 2018.

4. Presented paper (oral) on "Study of Non-Timber Forest Products: Phenological and Phytochemical Analysis of Selected Plant Species within Lunglei District, Mizoram" at the International Workshop on Research and Project Development (PUC Research Conclave) on January 24-25, 2023.

5. Presented paper (oral) on "An Evaluation of Anthropogenic impacts Using Remote Sensing Approach on Forest Coverage of Pualreng Wildlife Sanctuary" at the International Conference on Environment, Agriculture and Biotechnology (ICEABT) held in Bareilly, India on October 20, 20221.

Appendix IV

Conferences/ Seminars/ Workshops Attended

1. Participated in National level workshop on "Statistical and Computing Methods for Life-Science Data Analysis" held during 5th - 10th March, 2018 jointly organized by Biological Anthropology Unit, Indian Statistical Institute, Kolkata and Department of Botany, Mizoram University, Aizawl.

2. Participated in One Day National Workshop on "IPR and Plant Protection with special reference to NE India" jointly organized by Department of Botany, Mizoram University and Depart of Horticulture, Government of Mizoram on 18th December, 2019.

3. Participated in Two- Days National Workshop on "Biodiversity Loss and Climate Change" jointly organized by Department of Environmental Science, Pachhunga University College and Rajiv Gandhi National Institute of Youth Development Programme (YLSDP) in Higher Educational Institutions from 9th - 10th February, 2021.

4. Participated in Two- Days National Workshop on plant diversity of NE India with special reference to Mizoram organized by Department of Botany & Department of Life Sciences, Pachhunga University College, Mizoram University in collaboration with Govt. Zirtiri Residentail Science College from 23rd – 24th May, 2023.

5. Participated in "Introduction to Computational Drug Design (Theory-Demo-Hands-on)" co-organizes by Schrodinger & Pharmacy Council of India between 21st September – 23rd October, 2020.

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NAME OF THE CANDIDATE	: P.C. LALBIAKNII
DEGREE	: Ph.D
DEPARTMENT	: Botany
TITLE OF THESIS	: STUDY OF NON-TIMBER FOREST PRODUCTS: PHENOLOGICAL AND PHYTOCHEMICAL ANALYSIS OF SELECTED PLANT SPECIES WITHIN LUNGLEI DISTRICT, MIZORAM
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Head

Department of Botany

ABSTRACT

STUDY OF NON-TIMBER FOREST PRODUCTS: PHENOLOGICAL AND PHYTOCHEMICAL ANALYSIS OF SELECTED PLANT SPECIES WITHIN LUNGLEI DISTRICT, 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

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STUDY OF NON-TIMBER FOREST PRODUCTS: PHENOLOGICAL AND PHYTOCHEMICAL ANALYSIS OF SELECTED PLANT SPECIES WITHIN LUNGLEI DISTRICT, MIZORAM

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Submitted

In partial fulfillment of the requirements of the Degree of Doctor of Philosophy in Botany of Mizoram University, Aizawl

Abstract

Non-Timber Forest Products (NTFPs) constitute a diverse range of resources derived from forests, including plant and animal-based materials, often overlooked within formal economic frameworks. These products have historically served various purposes and hold cultural significance, deeply embedded in traditional beliefs, healthcare practices and sustenance of indigenous communities. The present study entitled "Study of Non-Timber Forest Products: Phenological and Phytochemical Analysis of Selected Plant Species within Lunglei District, Mizoram," was conducted within the confines of Thorangtlang Wildlife Sanctuary in Lunglei District, Mizoram. The aim was to explore the importance of NTFPs in the daily lives of rural communities residing near the sanctuary and assess their economic significance within local livelihood strategies. The study encompasses the following objectives:

1. Documentation and quantitative ethnobotanical study of important Ethnomedicinal NTFPs within the study area.

2. Phenological observation of selected Ethnomedical NTFPs.

3. Qualitative and quantitative phytochemical analysis and free- radical scavenging activity of selected Ethnomedicinal NTFPs.

