INVESTIGATION OF CERTAIN RARE ETHNOMEDICINAL PLANTS OF MEGHALAYA, INDIA: A PERSPECTIVE ON BIO-ACTIVITY AND PRODUCT SYNTHESIS

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

Submitted By

Mr. Samson Rosly Sangma

Ph.D. Regd. No. Ph.D/FRS/00438 w.e.f. 28/11/2020

Department of Forestry

School of Sciences, Nagaland University

Lumami-798627

Nagaland, India

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By

Mr. Samson Rosly Sangma

and

Dr. Mayur Mausoom Phukan

(Supervisor)

Submitted to

NAGALAND UNIVERSITY

In Partial Fulfilment of the Requirements for the Degree

Of

DOCTOR OF PHILOSOPHY IN FORESTRY

DEDICATION

Dedicated with heartfelt gratitude

to my **Father** and **Mother**,

to my **Sisters, Brothers** and my beloved **Fiancée**

whose unwavering love, support,

and encouragement

have been my constant source

of strength and inspiration

Samson Rosly Sangma

नागालैण्ड विश्वविद्यालय
NAGALAND UNIVERSITY
भौतिकी विभाग

(संसद द्वारा पारित अधिनियम 1989, क्रमांक 35 के अंतर्गत स्थापित केंद्रीय विश्वविद्यालय)

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DECLARATION

I, **Mr. Samson Rosly Sangma**, bearing Ph.D. Registration No. Ph.D./FRS/00438 (w.e.f. 28th November, 2020), hereby declare that the thesis entitled "***Investigation of Certain Rare Ethnomedicinal Plants of Meghalaya, India: A Perspective on Bio-activity and Product Synthesis***" is a record of original research work carried out by me. All sources of assistance have been assigned due acknowledgement. I also declare that neither this work as a whole nor a part of it has been submitted to any other University/Institute for any other degree, diploma or award.

This is being submitted to Nagaland University for the degree of Doctor of Philosophy in Forestry.

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CERTIFICATE

Certified that the thesis entitled "***Investigation of Certain Rare Ethnomedicinal Plants of Meghalaya, India: A Perspective on Bio-activity and Product Synthesis***" is an original and bonafide research work carried out by **Mr. Samson Rosly Sangma** under my supervision. This thesis is being submitted to the School of Sciences, Nagaland University, in partial fulfilment of the requirements for the award of degree of Doctor of Philosophy in Forestry. The matter embodied in this thesis has not been previously submitted to any University/Institution for the award of any degree, diploma or similar title.

All assistance and help received during the entire course of doctoral research has been duly acknowledged.

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LIST OF SYMBOLS AND ABBREVIATIONS

Abbreviation	Meaning
WHO	World Health Organization
IUCN	International Union for Conservation of Nature
TCM	Traditional Chinese Medicine
NAM	National Ayush Mission
TKDL	Traditional Knowledge Digital Library
NMPB	National Medicinal Plants Board
UNDP	United Nations Development Program
NBRI	National Botanical Research Institute
BHA	Butylated hydroxyanisole
BHT	Di butylhydroxytoluene
TBHQ	Tertiary butylhydroquinone
MDR	Multi-Drug Resistant
MRSA	Methicillin-Resistant *Staphylococci aureus*
VBL	Vinblastine
VCR	Vincristine
GIS	Geographic Information System
GMPGIS	Global Medicinal Plant Geographic Information System
DEM	Digital Elevation Model
FCC	False Colour Composite
NIR	Near-Infrared
C_2H_5OH	Ethanol
NaClO	Sodium hypochlorite
CH_3OH	Methanol
HCl	Hydrochloric acid
H_2SO_4	Sulphuric acid
Na_2CO_3	Sodium carbonate
Na_2HPO_4	Disodium hydrogen phosphate
NaH_2PO_4	Monosodium phosphate

Abbreviation	Meaning
NaOH	Sodium hydroxide
$CuSO_4$	Copper sulphate
$KNaC_4H_4O_6 \cdot 4H_2O$	Potassium Sodium Tartrate
$CHCl_3$	Chloroform
$FeCl_3$	Ferric Chloride
CH_3COOH	Acetic acid
NH_3	Ammonia
KBr	Potassium Bromide
NIST	National Institute of Standards and Technology
CTAB	Cetyltrimethylammonium Bromide
Tris-HCl	Tris(hydroxymethyl)aminomethane hydrochloride
EDTA	Ethylenediaminetetraacetic Acid
C_2H_6OS	β-Mercaptoethanol
$C_5H_{12}O$	Isoamyl alcohol
CH_3CO_2K	Potassium acetate
C_3H_8O	Isopropyl alcohol
TBE	Tris-Borate-EDTA
EtBr	Ethidium Bromide
$AlCl_3$	Aluminium chloride
$C_2H_3NaO_2$	Sodium acetate
$K_2S_2O_8$	Potassium persulfate
KCl	Potassium chloride
KH_2PO_4	Potassium dihydrogen phosphate
FTIR	Fourier Transform Infrared
GCMS	Gas Chromatography Mass Spectrometry
NCBI	National Center for Biotechnology Information
BLAST	Basic Local Alignment Search Tool
GAE	Gallic Acid Equivalent
QE	Quercetin Equivalent

Abbreviation	Meaning
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FRAP	Ferric Reducing Ability of Plasma/Power
TPTZ	2,4,6-Tripyridyl-s-triazine
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
DMSO	Dimethyl Sulfoxide
ZOI	Zone of Inhibition
MTCC	Microbial Type Culture Collection
MHA	Mueller Hinton Agar
MIC	Minimum Inhibitory Concentration
CFU/mL	Colony Forming Unit per milliliter
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
HT-29	Human colon adenocarcinoma cell line
NCCS	National Centre for Cell Science
DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal Bovine serum
SEM	Scanning Electron Microscope
TGA	Thermogravimetric Analysis
SGF	Simulated Gastric fluid
SIF	Simulated Intestinal fluid
PBS	Phosphate Buffered Saline
MD (DE-16)	Maltodextrin (Dextrose Equivalent -16)

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ABSTRACT

Ever since the days of antiquity mankind have relied extensively on plants. Plants offer vital resources such as food, fiber, clothing and most importantly, medicine. The use of plants for medicinal purposes predates recorded history, constituting the foundations of traditional healing systems such as *Ayurveda*, *Unani*, and *Traditional Chinese Medicine*. Even today, these ancient practices continue to influence healthcare globally, especially in rural and remote regions with little access to contemporary medical facilities The World Health Organization (WHO) estimates that about 60 % of the global population and nearly 80 % in developing countries rely primarily on traditional medicine, highlighting the relevance and necessity of ethnomedicinal knowledge.

In light of the increasing side effects and limitations of synthetic pharmaceuticals, public interest has grown significantly in herbal and plant-based remedies. This has spiked renewed interest in high value bioactive compounds from medicinal plants. The WHO advocates integrating traditional medicine into primary healthcare due to its accessibility, affordability, and cultural acceptance. Against this backdrop, the present study aims to bridge traditional wisdom and modern science by investigating selected rare ethnomedicinal plants from Meghalaya, India.

Meghalaya, a northeastern state of India, constitute a segment of the Indo-Burma biodiversity hotspot. It contributes approximately 18 % of India's total flora, including numerous rare, endemic, and medicinal plant species. The indigenous communities of Meghalaya have relied on traditional healthcare practices for generations, utilizing local flora for treating a wide range of ailments. Additionally, the collection and sale of medicinal plants provide a source of livelihood for many local communities. However, despite the high ethnobotanical value, many of these species remain scientifically unexplored. There is paucity of scientific information about their phytochemical profiles, pharmacological properties, and conservation status. There is a growing need to scientifically validate and sustainably utilize these plants to highlight their latent therapeutic potential in modern healthcare.

This study specifically focuses on three rare medicinal plant species native to Meghalaya, viz; *Goniothalamus simonsii* Hook. f. Thoms., *Viburnum odoratissimum var. odoratissimum*, and *Citrus latipes* (Swingle) Tanaka. Among these, *G. simonsii* and *C. latipes* are categorized as near-threatened and vulnerable according to the IUCN Red List (Version 2022-2). Despite their prevalent traditional use for treating gastrointestinal and other ailments, there is scanty of scientific evidence to substantiate their claimed medicinal properties. Therefore, this study undertakes a multidimensional approach to evaluate their spatial distribution, genetic identity, phytochemical content, biological activities (antioxidant, antimicrobial, and anticancer) and potential for product development.

The spatial distribution of the species under study was assessed using Geographic Information Systems (GIS). GIS technology allows for the precise mapping of medicinal plants and aids in ecological assessments and conservation strategies. The spatial analysis revealed that *G. simonsii* is typically found in subtropical forest environments characterized by moderate temperatures and high humidity. *V. odoratissimum var. odoratissimum* is primarily located in the central and southern Khasi Hills, suggesting a preference for moderately elevated and moist forested areas. *C. latipes*, on the other hand, demonstrates broader ecological adaptability.

Methodically, this doctoral research work integrates traditional knowledge with quantitative biochemical, phytochemical and spectroscopic analyses. Carbohydrate content ranged from 103 - 312 mg/g, and protein content ranged from 18 - 65 mg/g in various parts of the investigated plant species. The fruits of *V. odoratissimum var. odoratissimum* showed the highest levels of carbohydrates and proteins. Fourier Transform Infrared Spectroscopy (FTIR) analysis confirmed the presence of major phytocompounds including phenols, flavonoids, alkaloids, terpenoid, glycoside, saponin, etc. Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed the presence of several bioactive compounds including spathulenol, coniferyl alcohol, hexadecenoic acid, stigmasterol, β-sitosterol, neophytadiene, amyrin, ergostadienol, and stevioside. These phytochemicals are

known to possess varied bioactivities such as antioxidant, antibacterial, anticancer, anti-inflammatory, antidiabetic, and cardioprotective effects.

Molecular identification was carried out using DNA barcoding techniques involving the rbcL and ITS regions. The DNA sequences were submitted to the GenBank NCBI [accession numbers PV688311 (*G. simonsii*), PV749115 (*V. odoratissimum* var. *odoratissimum*), and PV737879 (*C. latipes*)]. BLAST analyses confirmed their genetic proximity to respective species within the same genus. A phylogenetic tree was constructed using the Maximum Likelihood method with the Tamura-Nei model in MEGA 12 software, reaffirming the genetic placement of these species thereby inferring their genealogical descent.

The biological activities of the selected plants were evaluated through standardized *in vitro* methods. Antioxidant activity was determined via the DPPH free radical scavenging and Ferric Reducing Antioxidant Power (FRAP) assay. The results showed strong antioxidant potential, with IC_{50} values ranging from 32 – 850 µg/ml. *G. simonsii* leaf extract demonstrated the highest phenolic content at 154.76 mg GAE/g, while bark extracts of *V. odoratissimum var. odoratissimum* and *C. latipes* recorded values of 356.71 mg GAE/g and 149.36 mg GAE/g, respectively. Antimicrobial efficacy was assessed using the agar well diffusion method. The extracts exhibited significant zones of inhibition (ZOI), ranging from 14 – 22 mm for *G. simonsii*, 9 – 18 mm for *V. odoratissimum var. odoratissimum*, and 10 – 15 mm for *C. latipes* against gram-positive bacteria (*Staphylococcus aureus* MTCC 11949 and *Bacillus cereus* MTCC 8361), gram-negative bacteria (*Escherichia coli* MTCC 593, *Salmonella enterica* MTCC 1166, and *Yersinia pestis*), and fungus (*Candida albicans* MTCC 13013). The variation in antimicrobial activity is attributed to differences in phytochemical composition and bacterial resistance mechanisms. Minimum inhibitory concentrations (MICs) ranged from 58 - 1250 µg/ml.

The anticancer potential was evaluated using the MTT assay on the HT-29 colon cancer cell line. *G. simonsii* stood out with a potent IC_{50} value of 8.82 µg/ml, indicating strong antiproliferative activity. *C. latipes* and *V. odoratissimum*

var. odoratissimum also exhibited promising cytotoxic effects with IC_{50} values of 52.39 µg/ml and 214.85 µg/ml, respectively. While these results are pre-clinical, they offer a valuable foundation for future clinical research and therapeutic development.

In addition to bioactivity screening, the study explored product development through microencapsulation technology. Extracts of the studied plants were encapsulated using maltodextrin, resulting in microcapsules with 5.05 ± 0.21 % moisture content, 90.61 ± 0.73 % solubility, and 88.18 ± 2.06 % encapsulation efficiency. Scanning Electron Microscopy (SEM) micrographs revealed that the microcapsules had irregular, creased surfaces with slight agglomeration but no fractures, indicating structural integrity. Thermal analysis demonstrated stability below 200 °C, supporting their application in nutraceutical formulations like herbal tea or health beverages. Further gastrointestinal simulation revealed that these microcapsules resisted degradation in the gastric phase and maximally released active compounds in the intestinal phase, suggesting targeted delivery. Two value-added products were developed: a fortified herbal green tea and a ready-to-consume juice. These products were tested for haemocompatibility and cytotoxicity against colon cancer cells, confirming both safety and efficacy. Nutritional analysis of the juice formulation indicated 64.2 kcal/100g energy content, 0.87 mg/100g calcium, 2.1 mg/100g vitamin C, 0.1 mg/100g sodium, 31.5 mg/100g carbohydrate and 10.4 mg/100g protein.

The experimental findings validate the therapeutic potential of these underutilized species and underscore their economic and conservation significance. The integration of traditional knowledge, advanced scientific techniques, and product development offers strong candidature for the broader application of these plants in pharmaceuticals and functional food industries. The study provides comprehensive insight into the phytochemical richness, pharmacological potential, and practical applicability of *G. simonsii*, *V. odoratissimum var. odoratissimum*, and *C. latipes*. These rare ethnomedicinal plants of Meghalaya represent an intriguing potential for novel drug discovery, nutraceutical product development, and sustainable healthcare solutions. Future research should focus on large-scale clinical validation, formulation

refinement, and long-term conservation strategies to harness the full potential of these plant resources for human health and well-being.

Keywords: Ethnomedicinal plants, GIS, Phytochemical analysis, Molecular characterization, Biological activities, Nutraceutical product development,

CHAPTER 1

INTRODUCTION

Chapter 1: Introduction

Much before the dawn of human civilization, medicinal plants have played a pivotal role in fulfilling both nutritional and healthcare needs (Nwachukwu et al., 2010). Their traditional use in disease prevention, therapeutic intervention, and the promotion of physical and spiritual well-being has rendered them indispensable across diverse cultures (Idu et al., 2007). The recent decades have witnessed a significant global resurgence in the use of medicinal plants, largely driven by their perceived efficacy, minimal side effects, and gentle pharmacological actions compared to synthetic drugs.

The use of plants for therapeutic purposes predates documented human history. The Chinese Emperor Shen Nung, in around 2000 BC, compiled a comprehensive text detailing the collection, preparation, and therapeutic applications of various plants. *Traditional Chinese Medicine* (TCM) remains one of the most extensive and well-documented traditional medical systems in human history. Similarly, ancient Greek scholars made significant contributions to herbal medicine. Notably, *De Materia Medica*, authored by Dioscorides around 100 AD, describes over 600 medicinal plants which served as a foundational text in pharmacology for centuries (Samuelsson, 1999). During the Dark and Middle Ages, it was the Arab scholars who preserved and expanded upon the Greek herbal knowledge, playing a pivotal role in the continuity and advancement of medical science. Alongside the contributions of Arab, Chinese, and Greco-Roman civilizations, the Indian subcontinent developed its own rich and sophisticated traditions of medicine. Systems such as *Ayurveda*, *Siddha*, and *Unani* are grounded in a highly evolved conceptual framework that predates many modern scientific approaches (Summner, 2003).

1.1. A Treatise on Ethnomedicinal Knowledge in India

Since time immemorial, the indigenous populations of India have relied on plants for both sustenance and medicinal purposes, cultivating a deep and dependable relationship with the natural world. This connection has given rise to a

rich legacy of traditional knowledge, characterized by a profound understanding of plant-based therapies employed for both preventive and curative healthcare (Chopra et al., 1956). Archaeological evidences reveals that ancient Indian civilizations (3300-1300 BC) utilized medicinal plants for therapeutic purposes. Excavations at Harappa and Mohenjodaro uncovered charred remnants of medicinal plants such as *Ficus religiosa* (Peepal) and *Acacia nilotica* (Babul), presumably utilized for antiseptic applications (Possehl, 2002). It also uncovered clay vessels containing herbal pastes, signifying a prehistoric pharmacopeia (Kenoyer, 1998). India's ethnomedicinal knowledge has historical roots, and finds specific mention even in sacred texts such as the *Vedas.* For instance, the *Rigveda* mentions about the utilization of medicinal herbs such as Soma (potentially *Ephedra* or *Amanita muscaria*) as a stimulant and Ashwagandha (*Withania somnifera*) as a restorative herb (Satayavati et al., 1988; Kamboj, 2000). *Ayurveda*, meaning "the science of life," is a thoroughly documented traditional system that prioritizes holistic healing via herbs, diet, and lifestyle (Qadry et al., 2004; Pandey et al., 2013). It encompasses a systematic approach to harmonious living, originating from ancient texts such as the *Rig* and *Atharva Veda*. The source of *Ayurveda* has been lost in ancient relic, yet its concepts and practices were idealized in India between 2500 and 500 BC (Mukherjee, 2001). The application of natural resources for treating human ailments through extensive experimentation and daily experiences have been a consistent practice in *Ayurveda* among the Indian populace. It is an extensive medical service system established on the principle that the human body consists of seven fundamental tissues (“Rasa,” “Rakta,” “Mansa,” “Meda,” “Asthi,” “Majja,” and “Shukra”) and the byproducts of bodily functions, such as excretion, urine, and sweat, which arise from the five fundamental elements: fire, water, air, ether, and earth, alongside three dynamic energies or functional principles known as “vata, pitta, and kapha” (*Tridosha*). Any irregularity or disruptive factor in these essential bodily elements results in illness (Mukherjee et al., 2001). The *Siddha system,* common in Tamil Nadu, combines spiritual and herbal medicine, whereas *Unani,* inspired by Persian and Arabic traditions, originated during the Mughal period (Kamboj, 2000). Around 1000 BC, the Charak Samhita and Sushruta Samhita

emerged as pivotal Indian texts on medical sciences. Charak and Sushruta systematically examined numerous plants for their therapeutic properties. Among the plants which they examined include Haritaki (*Terminalia chebula*) for digestion, Guduchi (*Tinospora cordifolia*) for immunity, Turmeric (*Curcuma longa*) for wound healing and Brahmi (*Bacopa monnieri*) for brain health (Bhishagratna, 1911; Satayavati et al., 1988). These systems collectively form the backbone of India's ethnomedicinal heritage.

Beyond these formal systems, tribal and indigenous communities across India have established unique ethnobotanical knowledge systems of their own (Singh et al., 2017). States such as Meghalaya, Odisha, Chhattisgarh, Jharkhand, Madhya Pradesh, and Andhra Pradesh are home to numerous tribal groups who continue to rely heavily on local flora for primary healthcare needs. These communities possess orally transmitted knowledge regarding the identification, collection, preparation, and administration of plant-based remedies. For instance, the Garo, Khasi, and Jaintia tribes of Meghalaya, India have constantly used various native plants to treat ailments ranging from wounds and fevers to digestive disorders and skin infections (Mir et al., 2014; Hazarika et al., 2023). Jagtap et al. (2006) documented the uses of 66 plant species by the Korku tribe of Maharashtra to address various health issues, including diarrhoea, skin diseases, wounds, jaundice, tuberculosis, migraine, stroke, menstrual and fertility problems, urinary issues, piles, and poison bites. Malik et al. (2015) documented the applications of 97 medicinal plants for treating digestive disorders, constipation, fever, cough, cold and menstrual disorders by the inhabitants of the Western Himalaya. Hajra & Baishya (1981) documented the uses of 29 plant species by the Miris (Mishings) of the Assam plains. Tiwari & Tiwari (1996) reported the uses of plants within the indigenous medicinal practices of the tribes in Arunachal Pradesh. The uses of 78 plant species by the local inhabitants of Rudraprayag in the Western Himalaya for treating several ailments, primarily targeting skin and gastrointestinal diseases were reported by Singh et al. (2017). Sharma et al. (2022) identified 73 ethnomedicinal plants used for various

neurological disorders in Himachal Pradesh and reported that majority of plants are used against epilepsy.

India's ethnomedicinal heritage is not only a testament to the country's biocultural wealth but also serves as a crucial resource for modern drug discovery and integrative medicine. Many contemporary pharmaceutical compounds have been derived from ethnobotanical leads, with traditional healers often acting as the primary source of information (Laldingliani et al., 2022). For instance, the anti-cancer drug vincristine, derived from *Catharanthus roseus*, and *reserpine*, an antihypertensive compound from *Rauvolfia serpentina*, were both identified through ethnomedicinal practices (Abdelfatah et al., 2015). Despite its significance, this heritage faces threats from modernization, habitat destruction, loss of traditional knowledge, and limited documentation. Consequently, it is crucial for systematic documentation, scientific validation, and conservation of the plants of therapeutic value and the indigenous knowledge systems associated with them. Initiatives such as the National Ayush Mission (NAM), the Traditional Knowledge Digital Library (TKDL), and various ethnobotanical surveys by research institutions have begun addressing these challenges (Mishra & Madhukar, 2024). TKDL serves as a comprehensive database of documented traditional medicinal formulations. It has proven effective in preventing biopiracy and protecting intellectual property rights by offering evidence of prior art to international patent offices. The National Medicinal Plants Board (NMPB) is actively engaged in promoting sustainable use and conservation of medicinal plant resources (Hazarika et al., 2023).

The surge in global demand for natural and plant-based therapeutics has opened new vistas in the ethnopharmacological research and herbal drug development. Indian pharmaceutical companies and research institutions are actively engaged in collaborative projects with global partners, applying advanced techniques in phytochemistry, genomics, and biotechnology to develop standardized, effective, and safe herbal formulations (Laldingliani et al., 2022).

1.2. Biodiversity and Ethnomedicinal Heritage of Meghalaya

Meghalaya, known as the 'Abode of Clouds', is one of the eight sister states in the Northeastern India. The region comprises the districts of East Garo Hills, West Garo Hills, South Garo Hills, East Khasi Hills, West Khasi Hills and Jaintia Hills, situated between 25°47' - 26°10' N latitude and 89°45' - 92°45' E longitude. Assam borders it to the north and northeast and Bangladesh to the south and southwest. The state's unique topography, characterized by rugged hills, deep valleys, high plateaus, and rich alluvial plains, creates diverse microclimatic zones that are highly conducive to the growth of medicinal plants (Barik et al., 1992; Jamir, 2000). The state's varied elevation, ranging from lowland tropical forests to high-altitude cloud-covered areas, supports a wide range of flora with therapeutic properties. High rainfall, dense forest cover, and fertile soil further enhance the region's capacity to nurture rare and endemic medicinal species. This ecological heterogeneity, combined with traditional knowledge systems of indigenous communities, makes the state a vital repository of ethnomedicinal biodiversity with immense potential for conservation and pharmacological exploration (Hooker, 1854; Kanjilal et al., 1934). The state is designated as a segment of the Indo-Burma Biodiversity Hotspot (Haridasan & Rao, 1985). It contributes approximately 18 % of the nation's total flora. It is home to 3128 species of ethnomedicinal plants, with 40 % of these being endemic in nature (Haridasan & Rao, 1985; Khan et al., 1997). Some of the rare, endemic and threatened species includes *Ceropegia angustifolia, Cyclea debiliflora, Diplomeris pulchella, Elaeagnus conferta, Gleditsia assamica, Goniothalamus simonsii, Mastixia arborea, Nepenthes khasiana, Saraca asoca,* and *Taxus baccata* (Upadhyay et al., 2013). *Nepenthes khasiana* (Pitcher Plant), a carnivorous plant was found only in Meghalaya. The state encompasses a variety of regions, each hosting a distinct array of rare and endemic plant species. Among the diverse regions, Nokrek exhibits the most significant proportion of endemic medicinal plants, standing at 37.8 %, followed by Jowai at 35.1 % and Raliang at 32.4 % (Haridasan et al., 2010). Fig. 1.1 illustrates the geographic location of the state, highlighting areas of plant endemism. The floral diversity of the state has also

garnered recognition from international organizations. In 2003, the United Nations Development Program (UNDP) identified Meghalaya as one among the seven Indian states for extensive research on medicinal plants (Telegraph, 2025).

Many researchers have explored the ethnomedicinal practices of various tribes within the state (Kharkongor & Joseph 1981; Rao 1981; Chaudhary & Neog, 2003) and have concluded that the traditional methods of treatment, based on medicinal plants, are still an important part of their social life and culture. For instance, Laloo & Hemalatha (2011) documented the uses of 58 plant species for treating diarrhea and dysentery. Rao (1981) have reported the uses of *Begonia josephi* for gastrointestinal issues, *Houttuynia cordata* for blood purification, *Rubus ellipticus* for dysentery, and *Plantago major* for treating burns and wounds. Similarly, Upadhaya et al. (2016) highlights the conventional applications of wild *Citrus species*, including *Citrus hystrix*, *Citrus latipes*, and *Citrus indica* for gastrointestinal issues, common cold, dermatological ailments, fever, and injuries. Mir et al. (2014) documented the traditional uses of endemic and endangered medicinal plants by the local populace for treating several ailments. A list of endangered and threatened medicinal plants used to treat common diseases in the state is furnished in Appendix (III). Medicinal plants have significantly contributed to the livelihoods of the state's populace by serving as a source of cash income. They are predominantly employed at the household level in a self-sustaining manner. The herbal practitioners use medicinal plants for both preventive and therapeutic purpose (Tynsong et al., 2012). The folk healers offer vital healthcare services to the populace, administering life-saving treatments in areas with difficult communication (Kharkongor & Joseph, 1981). The highest numbers of medicinal plants are used for gastrointestinal disorders than any other diseases (Mir et al., 2014). Fig. 1.2 depicted the major ailments treated with ethnomedicinal plants in the state. There are 33 rare plant species belonging to 28 genera and 24 families in the state used for the management of gastrointestinal complications. The local populace uses different formulations of medicinal plants. The most common formulation is decoction. Secondly, they use medicinal plants as vegetables in cooking or to make *chutney*

(Rao 1981; Singh & Borthakur 2011). For centuries, they relied on decoction and infusion methods of drug compound extraction later formulated into syrups (Singh et al., 2014). For internal diseases (mostly gastrointestinal complications) liquid medicine or syrup (formulated by boiling fresh plant materials) is most preferably given to the patients for oral consumption. The overview of the preparation and consumption of folk medicines by the indigenous populace of the state is furnished in Fig. 1.3. Majority of the folk healers prescribed the dosages of particular medicines based on the severity of illness and the age of patients. Since they don't have any standardized measuring system for herbal recipes, the doses for liquid medicines are prescribed in the pattern of half, full or one-fourth (¼) of the tea-cup, spoons and glasses.

The ethnomedicinal plants used in Meghalaya hold immense therapeutic potential, yet much of this traditional knowledge remains undocumented and scientifically unvalidated. The region's rich biodiversity, coupled with the deep-rooted indigenous knowledge of tribal communities, necessitates a systematic study to preserve, evaluate, and authenticate the therapeutic potential of these plants. Scientific exploration can facilitate the discovery of novel bioactive compounds and promote sustainable utilization. Moreover, systematic documentation helps protect indigenous intellectual property rights and prevents biopiracy. Consequently, the integration of age-old knowledge with contemporary research methodologies is crucial for ensuring the long-term conservation, validation, and utilization of the state's ethnomedicinal resources (Dolui et al. 2004; Singh et al., 2014).

1.3. Role of Ethnomedicinal Plants in Modern Therapeutics

Ethnomedicine refers to the study of traditional healthcare practices employed by diverse ethnic groups, particularly indigenous communities. The term is often used interchangeably with 'traditional medicine,' although it specifically emphasizes the cultural and ethnographic context of healing practices. The World Health Organization (WHO) defines traditional medicine as "the extensive corpus of knowledge, skills, and practices grounded in the theories, beliefs, and experiences

distinctive to diverse cultures, irrespective of their explicability, used in health preservation, as well as the prevention, diagnosis, enhancement, or treatment of physical and mental disorders" (WHO, 2025). Ethnomedicinal research encompasses indigenous categorizations and interpretative models of illness, including aetiologies, symptoms, disease progressions, and therapeutic approaches (Kleinman, 1980). Ethnomedicine fundamentally consists of valuable medicinal plants that are readily accessible. Medicinal plants are defined as species widely used for the treatment and prevention of specific ailments and diseases, including those recognized for their beneficial effects on healthcare and maintenance.

The impact of ethnomedicinal plants on the evolution of the human healthcare system is undoubtedly unquestionable. Nearly, 70 % of the pharmaceutical medications currently prescribed originates from compounds found in plants. Plants employed in folk medicine, especially those with traditional and ethnopharmacological relevance, have long served as fundamental sources for the discovery of medicines and drugs (Fabricant & Farnsworth, 2001). Approximately, 7000 medicinal compounds derived from plants have emerged as essential drugs within Western medicine (Nasim et al., 2022). A multitude of natural products play essential roles in contemporary drug development, particularly as antioxidants, antibacterial agents, anticancer, anti-inflammatory compounds, etc. For instance, quinine, derived from *Cinchona* tree serve as antimalarial drug. The alkaloids vincristine and vinblastine, derived from *Periwinkle* plant are used as anticancer drug in chemotherapy. Digoxin, derived from *Foxglove* plant is widely used to treat cardiac conditions and myocardial infarctions (Chaachouay et al., 2024). They have demonstrated better safety and efficacy when compared with synthetic pharmaceuticals. Numerous natural products offer direct advantages to human health, while others serve as chemical or molecular paradigms for the synthesis and design of novel pharmaceuticals. Plant metabolites can be classified into three primary groups: terpenoids, phenolics, and alkaloids. These metabolites, when considered together, contribute to the overall fitness of the plant within its natural habitat (Harborne, 1998).

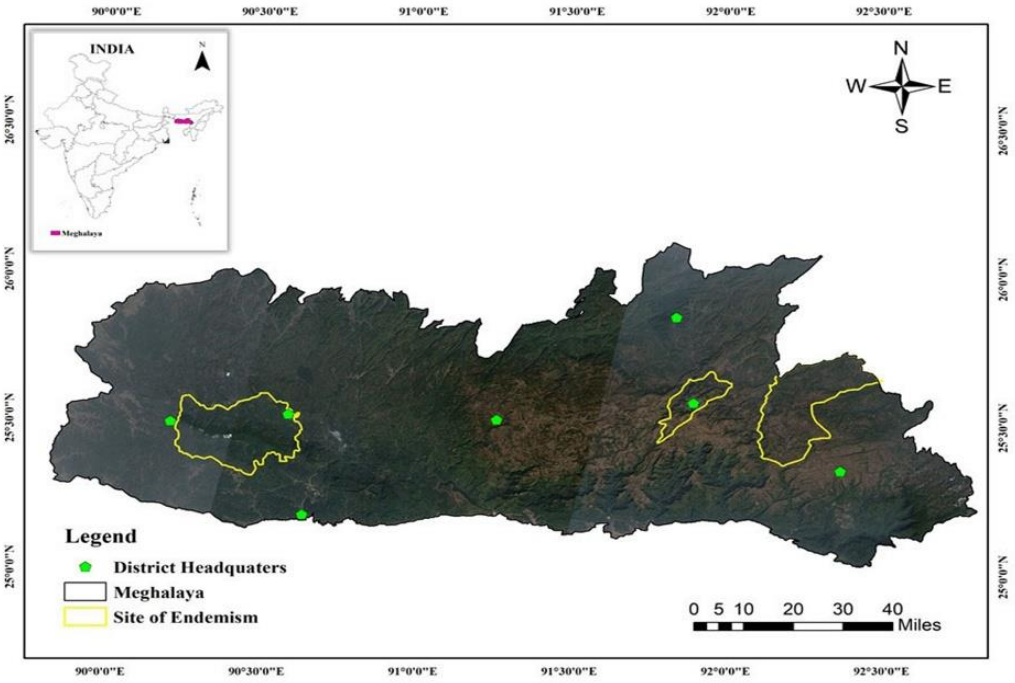

Fig. 1.1 Map illustrating the key regions within the state of Meghalaya that serve as hotspots for plant endemism

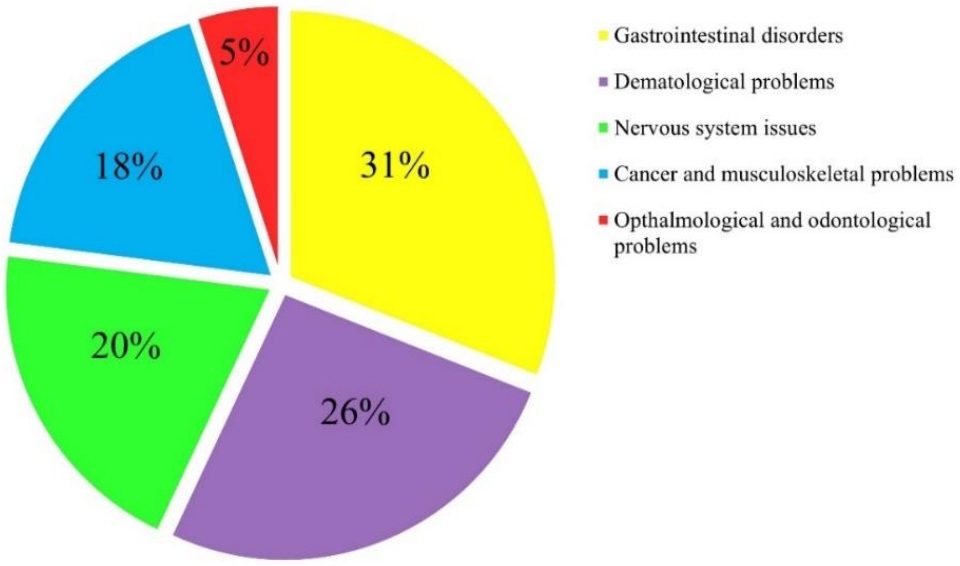

Fig. 1.2. Major ailments treated by ethnomedicinal plants in Meghalaya. (Source from Mir et al., 2014)

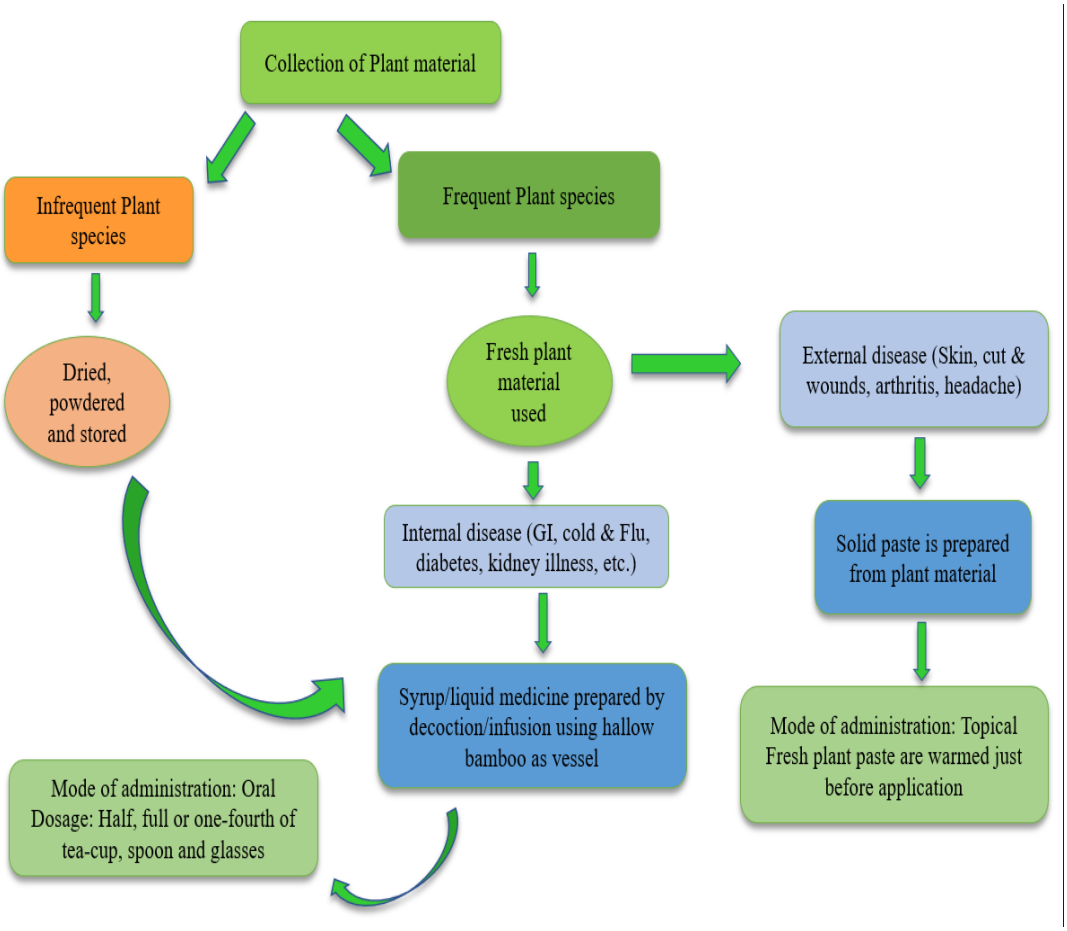

Fig. 1.3. Overview of the preparation and administration of folk medicines by the local populace of Meghalaya, India

They engage in communication, mitigate abiotic stress, and provide protection against herbivory and microbial diseases (Akula & Ravishankar, 2011). Hence, the crude or purified form of these metabolites have been extensively investigated by researchers for their biological roles in managing various diseases affecting both humans and animals. Currently, the antioxidant potential of secondary metabolites, particularly phenolics derived from plants, has been the subject of extensive study due to their advantageous effects on both humans and animals (Bukvicki et al., 2020). Natural compounds also offer considerable advantages as

antimicrobial and anticancer agents due to their diverse chemical structures, broad-spectrum efficacy, and potential for targeted therapies with reduced adverse effects relative to synthetic alternatives. Previous studies have validated the efficacy of natural compounds derived from medicinal plants in exhibiting antioxidant, antibacterial, and anticancer activities, as demonstrated through both *in vitro* and *in vivo* approaches (Tsai et al., 2017; Altay et al., 2018). These studies revealed the intriguing potential for plants to serve as a source of various health-enhancing metabolites. This may play an important role in the prevention and treatment of numerous diseases in the realm of 'Modern Therapeutics'.

1.4. High Value Bio-actives from Ethnomedicinal Plants with Special Reference to Antioxidants and Antimicrobials

Plants are known to possess a plethora of high-value bio-actives (phenols, flavonoids, steroids, glycosides, terpenoids, etc.), which are highly effective in neutralizing free radicals. A free radical is an independent molecule containing a single unpaired electron, which makes it highly reactive and capable of readily interacting with other molecules, often leading to significant biochemical alterations (Pizzino et al., 2017). They are generated naturally during vital metabolic processes in cells, including mitochondrial electron transport, enzymatic reactions, and immune responses. The body also produces these molecules in reaction to external factors such as ultraviolet radiation, pollution, smoking, and chemical exposure (Chandimali et al., 2025). Diseases such as cancer, cardiovascular disease, cataracts, asthma, rheumatoid arthritis, inflammation, burns, gastrointestinal diseases, progerias etc., are associated with oxidative stress (an imbalance of free radical generation and neutralization in the body) (Phaniendra et al., 2015).

Typically, cells are meticulously programmed to regulate the generation and processing of reactive oxygen species (ROS), ensuring their levels remain within a precise range essential for normal cellular function. The normal level of reactive oxygen species within the cell is predominantly maintained through both non-enzymatic agents (such as vitamin C, vitamin E, β-carotene, glutathione,

coenzyme Q, and bilirubin) and enzymatic mechanisms (such as catalase, peroxidase, superoxide dismutase, and glutathione reductase). However, with ageing and due to external stresses, this state of balance may be disturbed, resulting in the production of excess free radicals (Tang et al., 2011). In such circumstances, it is recommended to take antioxidant supplements to protect the body from the harmful effects of excessive free radical production. Antioxidant compounds can be derived from dietary supplements or synthetic pharmaceuticals. However, the detrimental effects of the extensive use of synthetic antioxidants have been reported. Certain synthetic antioxidants, such as butylated hydroxyanisole (BHA) and dibutylhydroxytoluene (BHT), have been shown to enhance carcinogenic activity potentially (Nasim et al., 2022). Tertiary butylhydroquinone (TBHQ) and propyl gallate have been reported to cause DNA damage, neurotoxicity, gastrointestinal irritation, and allergic reactions (Xu et al., 2021). The tendency to substitute such antioxidants with natural alternatives has been on the rise (Tajkarimi et al., 2010). Studies have been carried out on consumer perceptions regarding the risks of utilizing synthetic compounds for food coloring and preservation. Findings suggests that consumers are apprehensive about the presence of synthetic compounds in their daily diet, showing a strong preference for natural alternatives (Deshmukh & Gaikwad, 2024).

Natural antioxidants derived from plants can be categorized into three primary classes: phenolic compounds, vitamins, and carotenoids (Akbari et al., 2022). Phenolic compounds display remarkable structural diversity, encompassing simple molecules such as ferulic acid, vanillin, gallic acid, and caffeic acid, as well as more complex polyphenols like tannins and flavonoids (Tajkarimi et al., 2010). The most significant vitamins are Vitamins E and C. The majority of the natural antioxidants are derived from plant sources (Jiang & Xiong, 2016). Halvorsen & Holte (2002) reported that the plant families *Rosaceae*, *Empetraceae*, *Ericaceae*, *Grossulariaceae*, *Juglandaceae*, *Lamiaceae*, *Asteraceae*, *Punicaceae*, and *Zingiberaceae* encompass compounds with significant antioxidant properties. These plant families particularly include fruits such as strawberries, black currants, blackberries, pomegranates, blueberries, walnuts, etc. Aqueous tea extracts also serve

as sources of natural antioxidants due to their composition of various compounds, including catechins, tannins, and other flavonoids (Jiang & Xiong, 2016). Today, numerous natural antioxidants, such as chlorogenic acid, caffeic acid, curcumin, gallic acid, and ferulic acid, are recognized as pharmacologically significant compounds used in medications for treating several ailments caused by free radicals.

Likewise, in the context of antibiotics, the rise of microbial resistance has diminished their effectiveness, transforming the long-standing paradigm of their application. This significant trend has been recognized as an urgent concern by the WHO and has emerged as a formidable challenge for the global medical community (WHO, 2025). The significant rise in antibiotic resistance, due to the persistent existence of antibiotic-resistant bacteria, is emerging as a serious issue for global public health. The projections suggest a steady increase in fatalities associated with antimicrobial resistance (AMR) over the coming decades, with an expected annual toll of 1.91 million deaths due to AMR by 2050, representing a 67.5 % rise from the 1.14 million deaths recorded in 2021 (Bhattacharjee, 2019; Naghavi et al., 2024). Gram-negative bacteria exhibit greater resistance to antibiotics compared to gram-positive bacteria. The variation in cell wall structures may account for this phenomenon. Specifically, gram-negative bacteria possess an outer membrane composed of high-density lipopolysaccharides, which act as a barrier to numerous environmental substances, including antibiotics (Breijyeh et al., 2020). *Staphylococcus aureus*, a gram-positive bacterium, is recognized for causing various diseases, including food poisoning, skin infections, wound infections, and respiratory tract infections. Gram-negative bacteria, such as *Escherichia coli*, *Salmonella*, and *Shigella*, are widely recognized pathogens responsible for gastrointestinal tract infections. Common fungal skin infections encompass athlete's foot and ringworm, attributed to *Trichophyton rubrum*, as well as candidiasis, caused by *Candida albicans*, which affects the skin, oral cavity, vagina, and gastrointestinal tract (Breijyeh et al., 2020). The number of bacteria that have developed resistance to antimicrobial drugs has increased in recent decades. For example, methicillin-resistant *Staphylococci aureus* (MRSA), vancomycin-resistant enterococci, penicillin

and macrolides-resistant pneumococci, as well as multi-drug resistant (MDR) gram-negative bacteria (Ahmed et al., 2024). Bacteria developed antimicrobial resistance through several mechanisms. For instance, antibiotic inactivation can occur through the production of diverse enzymes, modifications in cell permeability, alterations of drug targets, the intrinsic expression of efflux pumps, and the formation of biofilms that act as a defence against drugs (Hoiby et al., 2010; Reygaert et al., 2018). Alongside mutations, mobile genetic elements such as plasmids, insertion sequences, transposons, and integrative conjugative elements significantly contribute to the proliferation of resistance across various bacterial groups (Beceiro et al., 2013).

Antibiotics derived from chemicals are typically used to inhibit microbial infections and prevent their transmission. Nevertheless, despite significant advancements in scientific research and modern medicine, the initial promise of antibiotics has faded. This can be attributable to the emergence of diverse resistance mechanisms, which present a considerable challenge to the effectiveness of conventional antibiotics. Furthermore, the extensive use of synthetic antibiotics has led to the emergence of opportunistic organisms that exhibit intrinsic resistance to existing pharmacological treatments, subsequently complicating the management of diseases in both hospital and community settings (AlSheikh et al., 2020). Under these circumstances, ethnomedicinal plants used by tribal peoples represent one potential alternative.

The rise in the use of plant medicine is believed to hold promise for the discovery of novel and effective drugs. Extensive research activities have confirmed that plants are highly effective in treating various ailments. Consequently, there is a persistent impetus to explore alternative sources, including natural products. For centuries, indigenous flora has been utilized in herbal medicine to treat various ailments (Essawi & Srour, 2000; Alzoreky & Nakahara, 2003). Extracts from garlic, cinnamon, curry, mustard, basil, ginger, and various other herbs demonstrates antimicrobial activity (Arora & Kaur, 1999). Essential oils from various aromatic plants, particularly those in the *Labiatae* family, exhibit antimicrobial properties (Elgayyar et al., 2001). For instance, oils derived from basil, bay, clove, thyme, and

rosemary exhibited bactericidal properties against *Listeria monocytogenes* and other pathogens (Smith-Palmer et al., 1998). Currently, there is a heightened focus on extracts and biologically active compounds derived from plant species used in herbal medicine. Some of the bioactive compounds used in contemporary medicine to combat antimicrobial resistance (AMR) include andrographolide from *Andrographis paniculata*; asiaticosides, indocentellosides, and thankunisides from *Centella asiatica*; guggulsterone and guggulsterol from *Commiphora mukul*; and triterpenoid saponins from Shatavari (*Asparagus racemosus*). Aspirin, atropine, ephedrine, digoxin, morphine, quinine, reserpine, and tubocurarine are notable examples of drugs discovered through knowledge derived from indigenous medical practices (Gilani & Rahman, 2005). Plant-derived antibiotics can safeguard against multi-drug-resistant (MDR) bacteria by employing distinct mechanisms compared to conventional antibiotics (Subramani et al., 2017). For example, they can improve the efficacy of conventional antibiotics or circumvent resistance mechanisms (Arip et al., 2022). Plant-derived compounds, such as alkaloids, can damage microbial membranes, leading to cell death. Specific plant metabolites can inhibit the synthesis of bacterial DNA, RNA, and enzymes or disrupt quorum sensing, a crucial communication mechanism in bacteria (Suganya et al., 2022). Specific phytochemicals, such as polyphenols, can counteract bacterial resistance mechanisms, thereby increasing their vulnerability to conventional pharmaceuticals (Guedes et al., 2024).

1.5. Natural Products and the Development of Futuristic Cancer Therapeutics

Cancer constitutes a major global health issue and the leading cause of death in the 21st century, according to the World Health Organisation (Ma & Yu, 2006). In 2020, nearly 10 million individuals worldwide succumbed to cancer, including breast, lung, rectal, prostate, colorectal, skin, and stomach cancers (WHO, 2025). The increasing global population and increased life expectancy will result in an annual rise in cancer cases. Consequently, the effective anticancer medications are critical. Chemotherapeutic agents are widely used in oncological treatment and have become an essential element of cancer pharmacotherapy. Various strategies have

been developed to mitigate the adverse effects of cancer therapeutics, including minimizing damage to surrounding cells and tissues, improving drug accumulation and efficacy at the target site, and creating novel drug delivery and targeting systems (Xia et al., 2021). Several additional approaches exist for cancer treatment, including surgical tumor excision, cancer vaccines, stem cell therapy, immunotherapy, chemotherapy, photodynamic therapy, radiation therapy, or a combination of these techniques, all of which often have adverse side effects (Chu et al., 2020). These detrimental effects encompass restricted metastasis, toxicity, insufficient specificity, poor bioavailability, and swift elimination (Lichota & Gwozdzinski, 2018). Cancer treatment options are dictated by the cancer's location, stage, and type. Chemotherapy agents, including alkylating agents (e.g., cyclophosphamide, oxaliplatin, carboplatin and melphalan), topoisomerase inhibiting agents (e.g., doxorubicin and irinotecan), and microtubule-targeting agents (e.g., vincristine), may induce adverse effects such as pulmonary toxicity, cardiotoxicity, gastrointestinal toxicity, coronary toxicity, nephrotoxicity, and hematologic toxicity (Kuroda et al., 2014).

Number of investigations demonstrated that natural compounds derived from medicinal plants exhibit significant chemo-preventive potential (Park et al., 2016; Huang et al., 2018; Newman & Cragg, 2020). These bioactive phytochemicals, either in their natural form or modified through physico-chemical processes, serve as a valuable reservoir for the development of effective anticancer agents. Their diverse structural complexity and biological activity offer promising avenues for novel therapeutic interventions in cancer prevention and treatment. In recent decades, considerable efforts have been made to isolate novel natural products from plants to evaluate their anticancer properties and investigate their mechanisms of action. These efforts resulted in the identification of some anticancer drugs. Between 1981 and 2019, it is estimated that around 25 % of newly approved anti-cancer drugs were derived from natural products (Huang et al., 2018; Newman & Cragg, 2020). Camptothecin and taxol are the two most prominent examples, both identified between 1950 and the 1960s during a campaign by the National Cancer

Institute (NCI) to explore the therapeutic potential of natural products (Wall & Wani, 1995; Ojo et al., 2022). Chinese researchers significantly advanced the integration of arsenic trioxide, a historical treatment in *Traditional Chinese Medicine* (TCM), into the standard care for acute promyelocytic leukemia (APL) (Sanz et al., 2019). Since then, plant-derived pharmaceuticals, including vinblastine (VBL), vincristine (VCR), etoposide, paclitaxel, docetaxel, topotecan, and irinotecan, have gained significant popularity and stand among the most effective cancer chemotherapeutics in the market (Pavithra et al., 2024). Notwithstanding their toxicities, adverse effects, and formulation challenges such as low solubility, these natural drugs have proven effective in cancer treatment (Škubník et al., 2021; Dhyani et al., 2022). The anticancer drugs derived from plants along with their mechanisms of action is presented in Appendix (IV).

The significance of natural products in future drug discovery is unequivocal. Plant derived phytochemicals provide promising and effective solutions to the complexities of cancer therapy. Their enduring relevance as a source of novel anticancer agents highlights the need to integrate these compounds into contemporary treatment strategies, positioning them as pivotal contributors to the advancement of modern oncology. Plant derived high value bio-actives are poised to serve as catalysts for innovation, heralding a new era in cancer treatment through the likely development of targeted, personalized, and more sustainable therapies in the ensuing future.

1.6. Herbal Products: An Attractive Facet in Health Care

Herbal product formulations represent the preparation and blending of medicinal extracts from plants and natural components into functional products for wellness and health applications. These formulations may exist as tablets, capsules, powders, teas, ointments, syrups, or oils (Bommakanti et al., 2023). The global market for herbal product formulations has witnessed substantial growth recently, propelled by rising customer preference for natural, plant-based substitutes in pharmaceuticals, nutraceuticals, cosmetics, and functional foods. This transition is

bolstered by scientific research, regulatory progress, and a growing preference towards holistic health. Consumers are progressively embracing herbal products owing to apprehensions regarding synthetic chemicals, adverse effects of conventional medications, and an interest for environmentally friendly and organic alternatives (Smith & Myers, 2021). A Grand View Research survey (2023) revealed that 65 % of consumers favour natural remedies over synthetic medications for minor ailments. The prevalence of traditional medicine systems such as *Ayurveda*, *Traditional Chinese Medicine* (TCM), and naturopathy has intensified this trend (WHO, 2024). Turmeric (curcumin), ashwagandha, ginger, and ginseng are extensively utilized in dietary supplements for their anti-inflammatory, adaptogenic, and immunostimulatory attributes (Mikulska et al., 2023; Yang et al., 2024). Herbal remedies are gaining recognition for the management of chronic conditions, including diabetes, arthritis, and cardiovascular diseases (Wang et al., 2023). Moreover, the global herbal nutraceuticals market is anticipated to expand at a compounded annual growth rate (CAGR) of 7.5 % from 2023 to 2030 (Market Research Future, 2023). In addition, functional foods enhanced with herbal extracts, including moringa-infused snacks and herbal teas, are increasingly popular for preventive healthcare (Kashyap et al., 2022).

In the world of skin care products, the demand for clean-label, chemical-free cosmetics has led to the incorporation of herbal ingredients such as aloe vera, neem, chamomile, and tea tree oil in skincare formulations (Market Reports World, 2024). A report by Allied Market Research (2022) indicates that the herbal cosmetics market is projected to attain $36.3 billion by 2027, with a CAGR of 5.8 %. Prominent trends encompass: Anti-aging formulations incorporating green tea and rosemary extracts; Haircare products featuring amla, bhringraj, and fenugreek; Natural preservatives such as grapefruit seed extract are substituting parabens. The emergence of herbal product formulations signifies a notable transition towards holistic health, integrating traditional knowledge with contemporary science. With rising demand, technological advancements, and an emphasis on sustainability, herbal formulations are expected to greatly influence the future of global healthcare.

1.7. Challenges and Opportunities in Ethnomedicinal Plant Research

Research on ethnomedicinal plants is of major significance to long-term health care and drug discovery. This field, grounded in traditional knowledge systems, particularly those of indigenous communities, investigates the medicinal properties of plants used throughout generations. Although ethnomedicinal plant research presents numerous opportunities, it has various challenges that impede its complete potential. A comprehensive understanding of both is crucial for the effective advancement and implementation of traditional knowledge within the realm of 'Modern Therapeutics' in general and 'Phytomedicine' in particular.

One of the major challenges is the swift erosion of indigenous knowledge caused by globalization, modernization, and the diminishing population of traditional healers. Younger generations' divergence from ancestral practices jeopardizes the preservation of valuable ethnobotanical knowledge (Damle et al., 2022). Secondly, in numerous regions, traditional knowledge is transmitted orally from one generation to another. This oral transmission restricts accessibility leading to inconsistencies or loss of information. Lack of documentation hampers researchers in validating and safeguarding ethnomedicinal practices. Thirdly, ethnomedicinal claims often lack scientific evidence. A gap between traditional usage and clinical evidence, hinders the incorporation of these practices into mainstream medicine. Moreover, challenges associated with the standardization of plant extracts, dosages, and active compounds complicate research findings (Damle et al., 2022). Fourthly, overexploitation, habitat destruction, and environmental degradation limit the availability of medicinal plant species. The challenges of conservation are exacerbated by lack of knowledge and poor oversight of wild plant collection. Last but not the least, biopiracy and the absence of equitable benefit-sharing with indigenous communities present significant ethical dilemmas. Pharmaceutical companies frequently exploit traditional knowledge without adequate acknowledgment or remuneration to the knowledge holders, violating upon intellectual property rights and indigenous sovereignty (Wanzala & Minyoso, 2024).

Despite these challenges, ethnomedicinal plant research presents significant opportunities for modern therapeutics. Ethnomedicinal plants offer a substantial reservoir of bioactive compounds that can be utilized as precursors for novel drug development. Continued studies may yield incremental advancements in the treatment of various human diseases. Scientifically validated ethnomedicinal practices can enhance modern medicine by providing holistic and personalized healthcare solutions (Alum, 2024). This integration is particularly beneficial in rural and resource constrained environments where traditional medicine frequently serves as the primary healthcare option. Advancing the cultivation and commercialization of medicinal plants can enhance rural economies and provide livelihoods for indigenous and local communities. This additionally promotes conservation by generating value. Tools such as genomics, metabolomics, and bioinformatics facilitates a more thorough analysis of plant characteristics and mechanisms of action. These technologies facilitate the integration of traditional knowledge with contemporary science, augmenting the credibility and applicability of ethnomedicinal research (Satrio et al., 2024). The growing global interest in natural and alternative therapies is enhancing the significance of ethnomedicinal plant research worldwide. This trend presents opportunities for global collaboration, financing, and knowledge transfer (Wanzala & Minyoso, 2024). Despite challenges concerning knowledge preservation, scientific validation, and ethical implications, it simultaneously offers exceptional prospects for healthcare, preservation of biodiversity, and community development. A collaborative, considerate, and rigorous scientific investigations can enable the achievement of this field's maximum potential.

Objectives

Based on the above background information, the present research work was carried out with the following specific objectives:

1) Evaluation of the population status and distribution of the selected rare ethnomedicinal plant species of Meghalaya
2) Biochemical characterization/profiling of the selected rare ethnomedicinal plants
3) Molecular characterization of the selected rare ethnomedicinal plant species
4) Assessment of bioactivity with specific focus on antimicrobial, antioxidant and anticancer properties
5) Investigation of the feasibility of product synthesis from the selected rare ethnomedicinal plants [Herbal tea, Ready to consume juice (RCJ)]

CHAPTER 2

REVIEW OF LITERATURE

Medicinal plants have played a pivotal role in healthcare since antiquity and continue to hold significant relevance in contemporary medicine. Despite considerable advancements in synthetic drug development, a substantial proportion of modern pharmaceuticals are either directly derived from or inspired by plant-based compounds. Furthermore, medicinal plants form the cornerstone of various integrative and complementary medical systems including *Ayurveda*, *Traditional Chinese Medicine* (TCM), *Kempo*, and naturopathy, which are increasingly gaining global recognition for their therapeutic potential. A specific compelling feature of medicinal plants is their complex composition of bioactive constituents, which often act synergistically to produce enhanced pharmacological effects. Medicinal plants offer a promising source of novel therapeutics and potential treatments for diverse human diseases, driving future innovations in healthcare.

2.1. Work done Abroad

2.1.1. GIS-Based Study of Ethnomedicinal Plant Distribution

Medicinal plants have always been fundamentally important to traditional healthcare systems worldwide. The growing demand for natural remedies, driven by the popularity of herbal medicine and the quest for bioactive compounds, necessitates a comprehensive understanding of the spatial distribution of medicinal plant species. Generally, medicinal plants are distributed unevenly over ecological and cultural landscapes. Conventional methods for analyzing these patterns predominantly depended on fieldwork and textual documentation, which frequently exhibited insufficient spatial accuracy (Zhang et al., 2011). GIS bridges this gap by facilitating the development of layered maps that integrate ecological, topographical, and climatic data (Yi et al., 2016).

The application of GIS tools in ethnobotany has revolutionized the techniques that allow researchers record, examine, and preserve medicinal plant resources. GIS integrates spatial data with ethnomedicinal knowledge, offering a dynamic framework for analyzing distribution patterns, identifying biodiversity

hotspots, and facilitating the sustainable management of plant-based healthcare resources (Wu et al., 2019). GIS facilitates the identification of appropriate habitats for medicinal plants and emphasizes areas with elevated species richness or endemism. Spatial analysis facilitates the mapping of current distribution patterns and the prediction of prospective regions where these plants could thrive under specific environmental conditions (Zhao et al., 2018). This is particularly crucial for rare and endangered species, as their conservation is vital for preserving biodiversity and traditional knowledge systems (Gamal et al., 2020).

In recent years, GIS has been used successfully in ethnobotanical research to record indigenous medicinal knowledge and associate it with geographic areas. It helps in recognizing regions where traditional usage of plant remains important and facilitates the prioritization of areas for conservation or additional ethnobotanical investigation.

Al-Bakri et al. (2011) examined the spatial distribution of medicinal plants in arid regions of Jordan using GIS technology. They investigated the correlations between the quantity of medicinal plant species and various land use types, altitude, slope, and aspect by generating GIS layers of land use and cover. The study area yielded 600 plant species, comprising 223 medicinal species. Intensive agricultural activities and urbanization, that affected the spatial distribution of medicinal plants were assessed in the study area. The quantity of medicinal plant species exhibited a marked decline with increasing altitude.

Wu et al. (2019) developed the Global Medicinal Plant Geographic Information System (GMPGIS) to assess environmental data of ecologically appropriate areas, thereby facilitating the conservation and introduction of medicinal plants. This system integrates theories and methodologies from various disciplines, including computer science, geoinformatics, ecology, and traditional herbal medicine. They assessed the previously identified ecologically suitable regions using a range-based method. The findings indicated that the results, corroborated by real-

world regions, demonstrated that GMPGIS is exceptionally precise in identifying ecologically suitable areas for the global cultivation of medicinal plants.

Liang et al. (2019) performed a study predicting the ecological suitability of *Panax quinquefolius* using the GIS for global medicinal plants (GMPGIS). Based on 476 occurrence points and 19 bioclimatic variables, they identified new ecologically suitable regions for *P. quinquefolius*, which includes East Asia and Eastern Europe, primarily encompassing China, Russia, Japan, Ukraine, Belarus, North Korea, South Korea, and Romania. Additionally, they asserted that, based on the current global climate change scenario, the ideal planting regions for *P. quinquefolius* would expand by 9.16 % – 30.97 %, extending northward and westward beyond the currently ecologically suitable regions by 2070.