4. Anti-bacterial activity of selected Ethnomedicinal NTFPs.

5. Documentation of RET species within the study area.

The selection of Thorangtlang Wildlife Sanctuary as the study site was driven by the absence of prior research aligning with the objectives of this study. Home to nine fringing villages, the communities residing within this region heavily depend on the sanctuary for their medical needs, given the limited availability of nearby healthcare facilities. As a result, traditional healthcare practices deeply rooted in local customs are prevalent. Employing a multifaceted approach including direct field observations, semi-structured interviews and questionnaires, a comprehensive survey was conducted. This methodology facilitated the documentation of 63 ethnomedicinal NTFPs, obtained from insights shared by 91 informants (69 male and 22 female) along with their respective ailments. The ethnomedicinal flora within the study area

was predominantly represented by the Asteraceae family. Herbaceous plants constituted the largest proportion encompassing 36% of the total documented ethnomedicinal NTFPs in this study followed by shrubs (28%), trees (21%), climbers (13%) and epiphytic ferns (2%).

The local informants demonstrated a variety of administration methods for herbal remedies reflecting a diverse array of traditional practices. These methods included raw consumption, powder formulation, paste application, cooking, decoction preparation and juice extraction. Among these approaches, juice extraction (36%) was the most frequently utilized followed by decoction (30%), cooking (17.50%), paste application (10%), raw consumption (4.60%) and powder ingestion (2.30%). This multifaceted approach highlights the extensive traditional knowledge and practices surrounding the utilization of ethnomedicinal plants within the community residing in the study area. The majority of ethnomedicinal NTFPs were primarily prepared from leaves (896 URs/39.33%), whole plant (519 URs/22.7%), root (340 URs/14.9%), rhizome (213 URs/9.3%), bark (158 URs/6.9%), stem (72 URs/3.16%), fruit (70 URs/3.07%) and latex (12 URs/0.53%).

Quantitative ethnobotanical indices including ICF, FL, FC, RFC, UR and UV were utilized to assess and evaluate the information provided by informants. *Melastoma malabathricum* emerged as the most frequently cited species with 74 citations underscoring its significant role in local traditional medicine. Following closely were *Vitex peduncularis* and *Flueggea virosa* each with 70 citations. Similarly, *Croton caudatus* received 69 citations while *Thunbergia grandiflora* and *Begonia roxburghii* were cited 67 times each. In contrast, *Aeginetia indica* and *Aganope thyrsiflora* were cited the least, with only 11 citations each suggesting limited utilization of these species. UV values ranged from 0.12 to 0.813 across observed species. *M. malabathricum* exhibited the highest UV value reaching 0.813 followed closely by *F. virosa* (0.78), *V. peduncularis* (0.76), *C. caudatus* (0.758) and *T. grandiflora* and *B. roxburghii* both registering at 0.736.

ICF values, indicating the degree of consensus among informants regarding the efficacy of specific plants for addressing particular health concerns ranged between 0

and 1. Particularly notable were the highest ICF values observed in the D-Digestive category, reaching 0.96, supported by 665 citations spanning 26 distinct plant species. R-Respiratory ailment category, also displayed an ICF of 0.96 supported by 476 citations across 20 plant species. Additionally, the S-Skin category recorded an ICF of 0.93 with 391 citations involving 25 different plant species. In contrast, the F-Eye category exhibited the lowest ICF value standing at 0.69 based on 14 citations referencing 5 plant species. This disparity in ICF values across different health categories underscores variations in informant consensus regarding the medicinal efficacy of plants for specific health concerns.

The phenological study serves as a crucial tool for comprehending plant reproductive success and acts as a vital bio-indicator providing valuable insights into climate change dynamics over time and space. The study meticulously documented the phenophases of six selected NTFPs over three consecutive years. Five healthy individuals of each species were monitored to elucidate the timing and duration of various phenological events.

The findings revealed that leaf growth initiation was predominantly observed in January 2018, comprising 66.6% of observations across all three years. Leaf fall, on the other hand was primarily observed from November to February coinciding with dry seasons. Notably, *B. roxburghii* exhibited the longest duration for flower bud formation spanning 6-7 weeks. Furthermore, the study uncovered fluctuating flowering percentages across different months and years. The highest flowering percentage was recorded in June 2018 (96.6%) followed by 83% in July 2019 and 77% in June 2021. These findings underscore the dynamic nature of plant phenology and its intricate relationship with seasonal variations and climatic patterns. Although a correlation between climatic variables and recorded phenological phases was observed, variability was noted across the three years of study.