Naghipour Borj et al. (2019) investigated the impact of climate change on the distribution of the endangered medicinal plant *Fritillaria imperialis* L. in central Zagros, using GIS technology. Two topographic variables and eight bioclimatic variables were used as inputs for the Maximum Entropy model (MaxEnt) for modeling its distribution based on correlation analysis. The findings indicated that temperature seasonality (55.1 %) and precipitation during the driest quarter (22.9 %) were significant determinants of suitable habitat for *F. imperialis*.

Gamal et al. (2020) developed a GIS model to assess the distribution of endangered plant species (*Ebenus armitagei* and *Periploca angustifolia*) in the Wadi Al-Afreet region of Egypt. The proposed model was predicated on identifying the correlations between established botanical phenomena and environmental variables. The model illustrates the distribution of environmental variables for the two endangered species. It predicts the locations where the two endangered plant species are anticipated to vanish along Wadi Al-Afreet. The model predicts that the most threatened locations for *Ebenus armitagei* were in the southeastern region of Wadi Al-Afreet and the eastern section of the midstream. Their findings asserted that the developed GIS-based model can assist in prioritizing plants for the restoration of their natural habitats, thereby enhancing plant conservation and restoration efforts.

GIS-based studies of medicinal plant distribution provide a scientific, efficient, and sustainable approach to managing plant resources. Integrating ecological data with geospatial technology enhances comprehension of plant-environment interactions, provides conservation strategies, and facilitates the sustainable use of medicinal plant biodiversity (Wu et al., 2019; Tshabalala et al., 2022). As technological advancements enhance the precision and availability of GIS tools, their application in ethnomedicinal plant research is expected to increase, substantially aiding the integration of traditional knowledge with modern scientific approaches.

2.1.2. Biochemical Profiling of Ethnomedicinal Plants

Medicinal plants have long been considered as significant reservoirs of therapeutic compounds. Their use in traditional systems of medicine across various cultures underscores the need for scientific validation and investigation of their bioactive components. The analysis of biochemical and phytochemical components is essential for understanding the therapeutic potential, nutritional significance, and pharmacological attributes of these plants.

Biochemical characterization involves the analysis of primary metabolites such as carbohydrates, proteins, amino acids, and lipids, all of which play essential roles in plant growth and development. These compounds also affect the nutritional quality of medicinal plants and may provide beneficial therapeutic effects (Pruteanu et al., 2018). For example, proteins and free amino acids contribute significantly to tissue repair and metabolic functions in the human body, whereas carbohydrates serve as fuel for the body (Hsu et al., 2021). Assessing these biochemical constituents contributes to a comprehensive understanding of the inclusive health benefits and potential supplementary uses of medicinal plants. Meanwhile, phytochemical profiling focuses on secondary metabolites including alkaloids, flavonoids, phenols, tannins, terpenoids, saponins, and glycosides. These compounds are primarily responsible for the therapeutic properties of plants,

including antioxidant, anti-inflammatory, antimicrobial, anticancer, antidiabetic activities, etc., (Vemuri et al., 2019; Agidew, 2022).

Over the past two decades, advancements in analytical techniques such as HPLC, GC-MS, LC-MS/MS, FTIR, and NMR have revolutionized the biochemical profiling process (Zaheer et al., 2021; Haddou et al., 2023). These tools not only enhance compound identification and structural elucidation but also support the standardization and quality control of plant-based formulations.

Erdogan et al. (2013) determined the phytochemical components of the *Phagnalon graecum* plant using HPLC and GC-MS techniques. They reported that HPLC analysis identified two phenolic acids: ferulic acid and O-coumaric acid. GCMS analysis identified 38 compounds, with the major compounds comprising germacrene, hexahydrofarnesyl acetone, β-caryophyllene, hexadecanoic acid, caryophyllene oxide, and δ-cadinene.

Bankole et al. (2016) conducted a qualitative and quantitative investigation of the principal bioactive compounds in plants used for malaria treatment in Nigeria. The qualitative analysis indicated the presence of alkaloids, phenols, flavonoids, steroids, glycosides, proteins, anthraquinones, saponins, and terpenes. The quantitative analysis revealed a substantial presence of the following compounds: alkaloids (1.43 – 6.48 mg/100g), phenols (22.73 – 41.41 mg/100g), flavonoids (4.41 – 13.08 mg/100g), tannins (29.24 – 46.93 mg/100g), glycosides (2.65 – 3.05 mg/100g), and saponins (8.46 – 10.64 mg/100g).

Riondato et al. (2019) performed a phytochemical analysis of the plant species used by the indigenous populations residing in the Maromizaha forest, Madagascar. The study employed spectrophotometric techniques and HPLC. A total of 117 plant species were evaluated and 22 distinct bioactive compounds were identified. The compounds including polyphenols, monoterpenes, organic acids, and vitamin C, were found in all the investigated plant species.

Mahnashi et al. (2021) investigated the phytochemical constituents of *Habenaria digitata* using GC-MS analysis. The study identified a total of 65 bioactive compounds. Some of the major compounds detected were, ethanimidic acid, methanediamine, 4H-pyran-4-one, humuladienone, 2-hexadecen-1-ol, etc.

Haddou et al. (2023) carried out a phytochemical investigation using HPLC-UV/GC-MS on various extracts of *Cannabis sativa* seeds from Morocco. The quantitative analysis of total polyphenols revealed that ethanolic and aqueous extracts contained the highest concentrations of polyphenolic compounds. The findings from GC–MS indicate that linoleic acid and 7-octadecenoic acid are the primary components. Moreover, the HPLC analysis of the organic extracts indicated the likely presence of catechin dihydrate, 6-hydroxyflavone, ferulic acid, 8-methoxyflavone, hesperidin acid, rutin, and cinnamic acid in the dichloromethane extract. In the ethanolic extract, naringin acid was the predominant component, constituting 41.92 %, followed by rutin, hesperidin acid, O-dianisidine, ferulic acid, chlorogenic acid, coumarin, and cinnamic acid.

2.1.3. Molecular Authentication of Ethnomedicinal Plants

The rising global demand for herbal medicines, coupled with overharvesting, habitat destruction, and unregulated trading, has resulted in widespread adulteration and replacement of plant materials. Such misidentifications may lead to diminished therapeutic efficacy or possibly harmful adverse effects (Sheidai et al., 2019). For instance, the adulteration of the roots of *Stephania tetrandra* S. Moore, an anti-inflammatory agent, with those of *Aristolochia fangchi* Y. C. Wu ex L. D. Chow & S. M. Hwang, led to kidney failure in nearly 100 women in China (El Beyrouthy et al., 2013). This also applies to *Cinnamomum verum* J. Presl bark that was contaminated with *Cinnamomum cassia* (L.) J. Presl and *Cinnamomum malabatrum* (Burm. f.) J. Presl. *Cinnamomum cassia* comprises 1 % coumarin, a naturally occurring flavouring agent associated with hepatotoxicity (Sheidai et al., 2019). Consequently, precise identification of medicinal plants is essential for guaranteeing the safety, efficacy, and consistency of herbal

formulations. Traditional techniques, dependent on morphological, anatomical, and phytochemical characteristics, frequently encounter limitations, particularly when plant material is desiccated, pulverized, or processed (Smillie & Khan, 2010). DNA barcoding, a molecular technique, has become a robust, rapid, and reliable method for authenticating medicinal plants, markedly decreasing misidentification and adulteration in herbal products.

DNA barcoding employs concise, standardized genomic regions for species identification. Numerous barcode loci in plants have been evaluated, with rbcL, matK, ITS, and trnH-psbA spacer regions being the most commonly utilized (Pang et al., 2012). The ideal barcode demonstrates significant interspecific variability and minimal intraspecific divergence, facilitating the differentiation of closely related taxa (Techen et al., 2014). For example, ITS2 is particularly effective in differentiating species within intricate genera like *Aconitum* and *Asparagus*, which are widely utilized in *Ayurveda* and traditional medicine (He et al., 2010).

Asahina et al. (2010) performed an investigation on the identification of medicinal *Dendrobium* species through phylogenetic analyses with matK and rbcL sequences. They investigated five medicinal *Dendrobium* species, viz. *D. fimbriatum*, *D. moniliforme*, *D. nobile*, *D. pulchellum*, and *D. tosaense*. The phylogenetic trees derived from matK data effectively differentiated each species from one another. Conversely, rbcL, as a single-locus barcode, exhibited lower species discrimination capability compared to matK, likely due to its limited variation. The integration of matK sequences of *D. officinale* from the DNA database revealed a close genetic relationship between *D. officinale* and *D. tosaense*, advancing the understanding of their taxonomic identity.

Liu et al. (2012) authenticated 38 *Rhododendron* species using four DNA barcodes, viz, rbcL, matK, psbAtrnH, and the ITS2 intergenic spacer. The findings indicated that the psbAtrnH barcode yielded a sequencing efficiency of 86.8 %, and highest interspecific divergence. Thus, their results indicated that the psbA-

trnH intergenic spacer was the most promising of the four markers for barcoding *Rhododendron* species.

Miao et al. (2019) performed a study to identify a universal DNA barcode for the classification of all toxic medicinal plants in the Chinese pharmacopoeia, along with their toxic relatives or adulterants. They selected four widely utilized regions as candidate DNA barcodes (ITS2, psbA-trnH, matK, and rbcL) and assessed their identification efficacy across 106 species from 27 families and 65 genera in total. The ITS2 sequence region exhibited significant variation, stable genetic distance, and relatively high identification efficiency. The topological structure of the Neighbor-Joining (NJ) phylogenetic tree indicated monophyly. The study's findings indicate that ITS2 can serve as a universal barcode for the identification of toxic medicinal plants in the Chinese pharmacopoeia, and their toxic relatives or adulterants.

Cahyaningsih et al. (2022) carried out DNA barcoding analysis on 61 Indonesian medicinal plant species from 30 families using ITS2, matK, rbcL, and trnL primers. The objective of the study was to examine how the four designated DNA barcoding regions (ITS2, matK, rbcL, and trnL) facilitate identification and conservation, as well as to assess their efficacy for DNA barcoding of the species. The study revealed 212 DNA barcoding sequences and identified novel ones. Although no optimal region exists for DNA barcoding of the target species, matK is recommended as the primary region for identifying Indonesian medicinal plants, with ITS2 and rbcL serving as alternative or supplementary regions.

2.1.4. Bioactivity Assessment of Ethnomedicinal Plants

The bioactivity evaluation of traditionally used medicinal plants serves as a critical bridge between ethnomedicine and modern pharmacology. Grounded in centuries-old indigenous wisdom, these plants embody a significant repository of potentially innovative therapeutic agents (Calixto et al., 2001). Bioactivity assessment denotes the methodical evaluation of the biological effects of plant extracts or isolated compounds, specifically regarding their antioxidant,

antimicrobial, anti-inflammatory, anticancer, and other pharmacological properties (Wangchuk et al., 2011; Tlili et al., 2019; Dubale et al., 2023). It establishes the scientific basis to authenticate traditional assertions, connect cultural practices with clinical significance, and promote the conservation and sustainable utilization of plant biodiversity.

Antioxidant potential is a frequently evaluated bioactivity, indicating a plant's capacity to neutralize free radicals and mitigate oxidative stress, a significant factor in aging and chronic diseases such as cancer, diabetes, and cardiovascular disorders. *In vitro* assays, including DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS, and FRAP, are frequently employed to measure antioxidant activity (Magalhães et al., 2008; Ramírez-García et al., 2022; Rumpf et al., 2023). Antimicrobial activity is a vital bioactivity, especially considering the rising antibiotic resistance. Conventional plants have been investigated for their ability to inhibit the growth of pathogenic bacteria, fungi, and viruses. Bioassays, including the disc diffusion method, broth microdilution, and agar well diffusion, are utilized to assess antimicrobial efficacy (Klančnik et al., 2010; Balouiri et al., 2016). The anticancer activity of specific plant-derived compounds is attracting attention due to their cytotoxic potential. Bioassays such as the MTT and SRB assays evaluate cell viability and proliferation across diverse cancer cell lines (Li et al., 2012).

Mothana et al. (2011) evaluated the antimicrobial, anticancer, and antioxidant properties of Yemeni medicinal flora. Cytotoxic activity was observed in the studied plants against the bladder cancer cell line (5637) and the breast cancer cell line (MCF-7). Antimicrobial activity with MIC values ≤ 125 µg/ml was detected against gram-positive bacteria.

Fatma et al. (2017) investigated extracts derived from Tunisian ethnomedicinal plants to evaluate their antioxidant, cytotoxic, and antimicrobial properties. Antioxidant activity was assessed using the DPPH radical scavenging assay and the β-carotene bleaching method, while cell viability was determined via the MTT assay. The study revealed that 11 plant species exhibited notable biological

activity. Moderate antibacterial effects were observed against one or more tested bacterial strains at concentrations of 300 mg and 3 mg per disc. In addition, certain extracts and hydrophobic fractions demonstrated strong DPPH radical scavenging activity, with EC_{50} values ranging from 6.78 to 8.55 µg/ml. Notably, specific extracts showed significant cytotoxicity against RAW 264.7 cells (a murine macrophage cell line derived from mouse tumor), with IC_{80} values of 0.36 and 1.55 µg/ml, respectively.

Tran et al. (2020) evaluated the antibacterial, anticancer, and antioxidant properties of *Euphorbia hirta* Linn. extracts prepared using various solvents, including methanol, petroleum ether, chloroform, ethyl acetate, and butanol. Among the tested extracts, the ethyl acetate fraction exhibited the highest antioxidant activity, with an IC_{50} value of 10.33 ± 0.01 µg/ml. In antibacterial assays, this extract demonstrated broad-spectrum efficacy against multiple bacterial strains. Additionally, it showed notable anticancer activity against the Hep G2 liver cancer cell line at a concentration of 100 µg/ml.

Korkmaz et al. (2021) examined the antioxidant, antimicrobial, and antiproliferative properties of *Galium aparine* extract. The agar dilution method was employed to assess the antimicrobial effect against bacteria and fungi. The A549 lung carcinoma cell line was used to assess antiproliferative activity. The findings reveal that the total antioxidant capacity of the plant extracts was measured at 5.147 ± 0.237, the total oxidant status (TOS) at 18.679 ± 0.245, and the oxidant stress index (OSI) at 0.346 ± 0.018. Plant extracts demonstrated efficacy against tested microorganisms at concentrations ranging from 50 to 200 µg/ml. Furthermore, it was established that the antiproliferative effect of the plant extract exhibited significant effects correlated with the elevation of extract concentration.

Khalid et al. (2022) conducted an evaluation of the antioxidant, antimicrobial, and anticancer properties of *Sisymbrium officinale* plant extract. The extract was evaluated against three bacterial strains (*Streptococcus spp., Staphylococcus aureus* and *Escherichia coli*) using the well diffusion technique. The

antioxidant capacity of the plant extract was assessed using the DPPH method, while the total phenolic and flavonoid contents were determined through a recognized chemical assay. The extract's anticancer activity was assessed against two cancerous cell lines: breast (MCF7) and colon (HCT116). The results indicated that the extract is abundant in polyphenols and flavonoids, exhibiting significant antioxidant activity as evidenced by its scavenging of free radicals (DPPH) at 193.7 ± 3.4 µg/ml. The extract demonstrated significant antimicrobial activity against both *Escherichia coli* and *Streptococcus* bacteria, with inhibition zones of 10 mm and 14 mm, respectively. The extract also demonstrated anticancer activity (approximately 6 %) against the MCF7 breast cancer cell line.

Jongrungraungchok et al. (2023) conducted a study to evaluate the antioxidant capacity, anticancer properties, and antimigration activities of Clear belongs Plus extract (CBL-P), which comprises five medicinal plants: *Piper nigrum*, *Alpinia galanga*, *Tiliacora triandra*, *Citrus aurantifolia*, and *Cannabis sativa*, on human colon cancer cell lines SW620 and HCT116, as well as human non-small cell lung cancer cell lines A549 and NCI-H460. The results indicated that CBL-P exhibited significant antiproliferative activity, with IC_{50} values demonstrating concentration- and time-dependent effects across all four cell lines. CBL-P exhibited significant antimigration efficacy against all examined cancer cell lines. CBL-P exhibited antimigration properties on four distinct cancer cell lines (A549, NCI-H460, HCT116, and SW620) following 48 h of incubation, with the most pronounced effect observed at the maximum concentration (15 µg/ml) in A549 cells (wound closure of 10.23 %) and NCI-H460 cells (9.16 % wound closure). CBL-P was also effective in diminishing movement in SW620 and HCT116 cells, with a closure area reduction ranging from 10 % to 50 %. Furthermore, CBL-P exhibited antioxidant activity with IC_{50} values of 8.549 ± 0.241 mg/ml for the DPPH assay and 2.673 ± 0.437 mg/ml for the ABTS assay.

2.1.5. Herbal Formulations for Healthcare

The past few decades have witnessed a renewed predilection for herbal products. This increase can be ascribed to a convergence of factors, including increased consumer awareness of natural remedies, the drawbacks and adverse effects of synthetic pharmaceuticals, and a revival of interest in traditional medical systems such as *Ayurveda*, *Traditional Chinese Medicine* (TCM), and *Unani* (Smith & Myers, 2021; WHO, 2024). Herbal products are now extensively accessible as dietary supplements, functional foods, cosmetics, personal care products, and pharmaceuticals. Consumers are increasingly aware of their dietary and topical choices, favouring natural and plant-based alternatives to synthetic products (Market Research Future, 2023). The COVID-19 pandemic further intensified this trend, leading many to adopt immunity-enhancing herbal formulations like Ashwagandha, Giloy, Tulsi, and Turmeric, thereby increasing demand in both domestic and global markets (Mikulska et al. 2023; Yang et al., 2024; Harfiani et al., 2025).

The herbal industry also benefited from the scientific validation of traditional knowledge. Multiple studies have validated the pharmacological properties of medicinal plants (Mothana et al., 2011; Fatma et al., 2017; Kebede et al., 2021; Kulaphisit et al., 2023). These evidences have bolstered consumer confidence on herbal products, often as alternative or complementary therapies. Additionally, the expansion of the nutraceutical and wellness industries has played a critical role. Herbal supplements containing extracts of Ginseng, Elderberry, Garlic, and Green tea have witnessed a marked rise in supermarkets and pharmacies (Shokri-Mashhadi et al., 2021; Gonçalves & Gaivão, 2024). These are promoted not only for disease management but also for improving vitality, cognitive function, and overall well-being.

Kiani et al. (2018) evaluated the antidiabetic efficacy of a polyherbal formulation (Tetraherb) in streptozotocin (STZ)-induced diabetic rats. Four herbs, namely Cinnamon (*Cinnamomum zeylanicum*), fenugreek (*Trigonella foenum-graecum*), shallot (*Allium hirtifolium Boiss*), and clove (*Syzygium aromaticum*), were

incorporated into the polyherbal formulation. Severe diabetic rats were administered orally with ethanolic extracts of cinnamon, fenugreek, shallot, and clove individually at a dosage of 75 mg/kg, or in a combined formulation (Tetraherbs) at doses ranging from 100 to 300 mg/kg once daily for a duration of 28 days. The impact on glucose concentrations, plasma lipid profiles, hepatic enzyme activity, and pancreatic histology were assessed. The results revealed that the blood glucose-lowering efficacy and pancreatic β cell regeneration of Tetraherbs were significantly superior to those of the individual plants used separately. No significant difference in the lipid-lowering and hepatoprotective efficacy of the herbs was observed, regardless of their use individually or in combination.

Bendaali et al. (2023) conducted a study to elucidate isotonic beverages enriched with bioactive compounds and antioxidant properties, utilizing organic ingredients and devoid of synthetic additives. Grape juice served as a natural source of sugars and phenolic compounds, combined with lemon juice and natural flavours derived from herb and spice extracts. The beverages exhibited a sugar concentration of 72.73 ± 0.23 to 78.43 ± 0.06 g/L, total soluble solids of 4.23 ± 0.06 to 4.83 ± 0.29 °Brix, and total acids ranging from 1.75 ± 0.02 to 2.39 ± 0.08 g/L. The antioxidant activity in beverages infused with herb and spice extracts was generally elevated, ranging from 3.28 ± 0.01 to 4.27 ± 0.09 µmols TE/ml. The sensory analysis results indicated that the flavoured beverages exhibited superior global perception values compared to the respective controls.

Maleš et al. (2023) conducted a study to examine the efficacy of sage (*Salvia officinalis* L.) and wild thyme (*Thymus serpyllum* L.) extracts, along with their combination (wild thyme:sage at a ratio of 3:1, v/v), in enhancing fruit juices (apple, pineapple, and orange). The acquired beverages were assessed for sensory characteristics, phenolic and headspace composition (via UPLC-MS/MS and HS-SPME/GC-MS analysis), and antioxidant capacity (using the ORAC assay). The addition of wild thyme extract to pineapple juice yielded the most balanced flavor and the greatest concentration of volatile compounds. The orange juice formulations exhibited the highest enrichment of phenolic and volatile compounds. The

formulation containing orange juice and sage extract exhibited the highest antioxidant capacity, measuring 22925.39 ± 358.43 µM TE. The research indicated that fortifying fruit juices with sage and wild thyme extracts could yield functional beverages with enhanced sensory attributes and health benefits.

Atlaw et al. (2024) conducted the formulation and characterization of herbal tea derived from Hibiscus (*Hibiscus sabdariffa* L.) and Lemon verbena (*Aloysia citrodora*). Dried *Hibiscus* calyces and lemon verbena leaves were blended in the following proportions: 90:5 (HL1), 90:10 (HL2), 85:15 (HL3), 80:20 (HL4), 75:25 (HL5), 70:30 (HL6), 65:35 (HL7), 60:40 (HL8), 55:45 (HL9), and 50:50 (HL10), with commercial tea and 100 % *Hibiscus* was used for comparative analysis. It was reported that *Hibiscus* calyces can be blended with 10 - 45 % dried Lemon verbena leaves to create functional tea with satisfactory sensory characteristics (color, flavor, taste, aftertaste, and overall acceptability), as well as optimal levels of total flavonoids, phenolics, and antioxidant scavenging capacities.

Noorulla et al. (2024) formulated a syrup incorporating green bean pod extract for its anti-urolithiatic properties. The syrup was formulated by blending and refined via a central composite design (CCD) incorporating two independent variables: the ratio of pod juice (PJ) to sugar solution (SS) varying from 1:0.5 - 1:1.5, and the concentration of carboxymethylcellulose (CMC) ranging from 0.2 % - 0.4 % w/v. The variables were examined for their influence on viscosity and sedimentation percentage, facilitating the identification of the optimal formulation among 13 variants. The optimized formulation was a green, viscous, transparent syrup with a pH of 5.8, a viscosity of 256.38 CP, a density of 1.31 g/ml, and a sedimentation rate of 0.69 %. The optimized formulation demonstrated stability, exhibiting no notable alterations in physicochemical and microbiological properties. The findings from the *in vitro*, *ex vivo*, and *in vivo* anti-urolithiatic studies demonstrated that the optimized formulation significantly prevented the aggregation of calcium oxalate. The acute toxicity studies indicated no mortality or adverse effects for both the optimized formulation and pure bean pod juice at a dosage of 2000 mg/kg body weight. Histopathological analysis indicated that rats administered with optimized

formulation demonstrated a marked decrease in both the quantity and dimensions of calcium deposits.

Avazsoofian et al. (2025) developed a herbal beverage through the spray drying of a mixture comprising basil extract and lemon juice in a 65: 35 (% v/v) ratio. Maltodextrin (MD) at varying concentrations (0 - 7.5 %) was used as the encapsulating material. GC-MS analysis indicated that the primary aromatic constituents in the final powder, lemon juice, and the reconstituted product were limonene and γ-terpinene; limonene; and γ-terpinene, linalool, and 1.8-cineole, respectively. The findings further indicated that spray drying (SD) with MD blends enhanced the integration of bioactive compounds in powdered form.

2.2. Work done in India

2.2.1. Distribution Study of Ethnomedicinal Plants using GIS

India, known for its extensive biodiversity and a centuries-old tradition of using medicinal plants, is among the largest repositories of ethnomedicinal knowledge in the World (Jain, 1994; Jain & Mudgal, 1999). Over 7000 plant species are utilized in traditional medicine systems such as *Ayurveda*, *Siddha*, and *Unani*. Understanding their distribution is crucial for sustainable use, conservation, and drug discovery (Jain & Mudgal, 1999; Arora et al., 2003). The diverse topography of India, which includes the Himalayan ranges in the North, the Western and Eastern Ghats, central plateaus, and coastal plains, fosters the growth of numerous medicinal plant species. Many of these valuable plants face threats from overharvesting, habitat loss, and climate change. GIS have become an indispensable tool for analyzing the distribution of medicinal plants within the varied ecological landscapes of India (Nimasow et al., 2016). GIS-based studies provide a systematic method for addressing these challenges through the identification of priority conservation areas and ecologically sensitive zones. In the Western Ghats and Northeastern states, GIS has assisted in the identification of species-rich zones and areas experiencing anthropogenic pressure, thereby enabling targeted conservation actions (Nimasow et al., 2016; Saadi et al., 2020).

GIS mapping in the Himalayan region has enabled the investigation of high-altitude medicinal plants, including *Lilium polyphyllum, Angelica glauca, Pleurospermum angelicoides,* and *Arnebia benthamii*. These species are recognized for their therapeutic properties but are classified as critically endangered. By overlaying species occurrence data with altitude, temperature, and rainfall patterns, researchers could model the potential distribution and suitable habitats for *in situ* and *ex situ* conservation (Kandari et al., 2011; Dhyani et al., 2021).

Amarnath et al. (2003) investigated the spatial distribution of evergreen forests in Tamil Nadu, India, an ecological hotspot in the Western Ghats, using remote sensing and GIS-based analytical techniques. A vegetation type map was prepared using Indian Remote Sensing Satellite Linear Imaging Self-Scanning Sensor III (IRS LISS III) satellite data to analyze patch characteristics, including patch size, number, shape, porosity, and land cover diversity (LD). The study reveals that evergreen forests in the Tirunelveli hills, covering 216.09 km², are fragmented into 306 patches, while in the Palni hills, with an area of 285 km², are divided into 1029 patches, demonstrating significant fragmentation. Landcover spatial heterogeneity, as indicated by LD, was significantly higher in the Nilgiri hills compared to the Tirunelveli hills.

Qayum et al. (2014) conducted an interdisciplinary study that integrates ethnomedicinal findings with GIS tools to create spatio-temporal maps of antimalarial plants found in three rural districts of Eastern Uttar Pradesh, India. A total of 48 plant species from 25 families were identified, with their geographical distribution depicted through a series of GIS maps. The map outlines the geographical distribution of antimalarial plants and enhances accessibility to their natural habitats.

Nimasow et al. (2016) carried out a remote sensing and GIS-based suitability assessment for the medicinal plant *Taxus baccata* in Arunachal Pradesh, India. The secondary metabolite Taxol (Paclitaxel) derived from *Taxus baccata* is broadly applied in chemotherapy to treat various cancers, including breast, ovarian,

lung, etc. The MaxEnt and Spatial Multi-Criteria Evaluation (SMCE) models were employed for suitability modelling of *Taxus baccata*. The occurrence points and environmental layers, including current global climate, altitude, slope, and aspect, were used for the MaxEnt analysis. The results indicate that 5.31 % of the region is classified as highly suitable or suitable, while 80.14 % is deemed not suitable.

Dwivedi et al. (2020) performed a GIS mapping of antimalarial plants based on traditional knowledge in Madhya Pradesh, India. A total of 19 antimalarial plants were recorded from 13 families and 19 genera. The coordinates of the plant locations were recorded using a handheld Global Positioning System (GPS), and geographical distribution maps were generated using GIS. The constructed multilayered database encompasses botanical information, spatial distribution, and plants used for antimalarial activity.

2.2.2. Biochemical Characterization of Ethnomedicinal Plants

Although the use and documentation of medicinal plants dates back thousands of years to ancient Ayurvedic texts like the *Charaka Samhita* and *Sushruta Samhita* (around 600–100 BC), the systematic and scientific biochemical analysis of ethnomedicinal plants in India commenced in the mid-20th century (Jain, 1994; Sreedevi et al., 2013). The formation of institutions such as the Council of Scientific and Industrial Research (CSIR) and the Central Drug Research Institute (CDRI) in the 1950s led to a more systematic approach to phytochemical and biochemical research (Rastogi, 1990). The increase in ethnobotanical surveys, particularly in tribal and biodiversity-rich areas such as the Western Ghats, Northeast India, and the Himalayas, has resulted in targeted phytochemical analysis of indigenous medicinal flora (Ved & Goraya, 2007). Meanwhile, modernized research commenced with sophisticated and analytical instrumentation facilities. Institutions like AYUSH, ICMR, NIPER, and various Indian Universities are actively engaged in research pertaining to traditional knowledge and their linkage with phytochemicals (Tandon & Yadav, 2017).

In recent decades, with the growth of herbal and pharmaceutical industries, GC-MS, LC-MS, HPLC, FTIR, and NMR spectroscopy became common tools in medicinal plant research in India (Tyagi & Agarwal, 2017; Fatima et a., 2021; Tiwari et al., 2021). For instance, Susanna et al. (2022) conducted metabolite profiling of the medicinal plant *Nothapodytes foetida* using FTIR, GC-MS, NMR, and LC-MS techniques. The presence of alcohols, phenols, alkanes, amino acids, nitro compounds, amines, and carboxylic acids in the extract was confirmed by FT-IR. Additionally, 33 volatile compounds, including viminalol, 9-methoxy-camptothecin, α and β-amyrins, β and γ-sitosterol, lupeols, and various di- and tri-terpenes in substantial amounts, were identified using the GC-MS. NMR analysis revealed well-resolved signals corresponding to flavonoids, organic acids, and several amino acids, including tryptophan, histidine, tyrosine, phenylalanine, alanine, isoleucine, glycine, glutamine, threonine, and valine. .

Das et al. (2014) estimated carbohydrate and protein contents of *Costus speciosus* by Anthrone's and Lowry's method, respectively. In addition, they performed preliminary phytochemical screening. The results revealed that total protein content of leaf, stem and rhizome were 99.45 mg/g, 86.938 mg/g and 57.94 mg/g, while total carbohydrate content were 25.97 mg/g, 20.72 mg/g and 27.63 mg/g, respectively. The presence of phytochemicals such as saponin, tannin, steroid, phenol and flavonoid were also reported.

Singh et al. (2023) conducted a study in which they prepared an ethanolic extract of a polyherbal formulation applying the cold maceration technique and identified bioactive compounds through GC-MS analysis. The polyherbal extract contained a total of 35 phytochemicals, of which 22 bioactive compounds were present in substantial quantities. Some of the identified compounds are 2-buten-1-ol, 2-ethyl-4-(2, 2, 3-trimethyl-3-cyclopenten-1-yl, 1, 2, 5, 6-tetrahydrobenzonitrile, 4-piperidinamine, 2, 2, 6, 6-tetramethyl, undecanoic acid, 5-chloro, and chloromethyl ester.

Furthermore, integration of biochemical knowledge with ethnopharmacological information has the potential to contribute in novel nutraceuticals and cosmeceuticals. Products like herbal teas, functional foods, and Ayurvedic skin cares are progressively seeking evidence-based validation for the bioactivity of their ingredients, a section where phytochemical profiling plays a crucial role (Singh et al., 2021).

2.2.3. Molecular Characterization of Ethnomedicinal Plants

Recently, India has made considerable progress in using DNA barcoding to verify its extensive collection of medicinal plants. For instance, research conducted by the National Botanical Research Institute (NBRI) and the Centre for DNA Fingerprinting and Diagnostics (CDFD) has facilitated the identification of adulterants in commercial samples of *Withania somnifera* (Ashwagandha), *Bacopa monnieri* (Brahmi), and *Centella asiatica* (Gotu kola) (Thakur et al., 2019; Amritha et al., 2020; Shah et al., 2023). The differentiation of closely related or morphologically similar species, such as *Terminalia chebula* and *Terminalia bellirica*, both integral to the traditional formulation *Triphala*, has been effectively achieved through DNA barcoding (Sharma & Shrivastava, 2016).

Researchers have accurately identified plant species, particularly those listed in the Ayurvedic Pharmacopoeia using standardized DNA barcode regions, such as rbcL, matK, and ITS (Vassou et al., 2016). Numerous barcoding studies have effectively distinguished closely related or morphologically identical species that were frequently substituted in traditional markets. For instance, DNA barcoding has been employed to differentiate *Asparagus racemosus* from its adulterants and *Withania somnifera* (Ashwagandha) from the species that are closely associated (Shanmughanandhan et al., 2016). This has significantly aided in quality monitoring and the prevention of toxic misidentifications.

Barcoding efforts specific to regions have also gained momentum. In biodiversity hotspot such as the Western Ghats, Northeast India, and the Himalayan belt, DNA barcoding is being used to catalog and authenticate the endangered and

endemic species (Mishra et al., 2017). For example, in Meghalaya and Arunachal Pradesh, where traditional ethnomedicinal knowledge is rich but vulnerable, researchers are applying ITS2 and matK barcodes to authenticate species such as *Paris polyphylla* (Islam et al., 2021), *Clerodendrum sp*. (Gogoi et al., 2020), and *Ilex venulosa* (Nongbet & Chrungoo, 2022). These plants are often subject to illegal trade and overharvesting.

Saha et al. (2020) conducted DNA barcoding on selected *Zingiberaceae* species from North-East India. They assessed the effectiveness of four candidate barcoding loci (ycf1b, rbcL, ITS, and ITS2) on thirteen species from four genera of *Zingiberaceae*. The findings demonstrated that the conserved sites were most abundant in ycf1b and least abundant in ITS. In contrast, ITS demonstrated greater interspecific and intraspecific variations than the other loci. The mean interspecific divergence of ycf1b exceeded the intraspecific divergence, exhibiting a barcode gap that enabled the differentiation of all samples in a species-specific manner within the single-locus tree-based analysis. The results suggest that ycf1b may serve as a potential DNA barcode for the precise identification of *Zingiberaceae* plant species.

Devi et al. (2022) conducted a study on the identification of *Zanthoxylum armatum* DC. from Manipur using molecular markers. Molecular markers, including ITS region and DNA barcoding genes such as matK, rbcL, psbA-trnH, and trnL-trnF, were analyzed to determine the most potent DNA barcode for species identification. The study indicated that the ITS sequence, when combined with DNA barcoding sequences of rbcL, trnH-psbA, and trnL-trnF, effectively identified *Z. armatum* and distinctly differentiated it from other species in phylogenetic analysis. The ITS region emerged as the most appropriate DNA barcode, forming a distinct monophyletic clade of the species in phylogenetic analysis.

2.2.4. Ethnomedicinal Plants and their Bioactivity

Traditionally used medicinal plants have constituted the foundation of rural healthcare and cultural therapeutic practices throughout the nation. In recent decades, there has been a notable transition from informal usage to systematic scientific validation employing bioactivity assessments. India's ethnomedicinal flora, extensively recorded in ancient texts such as the Charaka Samhita and Sushruta Samhita, have been scientifically validated; additionally, their traditional therapeutic claims and novel therapeutic agents were identified (Baliga et al., 2012; Mukherjee et al., 2016).

Singh et al. (2016) examined the antioxidant capacity, cytotoxic effects on HepG2 (human hepatocellular carcinoma) cell lines, and antimicrobial properties of the methanol extract from traditional medicinal plants sourced from Mizoram, India. The antioxidant capacity was assessed using DPPH (IC_{50} values ranging from 34.22 to 131.4 µg/ml), ABTS (IC_{50} values ranging from 24.08 to 513.4 µg/ml), and reducing power assays. The antimicrobial activity was evaluated against gram-positive (*Staphylococcus aureus*), gram-negative (*Escherichia coli, Pseudomonas aeruginosa*), and yeast (*Candida albicans*), indicating that the methanol extracts of certain plants were effective antimicrobial agents. Cytotoxicity was evaluated on human hepatocellular carcinoma (HepG2) cell lines, revealing that the extracts of *Albizia lebbeck*, *Dillenia indica*, and *Bombax ceiba* markedly reduced cell viability at low concentrations, with IC_{50} values of 24.03, 25.09, and 29.66 µg/ml, respectively.

Palani et al. (2020) conducted a study to evaluate the antimicrobial efficacy against pathogens, antioxidant properties, and larvicidal activities of *Goniothalamus wightii* leaf extract. The extract significantly inhibited the proliferation of bacterial species such as *Streptococcus pyogenes, Staphylococcus aureus, Klebsiella pneumoniae*, and *Proteus vulgaris*, as well as fungal species (*Candida albicans, Aspergillus fumigatus, Fusarium oxysporum*, and *Trichoderma virideae*). *In vitro* antioxidant assays demonstrated a 70 % inhibition of free radicals.

Paw et al. (2020) examined the antioxidant, anti-inflammatory, genotoxic, and antimicrobial properties of the essential oil derived from the rhizome of *Curcuma caesia* Roxb, sourced from Northeast India. The standard antioxidative tests conducted include the DPPH assay, reducing power assay, and *in vitro* anti-inflammatory activities, specifically egg albumin denaturation and protease inhibitory assays. Antimicrobial activity was assessed using the disc diffusion method and MIC, while genotoxicity was evaluated using the *Allium cepa* assay. The essential oil exhibited superior antioxidant (IC_{50} = 48.08±0.003 µg/ml), anti-inflammatory (IC_{50} = 121.7 ± 0.0013 µg/ml), and antimicrobial activities compared to the standard drugs fluconazole for fungal infections and ciprofloxacin for bacterial infections. The essential oil exhibited potent antibacterial effect against the two bacterial strains, *B. subtilis* and *B. cereus*, with MIC of 7.5 µg/ml. Additionally, it demonstrated the highest efficacy against the yeast strain *S. cerevisiae*, with a MIC of 2.5 µg/ml.

Al-Qahtani et al. (2022) assessed the phytochemical and biological properties of *Myristica fragrans*, an Ayurvedic medicinal plant native to Southern India. The study identified twenty-three phytoconstituents, with elemicin constituting 24.44 % as the predominant component. The lipid peroxidase, catalase, and DPPH assays demonstrated substantial antioxidant activity. The antibacterial investigation demonstrated that elemicin exhibited a minimum inhibitory concentration (MIC) of 31.25 µg/ml against *Escherichia coli, Pseudomonas aeruginosa*, and *Salmonella typhi*, and 62.5 µg/ml against *Klebsiella pneumoniae* and *Staphylococcus aureus*.

2.2.5. Herbal Products from Ethnomedicinal Plants and the Future of Herbal Industry

India is renowned for its rich heritage of medicinal plants and traditional healing systems, such as *Ayurveda*, *Siddha*, and *Unani*. Herbal products derived from medicinal plants/herbs today lies in the vanguard of alternative health therapies. Rooted in ancient wisdom and sustained by generations of traditional knowledge, herbal products in India are experiencing a significant resurgence, driven

by a global shift toward natural and holistic well-being. The enduring significance of herbal products in India can be attributed to a profound trust in traditional medicine. *Ayurveda*, practiced for over 5000 years, constitutes the foundation of India's herbal health system. The emergence of *Ayurveda* between 1000 and 500 BC notably systematized and broadened the application of herbal remedies (Singh et al., 2022). Foundational Ayurvedic texts such as the Charaka Samhita and Sushruta Samhita provide comprehensive descriptions of plant-based medicines, including their classifications, formulations, dosages, and applications (Verma et al., 2024). These works highlighted a personalized and balanced approach to health, incorporating physical, mental, and spiritual well-being. Herbal preparations, including decoctions (Kashayas), powders (Churnas), oils (Tailas), and pastes (Lehyas), were formulated from locally sourced medicinal plants to meet specific individual requirements (Kakodkar et al., 2021). As for instance, Chyawanprash (a millennia old herbal formulation) is highly revered herbal formulations in Indian traditional medicine. Its origins are deeply rooted in ancient Indian history, mythology, and medical literature, dating back over 2000 years. It is believed that Maharishi Chyawan having grown old, was rejuvenated by consumption of a herbal health supplement which later on became known as Chyawanprash (Suryavanshi et al., 2021). Even today, it remains one of the most extensively used Ayurvedic formulations in India. Apart from India, Chyawanprash has gained global acknowledgment for its multitude of health benefits. The present market value of Chyawanprash is approximately $100–$120 million, with exports extending to over 30 countries (Newedge Overseas, 2025). The principal companies engaged in the production of this product include Dabur, Baidyanath, Zandu, Patanjali, and Himalaya.

Triphala (another common Indian herbal product), is a blend of the dried fruits from three medicinal plants: Amalaki (*Emblica officinalis*), Bibhitaki (*Terminalia bellirica*), and Haritaki (*Terminalia chebula*). Triphala is classified as a Rasayana, which refers to a category of therapies aimed at rejuvenation and anti-aging. Triphala is widely consumed by the Indian populace for proper maintenance of digestive health, and as an immunity booster. The present market value of

Triphala is approximately $35–$60 million, with Organic India, Himalaya, Dabur, Baidyanath, and Patanjali identified as the principal companies (Newedge Overseas, 2025).

Herbal products play a vital role in health and wellness and significantly impact the Indian economy. The herbal industry in India has experienced significant growth in recent years, as more individuals are opting for herbal alternatives in cosmetics, dietary supplements, personal care, and household remedies (Yadav et al., 2024). The Government has shown keen interest in promoting the herbal products industry in India and abroad. Initiatives such as the National AYUSH Mission (NAM), the formation of the Ministry of AYUSH, and policies promoting the cultivation of medicinal plants have fostered a conducive environment for the development of the herbal industry (Kumar et al., 2022a). These measures promote scientific validation, standardization, and quality assurance, thereby enhancing the credibility and global competitiveness of Indian herbal products. The Indian herbal market is projected to reach USD 10 billion by 2030, influenced by increasing consumer awareness, governmental support, and a heightened preference for natural and chemical-free products (CMI, 2025). The COVID-19 pandemic further underscored the significance of herbal products in immunity building and preventive healthcare. Products like *Kadha*, *Giloy juice* (*Tinospora cordifolia*), and herbal teas witnessed a surge in demand during this pandemic as people sought natural ways to boost their immunity. This shift highlighted the relevance of herbal remedies in contemporary healthcare (Kumar et al., 2022b).

Brindavanam et al. (2003) developed a novel oral liquid herbal formulation for the treatment of bronchial asthma. Each 100 ml of the formulation contained the following plant extracts: Kantakari (*Solanum xanthocarpum* - whole plant) 3 - 12 g, Shireesh chhal (*Albizia lebbeck* - bark) 5 - 15 g, Gokshur (*Tribulus terrestris* - seeds) 3 - 12 g, Yashthi madhu (*Glycyrrhiza glabra* - rhizome) 1 - 10 g, Karkatashringi (*Pistacia integerrima* - fruit) 1 - 5 g, Vasaka (*Adathoda vasica* - leaves) 1 - 5 g, *Woodfordia fruticosa* (flower) 1 - 7 g, *Piper longum* 0.1 - 0.3 g,

Elettaria cardamomum 0.1 - 0.3 g, *Syzygium aromaticum* 0.1 - 0.3 g, and *Mesua ferrea* 0.1 - 0.3 g. The incorporation of herbs *Piper longum*, *Elettaria cardamomum*, *Syzygium aromaticum*, and *Mesua ferrea* into the formulation augmented their efficacy as drug potentiators and significantly contributed to enhancing bioavailability. The herbal formulation was most efficacious solely in its liquid form rather than in any alternative physical form. The formulation demonstrated substantial antihistaminic efficacy and spasmolytic activity on animal models.

Reddy et al. (2007) developed a topical ointment formulation for the safe and effective reduction of dermal vessel tortuosity. The formulation comprised a non-aqueous extract of *Wrightia tinctoria* (5 %), *Cocos nucifera* (65 %), beeswax (6 %), liquid paraffin (24 %), with a coloring agent and fragrance as required. Clinical studies were conducted on twenty patients over a duration of 8 weeks, with two groups of ten patients each. The results clearly showed that the herbal formulation treatment significantly reduced dermal vessel tortuosities and eliminated dermal infiltrate compared to the initial condition prior to treatment. The tortuosity values of the dermal vessels diminished over time, indicating a favorable response to herbal treatment. Nonetheless, the allopathy control formulation demonstrated no statistically significant alteration in vital sign measurements over time as a result of treatment with the herbal formulation.

Goyal & Patel (2019) formulated a beverage to alleviate and avert the detrimental effects of alcohol consumption, including hangovers and hepatic cellular damage. The drink contains *Curcuma longa* rhizome extract, L-ornithine amino acid, and inositol as active components. The curcumin extract is standardized to >95 % pure curcuminoids, containing at least 12 % in the form of water-dispersible micelle liquid. A beverage composition method involves a specified quantity of curcumin extract, L-Ornithine, Inositol, and sugar syrup, wherein the curcumin, L-Ornithine, and Inositol powder are incorporated into the separately prepared sugar syrup while maintaining continuous stirring. The results demonstrated the *in vivo* breakdown of alcohol post-consumption, thereby aiding in the alleviation and prevention of detrimental effects associated with alcohol, including hangover and hepatic damage.

Dey et al. (2020) formulated BV-7310 for the treatment of alcoholic liver disease (ALD). BV-7310 is a standardized mixture of four Ayurvedic herbs: *Phyllanthus niruri*, *Tephrosia purpurea*, *Boerhavia diffusa*, and *Andrographis paniculata*. In various traditional medicine systems, each of these plants is recognized for its application in gastrointestinal disorders. They examined the hepatoprotective efficacy of BV-7310 against alcohol-induced toxicity in human liver HepG2 cells. Ethanol treatment (120 mM for 48 h) exhibited significant toxicity (approximately 42 %) in these cells, while coincubation with BV-7310 mitigated ethanol-induced cell death in a dose-dependent manner. Notably, the formulation BV-7310 exhibited greater synergistic activity than any individual extract evaluated in this assay. BV-7310 demonstrated significant antioxidant activity in the DPPH assay. BV-7310 mitigates alcohol-induced toxicity in both *in vitro* and *in vivo* models and may be advantageous for the treatment of alcoholic liver disease or other conditions that can lead to liver toxicity.

CHAPTER 3

MATERIALS AND METHODS

Chapter 3: Materials and Methods

Meghalaya, situated in Northeast India, is renowned for its vast biological diversity and indigenous ethnomedicinal knowledge. The present study focusses on rare ethnomedicinal plants used by indigenous populations of Meghalaya for the treatment of various ailments. A multitude of these plants remains scientifically unexplored, rendering them significant prospects for pharmacological evaluation and potential product development.

This study employed GIS to analyze the spatial distribution of plants, mapping current patterns and predicting potential regions for their growth under defined environmental conditions. Biochemical characterization was conducted to elucidate the qualities and quantities of primary metabolites (carbohydrates, proteins, and lipids) and secondary metabolites (phytocompounds). Molecular characterization was performed to identify and authenticate the selected rare ethnomedicinal plants at the molecular level. The biological activities, including antioxidant, antimicrobial, and anticancer properties, were investigated to assess their pharmacological potential. The study also includes an assessment of the viability of product synthesis.

3.1. Plastic/Glassware

All the sterilized polystyrene tubes were acquired from Torson, India. The beakers, flasks and test tubes were purchased from Borosil, Mumbai, India.

3.2. Chemicals Used

The chemicals and reagents used in the present study were of the analytical grade and were procured from Merck India Ltd, SRL, Qualigen, Himedia and Sigma-Aldrich. The reagents used for Gas Chromatography-Mass Spectrometry (GC-MS) analysis were of chromatography-grade.

3.3. Equipments Used

The equipment used in the present study is detailed below

1. Laminar Hood - RSI/VLF-16, Reico, Kolkata, India

2. Autoclave – LAC-3011V, Dainan Labtech, Co. Ltd, Gyeonggi-do, South Korea
3. Digital weighing balance – ME54, Metler Toledo, Ohio, United States
4. Water bath – 1201D, JSGW, India
5. Lyophilizer –Mini-Lyodel, Delvac Pumps Pvt Ltd, Chennai, India
6. Hot air oven – RDHO-50, Remi, Mumbai, India
7. Vortexer – Cat. No. 1228, JSGW, India
8. pH meter – Cyberscan 500, Eutech Instruments, Singapore
9. Heating mantle – Rivotech, India
10. Incubator – EN500, Labtech, Singapore
11. Incubator shaker – LT Part No. LT-TT-BH, Scigenics, Orbitek
12. Centrifuge – R4c, Remi
13. Cooling centrifuge – CM-8 Plus, Remi
14. Deep freezer – C34085, New Brunswick Scientific,
15. Sonicator – Ultrasonic homogenizer, OMNI International
16. UV spectrophotometer – Beckman DU530 and CECIL 7400

3.4. Distribution Mapping of the Species

The distribution mapping of *Goniothalamus simonsii* Hook.f. & Thoms., *Viburnum odoratissimum var. odoratissimum* and *Citrus latipes* (Swingle) Tanaka was conducted in five districts of Meghalaya. Table 3.1 and Fig. 3.1 outline the study region, encompassing its geographical coordinates.

The designated regions for the study were selected due to their reported high concentrations of rare and endemic species (Upadhaya et al., 2003; Mir et al., 2019). A field survey was conducted in the selected five districts from January-2021 to November-2023.

GPS coordinates were obtained for specified locations, and within a GIS framework, maps were generated to demonstrate the geographical distribution of the selected rare ethnomedicinal plants. The 30 m Digital Elevation Model (DEM) STRM was obtained from Open Topography (Acquisition date: 15-05-22). Landsat 8 – 9 TM, 30 m was obtained from USGS (Acquisition dates: 09-02-22, 14-02-22 and 24-02-22). The selected data offered cloud-free conditions, enabling the attainment of highly precise results. The image was processed with ArcGIS 10.8. The entire process of distribution mapping construction is illustrated in Fig. 3.2.

Table 3.1. Study areas and their geographical coordinates

Sl. No.	Study Area	Geographical coordinates
1	West Garo Hills	25°28'46.8" N, 90°18'48.3" E
2	South Garo Hills	25°15'12.3" N, 90°34'38.8" E
3	Ri-Bhoi	25° 55' 12" N, 91° 52' 27" E
4	East Khasi Hills	25°24'07.8" N, 91°46'19.4" E
5	Jaintia Hills	25°37'56.4" N, 92°26'11.5" E

3.5. Collection and Identification of the Plant Species

The fresh plant materials of the selected rare medicinal plants viz. *G. simonsii*, *V. odoratissimum var. odoratissimum* and *C. latipes* were collected from West Garo Hills [25°38'48" N, 90°18'40" E], East Khasi Hills [25°24'07.8" N, 91°46'19.4" E] and Nokrek Biosphere Reserve [25°27´42.5´´N, 90°19´20.3´´E], Meghalaya, India, respectively. The plant specimens were gathered during the flowering period, notably from April to June. The plant species' herbarium specimens were made using the procedure outlined by Seshagirirao et al. (2016). The plants were identified and authenticated at the Botanical Survey of India, Eastern Regional Centre, Shillong, Meghalaya, India [Reference No. BSI/ERC/Tech/2023-24/1847].

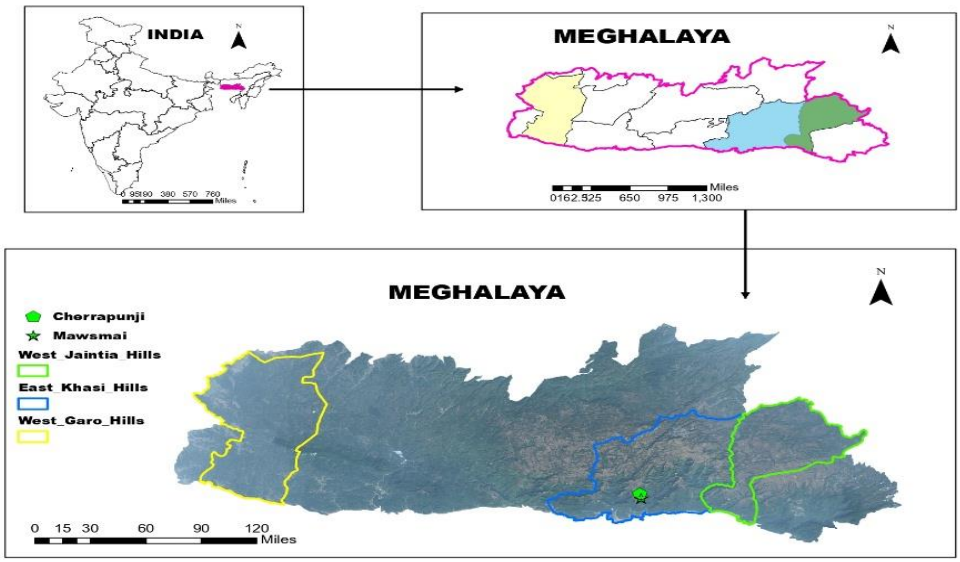

Fig. 3.1. Map outlining the study area

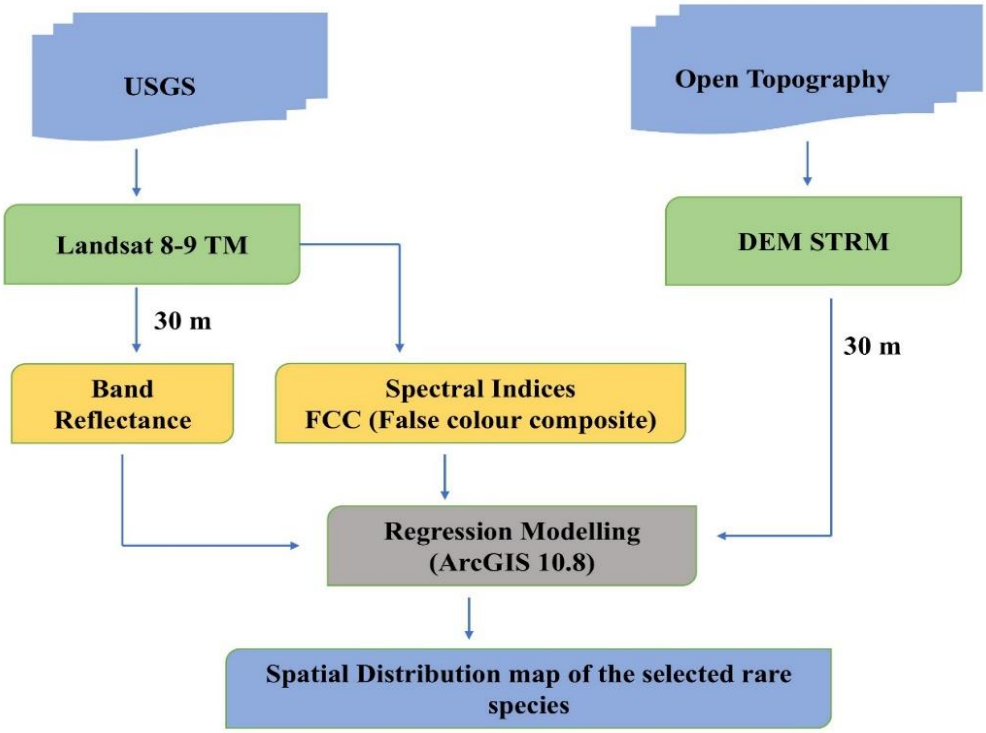

Fig. 3.2. Flowchart depicting the workflow for the construction of spatial distribution maps

3.6. Preparation of the Extracts

The collected fresh plant materials (leaves, fruits, barks, and roots) were washed multiple times using distilled water. Surfaces sterilization was done using 70 % C_2H_5OH and 1 % NaClO solution. The clean plant materials were desiccated in an oven at 32 ℃. The desiccated samples were properly pulverized using an electric grinder and macerated in hydro-methanolic solution (80 % CH_3OH) for 72 h at 35 ℃ with constant stirring. The extracts were finally concentrated using a rotary vacuum evaporator. The concentrated extracts were kept at 4 ℃ for subsequent analysis.

3.7. Biochemical Characterization

3.7.1. Estimation of Carbohydrate Content

The total carbohydrate content of various plant parts (leaf, fruit, bark, and roots) was assessed using a modified version of Anthrone's method (Yemm & Willis, 1954). In brief, the 100 mg of powdered plant material was acid hydrolyzed for 3 h with HCl (2.5 N), followed by filtration through Whatman No. 4 filter paper (20 µm pore size). A volume of 13 ml of the filtrate was transferred to a test tube and subsequently cooled in an ice-water bath. Following cooling, 4 ml of Anthrone's reagent was added to the tube, which was then maintained at 100 °C for 10 min in a water bath. Similarly, 4 ml of Anthrone's reagent was combined with 1 ml of each concentration of standard glucose solutions (20 – 100 µg/ml) and left in a water bath for 10 min. Using a UV-Vis spectrophotometer, the mixture's absorbance was determined at 620 nm. Using the absorbance value of standard glucose solutions, a standard calibration curve was produced. Equation (1) was utilized to determine the sample's carbohydrate content. The results displayed are the mean values of triplicates and are presented as mg/g of the powdered sample.

$$Csample = \frac{1}{0.0062} (Abs620 - 0.0168) \quad (1)$$

Where, Abs_{620} is the sample's absorbance value at 620 nm and *Csample* is the sample's carbohydrate content.

3.7.2. Estimation of Protein Content

The protein content of the samples was estimated using Lowry's method (Lowry et al., 1951) with minor modifications. Using a mortar and pestle, 500 mg of plant material was homogenized in 10 ml of phosphate buffer solution. After centrifuging the mixture, the supernatant was gathered for the test. Reagent A (2 % Na_2CO_3 in 0.1 N NaOH) and reagent B (solution containing 0.5 % $CuSO_4$ and 1 % $KNaC_4H_4O_6 \cdot 4H_2O$) were combined in a 50:1 ratio to make the alkaline reagent. 4 ml of alkaline reagent and 1 ml of distilled water were combined with 1 ml of the collected supernatant. For 10 min, the mixture was incubated at room temperature. After incubation, the mixture was vortexed, 0.5 ml of Folin-Phenol reagent was added, and it was incubated for 30 min at 37 °C. A UV-Vis spectrophotometer was used to measure the mixture's absorbance at 660 nm. A standard calibration curve was created using standard protein solutions (bovine serum albumin) with different concentrations (200 – 1000 µg/ml). Equation (2) was used to determine the sample's protein content. The triplicate mean values are displayed in result and presented as mg/g.

$$P\text{sample} = \frac{1}{0.04} (Abs_{660} - 0.0094) \qquad (2)$$

Where, Abs_{660} is the sample's absorbance value at 660 nm and P_{sample} is the sample's protein content.

3.7.3. Determination of Lipid Content

The lipid content of the plant materials was determined using the Bligh & Dyer (1959) method. Briefly, 100 g of plant material was homogenized for 1 min in a 1:2 (v/v) chloroform: methanol mixture (100 ml $CHCl_3$ and 200 ml CH_3OH). Subsequently, an additional 100 ml of $CHCl_3$ was added, and the mixture

was further homogenized for 30 s. This was followed by the addition of 100 ml of distilled water and another 30 s of homogenization. The resulting mixture was filtered through Whatman No. 4 filter paper (20 µm) and allowed to stand for several min to facilitate phase separation. The upper aqueous layer was carefully removed using a micropipette, and the organic (lower) phase was subjected to vacuum distillation for drying. Lipid content was calculated using Equation (3).

$$Lc\ (\%\ \text{w/w}) = \frac{Wf}{Wi} \times 100 \qquad (3)$$

Where, Lc is lipid content, W_i is the weight of the sample in grams, W_f is the weight of the extracted lipids in grams

3.7.4. Preliminary Screening for Phytochemical

Preliminary screening for phytochemical was done according to the procedure described by Harborne (1998). The sample for the test was prepared by dissolving the crude extract in 95 % CH_3OH and filtered using Whatman No. 4 filter paper (20 µm). The filtrate was used for the test.

3.7.4.1. Test for Alkaloids

2 ml of the filtrate was mixed with 1 ml of Wagner's reagent. The appearance of a brown precipitate indicates the presence of alkaloids.

3.7.4.2. Test for Phenols

A Biuret reagent was combined with 1 ml of filtrate. The presence of phenols is indicated by the appearance of a blue colour.

3.7.4.3. Test for Flavonoids

A few drops of conc. HCl were added to 2 ml of filtrate along with a few pieces of magnesium ribbon. The presence of flavonoids was confirmed by the appearance of a pink or tomato-red colour.

3.7.4.4. Test for Tannins

2 ml of the filtrate were mixed with a few ml of 5 % $FeCl_3$. The presence of tannins is indicated by the appearance of a dark blue or greenish-black colour.

3.7.4.5. Test for Glycosides

2 ml of $CHCl_3$ and 2 ml of CH_3COOH were combined with a few mg of crude extract. Ice-cold water was used to cool the mixture. The ice-cold mixture was mixed with a few drops of conc. H_2SO_4. The presence of glycosides is confirmed when there is a colour shift from blue to green.

3.7.4.6. Test for Cardiac Glycosides

2 ml of CH_3COOH, 2 ml of 2 % $FeCl_3$, and 2 ml of conc. H_2SO_4 were combined with a few mg of the crude extract. The presence of cardiac glycosides is indicated by the development of a brown ring colour at the solution's interface.

3.7.4.7. Test for Steroids

The crude extract was mixed with a few ml of conc. H_2SO_4 and shaked vigorously. The mixture was allowed to stand for 2 min. The appearance of a red colour on standing indicates the presence of steroids.

3.7.4.8. Test for Terpenoids

2 ml of $CHCl_3$ and a few drops of conc. H_2SO_4 were combined with the crude extract. The presence of terpenoids is indicated by the appearance of a reddish-brown colour at the interface between $CHCl_3$ and H_2SO_4.