The six selected ethnomedicinal NTFPs underwent comprehensive research investigation including the preparation of methanolic and aqueous extracts for experimentation. Methanolic extracts consistently exhibited more promising outcomes compared to aqueous extracts. Analysis of these extracts revealed the

presence of eight phytoconstituents including alkaloids, carbohydrates, saponins, phenols, tannins and flavonoids in all six ethnomedicinal NTFPs methanolic extracts. Both methanolic and aqueous extracts tested negative for phytosterols and amino acids. Quantitative analysis of methanolic plant extracts revealed remarkably high levels of phenols and flavonoids with the total phenolic content being particularly noteworthy.

Among the methanol extracts, *C. caudatus* exhibited the highest total phenolic content at 155 ± 0.5 mg GAE/g while *M. malabathricum* displayed the highest flavonoid content at 139.6 ± 1.8 mg QE/g. In contrast, among the aqueous extracts, *V. peduncularis* demonstrated the highest phenolic content at 139 ± 2.5 mg GAE/g while *M. malabathricum* showcased the highest flavonoid content at 126 ± 2 mg QE/g. These findings underscore the remarkable efficacy of these selected plants and their potential antioxidant properties. The methanol and aqueous extracts from various plants exhibited robust antioxidant activity demonstrating significant scavenging effects on DPPH and ABTS radicals. Furthermore, the DPPH and ABTS radical scavenging activities of the methanol extracts from all nine samples were observed to follow a dose-dependent pattern. Out of the six selected ethnomedicinal NTFPs, methanolic and aqueous extracts of *B. roxburghii* and *V. peduncularis* exhibited the strongest DPPH scavenging activity with IC50= 19.5 ± 0.14 µg/ml and 50 ± 0.13 µg/ml respectively. Additionally, methanolic and aqueous extracts of *V. peduncularis* and *M. malabathricum* showed the highest ABTS scavenging activity with IC50= 32.47 ± 0.11 µg/ml and 59 ± 0.11 µg/ml respectively.

In the methanolic extract, a robust positive correlation of statistical significance was observed between Total Phenolic Content (TPC) and DPPH activity ($R^2 = 0.97$, p = 0.001**). Similarly, a significant correlation was found between Total Flavonoid Content (TFC) and ABTS activity ($R^2 = 0.83$, p = 0.039*). Moreover, in the aqueous extracts similar significant correlation was identified between TPC and DPPH activity ($R^2 = 0.82$, p = 0.022*) as well as between TFC and DPPH activity ($R^2 = 0.75$, p = 0.043*). A strong positive correlation between TPC and DPPH scavenging activity, and between TFC and ABTS scavenging activity indicates that higher levels of phenolic compounds and flavonoids in the extract are associated with greater

antioxidant activity. This correlation suggests that phenolic compounds and flavonoids could be the major contributors to the antioxidant potential of the plant extract and their presence in higher concentrations enhances the ability of the extract to scavenge free radicals and protect against oxidative damage.

Antibacterial screening was conducted against five bacterial pathogens comprising two gram-positive and three gram-negative bacteria: *Bacillus subtilis* (ATCC-11774), *Micrococcus luteus* (ATCC- 10240), *Escherichia coli* (ATCC- 10145), *Klebsiella pneumoniae* (ATCC- 10031) and *Salmonella typhimurium* (ATCC 51812). Both methanolic and aqueous plant extracts demonstrated potential zone of inhibition against the bacterial strains. ANOVA analysis, with a significance level set at $P < 0.05$ was employed to assess the results. The analysis revealed that the methanolic extracts of *F. virosa* and *T. grandiflora* exhibited statistically significant inhibition against all five bacterial strains whereas the remaining four plant species showed insignificant results. Furthermore, the aqueous extracts of *F. virosa*, *M. malabathricum* and *B. roxburghii* displayed significant variation while the remaining three were deemed insignificant.

A total of six Rare, Endangered and Threatened (RET) species were documented within the study area. Among these, *Saraca asoca* (Roxb.) W.J.de Wilde and *Globba spathulata* Roxb. were classified as Vulnerable. Additionally, *Sophora wightii* Baker and *Prunus ceylanica* (Wight) Miq. were categorized as Endangered. Finally, *Saurauia punduana* Wall. and *Ilex khasiana* Purkay. were identified as Critically Endangered.

The research aims to document ethnomedicinal NTFPs utilized by indigenous informants residing in the peripheral villages of Thorangtlang Wildlife Sanctuary. Recognizing the broad scope of NTFPs, the study deliberately focuses on ethnomedicinal NTFPs to establish precise objectives. It highlights the significant reliance of local communities on ethnomedicinal NTFPs to address their healthcare needs, deeply rooted in traditional practices and a diverse reservoir of knowledge aimed at managing various ailments. This investigation illustrates the crucial role of ethnomedicinal NTFPs in meeting the medical and healthcare needs of local

populations. The dependence on these resources is intricately linked with traditional practices indicating a symbiotic relationship between human communities and their natural environment. Moreover, the utilization of ethnomedicinal NTFPs highlights the adaptation of indigenous knowledge systems to local ecological contexts thereby enhancing community resilience and self-sufficiency in healthcare provision.

The research findings suggest the presence of potential bioactive compounds within the investigated plant species. However, a comprehensive phytochemical investigation is essential to isolate bioactive molecules with diverse pharmacological activities. This study advocates for the integration of modern methodologies with traditional knowledge systems aiming to harness NTFPs for the development of novel pharmaceutical agents. The incorporation of contemporary techniques in conjunction with indigenous wisdom holds promise in enhancing the efficacy and sustainability of drug discovery efforts. This approach not only underscores the importance of preserving traditional knowledge but also highlights its capacity to inform and enrich modern scientific practices fostering collaborative partnerships between indigenous communities and scientific researchers.

Phenological data serves as a valuable tool for acting as a significant biological indicator in predicting forthcoming climatic fluctuations and alterations in biodiversity. These observations provide insights into the timing of natural events in plant life cycles such as flowering, leaf emergence and fruiting which are influenced by environmental cues such as temperature, precipitation and photoperiod. Analysing short-term phenological trends allows researchers to extract crucial information about how plants respond to immediate environmental fluctuations, offering early warnings of ecological shifts and potential impacts on plant survival and reproductive success. However, for a comprehensive understanding of plant stability in response to environmental factors, long-term phenological studies are indispensable. Long-term observations enable the detection of subtle trends and patterns that may not be evident in short-term datasets. Furthermore, they facilitate the assessment of plant population resilience to prolonged environmental changes and the elucidation of complex interactions between phenological shifts and ecosystem dynamics. Therefore, a combination of short-term and long-term

phenological studies is recommended to capture both immediate responses and enduring trends thereby facilitating informed conservation and management strategies in the face of environmental change.

Given the persistent reliance of local communities on traditional medicines, our interviews revealed informants profound interest in understanding the therapeutic properties of the plants they have been using for various ailments. As such, I have a significant obligation to disseminate the findings of my research, thereby raising awareness regarding the efficacy of these plants and emphasizing the critical need for their conservation. This engagement serves a dual purpose: firstly, it provides valuable insights to the local population empowering them with knowledge about the medicinal properties of indigenous flora. Secondly, it fosters a sense of stewardship and promotes the sustainable utilization of these plant resources aligning with principles of ethnomedicinal preservation and biodiversity conservation.

It is highly advisable to undertake thorough documentation and inquiry into traditional knowledge concerning the utilization of medicinal plants. These efforts constitute an essential initial step in advancing pharmacological research and the quest for novel drug development. Consequently, there exists a pressing need for further scientific exploration into these medicinal plant species encompassing investigations into their phytochemical compositions, biological attributes and subsequent clinical assessments. Such endeavours hold the potential to unlock valuable therapeutic agents and contribute significantly to healthcare innovation and public health improvement.