3.7.4.9. Test for Anthraquinones

2 ml of 10 % NH_3 and 1 ml of benzene were combined with 1 ml of the filtrate. The presence of anthraquinones is indicated by the appearance of a pink or red colour.

3.7.4.10. Test for Coumarins

2 ml of filtrate was mixed with 1 ml of 10 % NaOH and observed for the appearance of a yellow colour.

3.7.4.11. Test for Saponins

The crude extract was mixed with distilled water, shaken vigorously, and observed for the formation of foams.

3.7.5. Fourier Transform Infrared Spectroscopy

The Fourier Transform Infrared **(**FTIR) analysis of the plant samples was performed using a Perkin Elmer's Infrared Spectrometer (Spectrum 100). A small amount of the sample was mixed with KBr and pressed into a pellet. The spectra were obtained in the mid-infrared range (4000 - 400 cm^{-1}) using transmittance mode at ambient temperature (28 ± 2 °C). The spectral data were analyzed using a standard IR chart (Pavia et al., 2015).

3.7.6. Gas Chromatography-Mass Spectrometry Analysis

A Thermo-Fisher Scientific Gas Chromatograph-Mass Spectrophotometer (GC-MS) coupled with an ISQ7000 mass spectrophotometer, equipped with a TG-5MS fused silica capillary column (30 m x 0.25 mm, 0.25 mm) was used for the GC-MS analysis. Ultrapure Helium (99.99 %) gas was used as the carrier gas, with a flowing rate of 1 ml/min. The oven temperature was adjusted from 60 °C to 230 °C at a rate of 5 °C/min. The injector temperature was maintained at

250 ℃, with an injection volume of 1 µl and a split ratio of 1/100. The temperatures of the MS transfer line and ion source were set at 280 ℃ and 230 ℃, respectively. The retention time (RT) index and mass spectrum were compared and components were identified and confirmed as per the authentic reference given in the NIST library.

3.8. Molecular Characterization

3.8.1. DNA Isolation

The isolation of DNA from fresh plant materials was carried out using the CTAB method, following the protocol outlined by Allen et al. (2006) with minor modifications.

i. The 3X extraction buffer was preheated to 65 ℃ in a water bath and 0.3 % C_2H_6OS was added into the 3X CTAB extraction solution immediately prior to use.
ii. 50 mg of the plant sample were pulverized in liquid nitrogen using a pre-chilled mortar and pestle. While remaining in the mortar, 800 µl of the preheated 3X CTAB extraction buffer was put into the ground plant sample and mixed gently with the pestle.
iii. The sample mixture was placed in a 2 ml microcentrifuge tube and kept in a water bath at 65 ℃ for 1 h, subsequently cooled to room temperature. An equal volume of $CHCl_3$:$C_5H_{12}O$ (24:1 v/v) was added and mixed by gentle inversion.
iv. The mixture was centrifuged at 13000 rpm for 15 min at ambient temperature.
v. The top aqueous phase, which contains DNA, was carefully transferred to a new 1.5 ml Eppendorf tube using a wide-bore pipette.
vi. Half the volume of the aqueous phase of 6 M NaCl was introduced into the 1.5 ml Eppendorf tube containing DNA. Subsequently, 1/10

of the aqueous phase volume of 3 M CH_2CO_2K and 500 µl of ice-cold absolute C_3H_8O were mixed and gently inverted to facilitate DNA precipitation.

vii. The solution was incubated at −20 ℃ for 30 min and subsequently subjected to centrifugation at 13000 rpm for 5 min.

viii. The supernatant was discarded.

ix. The resulting DNA pellet was rinsed with 500 µl of 70 % C_2H_5OH. The sample was centrifuged at 13000 rpm for 5 min, after which the supernatant was discarded, and the pellets were air-dried at room temperature.

x. The DNA pellet was resuspended in 50 µl of 1X TE buffer and incubated at 50 ℃ for 1 h to achieve thorough resuspension. The resultant DNA was preserved at −20 ℃ for subsequent analysis.

3.8.2. DNA Amplification and Sequencing

DNA amplification was performed using the polymerase chain reaction (PCR) method with a T100 Thermal Cycler (Bio-Rad) for sequencing and phylogenetic analysis. It was performed using the primers rbcLa_F, rbcLa_R, matK-413f-1, matK-1227r-3, and ITS2. The primers were sourced from MOLBIOGEN, and the details are presented in Appendix (V). The PCR product comprises 12.5 µl of Taq DNA Polymerase Master Mix RED, 8.5 µl of deionized H_2O, 1 µl of each primer, and 2 µl of DNA template, all contained in a 0.2 ml Eppendorf tube. The amplification protocol includes a pre-denaturation step at denaturation at 95 ℃ for 1 min, annealing at 52 ℃ for 1 min, extension at 72 ℃ for 1 min, and a final extension at 72 ℃ for 5 min. The cycle was run 30 times (Leontidou et al., 2020). The efficacy of the PCR product was evaluated via electrophoresis (Mini Electrophoresis System/Mini ES-1), using a 1.5 % (w/v) agarose gel in TBE (Tris-boric acid-EDTA) once. Each well contained 2 µl of the PCR product and a 100 bp ladder marker. Electrophoresis was conducted at a voltage of 50 V for a duration of 50 min. The

resultant gel was immersed in ethidium bromide (EtBr) for 10 min and subsequently rinsed with distilled water. Following that, the gel was visualized with a UV transilluminator (E3000-E). A sequencing reaction was conducted using a PRISM BigDye Terminator v3.1 Cycle Sequencing Kit. The DNA samples with extension products were incorporated into Hi-Di formamide (Applied Biosystems, Foster City, CA). The solution was incubated at 95 °C for 5 min, subsequently placed on ice for 5 min, and then analyzed using the ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA). The sequenced DNA were analyzed using MEGA 12 software, and alignment was carried out using the Basic Local Alignment Search Tool (BLAST) available on the National Center for Biotechnology Information (NCBI) website (https://www.ncbi.nlm.nih.gov). Sequence similarity was assessed by comparing the obtained results with reference sequences archived in the GenBank database, ensuring accurate identification and validation.

3.8.3. Phylogenetic Analysis

Phylogenetic analysis was performed by comparing the consensus DNA sequences of *G. simonsii, V. odoratissimum var. odoratissimum*, and *C. latipes* with consensus data from related species within the respective genera and outgroups, using accession numbers from NCBI. Alignment was performed using ClustalW in MEGA 12 software. A phylogenetic tree was constructed employing the Maximum Likelihood (InL) method with 100 bootstrap replications and Tamura-Nei model (TN93).

The Tamura-Nei model is a nucleotide substitutions model employed in phylogenetic tree construction to calculate genetic distances among DNA sequences. It considers varying rates of transitions and transversions, differential base frequencies, and G+C content bias, providing a more precise representation of evolutionary processes. The TN93 model enhances simpler models such as Jukes-Cantor or Kimura 2-Parameter by integrating these factors. This methodology is

extensively employed in techniques like Maximum Likelihood and Neighbor-Joining and for the construction of phylogenetic trees, particularly in DNA barcoding, species identification, and molecular evolution research, thereby augmenting the reliability of deduced evolutionary relationships (Tamura, 1992).

3.9. Bioactivity Assessment

3.9.1. Antioxidant Activity

3.9.1.1. Determination of Total Phenolic Content

The phenolic content (TPC) was determined using the Folin-Ciocalteu (FC) method (Singleton & Rossi, 1965) with minor modifications. The FC reagent is a complex combination of heteropolyphosphotungstate-molybdate, functioning as an oxidizing agent in the experiment. Phenolic chemicals in the sample interact with the FC reagent, resulting in its reduction and the formation of a blue-coloured complex. The amounts of phenolic compounds present in the sample is directly correlated with the blue color's intensity.

Briefly, CH_3OH was used to dissolve the crude extract. After 45 min of sonication at 40 ºC, the mixture was centrifuged for 10 min at 1000 rpm. For the analysis, the clear supernatant was gathered. 6 ml of distilled water were added to a 15 ml test tube containing 100 µl of the collected supernatant. After adding 0.5 ml of FC reagent and properly vortexing the mixture, it was allowed to stand at room temperature for 4 min. After adding 1.5 ml of Na_2CO_3, the mixture was vortexed. After adding 1.9 ml of distilled water, the mixture was properly vortexed and incubated at room temperature in a dark chamber for 2 h. A UV-Vis spectrophotometer was used to measure the mixture's absorbance at 765 nm. The absorbance values of gallic acid solutions at different concentrations (250, 500, 750, 1000, and 1250 µg/ml) were used to generate the standard calibration curve.

Equation (4) was used to calculate the sample's TPC, and expressed as mg GAE/g of the crude extract.

$$TPC\ (mg\ GAE/g) = \{(AB_{sample} - b_{cal})/m_{cal}\} * (v/w) * 1000 \quad (4)$$

Where, AB_{sample} is the absorbance value of the sample, b_{cal} and m_{cal} are the intercept and slope of a standard calibration curve, v is the volume of the tested sample, and w is the extract's weight in the tested volume

3.9.1.2. Determination of Total Flavonoid Content

The total flavonoid content (TFC) was determined using the method outlined by Shen et al. (2009) with slight modifications.

The structural basis of flavonoids, a type of polyphenol, is a pyran ring connecting two benzene rings. $AlCl_3$ can combine with the C-4 keto groups and flavone and flavonol hydroxyl groups on the C-3 or C-5 positions to form acid-stable compounds. It creates acid-labile complexes when coupled with the ortho-dihydroxyl groups present on the A or B rings of flavonoids. As a result of this complicated creation, a spectrophotometer can detect a change in colour, most commonly a shift towards yellow. An acidic pH is optimal for $AlCl_3$ to form complexes with flavonoids, and $C_2H_3NaO_2$ aids in maintaining and adjusting this pH while also improving the complexation of $AlCl_3$ with flavonoids.

The sample for the analysis was prepared following the procedures described in the section 3.9.1.1. A 1 ml test sample was mixed with 4 ml of CH_3OH and 1 ml of 10 % $AlCl_3$, followed by incubation for 6 min at room temperature. Subsequently, 1 ml of $C_2H_3NaO_2$ solution was added into the mixture, followed by thorough vortexing and incubation for 45 min at room temperature in a dark room. The absorbance of the mixture was measured with a UV-Vis spectrophotometer at 415 nm. A calibration curve was established using the absorbance values from a standard series of quercetin solutions at concentrations of 25, 50, 100, 150, and 200

µg/ml. TFC was determined using equation (5), with results expressed as mg QE/g of crude extract.

$$TFC\ (mg\ QE/g) = \{(A_{sample} - b_{cal})/m_{cal}\} * (v/w) * 1000 \quad (5)$$

Where, A_{sample} is the absorbance value of the sample, b_{cal} and m_{cal} are the intercept and slope of a standard calibration curve, v is the volume of the tested sample, and w is the extract's weight in the tested volume

3.9.1.3. DPPH Free Radical Scavenging Assay

The plant samples were evaluated for their free radical scavenging activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, following the protocol described by Xiao et al. (2020) with minor modifications. DPPH is a stable free radical that exhibits a deep purple colour in its powdered form, which turns yellow upon reduction by an antioxidant. This colour changes forms the basis of the assay, serving as an indicator of free radical scavenging activity. The reaction between DPPH and an antioxidant (denoted as H – A) can be represented as follows.

$$(DPPH) + (H - A) \rightarrow DPPH\text{-}H + (A)$$

Antioxidants react with DPPH, reducing it to DPPH-H, which results in a decrease in absorbance. The degree of discolouration corresponds to the scavenging capacity of the antioxidant compounds or extracts, reflecting their hydrogen-donating ability.

Briefly, the crude extract was dissolved in CH_3OH and subsequently diluted to generate a series of concentrations (10, 20, 30, 50, 100, 250, and 500 µg/ml). As a standard free radical eliminating agent, ascorbic acid in different concentrations (10, 20, 30, 50, and 100 µg/ml) was also used in the test. 1 ml of the prepared sample was combined with 3 ml of DPPH reagent, vortexed thoroughly,

and kept for 30 min in dark. At 517 nm, the absorbance was measured. Control was prepared by mixing 1 ml of CH_3OH and 3 ml of DPPH reagent.

The formula (6) provided below was used to determine the radical scavenging percentage (% I).

$$\% I = \{(A(control) - A(sample))/A(control)\} \times 100 \quad (6)$$

Where, % I is free radical scavenge percentage, *A(control)* is absorbance of the control (DPPH + CH_3OH), *A(sample)* is absorbance of the sample (Sample + DPPH + CH_3OH).

IC_{50} (Inhibitory Concentration) was calculated from the free radical scavenging percentage (% I).

3.9.1.4. Ferric Reducing Antioxidant Power Assay

The ferric-reducing capacity (FRAP) of the plant sample was assessed using the method given by Benzie & Strain (1996) with minor modifications. The FRAP reaction occurs at an acidic pH of 3.6 to preserve iron solubility. The low pH reduces ionization potential, facilitating hydrogen atom transfer and enhancing redox potential, which is the primary reaction mechanism. Antioxidants function as reducing agents by donating electrons to the Fe^{3+} ion, thereby reducing it to Fe^{2+}. The resultant Fe^{2+} ion interacts with the probe tripyridyltriazine (TPTZ), yielding a blue-coloured compound. The intensity of the blue colour is quantified with a spectrophotometer, generally at 593 nm. The decrease in absorbance is directly proportional to the antioxidant activity of the sample. The quantity of Fe^{3+} reduced serves as an indicator of the substance's antioxidant capacity, typically represented as Fe^{2+} equivalents or in comparison to a standard antioxidant such as ascorbic acid.

The sample for the analysis was prepared following the procedures described in the section 3.9.1.1. A 3 ml working solution of FRAP and 100 µl of the

sample were mixed. The mixture was shaken thoroughly and incubated at 37 ℃ for 15 min in the dark. Absorbance was recorded at a wavelength of 593 nm. Ascorbic acid served as the standard, while CH_3OH was used as the blank control in the assay. A standard calibration curve was generated using the absorbance values of a series of ascorbic acid solutions at concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mM/L. The FRAP value of the sample was determined using equation (7), with results presented as micromoles (µM) of ascorbic acid equivalent per gram of crude extract.

$$\text{FRAP value } (\mu M \text{ AAE}/g) = c \times V \times t / m \quad (7)$$

Where, c is the ascorbic acid concentration (µmol/ml) of the corresponding standard curve of the diluted sample, V is the sample volume (ml), t is the dilution factor, and m is the weight of the sample dry matter (g).

3.9.1.5. $ABTS^+$ Assay

The plant samples were evaluated for their potential to reduce the $ABTS^+$ radical cation using the method described by Xiao et al. (2020) with minor modifications. ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) is oxidized to its radical cation ($ABTS^{\bullet+}$) using a reagent such as $K_2S_2O_8$. The $ABTS^{\bullet+}$ radical cation exhibits a blue-green colour and absorbs light at 734 nm. In the presence of antioxidants, electrons are donated to $ABTS^{\bullet+}$, reverting it to its original colourless form. The decolourisation of $ABTS^{\bullet+}$ correlates with antioxidant activity and can be quantified spectrophotometrically by observing the reduction in absorbance at 734 nm. Increased antioxidant activity correlates with a greater decrease in $ABTS^{\bullet+}$ and a corresponding reduction in absorbance at 734 nm.

The crude extract was dissolved in CH_3OH and sonicated for 45 min at 40 ℃. The mixture was then centrifuged at 1000 rpm for 10 min, and the clear supernatant was collected for further analysis. A volume of 100 µl of the supernatant was added to 2.0 ml of the $ABTS^+$ working solution, mixed thoroughly, and

incubated in the dark at room temperature for 20 min. The absorbance was measured at 734 nm. Ascorbic acid, prepared at varying concentrations (0.2–1.0 mM), was used as the standard to construct a calibration curve, while distilled water served as the blank control. The steps listed below were used to calculate the plant sample's final ABTS value:

- First, the $ABTS^+$ scavenging percentage of the sample and standard series was calculated using equation (8)

$$ABTS^+ \text{ scavenging percentage } (\%) = \{(Ab - As)/Ab\} \times 100 \quad (8)$$

Where, *Ab* is the absorbance of the blank, *As* is the absorbance of the sample

- The percentage derived from different standard concentrations was then used to create the standard graph.
- The final ABTS value of the sample was then calculated from the standard calibration curve, and the results were expressed as micromoles (µM) of ascorbic acid equivalent per gram of crude extract.

3.9.2. Antimicrobial Activity Assay

3.9.2.1. Test Organisms

The present study employed five bacterial species as test microorganisms: two gram-positive (*Staphylococcus aureus* MTCC 11949 and *Bacillus cereus* MTCC 8361), three gram-negative (*Escherichia coli* MTCC 593, *Salmonella enterica* MTCC 1166, and *Yersinia pestis*), and a fungus (*Candida albicans* MTCC 13013). The freeze-dried microbes were sourced from IMTECH, Chandigarh, India. The microbes were revived using suitable culture medium and preserved in agar slants at 4 ºC. The strains were activated through subculturing at 37 ºC for 24 h on a new suitable agar plate (Nutrient agar for bacteria and Sabourand dextrose agar for *C. albicans*) before conducting any antimicrobial assays.

3.9.2.2. Determination of Inhibition Zone

The agar well diffusion method was employed to measure the diameter of the zones of inhibition, following the procedure described by Holder & Boyce (1994) with minor modifications. The test samples were prepared by dissolving crude hydro-methanolic extracts in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/ml. The inoculum was prepared by taking a loopful of culture from the stored culture and inoculating it into pre-sterilized Mueller-Hinton broth, followed by incubation at 37 ºC for 24 h (for bacteria) and 48 h (for fungus). The cell concentration of 1 x 10^8 CFU/ml was obtained by diluting the incubated broth culture with 0.9 % normal saline and matching the turbidity to a 0.5 McFarland standard. 0.1 ml of the prepared inoculum was evenly spread onto pre-sterilised Mueller-Hinton agar. An 8 mm well was bored with a sterile cork borer. 0.1 ml of the prepared sample and DMSO were placed into the wells as the test samples and a negative control, respectively, using a micropipette. A standard antibiotic disc containing ciprofloxacin (5 µg/disc) for bacteria and fluconazole (25 µg/disc) for fungus was also appropriately placed. The test plates were incubated at 37 °C for 24 h (for bacteria) and 48 h (for fungi). Following the incubation, the diameter of the inhibition zone was measured and recorded.

3.9.2.3. Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) was measured using the micro broth dilution method, following the procedures described by Bussmann et al. (2010) with minor modifications. Inoculum was prepared by transferring multiple colonies of microorganisms to 5 ml of sterile distilled water. The suspensions were agitated for 15 s and then diluted to achieve a turbidity equivalent to the 0.5 McFarland standard (1 × 10^8 CFU/ml).

Stock solutions of the plant extracts were prepared in 100 % DMSO at a concentration of 100 mg/ml. The working solutions (3000 µg/ml) were prepared by diluting the stock solutions in sterile Mueller-Hinton broth.

The test was carried out in sterile 96-well microplates. All the wells received 100 µl of Mueller-Hinton broth supplemented with 10 % glucose and 0.5 % phenol red. 100 µl of the working solution (3000 µg/ml) of the extracts was dispensed into the wells in rows A to H of column 1. Using a multichannel pipette, 100 µl was transferred from column 1 to column 2, and the well contents were thoroughly mixed. Identical serial 1:2 dilutions were continued up to column 10, and 100 µl of surplus medium was removed from the wells in column 10. 5 - 10 µl of the inoculum suspension was added to the wells in rows A - H, columns 1 - 11. Column 11 and 12 served as drug-free controls. Two-fold serial dilutions of ciprofloxacin (Stock concentration 100 µg/ml) and fluconazole (Stock concentration 100 µg/ml) were used as positive controls against bacteria and fungi, respectively. Each microplate was covered and incubated for 24 h at 37 °C. The red colour of the well was interpreted as no growth, and wells with a defined yellow colour were scored as positive due to the formation of acidic metabolites corresponding to microbial growth.

3.9.3. Antiproliferative Activity Assessment

The crude extracts were evaluated for their potential antiproliferative activity. The human colorectal cancer cell line (HT-29) was employed in the present investigation.

The HT-29 colorectal cancer cell line was procured from the National Centre for Cell Science (NCCS), Pune, India, and cultured in DMEM enriched with 10 % fetal bovine serum (FBS) under a 5 % CO_2 environment at 37 °C.

The MTT assay was employed to assess the antiproliferative efficacy of the crude extract on HT-29 cells (Khodavirdipour et al., 2020). The cells were inoculated in 96-well culture plates at a density of 1×10^6 cells per plate in DMEM enriched with 10 % fetal bovine serum (FBS) and kept at 37 °C in a 5 % CO_2 incubator. Following a 24 h, the medium was substituted with different concentrations of the extract (10, 50, 100, 250, 500, 1000 µg/ml) and incubated at 37 °C for an additional 24 h. Subsequently, 50 µl of MTT solution (5 mg/ml in PBS) was mixed with an equal volume of DMEM and added to each microtiter plate well, followed by a 4 h incubation of the samples at 37 °C. The precipitated formazan dye (an indicative of the viable cells) were subsequently solubilized in MTT solvent for 15 min in a shaker incubator. The absorbance at 590 nm was measured using a Multiskan GO spectrophotometer (Thermo Fisher Scientific, ESW version 1.00.40, Finland). The viability of cells was determined through comparative analysis using the formula provided below. The IC_{50} was estimated using Origin 2024b (version 10.15).

$$\text{Cell viability (\%)} = (OD_{tc}/OD_{uc}) \times 100 \qquad (9)$$

Where, OD_{tc} is the OD value of treated cells, OD_{uc} is the OD value of untreated cells (control)

3.10. Product Synthesis and Characterizations

3.10.1. Preparation of Ingredients for Microencapsulation

The present study samples viz. *Goniothalamus simonsii* (leaf), *Viburnum odoratissimum var. odoratissimum* (leaf and bark) and *Citrus latipes* (bark) were selected as primary ingredients based on their notable bioactivity results. The samples of other potential medicinal plants including *Citrus aurantium* (fruit pulp), *Tinorpora cordifolia* (stem), *Piper nigrum* (seed), *Acorus calamus* (whole plant), *Curculigo orchioides* (root), and *Kaempferia parviflora* (root) were also

included as secondary ingredients to enhanced the therapeutic efficacy of the products. All the ingredients were washed properly and shade dried. Subsequently, they were ground to fine powder, and aqueous extracts were obtained through the process of maceration. The aqueous extracts were subsequently separated from the solvent, using a vacuum rotary evaporator. The final concentrated aqueous extracts were kept at 4 ºC for subsequent formulation processes.

3.10.2. Preparation of Microcapsules

The ingredients i.e. aqueous extracts [*Goniothalamus simonsii* (50 mg), *Viburnum odoratissimum var. odoratissimum* (50 mg), *Citrus latipes* (50 mg), *Citrus aurantium* (100 mg), *Tinorpora cordifolia* (50 mg), *Piper nigrum* (50 mg), *Acorus calamus* (80 mg), *Curculigo orchioides* (100 mg), and *Kaempferia parviflora* (80 mg)] were mixed to form a solution using deionized water and used as a core material. Maltodextrin (DE 10-16), sourced from Sigma-Aldrich (St. Louis, Missouri, USA) was used as the encapsulant for the microcapsules.

The microcapsules were prepared by spray drying method following the procedure outlined by Alaşalvar et al. (2020) with slight modification. Briefly, the liquid feed was made by dissolving the wall material (maltodextrin) at a 10 % concentration in deionized water. The solution was kept overnight at ambient temperature with constant stirring at 1000 rpm to ensure complete saturation of the polymer molecules. The following day, the prepared solution of ingredients was gradually added to the wall material solution and stirred at 3500 rpm for 10 min using a rotor-stator blender to form an emulsion. The mixture solution was then adjusted to 1 L volume by adding deionized water and kept stirring for 5 min.

The 1 L liquid feeds were dried using a nanospray dryer (BÜCHI B-90, Flawil, Switzerland) equipped with a two-fluid nozzle atomizer. The drying conditions were as follows: inlet air temperature of 160 ºC, aspiration rate of 100 %, air flow rate of 600 L/h and feed rate of 8 ml/min (Alaşalvar et al., 2020). The dried

powders were collected and stored in opaque, airtight containers at 4 ℃ until subsequent analysis.

3.10.3. Characterization of Microcapsules

3.10.3.1. Determination of Moisture Content

The revised method of Quek et al. (2007) was used for evaluating the moisture content of the spray-dried microcapsule powder. A 0.20 g sample was weighed in a pre-weighed, clean, dried crucible and subsequently dried at 105 ºC in an oven until a constant weight was achieved. The moisture was determined using the equation provided below.

$$M_c\ (\%) = [(W_i - W_f)/W_i] \times 100 \qquad (10)$$

Where, M_c represents the moisture content, W_i denotes the initial weight of the sample before drying, and W_f signifies the weight of the sample following drying

3.10.3.2. Determination of Wettability and Solubility

The microcapsule's wettability was assessed using the methodology outlined by Fuchs et al. (2006). 1 g of microcapsule was dispersed over the surface of 100 ml of distilled water at 20 ℃ without agitation. The time required for the powder particles to sediment, submerge, and disappear from the water's surface was recorded and used to compare the wettability of the samples.

The solubility of microencapsulated powders was determined using a method that involves mixing the powder with distilled water, centrifuging the solution, and drying the supernatant (Fuchs et al., 2006). Briefly, 25 ml of distilled water were mixed with 0.5 g of the powder. The mixture was agitated with a magnetic stirrer for 10 min. For 15 min, the solution was centrifuged at 3000 rpm. 25 ml of the supernatant was aliquoted into a pre-weighed petri dish. The supernatant

was dried in an oven set to 105 ℃ for 24 h. The percentage of the water solubility index (WSI) was calculated following the equation (11).

$$\text{WSI \%} = \frac{TDS}{TDP} \times 100 \qquad (11)$$

Where, TDS is the total weight in grams of the dried supernatant, TDP is the total weight in grams of the micro-capsulated powder used

3.10.3.3. Determination of Encapsulation Efficiency

The quantity of unencapsulated extracts (i.e., free extracts or surface extracts) on the powder's surface was determined using the method outlined by Tan et al. (2005) with minor modifications. 15 ml of CH_3OH were added to 2 g of powder in a 30 ml glass vial with a screw cap, and the mixture was agitated using a vortex mixer for 2 min at room temperature. The solvent mixture was subsequently decanted and filtered using Whatman No. 1 filter paper (10 µm). 20 ml of CH_3OH was passed through the powder three times to rinse the collected washed powder on the filter paper. To eliminate any remaining solvents, the leftover powder was subsequently dried at 60 ºC until its weight remained constant. The percentage of the powder's weight difference before and after extraction and CH_3OH washing was then used to determine the free extract content.

The following formula was used to determine encapsulation efficiency (EE).

$$\text{EE \%} = \frac{(Total\ extract - Surface\ extract)}{(Total\ extract)} \times 100 \qquad (12)$$

3.10.3.4. Morphological Analysis

The morphology and surface properties of microencapsulated powders were analyzed via a scanning electron microscope (SEM). The powder samples were attached to SEM stubs using double-sided adhesive tape. The sample specimens were then coated with a thin coating of gold in a vacuum evaporator using a sputter coater (ZEISS, SCD-050, Jena, Germany). The applied coating current and voltage were 5 –

10 mA and 1.1 kV, respectively. The sputter-coated samples were subsequently visualized at a voltage of 5 kV using an SEM (SEM EVO HD15, Carl Zeiss Microscopy GmbH, Jena, Germany). The SEM micrographs depicting the microstructure of the powders were acquired using software installed on a PC linked to the system.

3.10.3.5. Thermogravimetric Analysis (TGA)

The thermal characteristics of the micro-encapsulated powder were determined through thermogravimetric analysis (TGA). The TGA of the microcapsules was carried out using a Mettler Toledo thermogravimetric analyzer (TGA-2, Mettler Toledo, Columbus, Ohio, United States), following the methodology outlined by Ballesteros et al. (2017). Briefly, 5 mg sample was placed on a small aluminum plate and heated from 25 ºC to 800 ºC at a rate of 10 ºC/min, in nitrogen atmosphere with a flow rate of 50 cm^3/min. The weight loss was determined using the data generated from the analysis.

3.10.3.6. FTIR Analysis

The FTIR analysis of the microcapsule powder was performed following the procedure mentioned in the section 3.7.5.

3.10.3.7. GC-MS Analysis

The GC-MS analysis of the synthesized microcapsules was carried out following the procedure and the operating conditions detailed in the section 3.7.6.

3.10.3.8. *In vitro* Release Study

The micro-encapsulated powders were subjected to *in vitro* digestion models that replicate the digestive processes in the stomach and intestine, following the methodologies outlined in Parvez et al. (2022) with some modifications.

3.10.3.8.1. Preparation of Simulated Gastric Fluid (SGF)

Simulated gastric fluid was prepared by dissolving pepsin (obtained from Sigma-Aldrich, St. Louis, Missouri, United States), NaCl, and HCl in deionized water. 3.2 g of pepsin (0.017 mol/L) and 2 g of NaCl (0.034 mol/L) were first dissolved in 500 ml of deionized water. Then, the pH of the fluid was adjusted to 2.0 using 0.1 M HCl, and the volume was brought to 1000 ml.

3.10.3.8.2. Preparation of Simulated Intestinal Fluid (SIF)

Simulated intestinal fluid was formulated by dissolving bile salts (sourced from SRL, Mumbai, India) and pancreatin (sourced from Sigma-Aldrich, St. Louis, Missouri, United States) in phosphate-buffered saline. The phosphate-buffered saline was first prepared by dissolving NaCl (0.8 %), Na_2HPO_4 (0.144 %), KCl (0.02 %), and KH_2PO_4 (0.024 %) in deionized water. The bile salt (3.0 g/L) and pancreatin (10.0 g/L) were dissolved in the prepared phosphate buffer saline. The pH of the solution was adjusted to 7.5 using 0.1 M NaOH.

3.10.3.8.3. Procedure

The microencapsulated powder, weighing 300 mg, was placed inside a centrifuge tube containing 15 ml of SGF. The tubes were incubated inside an orbital shaker with 100 rpm at 37 ºC. 2 ml aliquots of the supernatant were drawn out at specified intervals viz. 0, 20, 60, 100, and 180 min. All aliquots were immediately cooled after collection and maintained at -20 ºC until further use. Following the gastric phase, the mixture was centrifuged at 1200 rpm for 5 min. The supernatant was subsequently collected, and 15 ml of SIF was added to the remaining residue. The tubes were again placed in the orbital shaker at 37 ºC and a speed of 100 rpm. Aliquots of 2 ml were collected at various intervals viz. 0, 60, 180, and 240 min. The incubation period for the gastric and intestinal phases was 3 h (180 min) and 4 h (240 min), respectively. All aliquots were immediately cooled after collection and

maintained at -20 ºC until further use. The collected samples from different intervals of SGF and SIF phases were analyzed for phenolic content and DPPH radical scavenging activities.

3.10.3.9. Storage Stability Analysis

The storage stability of the microencapsulated powder was evaluated using the procedures outlined by de Sena Andrade et al. (2023), with minor modifications. The powder of 1.5 g was packed in biodegradable tea bags (sourced from IndiaMART, Noida, India) and stored under different temperature conditions (4 °C and 40 °C) for 8 weeks. 100 mg of the powder was extracted and tested weekly for TPC and DPPH scavenging capacity retained. As a control, an aqueous extract sample of the ingredients without encapsulation was utilized.

3.10.4. Formulation of Fortified Herbal Green Tea and Characterization

The commercially available organic green tea was used as base material for the formulation of fortified herbal green tea. The organic green tea weighing 2 g was mixed with 100 mg of microencapsulated powders derived from polyherbal aqueous extracts and packed in a biodegradable tea bag (sourced from IndiaMART, Noida, India). For analysis, each tea bag was steeped in 100 ml of potable water at 90 ºC for 2 min, and the resulting liquid was used for subsequent analysis. Fig. 3.3. illustrates the procedures of the formulation of fortified herbal green tea.

3.10.4.1. Physicochemical Analysis

The fortified herbal green tea was evaluated for its pH, total soluble solid and colour. pH was measured using a standard pH meter (Cyberscan 500, Eutech, Singapore). A refractometer (Labart Copper Refractometer with ATC, Gdansk, Poland) was used to measure the soluble solid content. The values obtained

from the refractometer were expressed in grams per 100 ml. The colour was determined calorimetrically.

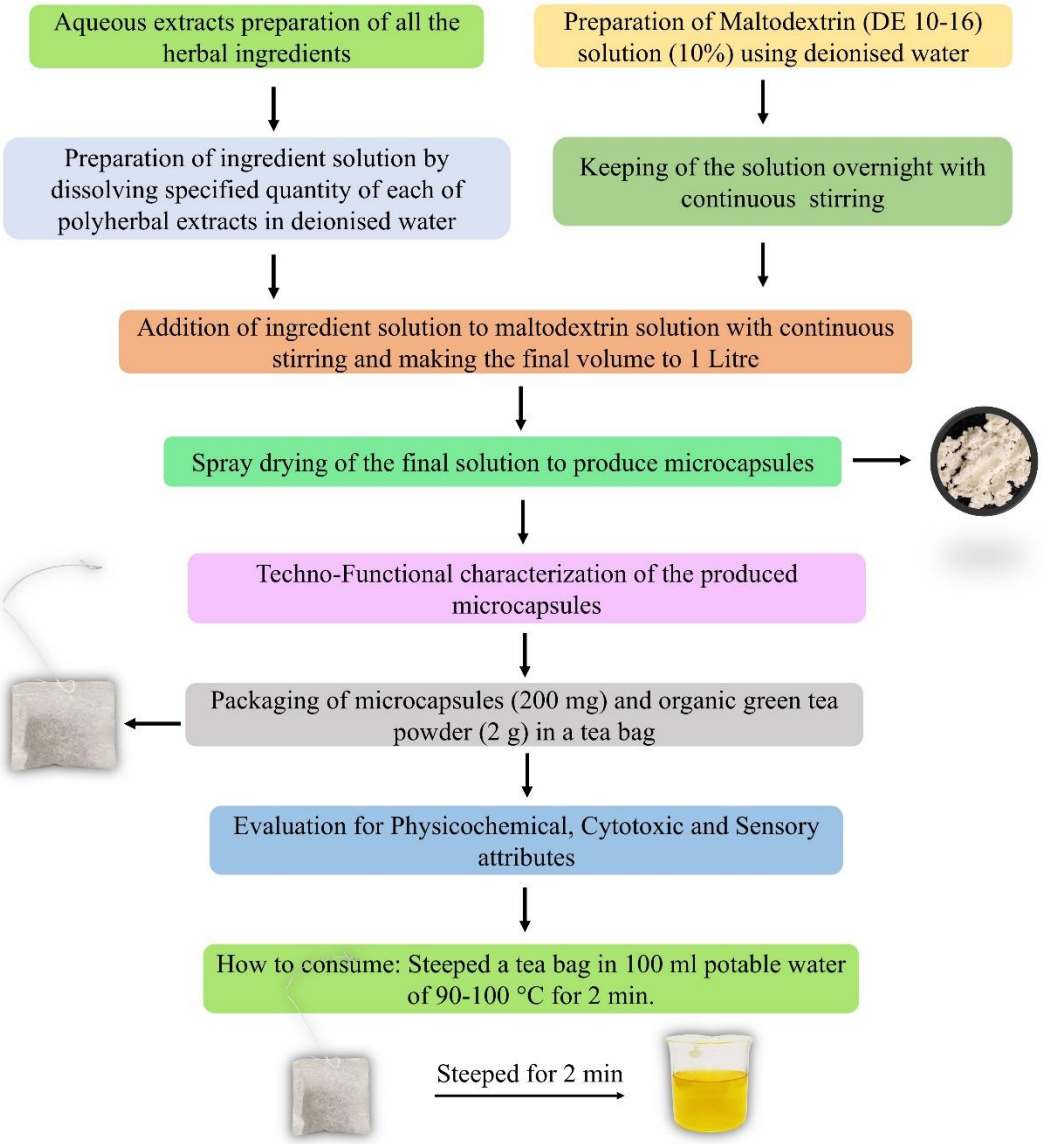

Fig. 3.3. Flowchart illustrating the preparation of the polyherbal extract microcapsules and subsequent herbal green tea formulation

3.10.4.2. Cytotoxicity Assay

3.10.4.2.1. Haemolytic Activity

The *ex vivo* haemolytic activity assay was performed according to the method described by Yang et al. (2005), with slight modifications. In brief, 1000 µl of defibrinated sheep blood was collected and centrifuged at 1000 rpm for 5 min at 4 °C. The resulting red blood cell (RBC) pellet was washed three times with phosphate-buffered saline (PBS), followed by centrifugation under the same conditions. Test samples at different concentrations (5 mg/ml, 10 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml) were then mixed with the RBC suspension in 96-well microplates and incubated at 37 °C for 1 h. After incubation, the plates were centrifuged at 1500 rpm for 5 min. The supernatants were carefully transferred to fresh wells, and haemolysis was assessed by measuring the absorbance at 540 nm using a microplate reader. A 1 % Triton X-100 solution was used as the positive control to represent 100 % haemolysis.

3.10.4.2.2. Antiproliferative Activity

The fortified herbal green tea was assessed for its potential antiproliferative activity. The human colorectal cancer cell line (HT-29) was employed in the present investigation. The HT-29 colorectal cancer cell line was procured from the National Centre for Cell Science (NCCS) in Pune, India, and cultured in DMEM enriched with 10 % FBS in a 5 % CO_2 atmosphere at 37 °C.

For analysis, the brewed tea sample in liquid was converted to powder using a Freeze dryer (Delvac's Maxi Lyodel Freeze Dryer). The antiproliferative activity of the fortified herbal green tea was assessed by MTT assay following the procedures mentioned in the section 3.9.3.

3.10.4.3. Sensory Evaluation

A trained panels (n = 12) conducted a sensory analysis of fortified herbal green tea, employing a 9-point hedonic scale to evaluate factors such as colour, appearance, flavour, texture, taste, and overall acceptability. The samples were presented to the trained panellists in white cups, each labelled with a unique 3-digit random code. The mean of the twelve observations recorded by the trained panellists (n = 12) was used to present each value of the sensory attributes data.

3.10.5. Formulation of Ready-to-Consume (RTC) Juice

Pomelo (*Citrus maxima*) juice was used as a base material for the formulation of ready-to-consume (RTC) juice. The juice was formulated using microencapsulated powder derived from the polyherbal aqueous extract following the standard protocol as per the flowchart displayed in Fig. 3.4. The concentration of pomelo juice in the final RTC was maintained at 15 %, while soluble solid content and citric acid was at 10 °Brix and 0.3 %, respectively.

3.10.5.1. Physicochemical Analysis

The formulated RTC juice was assessed for its physicochemical properties, including pH, soluble solid content, titratable acidity (TA), moisture content, ash content, and colour.

The pH, soluble solid contents and colour of RTC juice were determined following the procedures mentioned in section 3.10.4.1. The evaluation of the acidity in the RTC juice samples was conducted using a 0.01 N NaOH solution, with the results expressed as a percentage of anhydrous citric acid, following the formula provided below.

$$\text{TA (\%)} = \frac{Titre\ value\ (mL)\ x\ N\ NaOH\ x\ Vol.(mL) x\ Eq.weight\ (citric\ acid)}{Sample\ weight\ (g) x\ Aliquot\ taken\ for\ titration\ (m) x\ 1000} \text{ x } 100 \qquad (13)$$

The moisture content of the juice assessed following the procedure detailed in the section 3.10.3.1. The ash content was quantified according to the AACC method (Muthukumaran et al., 2020). A measured volume of juice sample was put in a pre-weighed crucible, which was subsequently subjected to incineration in a muffle furnace at 820 ℃ for 4 hours. The crucible was subsequently cooled in a desiccator and weighed.

$$\text{Ash content (\%)} = \frac{Weight\ of\ ash}{weight\ of\ sample} \text{ x } 100 \quad (14)$$

3.10.5.2. Nutritional Profiling

The nutritional constituents of the RTC juice, encompassing calories, carbohydrates, proteins, fats, calcium, sodium, potassium, vitamin C, and vitamin B, were assessed in accordance with the procedures outlined in the AOAC analytical method (Baur & Ensminger, 1977).

3.10.5.3. GC-MS Analysis

The GC-MS analysis of the formulated ready-to-consume juice was performed following the procedure and the operating conditions detailed in the section 3.7.6.

3.10.5.4. Determination of Antioxidant Activity

The antioxidant activity of the formulated RTC juice was determined by evaluating the phenolic content and free radical scavenging activity. The TPC was determined following the procedure outlined in the section 3.9.1.1. The free radical scavenging capacity was evaluated by DPPH assay following the procedure described in the section 3.9.1.

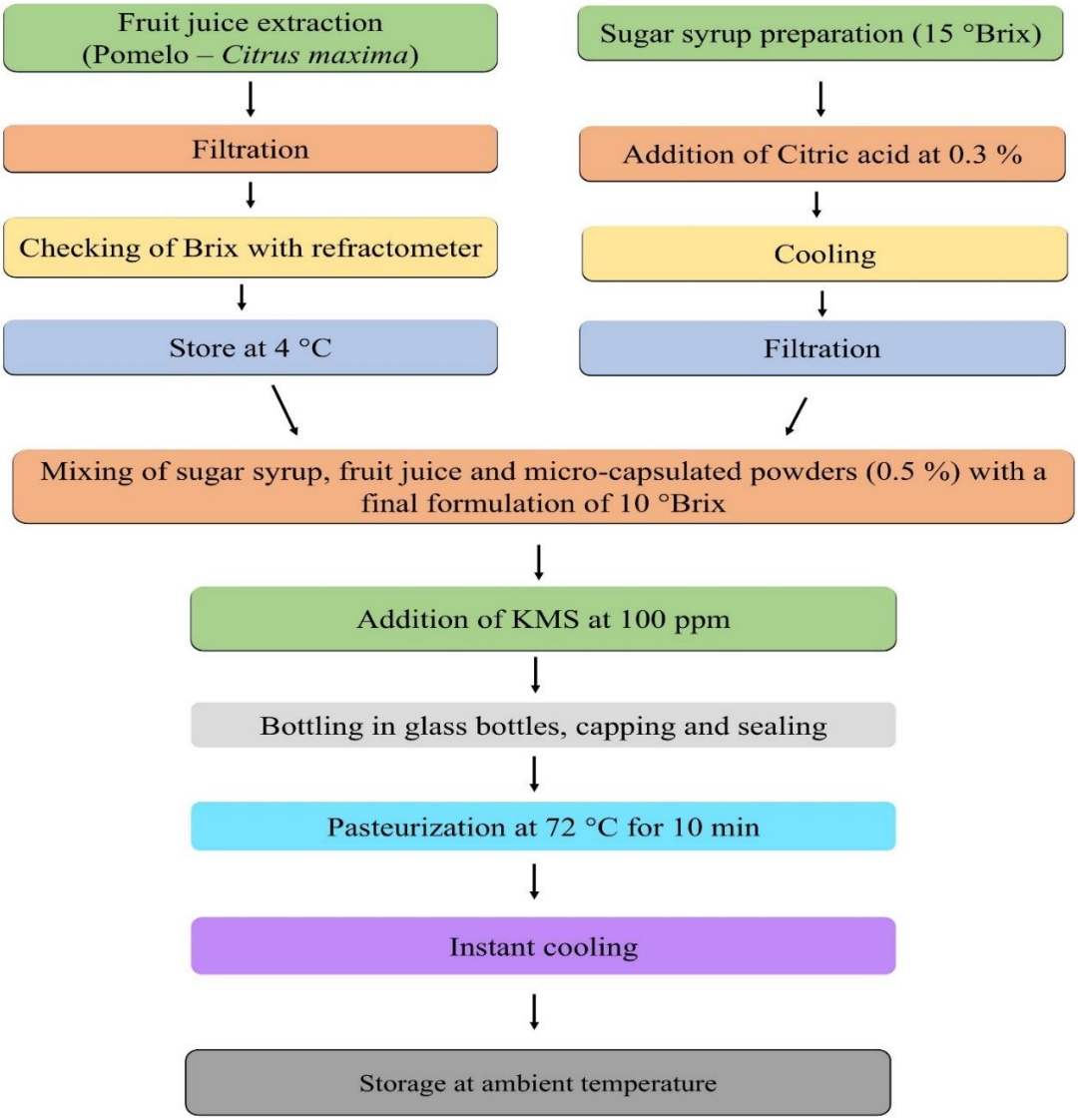

Fig. 3.4. Flowchart showing the procedure of the formulation of Ready-to-Consume (RTC) juice

3.10.5.5. Cytotoxicity Assay

The cytotoxic activity of RTC juice was assessed to ascertain its potential toxicity to human cells. Assessments of haemolytic activity and antiproliferative activity against cancer cell lines were performed. The evaluation of both activities was carried out in accordance with the procedures outlined in the sections 3.10.4.2.1 (Haemolytic activity) and 3.10.4.2.2 (Antiproliferative activity).

3.10.5.6. Sensory Evaluation

The sensory analysis of the RTC juice was conducted according to the procedure outlined in the section 3.10.4.3.

3.11. Statistical Analysis

A statistical analysis of the experimental results was conducted using one-way analysis of variance (ANOVA), Tukey-Kramer multiple comparisons test and Pearson's correlation analysis using a software OriginPro, version 2024b (OriginLab Corporation, Northampton, MA, USA). *p*-values below 0.05 were considered statistically significant. The presented results are the mean values of the triplicates.

CHAPTER 4

RESULTS AND DISCUSSION

For centuries, the indigenous Garo community of Meghalaya have relied extensively on medicinal plants for the management of various ailments. However, a considerable fraction of these ethnomedicinal plants remain scientifically undocumented and lack comprehensive pharmacological validation. Therefore, the present study was undertaken to address these gaps by focusing on selected rare ethnomedicinal plant species, traditionally used in folk medicine by the local Garo populace of Meghalaya.

The doctoral research work aims to highlight three traditionally used, but scientifically undocumented species, viz. *Goniothalamus simonsii, Viburnum odoratissimum var. odoratissimum,* and *Citrus latipes* with unexplored therapeutic potential. The cardinal focus of the investigation was to elucidate and substantiate their biological activities, with particular emphasis on their phytochemical, antioxidant, antimicrobial and anticancer activities. Additionally, the study aims to synthesize products from these plants (herbal green tea, ready-to-consume juice) for potential commercial use.

4.1. Spatial Distribution of the Species

The assessment of the spatial distribution of the rare species, namely *G. simonsii, V. odoratissimum var. odoratissimum* (Synonym *V. simonsii*), and *C. latipes*, was conducted using Geographic Information Systems (GIS) technology. GIS have transformed the analysis of medicinal plants through accurate mapping, ecological assessment, and conservation strategy development. In biodiversity-rich area like Meghalaya, GIS technology has become crucial for the identification, monitoring, and conservation of endangered medicinal species (Qayum et al., 2014).

The spatial distribution map depicted in Fig. 4.1, presents vital information on the geographical range and preferred habitats of the investigated species. The map (Fig. 4.1) incorporates satellite imagery, with georeferenced species distributions presented using a False Colour Composite (FCC) model. In FCC map, bands from the Red, Green, and Near-Infrared (NIR) portions of the

spectrum are usually mapped to RGB channels. This makes differences in land use and vegetation cover more visible (Javed & Khan, 2012). The FCC colour appearance and interpretations are furnished in Table 4.1. Dense vegetation is indicated by bright red/pink, sparse vegetation by light red/pink, and built-up areas (characterized by reduced vegetation in urban regions) are represented by blue/ white (Patra et al., 2006). These maps play an essential role in ecological research by differentiating land cover types and elucidating the ecological preferences of different species.

G. simonsii as depicted in Fig. 4.1, is found in the western part of the state, particularly in the Garo Hills area. This distribution corresponds with subtropical forest regions characterized by medium temperature and high humidity. The dispersed yet concentrated occurrence points indicate a fragmented population, probably affected by habitat degradation (Upadhaya et al., 2013). The distribution of *V. odoratissimum var. odoratissimum* is predominantly located in the central and southern areas of the Khasi Hills region (Fig. 4.1). The clustering of occurrence points indicate that the species proliferates in moderately elevated, forested, and potentially moist habitats. This limited range highlights its ecological sensitivity and potential susceptibility to environmental disruptions. The species *C. latipes* flourish in the eastern and southeastern regions of Meghalaya, particularly the Jaintia Hills and surrounding areas. These regions exhibit dense forest cover and substantial precipitation, indicating that *C. latipes* may prosper in humid evergreen forest ecosystems. The abundance of numerous points across a broad region signifies enhanced ecological adaptability relative to the other two species.

These distribution maps are crucial for biodiversity inspection, conservation strategy, and species habitat prediction. By visually correlating the locations of plant species with the environmental conditions depicted in the FCC background, investigators can deduce habitat preferences and pinpoint biodiversity hotspots or areas of conservation priority. The FCC background facilitates the identification of land use alterations and the evaluation of risks to natural habitats, including deforestation and agricultural expansion (Khadijat et al., 2021).

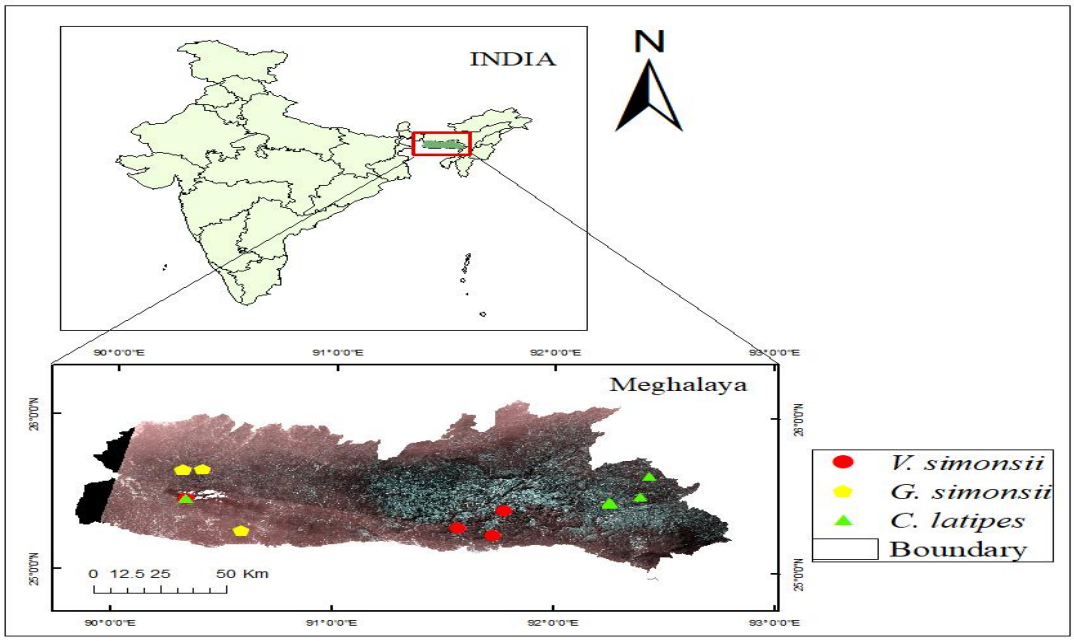

Fig. 4.1. False Colour Composite map showing spatial distribution of the species at various locations. Red/pink colour denotes high vegetation, Blue/white colour denotes areas with less vegetation

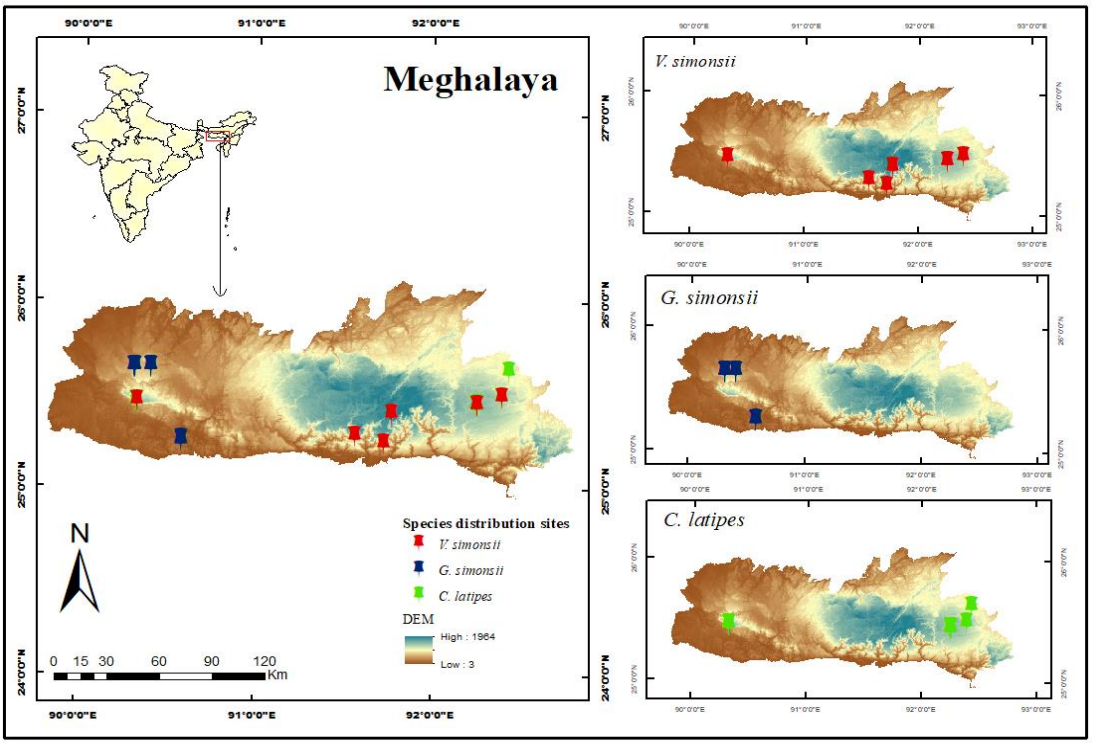

Fig. 4.2. DEM showing the distribution pattern of the species along the elevation gradients

Table 4.1. FCC colour interpretations

FCC Colour	Typical Appearance	Explanation/Interpretations
Bright Red/Pink	Dense, healthy vegetation	High reflectance in Near-Infrared (NIR), absorbed in red, indicates lush forests or crops
Dull Red/Brownish Red	Sparse or stressed vegetation	Weaker NIR reflectance, degraded forests, shrubs, or dry grasslands
Light Pink/Pale Red	Agricultural fields /grasslands	Moderately healthy vegetation, young crops or seasonal grass
Blue/White	No vegetation	Water bodies, concrete roads, buildings

The distribution pattern was further demonstrated using a Digital Elevation Model (DEM) map (Fig. 4.2). In ecological and biodiversity investigations, DEM are essential for: determining the altitudinal distribution of flora and fauna; forecasting prospective habitats based on the elevation, the slope, and aspects; and assessing ecological niches and the impact of elevation on the diversity of species (Lassueur et al., 2006). The DEM map as shown in Fig. 4.2, encompass elevations of 3 - 1984 m above sea level, with gradients displayed from dark brown (low) to blue (high). The observed distribution patterns of the species are intricately linked to the region's topography and ecological zones. *G. simonsii* appears to be confined, with all recorded sites located in the western part of the state (Garo Hills region). These regions exhibit relatively lower elevations, indicating the species' preference for subtropical lowland forest ecosystems. The distribution sites of *V. odoratissimum var. odoratissimum* is predominantly focused in the central and eastern regions of the state. These locations are primarily situated in mid to high elevation regions, especially surrounding the Khasi and Jaintia Hills. Additionally, they are noted for their cooler temperatures and high precipitations. The distribution of *C. latipes* is considerably dispersed, yet it exhibits a significant preference for the southeastern region of the state. This species appears to inhabit a broader elevation gradient, extending from lowland to mid-elevation forests. Consequently, the species' adaptability to wider elevation range demonstrates its ecological versatility.

The spatial distribution of these species across the various ecological zones of the state highlights the significance of microhabitat requirements for their survival. Habitat fragmentation resulting from shifting cultivation, forest clearing, and infrastructure development may present substantial threats to these geographically confined species.

4.2. Biochemical Characterization

Biochemical characterization of medicinal plants is essential for comprehending the therapeutic prospects, safety, and efficacy of compounds derived from plants. This entails the identification, quantification, and analysis of diverse phytochemicals, including alkaloids, phenolic, flavonoids, glycosides, saponins, tannins and terpenoids compounds, which often account for the medicinal properties of plants. This process is essential for validating traditional knowledge, advancing the scientific application of herbal medicines, and facilitating the development of novel pharmaceutical products (Davis et al., 2024). Plant-derived products are gaining global attention as an increasingly attractive option for phytochemistry research. Consequently, the discovery of new phytocompounds with potential bioactivity (antioxidant, antimicrobial, anticancer, anti-inflammatory, etc.) is a top-notch research area in the domain of Plant Sciences (Bhat, 2021).

4.2.1. Carbohydrate, Protein and Lipid contents

It is important to note that most plants with therapeutic value are used in the formulation of nutraceutical products. Consequently, an investigation of the nutritional composition of plants, including carbohydrates, proteins, fats, and minerals, is essential. The nutritional compounds play a crucial role in the development of healthy organ systems in humans (Rahmatollah et al., 2010). Furthermore, they represent essential factors in the choice of plant species for their nutraceutical importance (Nisar et al., 2018). Accordingly, the carbohydrate, protein, and lipid contents of the different parts (leaf, fruit, bark, and root) of *G. simonsii*, *V. odoratissimum var. odoratissimum*, and *C. latipes* were determined.

The results of carbohydrate, protein and lipid content assessment of *G. simonsii*, *V. odoratissimum var. odoratissimum*, and *C. latipes* are furnished in Table. 4.2. In *G. simonsii*, the fruit exhibited the highest quantities of carbohydrates, proteins, and lipids, measuring 288.9 ± 5.23 mg/g, 42.5 ± 2.3 mg/g, and 21.47 ± 0.1 %, respectively, compared to other plant parts. The leaf and bark displayed a carbohydrate content of 103.1 ± 4.3 mg/g and 145.2 ± 2.37 mg/g, protein content of 27.8 ± 2.51 mg/g and 18.5 ± 1.77 mg/g and lipid content of 3.57 ± 0.4 % and 5.25 ± 0.07 %, respectively.

The results presented in Table 4.2 shows that *V. odoratissimum var. odoratissimum* leaf, fruit, bark and root have a carbohydrate content of 232.9 ± 6.64 mg/g, 312.6 ± 10.74 mg/g, 252.18 ± 13.22 mg/g and 156.69 ± 12.78 mg/g, respectively. The observed protein content was 34.6 ± 4.57 mg/g (leaf), 65.9 ± 3.79 mg/g (fruit), 41.84 ± 1.45 mg/g (bark), and 18.17 ± 4.75 mg/g (root). The fruit exhibited the highest concentrations of carbohydrates and proteins in comparison to other components. In terms of lipid content, the root exhibited the highest content, measuring at 11.38 ± 0.57 %, while 11.06 ± 0.89 % (leaf), 7.04 ± 0.80 % (fruit) and 11.15 ± 0.38 % (bark) were observed for other parts of the species. In contrast to other related species, for instance *V. mullaha* (Maikhuri et al., 2012), the carbohydrate content of *V. odoratissimum var. odoratissimum* was higher. However, it was lower than the wild vegetables such as *Dryopteris filixmas*, *Corchorus capsularis*, and *Ipomoea aquatica*, as well as wild edible plants like *Gnetum gnemon*, *Prenanthes hookeri*, *Smilax perfoliata*, and *Blumea lanceolaria*, which are consumed by the indigenous tribes of Meghalaya (Satter et al., 2016; Seal & Chaudhuri, 2016). The protein content was in close proximity with earlier findings on *V. opulus* (Polka et al., 2019) and *V. mullaha* (Maikhuri et al., 2012).

The carbohydrate, protein, and lipid contents of the various parts (leaf, fruit, bark and root) of *C. latipes* are furnished in Table 4.2. The fruit showed 249.55 ± 9.04 mg/g of carbohydrate, 42.51 ± 1.25 mg/g of protein and 10.25 ± 0.76 % (w/w) of lipid content. The root exhibited a lipid content of 15.08 ± 0.53 %, surpassing that of other parts of the species, whereas the leaf and bark showed a lesser quantity with

3.0 - 3.25 %. All parts of the species exhibited a comparable protein content, ranging from 30 - 42 mg/g. These findings are concurrent with the results of previous investigations conducted by Liu et al. (2012), Lu et al. (2021) and Panwar et al. (2023) on the related species. For instance, the carbohydrate content in the fruit of *C. sinensis*, *C. limon*, *C. paradisi* and *C. reticulata* were 117.5 mg/g, 93.2 mg/g, 106.6 mg/g and 133.4 mg/g, respectively (Liu et al., 2012). Nevertheless, the peels of *C. limetta* exhibited higher carbohydrate content (640.8 mg/g) than *C. latipes* (Panwar et al., 2023). In terms of protein content, *C. latipes* exhibited a higher protein content (30 - 42 mg/g) compared to previously examined citrus plants such as *C. sinensis* (9.4 mg/g), *C. limon* (11.0 mg/g), *C. paradisi* (7.7 mg/g) and *C. reticulata* (8.1 mg/g) (Liu et al., 2012).

Table 4.2. Carbohydrate, protein and lipid contents of *G. simonsii*, *V. odoratissimum var. odoratissimum* and *C. latipes*

Plant species	Parts of the plant	Total carbohydrate content (mg/ g)	Total Protein content (mg/ g)	Total Lipid content (% w/w)
G. simonsii	Leaf	103.1±4.3*	27.8±2.51	3.57±0.4
	Fruit	288.9±5.23	42.5±2.3	21.47±0.1
	Bark	145.2±2.37	18.5±1.77	5.25±0.07
V. odoratissimum	Leaf	232.9±6.64	34.6±4.57	11.06±0.89
var. odoratissimum	Fruit	312.6±10.74	65.9±3.79	7.04±0.80
	Bark	252.18±13.22	41.84±1.45	11.15±0.38
	Root	156.69±12.78	18.17±4.75	11.38±0.57
C. latipes	Leaf	177.4±2.18	38.63±1.29	3.03±0.08
	Fruit	249.55±9.04	42.51±1.25	10.10±0.20
	Bark	178.01±8.85	30.63±1.05	3.01±0.15
	Root	144.48±6.97	31.92±1.25	15.05±0.27

***mean±SD**

Among the three species investigated, the fruit of *G. simonsii* had the highest carbohydrate and lipid content, while the fruit of *V. odoratissimum var. odoratissimum* had the highest protein content. Owing to the presence of an appreciable content of biomolecules (carbohydrate, protein and lipid), these plant species can be further investigated for their prospective applicability in dietary supplements.

4.2.2. Phytochemical screening

Bioprospecting of medicinal plants necessitates a comprehensive pharmacognostic assessment of raw plant materials. These investigations encompass the screening of phytochemicals and their biological activities (Bhat, 2021). The presence or absence of phytochemicals is pivotal in the biological activities of particular plant species (Sureshkumar et al., 2021). The phytochemical screening identified significant phytochemicals, including alkaloid, phenol, flavonoid, steroid, glycoside, etc. The findings of the preliminary phytochemical screening are presented in Table. 4.3 and Fig. 4.3. Previous studies have confirmed that phenols, flavonoids, alkaloids, terpenoids, saponins, etc., are primarily responsible for various bioactivities, including antioxidant, antimicrobial, anticancer, anti-inflammatory, etc., (Barbieri et al., 2017; Shukla et al., 2020). This finding implies that the investigated species may demonstrate the above-mentioned bioactivities.

4.2.3. Fourier Transform Infrared Spectroscopy (FTIR) analysis

FTIR analysis was performed to identify the functional groups present in the methanolic extracts of *G. simonsii*, *V. odoratissimum var. odoratissimum* and *C. latipes*. The wavenumber with their corresponding functional groups is presented in Table 4.4 - 4.6. The IR spectra are displayed in Fig. 4.4 - 4.6.

The different parts of these three plant species showed absorption at approximately 3465-3437 cm^{-1}, 2939-2922 cm^{-1}, 2853-2851 cm^{-1}, 1735-1710 cm^{-1}, 1642-1635 cm^{-1}, 1457-1443 cm^{-1}, 1257-1245 cm^{-1}, 1051-1022 cm^{-1} and 668-602 cm^{-1}, resulting in a total of seven to nine (7-9) peaks.

Table 4.3. Results of the preliminary phytochemical screening

Compounds	*G. simonsii*			*V. odoratissimum var. odoratissimum*				*C. latipes*			
	Leaf	Fruit	Bark	Leaf	Fruit	Bark	Root	Leaf	Fruit	Bark	Root
Alkaloids	+	+	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	-	+	+	+	+	-	+	+	+
Cardiac glycosides	+	-	-	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+	+

*'+' indicate presence, '-' indicate absence of the respective compound

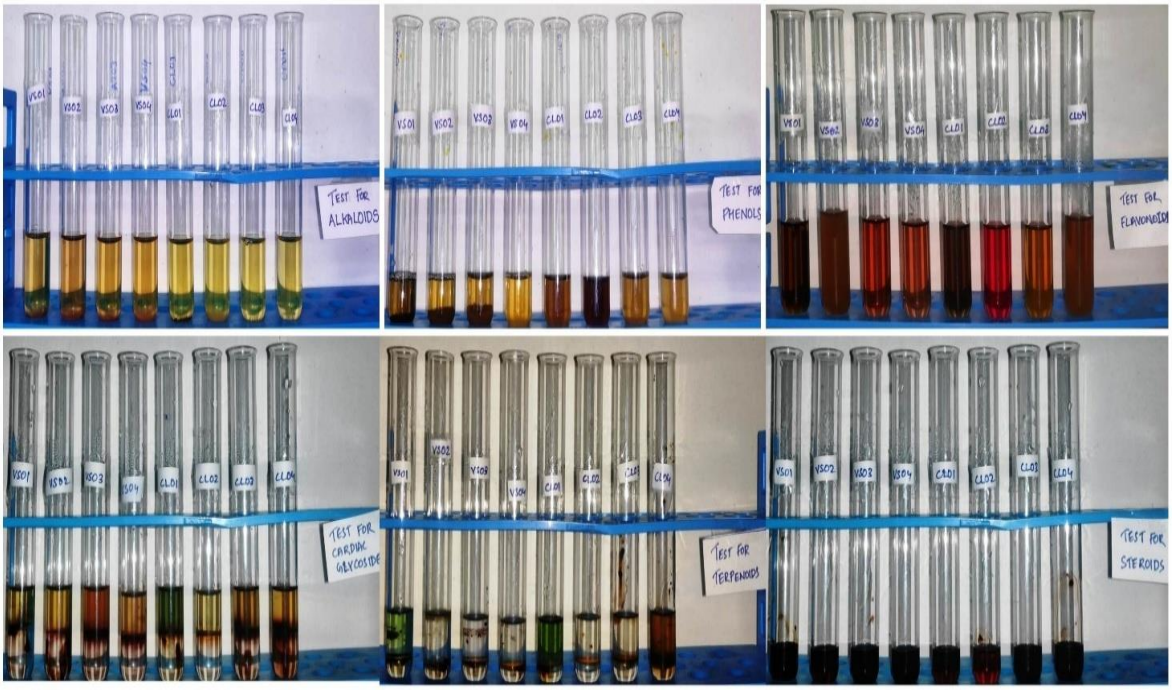

Fig. 4.3. Results of the preliminary phytochemical screening. The corresponding colour change in the specific compound's test signifies its presence

The stretching vibration of OH groups, such as those in alcohols and phenols, corresponds to the peak at 3465-3437 cm^{-1}. The significant peaks at 2939-2922 cm^{-1} and 2853-2851 cm^{-1} implies the presence of alkanes (C-H) (Pavia et al., 2015). The presence of ketone group (C=O) is attributed to the absorption band at 1735-1710 cm^{-1}. The moderate impulses at 1642-1635 cm^{-1} and 1257-1245 cm^{-1} indicate the stretching vibration of imine (C=N) and amine (C-N), respectively (Sutariya et al., 2023; Selvaraju et al., 2021). The presence of ether (C-O) is revealed by the stretching vibration at 1051-1022 cm^{-1} and a halo compound at 668-602 cm^{-1} (Sutariya et al., 2023).

The FTIR spectra of the *G. simonsii* leaf, fruit, and bark displayed similar absorption patterns, with minimal differences in intensity (Fig. 4.4). The absorption pattern of *V. odoratissimum var. odoratissimum*'s leaf resembles that of its fruit, whereas the bark resembles to that of the root (Fig. 4.5). All the four samples of *V. odoratissimum var. odoratissimum* (leaf, fruit, bark and root) exhibited variations in intensity. However, both root and bark demonstrated a notable similarity in their absorption intensity. Regarding *C. latipes* FTIR spectra, the fruit, bark and root showed similar absorption pattern with negligible variations in the intensity (Fig. 4.6). The differences in absorption patterns and intensity among the species can be attributed to the varying concentrations of phytocompounds in various parts of the plant. Nevertheless, the FTIR analysis confirmed the presences of phenolic compounds, ethers, amines, alkanes, aldehydes, etc., in all the parts of the investigated plant species.

4.2.4. Gas Chromatography Mass Spectrometry (GCMS) Analysis

Medicinal plants provide an array of key bioactive chemicals crucial for the development of new medicinal products. Many contemporary pharmaceuticals are indirectly sourced from medicinal plants (Panwar et al., 2023). As a result, they have significantly contributed to global health by providing diverse resources to mitigate various diseases and conditions.

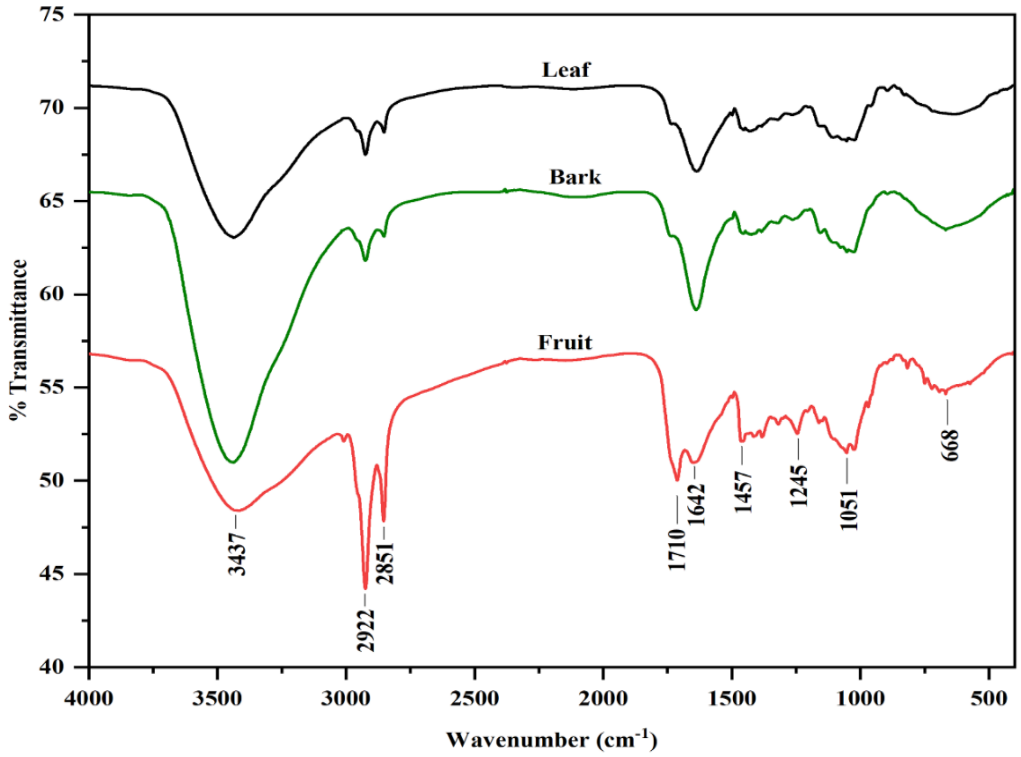

Fig. 4.4. FTIR spectra of leaf, fruit and bark of *G. simonsii*

Table 4.4. FTIR spectral values and the corresponding functional groups of *G. simonsii*

Wavenumber (cm^{-1})	Appearance	Group	Compound class
3437	Stretch	O-H	Alcohol
2922	Stretch	C-H	Alkane
2851	Stretch	C-H	Alkane
1710	Stretch	C=O	Ketone
1642	Stretch	C=N	Imine/oxime
1457	Bend	C-H	Alkane (Methyl group)
1245	Stretch	C-N	Amine
1051	Stretch	C-O	Ether
668	Stretch	C-Br	Halo compound

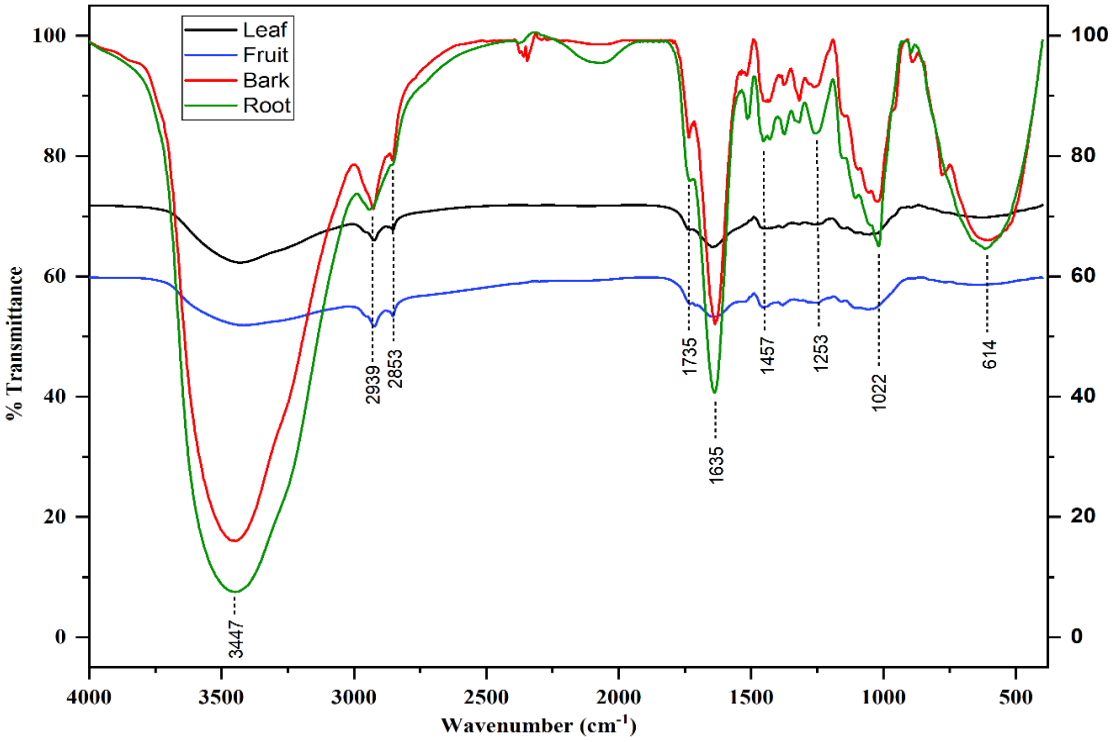

Fig. 4.5. FTIR spectra of leaf, fruit, bark and root of *V. odoratissimum var. odoratissimum*

Table 4.5. FTIR spectral values and the corresponding functional groups of *V. odoratissimum var. odoratissimum*

Wave number (cm^{-1})	Appearance	Group	Compound Class
3465	Stretch	O-H	Alcohol
2932	Stretch	C-H	Alkane
1638	Stretch	C=C	Alkenes
1443	Bend	$-CH_3$	Alkane
1257	Stretch	C-O	Ether
1018	Stretch	C-N	Aliphatic amines
602	Stretch	C-Br	Alkyl halides

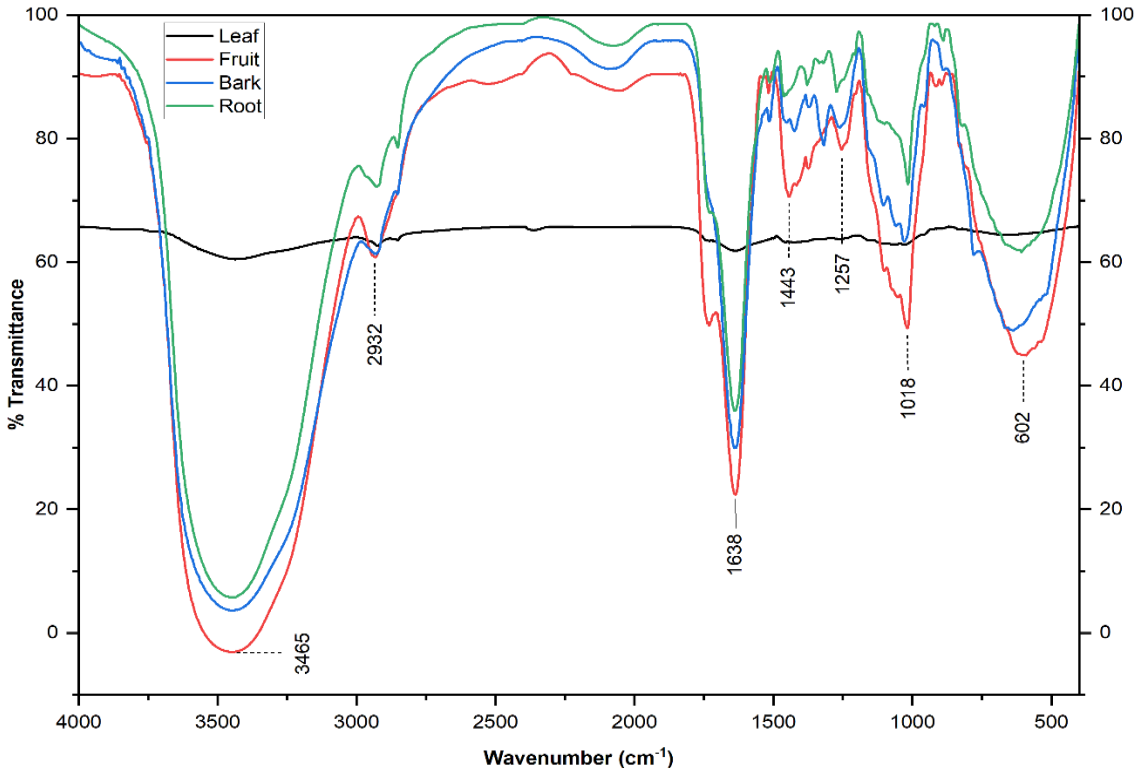

Fig. 4.6. FTIR spectra of leaf, fruit, bark and root of *C. latipes*

Table 4.6. FTIR spectral values and the corresponding functional groups of *C. latipes*

Wavenumber (cm^{-1})	Appearance	Group	Compound class
3447	Stretch	O-H	Alcohol
2939	Stretch	C-H	Alkane
2853	Stretch	C-H	Alkane
1735	Stretch	C=O	Esters
1635	Stretch	C=C	Alkene
1457	Bend	C-H	Alkane (Methyl group)
1253	Stretch	C-N	Amine
1022	Stretch	C-O	Esters
614	Stretch	C-Br	Halo compound

The pursuit of new bioactive compounds and their extraction from medicinal plants have become essential for the advancement, modernization, and quality assurance of herbal formulations (Panwar et al., 2023). A total of 24 compounds were identified in *G. simonsii* extracts. The details of identified compounds with their retention time and peak area (%) are furnished in Table 4.7 - 4.9. The chromatograms are depicted in Fig. 4.7. In leaf extract, 2-Cyclohexen-1-ol and cis-Vaccenic acid were detected with highest peak area of 25.43 % and 20.45 %, respectively. The fruit and bark extract displayed a considerable peak area of Kavain (19.05 %) and Phenol, 3-methoxy-2-(quinoxalin-6-yliminomethyl) (2.18 %). The other potential phytocompounds detected includes (-)-Spathulenol, Neointermedeol, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, n-Hexadecanoic acid, Aziridine, Stigmasterol, 4,22-Stigmastadiene-3-one, Cholest-4-en-3-one, 2-Methoxy-4-vinylphenol, ß-Sitosterol, 3-[5-(3,4-Dimethoxyphenyl)-4-methylimidazol-1-yl] aniline, trans-Sinapyl alcohol, etc. Compounds such as Spathulenol, neointermedeol, hexadecenoic acid and ß-Sitosterol were also reported from the related species including *G. tortilipetalus* and *G. sesquipedalis* (Wiart, 2007; Anatachodwanit et al., 2024). These compounds demonstrated numerous bioactivities including antioxidant, antimicrobial, anti-cancer, anti-inflammatory, anti-obesity, antiproliferative, cysteine protease, tuberculosis inhibition, antimycobacterial, hypolipidemic properties, etc., (Kowalczyk et al., 2017; Lee et al., 2025). For instance, Semwal et al. (2018) demonstrated that cis-Vaccenic acid play a role in regulating immune response, particularly by influencing the activity of immune cells like $CD8^+$ T cells. Similarly, Nascimento et al. (2018) demonstrated that Spathulenol displayed antiproliferative efficacy against a wide range of human cancer cell lines, including breast (MCF-7), prostrate (PCO-3), ovarian (OVCAR-2), colon (HT-29), etc. These findings indicate the prospective antioxidant, antimicrobial, and antiproliferative properties of *G. simonsii* extracts.

In *V. odoratissimum var. odoratissimum*, 21 compounds were identified. The chromatograms are shown in Fig. 4.8, whereas the phytocompounds with their retention time (RT) and concentration (Peak area %) are presented in

Table. 4.10 - 4.13. 1,2,3,4-Cyclohexanetetrol, a polyol, demonstrates the largest peak area percentage (16.86 %) in leaf extract, serving as a glucosidase inhibitor (Ogawa et al., 2005). Lup-20(29)-en-3-ol, acetate, (3ß)-, present in both leaf (9.22 %) and fruit (35.12 %) extracts, exhibits anticancer, anti-inflammatory, antituberculosis, antimalarial, antibacterial, antinociceptive, and antioxidant properties (Prachayasittikul et al., 2010; Liu et al., 2021). ß-Sitosterol (5.70 %, 8.96 %) exhibits anticancer properties against breast, lung, prostate, and colon cancers (Khan et al., 2022). α and ß-Amyrins (15.95 % and 4.41 %, respectively) have been documented to possess anti-nociceptive, anti-inflammatory, antioxidant, anti-diabetic, anticancer, antihyperglycemic, gastroprotective, and anticonvulsant effects (Ghosh et al., 2015; Alam et al., 2023). Neophytadiene (4.96 %) possesses anti-inflammatory, antimicrobial, antioxidant, antipyretic, and analgesic activities (Bhardwaj et al., 2020; Gonzalez-Rivera et al., 2023). Additionally, 4-ethoxybenzoic acid ethyl ester, present in both leaves and fruit, exhibits cardioprotective, antibacterial, antioxidant, and anti-inflammatory activities (Lipińska et al., 2023). Pentadecanoic acid, 14-methyl-, methyl ester; Tridecanoic acid, 12-methyl-, methyl ester; Hexadecanoic acid, methyl ester; n-Hexadecanoic acid; 11-Octadecenoic acid, methyl ester; and 1-Heptatriacotanol have been documented to exhibit antimicrobial, anti-inflammatory, hypocholesterolemic, cancer-preventive, antioxidant, hepatoprotective, and antiarthritic properties (Aparna et al., 2012; Ravi et al., 2017). cis-Z-a-Bisabolene epoxide enhances sex hormone activity and possesses anticancer properties (Ganesh et al., 2017). Previous research on related species of *Viburnum* identified and reported the isolation of dehydrovibsanin G (a diterpenoid) and (+)-9'-O-senecioyllariciresinol (a lignan), both exhibiting anticancer potential against breast cancer cell lines (human A431, T47D) (Li et al., 2015). Five novel terpenoids (two vibsane-type diterpenoids and three iridoid allosides) were recently discovered from *V. odoratissimum var. sessiliflorum*, demonstrating significant anti-inflammatory and anticancer properties against colon cancer (Yang et al., 2023).

A total of 22 compounds were identified in *C. latipes* extracts. The identified phytocompounds, along with their retention times (RT) and relative

concentrations (Peak area %), are summarized in Table 4.14 - 4.17. The chromatograms are depicted in Fig. 4.9. A significant peak area of the compound cis-Vaccenic acid is detected in methanolic extract of *C. latipes* leaf, fruit and bark. Meanwhile, in root extract, a compound osthole is found with the highest peak area. Some compounds such as n-Hexadecanoic acid, cis-Vaccenic acid, 2-[5-(2-Methyl-benzooxazol-7-yl)-1H-pyrazol-3-yl]-phenol and trans-13-Octadecenoic acid are present in all the four extracts with varied peak areas. Some organic compounds were detected only in specific extract viz. Phenol, 4-ethenyl-2,6-dimethoxy- (bark), stevioside (bark), 2H,8H-Benzo[1,2-b:5,4-b'] dipyran-2-one, 8,8-dimethyl- (root), Ricinoleic acid (leaf), Osthole (root), d-Tocopherol (leaf) and Stigmasterol (leaf). Other compounds that are found in the *C. latipes* extracts include ß-Sitosterol, 2,4,6-Tri-tert-butylphenol, 1-Glyceryl ricinoleate, Cholesta-22,24-dien-5-ol, 4,4-dimethyl-, 8-(2,3-Dihydroxy-3-methylbutyl)-7-methoxy-2H-chromen-2-one. These chemicals demonstrate numerous pharmacological properties including antibacterial, anti-inflammatory, anti-cancer, antioxidant, hypolipidemic activity, antipyretic, analgesic, neuroprotective, cardioprotective and anti-diabetic (Sun et al., 2021; Aware et al., 2022). The bark extract exhibited a higher concentration of phytocompounds compared to other extracts (leaf, fruit and root). Consequently, this aspect suggests that the bark extract may exhibit enhanced antioxidant and antimicrobial activities compared to the other extracts. Similarly, owing to the higher concentrations of cis-Vaccenic acid (51.74 %), n-Hexadecanoic acid (9.12 %), pyrazol-phenol (14.13 %), osthole (35.85 %) and Indolo [2,1-a] isoquinoline (1.80 %), fruit and root extract could exhibit considerable antioxidant and antimicrobial activities (Ravi et al., 2017; Sun et al., 2021; Scott et al., 2024). Further, the anti-cancer, anti-TB, anti-COVID-19 and antileukemic activities of the phytocompound Indolo[2,1-a] isoquinoline were validated by Verma et al. (2021). This suggests that the extracts may also exhibit these pharmacological features.

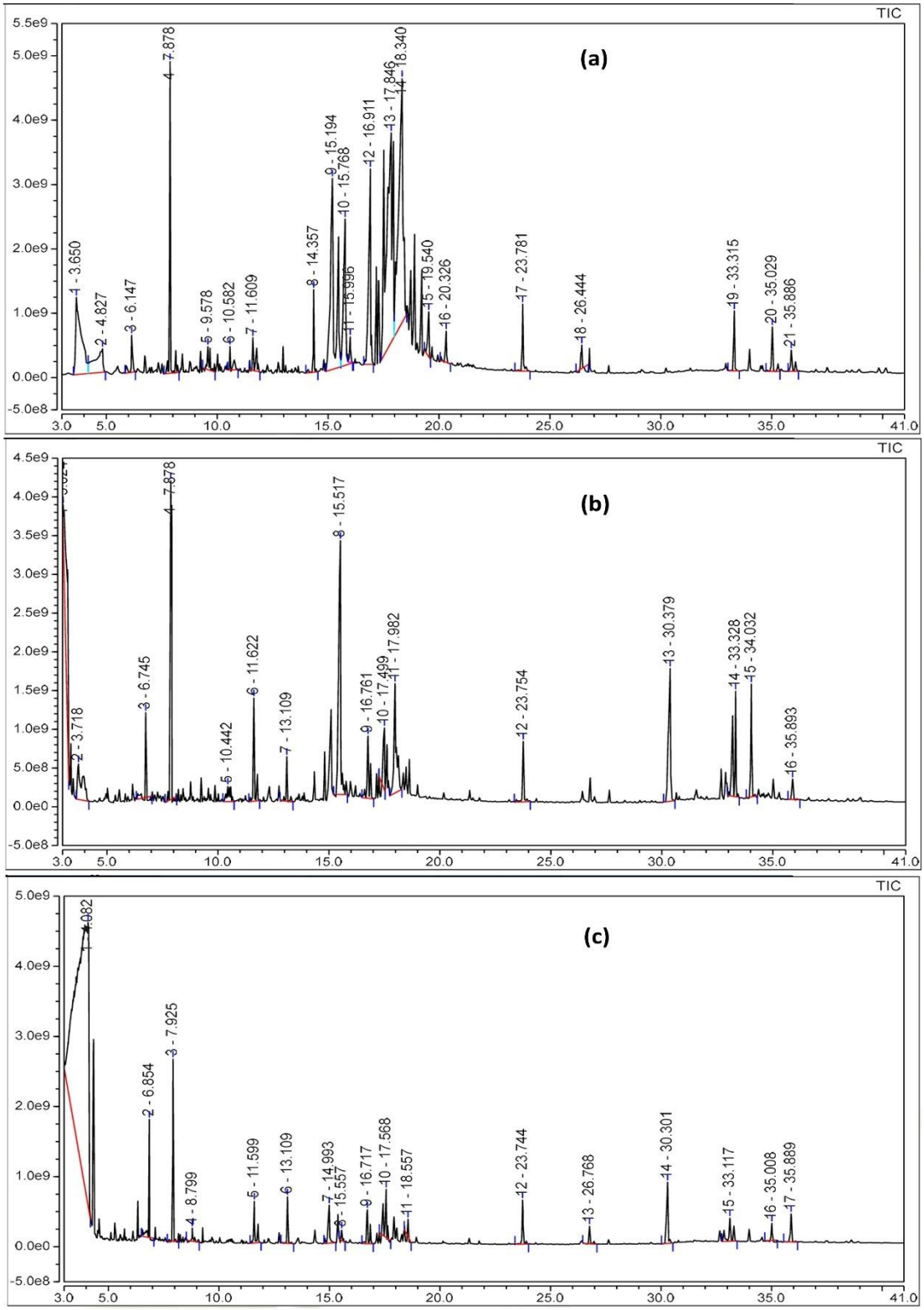

Fig. 4.7. TIC of *G. simonsii* extracts (a) leaf, (b) fruit, and (c) bark

Table 4.7. Chemical constituents of *G. simonsii* leaf extract

R.T (min)	Name of the compound	Peak area (%)
3.650	Benzaldehyde	8.09
4.827	Erythritol	3.54
7.878	Benzene, (1,4-cyclohexadien-1-yl)	4.81
9.578	(-)-Spathulenol	0.85
10.582	Neointermedeol	0.68
11.609	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	1.23
15.194	n-Hexadecanoic acid	13.18
15.768	Aziridine, 1-(1,2,3,4-tetrahydro-2-naphthyl)	5.85
15.996	2-Phenyl-3-(2-furyl)-propenal	0.48
16.911	ß-Ethylphenethyl alcohol	7.14
17.846	2-Cyclohexen-1-ol, 1-butyl-	25.43
18.340	cis-Vaccenic acid	20.45
19.540	Hydrocinnamic acid	1.03
33.315	Stigmasterol	1.03
35.029	4,22-Stigmastadiene-3-one	1.08
35.886	Cholest-4-en-3-one	0.69

Table 4.8. Chemical constituents of *G. simonsii* fruit extract

R.T (min)	Name of the compound	Peak area (%)
3.718	Benzaldehyde	5.61
6.745	2-Methoxy-4-vinylphenol	2.42
7.878	Benzene, (1,4-cyclohexadien-1-yl)	14.32
10.442	2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethyl	1.65
11.622	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	3.88
13.109	2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester	1.38
15.517	Kavain	19.05
16.761	ß-Ethylphenethyl alcohol	3.47
17.499	2-Cyclohexen-1-ol, 1-butyl	2.16
17.982	Hydrocinnamic acid	8.63
23.754	Hexadecanoic acid	2.16
30.379	Phenol, 3-methoxy-2-(quinoxalin-6-yliminomethyl)-	9.66
33.328	Stigmasterol	6.50
34.032	ß-Sitosterol	3.32
35.893	Cholest-4-en-3-one	0.90

Table 4.9. Chemical constituents of *G. simonsii* bark extract

R.T (min)	Name of the compound	Peak area (%)
6.854	2-Methoxy-4-vinylphenol	1.54
7.925	Benzene, (1,4-cyclohexadien-1-yl)-	2.17
8.799	Benzoic acid, 4-ethoxy-, ethyl ester	0.31
11.599	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	1.32
13.109	2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester	0.91
14.993	n-Hexadecanoic acid	1.53
15.557	trans-Sinapyl alcohol	0.16
16.717	ß-Ethylphenethyl alcohol	1.29
17.568	8-Hydroxy-2,2-dimethyl-8-phenyl-oct-5-en-3-one	1.83
23.744	Hexadecanoic acid	1.04
26.768	Octadecanoic acid,	0.46
30.301	Phenol, 3-methoxy-2-(quinoxalin-6-yliminomethyl)-	2.18
33.117	3-[5-(3,4-Dimethoxyphenyl)-4-methylimidazol-1-yl] aniline	1.30
35.008	4,22-Stigmastadiene-3-one	0.17
35.889	Cholest-4-en-3-one	0.91

Table 4.10. Chemical constituents of *V. odoratissimum var. odoratissimum* leaf extract

R.T (min)	Name of the compound	Peak area (%)
7.174	8-Methyl-3-oxo-2-oxabicyclo (4.4.0) deca-4,9-diene-6,8-carbolactone	1.32
8.850	Benzoic acid, 4-ethoxy-, ethyl ester	2.51
9.847	1,2,3,4-Cyclohexanetetrol	16.86
11.333	Tridecanoic acid, 12-methyl-, methyl ester	1.59
13.078	Neophytadiene	4.96
13.762	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	2.23
14.455	Hexadecanoic acid, methyl ester	4.04
15.034	n-Hexadecanoic acid	2.22
17.363	11-Octadecenoic acid, methyl ester	0.92
18.493	1-Heptatriacotanol	2.07
18.833	Doconexent	2.41
21.608	cis-Z-a-Bisabolene epoxide	2.50
28.046	Ergosta-5,22-dien-3-ol, acetate, (3ß,22E)-	1.70
31.464	W-18	1.45
31.981	a-Tocopheryl acetate	1.48
34.138	ß-Sitosterol	5.70
34.644	ß-Amyrin	4.41
35.291	a-Amyrin	15.95
36.811	Lup-20(29)-en-3-ol, acetate, (3ß)-	9.22

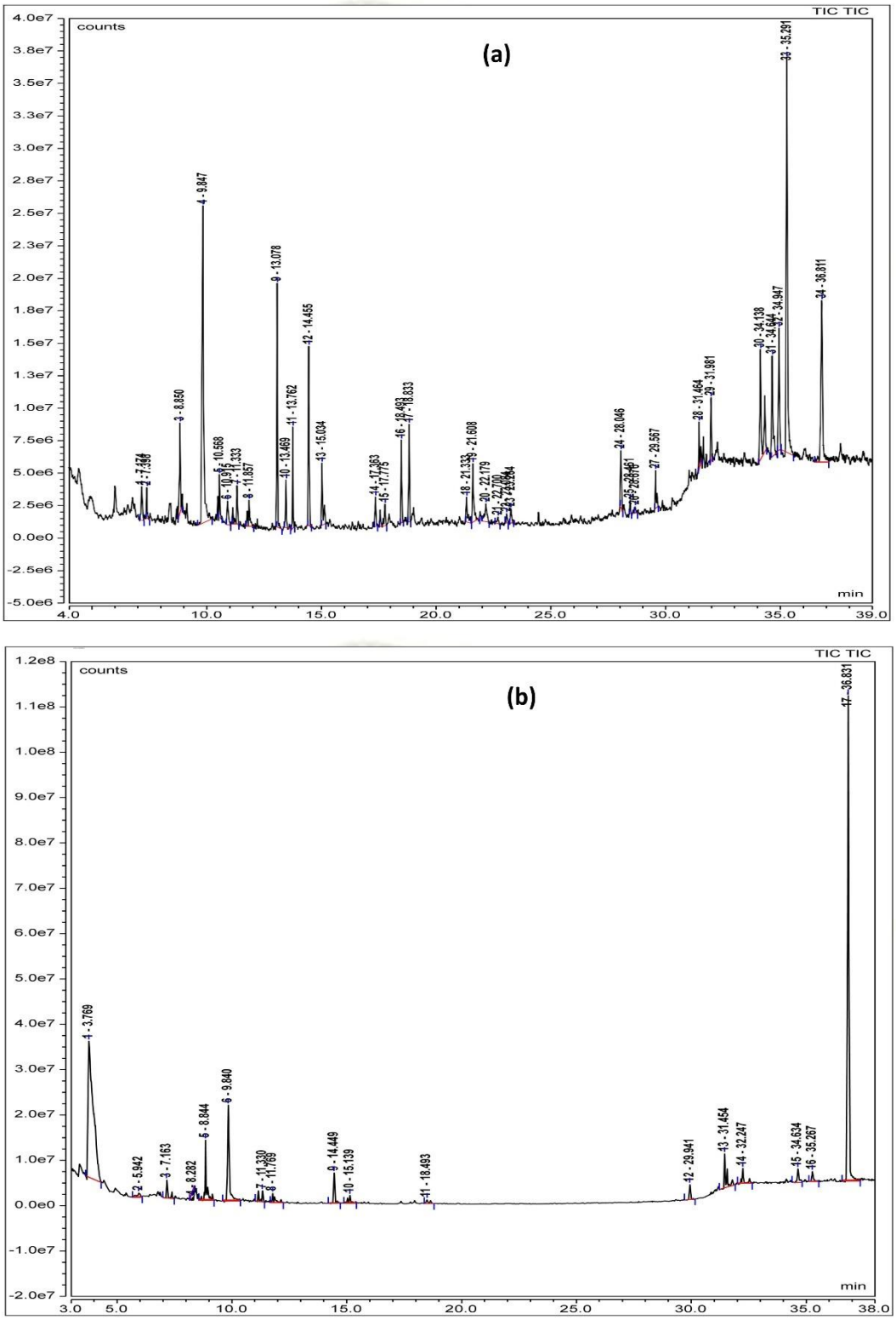

Fig. 4.8. TIC of *V. odoratissimum var. odoratissimum* extracts (a) leaf, (b) fruit, (c) bark, and (d) root

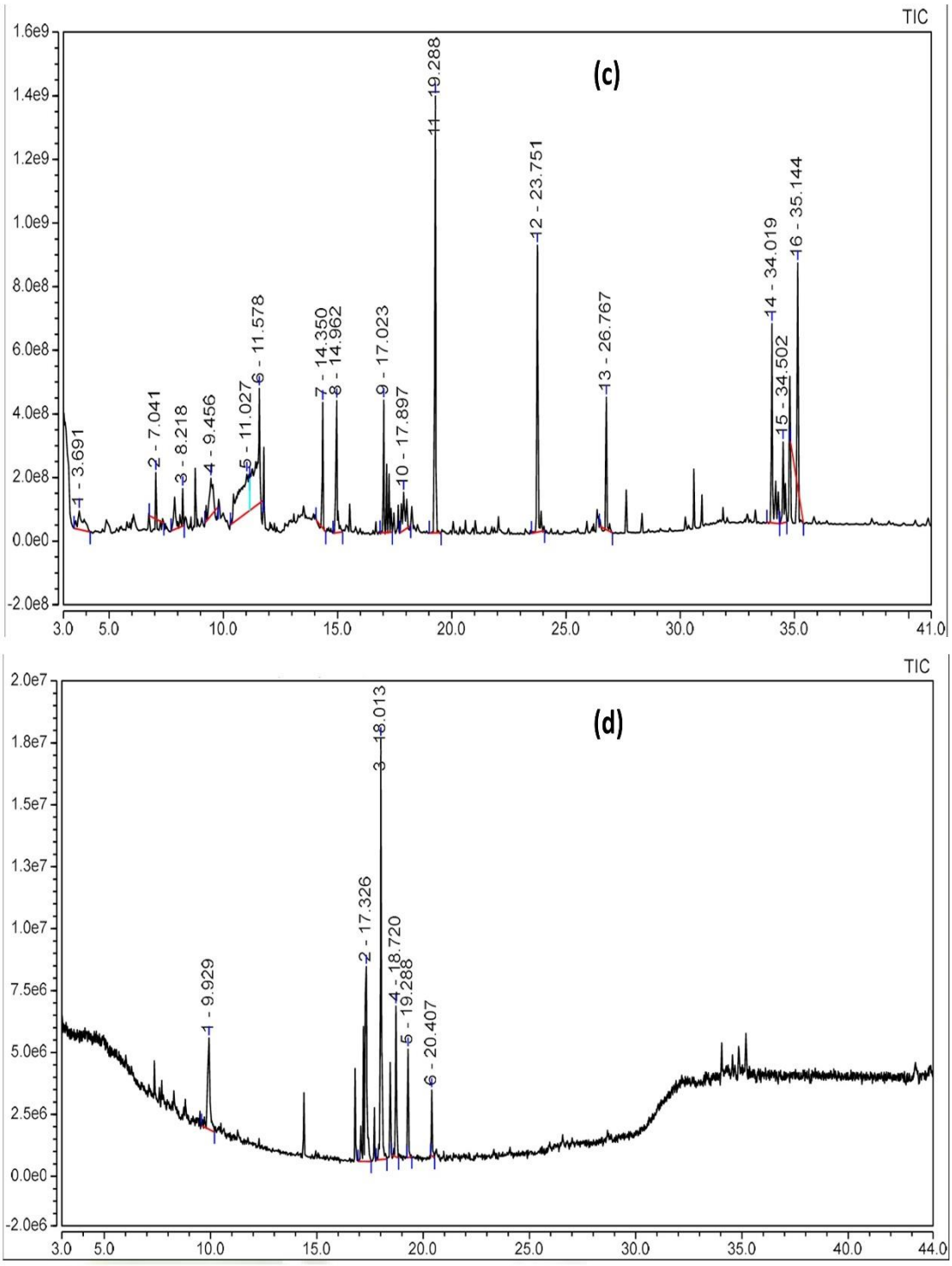

Fig. 4.8. ***continued***

Table 4.11. Chemical constituents of *V. odoratissimum var. odoratissimum* fruit extract

R.T (min)	Name of the compound	Peak area (%)
3.769	Glycerin	37.38
7.163	Benzeneacetaldehyde, a,2,5-trimethyl-	1.33
8.282	3-Pyridinecarboxylic acid, 1,6-dihydro-4-hydroxy-2-methyl-6-oxo-, ethyl ester	1.13
8.844	Benzoic acid, 4-ethoxy-, ethyl ester	3.90
9.840	Propanoic acid, 2-methyl-, 2-ethylhexyl ester	8.20
11.330	Cyclopentanetridecanoic acid, methyl ester	1.00
11.769	Eicosane, 2-methyl-	1.27
14.449	Pentadecanoic acid, 14-methyl-, methyl ester	1.49
15.139	Decane, 2,3,5,8-tetramethyl-	0.91
29.941	Lupeol, trifluoroacetate	0.76
31.454	Lupeol	2.84
32.247	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	1.37
36.831	Lup-20(29)-en-3-ol, acetate, (3ß)-	35.12

Table 4.12. Chemical constituents of *V. odoratissimum var. odoratissimum* bark extract

R.T (min)	Name of the compound	Peak area (%)
7.041	Phenol, 2,6-dimethoxy	1.49
8.218	1-(4-Ethoxyphenyl) propan-1-ol	3.18
9.456	d-Mannose	3.82
11.027	3-O-Methyl-d-glucose	13.13
11.578	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	11.87
14.962	n-Hexadecanoic acid	5.54
17.023	2H,8H-Benzo[1,2-b:5,4-b'] dipyran-2-one, 8,8-dimethyl	7.35
17.897	Oleic Acid	3.45
19.288	Osthole	13.96
23.751	Hexadecanoic acid, 2-hydroxy-1-	8.75

	(hydroxymethyl)ethyl ester	
34.019	ß-Sitosterol	8.96
34.502	ß-Amyrin	4.23
35.144	a-Amyrin	4.60

Table 4.13. Chemical constituents of *V. odoratissimum var. odoratissimum* root extract

R.T (min)	Name of the compound	Peak area (%)
9.929	3-O-Methyl-d-glucose	15.91
17.326	9,12-Octadecadienoic acid, methyl ester	31.08
18.013	Methyl 9-cis,11-trans-octadecadienoate	31.79
19.288	Osthole	6.36
20.407	9-Octadecenoic acid	3.75

Table 4.14. Chemical constituents of *C. latipes* leaf extract

R.T (min)	Name of the compound	Peak area (%)
9.857	Epicurzerenone	0.60
11.762	7-Methyl-Z-tetradecen-1-ol acetate	0.55
15.006	n-Hexadecanoic acid	8.35
18.061	cis-Vaccenic acid	43.57
20.401	9-Octadecenoic acid, 12-hydroxy-, methyl ester	4.40
21.033	Ricinoleic acid	1.55
23.370	Oxypeucedanin	0.76
25.570	2-[5-(2-Methyl-benzooxazol-7-yl)-1H-pyrazol-3-yl]-phenol	9.35
26.451	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	3.79
29.199	1-Glyceryl ricinoleate	12.08
29.961	d-Tocopherol	1.05
30.665	3-(3,4-Dimethoxyphenyl)-6-methoxy-4-methylcoumarin	1.08
33.328	Stigmasterol	0.67
34.036	ß-Sitosterol	1.92

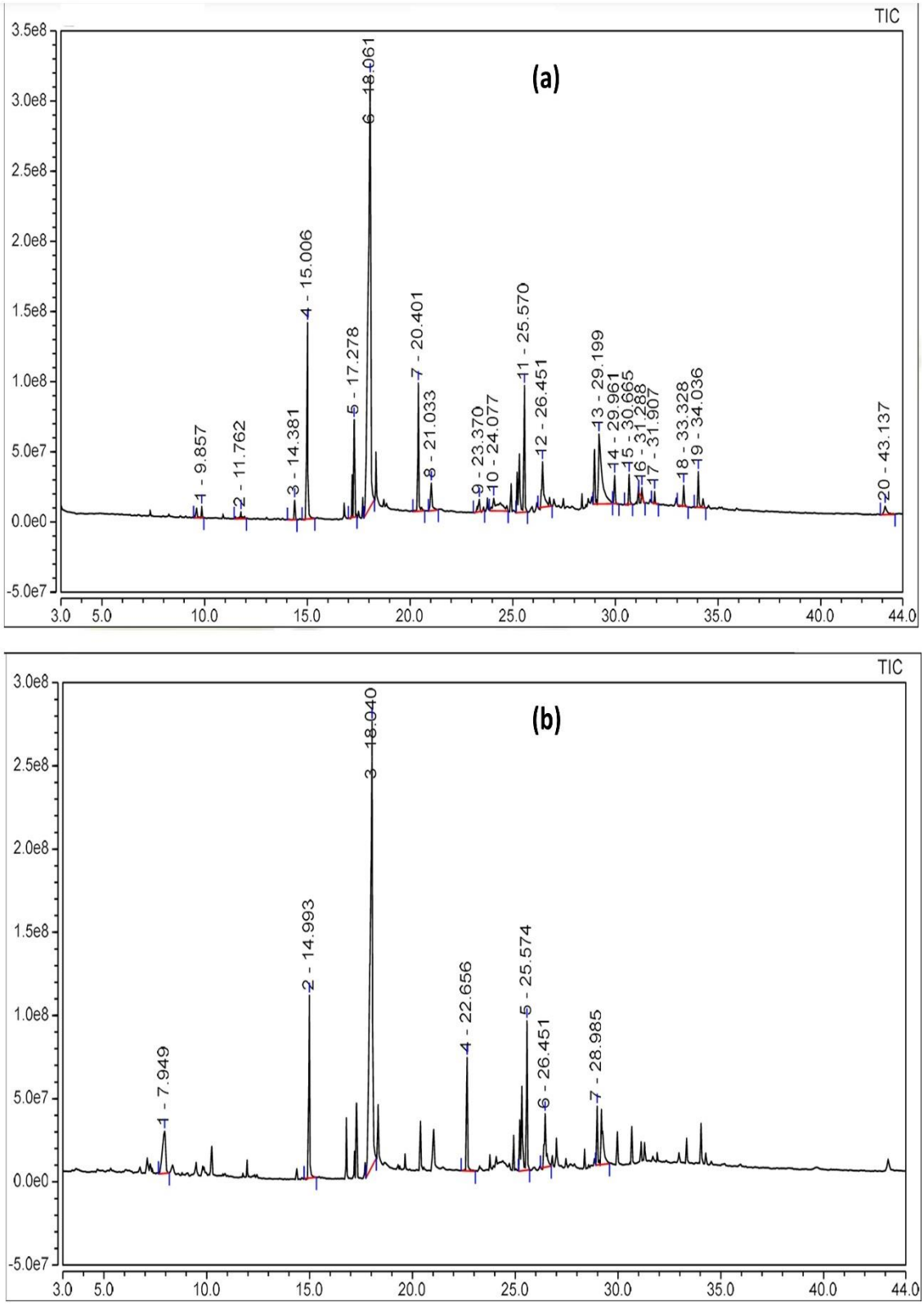

Fig. 4.9. TIC of *C. latipes* extracts (a) leaf, (b) fruit, (c) bark, and (d) root

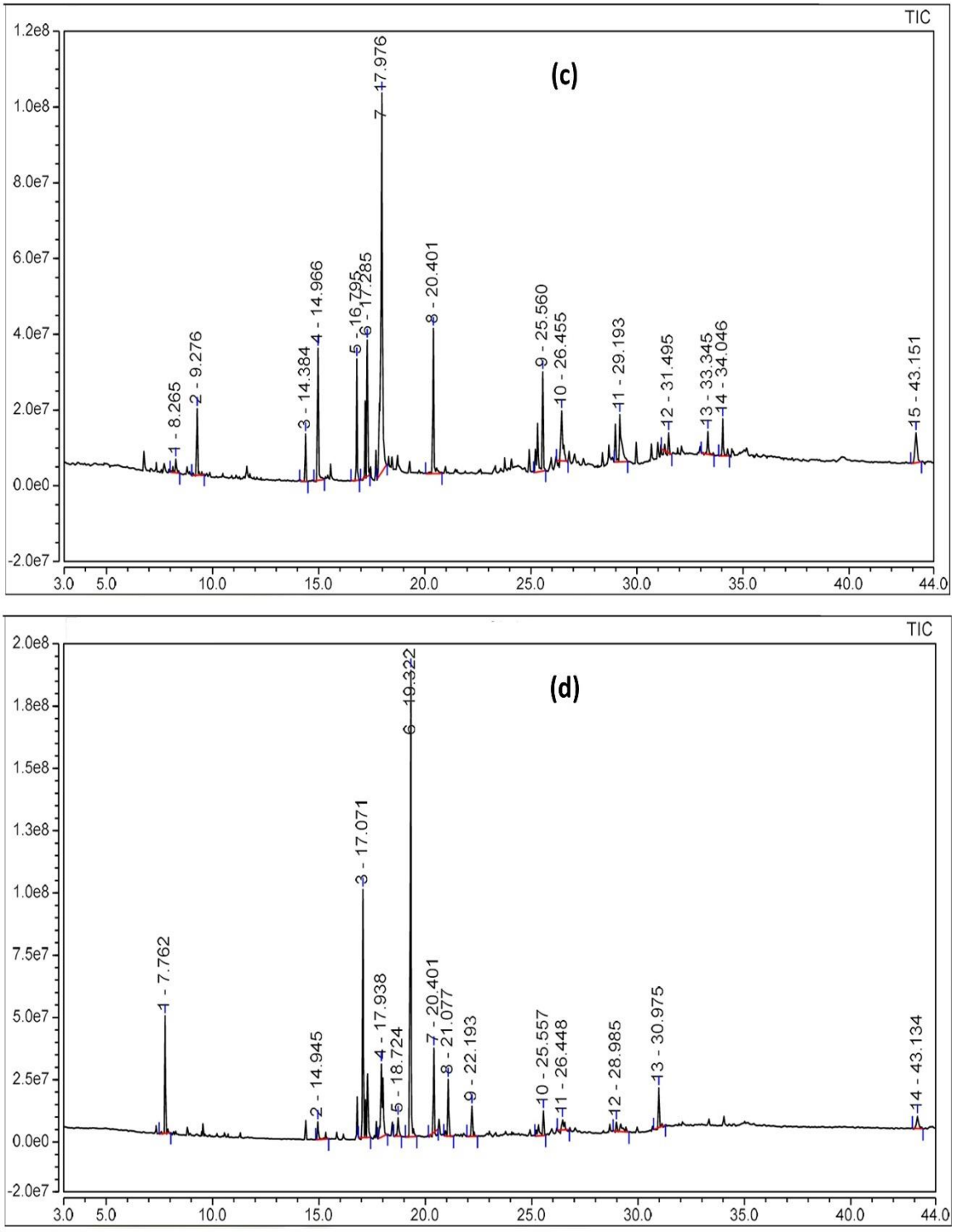

Fig. 4.9 ***continued.***

Table 4.15. Chemical constituents of *C. latipes* fruit extract

R.T (min)	Name of the compound	Peak area (%)
7.949	Sucrose	6.88
14.993	n-Hexadecanoic acid	9.12
18.040	cis-Vaccenic acid	51.74
22.656	8-(2,3-Dihydroxy-3-methylbutyl)-7-methoxy-2H-chromen-2-one	5.42
25.574	2-[5-(2-Methyl-benzooxazol-7-yl)-1H-pyrazol-3-yl]-phenol	14.13
26.451	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	4.51
28.985	2,4,6-Tri-tert-butylphenol, trimethylsilyl ether	8.21

Table 4.16. Chemical constituents of *C. latipes* bark extract

R.T (min)	Name of the compound	Peak area (%)
8.265	Stevioside	1.18
9.276	Phenol, 4-ethenyl-2,6-dimethoxy-	2.58
14.384	Hexadecanoic acid, methyl ester	1.92
14.966	n-Hexadecanoic acid	6.08
16.795	13-Hexyloxacyclotridec-10-en-2-one	5.30
17.285	trans-13-Octadecenoic acid, methyl ester	9.76
17.976	cis-Vaccenic acid	34.71
25.560	2-[5-(2-Methyl-benzooxazol-7-yl)-1H-pyrazol-3-yl]-phenol	10.19
26.455	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	4.33
29.193	Ricinoleic acid	8.01
33.345	Cholesta-22,24-dien-5-ol, 4,4-dimethyl-	1.61
34.046	ß-Sitosterol	2.70
43.151	Isopropyl linoleate	3.89

Table 4.17. Chemical constituents of *C. latipes* root extract

R.T (min)	Name of the compound	Peak area (%)
7.762	Tricyclo [2.2.1.0(2,6)] heptane, 1,7-dimethyl-7-(4-methyl-3-pentenyl)-, (-)-	6.29
14.945	n-Hexadecanoic acid	1.68
17.071	2H,8H-Benzo[1,2-b:5,4-b'] dipyran-2-one, 8,8-dimethyl-	22.47
17.938	cis-Vaccenic acid	11.36
18.724	Methyl 9-cis,11-trans-octadecadienoate	1.53
19.322	Osthole	35.85
20.401	9-Octadecenoic acid, 12-hydroxy-, methyl ester, (Z)-	3.31
22.193	2H-1-Benzopyran-2-one, 6-(3-hydroxy-3-methyl-1-butenyl)-7-methoxy-, (E)-	2.28
25.557	2-[5-(2-Methyl-benzooxazol-7-yl)-1H-pyrazol-3-yl]-phenol	3.73
26.448	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	1.20
28.985	2,6-Di-tert-butyl-4-mercaptophenol, S-trifluoroacetyl-	2.20
30.975	(1H) Indolo [2,1-a]isoquinoline, 11,12-dihydro-2,3,8,9-tetramethoxy-	1.80

The bioactivities of the major phytocompounds detected in *G. simonsii, V. odoratissimum var. odoratissimum* and *C. latipes* are furnished in Table 4.18. Nevertheless, greater research capacities are warranted for all the investigated species (*G. simonsii, V. odoratissimum var. odoratissimum* and *C. latipes*) to comprehend their pharmacological properties and validate them as a potential source for future drug development under the aegis of 'Natural Product Research'

Table 4.18. The bioactivities of the major phytocompounds detected in *G. simonsii, V. odoratissimum var. odoratissimum* and *C. latipes* extracts

Phytocompounds	Class of compounds	Bioactivities	References
Benzaldehyde	Glycoside	Antimicrobial, antioxidant, tyrosinase inhibition, insecticidal	Neto et al., 2021
Spathulenol	Terpenoid	Antioxidant, antimicrobial, anti-inflammatory, neuroprotective, anticancer	Nascimento et al., 2018; Manjima et al., 2021
Coniferyl alcohol	Polyphenol	Antioxidant, antimicrobial, anti-inflammatory, antiviral	Taher et al., 2023
n-Hexadecanoic acid	Fatty acid	Antioxidant, antimicrobial, anti-inflammatory, antiandrogenic, anticancer	Aparna et al., 2012; Ravi et al., 2017
Aziridine	Alkaloid	Anticancer, antioxidant, antimicrobial,	Ismail et al., 2009
ß-Ethylphenethyl alcohol	Primary alcohol	Antimicrobial, anti-tyrosinase, preservative	Corre et al., 1990
cis-Vaccenic acid	Fatty acid	Antibacterial, anti-inflammatory, antidiabetic, anticancer, anti-CVD effects, improved insulin sensitivity and lipid profiles,	Jacome-Sosa et al., 2016; Scott et al., 2024
Stigmasterol	Steroid	Anticancer, anti-inflammatory, anti-diabetic, neuroprotective, antimicrobial, antioxidant, immunomodulatory	Zhang et al., 2022; Valitova et al., 2024; Goswami et al., 2023
4,22-Stigmastadiene-3-one	Steroid	Antimicrobial, antioxidant, anti-inflammatory, anti-tubercular, anticancer, cardiovascular protection	Singariya et al., 2013;
Cholest-4-en-3-one	Steroid	Anticancer, anti-obesity, anti-leishmaniasis	Ma et al., 2016
2-Methoxy-4-vinylphenol	Phenolic	Antimicrobial, anticancer, anti-inflammatory,	Rubab et al., 2020

Kavain	Alkaloid	Anxiolytic, analgesic, anti-inflammatory, antioxidant, neuroprotection, anticonvulsive	Chamoli et al., 2020; Vale et al., 2022
Phenol, 3-methoxy-2-(quinoxalin-6-yliminomethyl)-	Phenolic	Antioxidant, enzyme inhibition, antimicrobial	Artunc et al., 2020
ß-Sitosterol	Steroid	Antioxidant, anti-inflammatory, anticancer, antimicrobial, immunomodulator, antiviral, cardiovascular protection	Nandi et al., 2024; Babu et al., 2020
Neophytadiene	Terpenoid	Analgesic. Antipyretic, anti-inflammatory, antimicrobial, antioxidant, anticonvulsant, anticancer, cardioprotective	Gonzalez-Rivera et al., 2023; Bhardwaj et al., 2020
Ergosta-5,22-dien-3-ol	Steroid	Antioxidant, anti-inflammatory, antidiabetic, anticancer	Hazra et al., 2023; Ray et al., 2022
ß-Amyrin	Terpenoid	Anti-inflammatory, anti-Parkinsonian, antimicrobial, antioxidant, antinociceptive, gastroprotective, anxiolytic	Alam et al., 2023; Thirupathi et al., 2017
Lup-20(29)-en-3-ol	Terpenoid	Anticancer, anti-inflammatory, antioxidant, antimicrobial,	Liu et al., 2021
Phenol, 2,6-dimethoxy	Polyphenol	Antioxidant, antimicrobial, cytotoxic	Park et al., 2014
2H,8H-Benzo[1,2-b:5,4-b'] dipyran-2-one, 8,8-dimethyl	Terpenoid	Anti-inflammatory, antioxidant, vasorelaxant, antinociceptive	Singh et al., 2020;
Oleic Acid	Fatty acid	Antimicrobial, antioxidant, anti-inflammatory	Choulis, 2011
Osthole	Polyphenol	Anticancer, anti-inflammatory, neuroprotective, cardiovascular protective, antimicrobial, antioxidant, anti-diabetic, immunomodulator	Sun et al., 2021; Zhang et al., 2015

Epicurzerenone	Terpenoid	Antimicrobial, antitumor, anti-inflammatory, insecticidal, hepatoprotective, antiviral, anti-allergic, anti-convulsant	Anjum et al., 2023; Lai et al., 2004
Ricinoleic acid	Fatty acid	Laxative, analgesic, anti-inflammatory	Vieira et al., 2000
Oxypeucedanin	Polyphenol	Antimicrobial, antioxidant, anticancer, anti-HIV	Park et al., 2020; Du et al., 2022
d-Tocopherol	Terpenoid	Antioxidant, immunomodulator, neuroprotection, anti-inflammatory	Szewczyk et al., 2021
3-(3,4-Dimethoxyphenyl)-6-methoxy-4-methylcoumarin	Polyphenol	Antimicrobial, antioxidant, anticancer, anti-inflammatory, anti-diabetic, anti-HIV	Lake et al., 1994; Sahoo et al., 2021
Stevioside	Diterpenoid glycoside	Anti-diabetic, anti-inflammatory, antioxidant, anticancer, anti-hypersensitive, antiviral	Peteliuk et al., 2021; Chatsudthipong et al., 2009
2,6-Di-tert-butyl-4-mercaptophenol, S-trifluoroacetyl	Polyphenol	Antimicrobial, antifungal, anti-inflammatory	Eltai et al., 2022
Indolo [2,1-a]isoquinoline,	Alkaloid	Anticancer, antioxidant, antimicrobial	Verma et al., 2021

4.3. Molecular Characterizations

Molecular characterization is pivotal for enhancing medicinal plant research by offering insights into molecular taxonomy and genetic and biochemical aspects. Molecular markers, including DNA barcoding, RAPD, AFLP, and SSR, facilitate the precise identification of medicinal plants, particularly those that exhibit morphological similarities (Cahyaningsih et al., 2022). It also facilitates the prevention of adulteration and misidentification, thereby ensuring the quality and safety of herbal products. Molecular profiling also facilitates the screening of plant

species for novel genes and compounds with potential pharmacological applications, thereby expediting the discovery of new drugs by targeting specific genetic traits associated with medicinal properties (de Boer et al., 2022; Li et al., 2023).

In the present study molecular identification was performed through DNA barcoding approach using rbcl and ITS2 genes. The sequences were submitted to the GenBank NCBI database, and their accession numbers are PV688311 (*Goniothalamus simonsii*), PV749115 (*Viburnum odoratissimum var. odoratissimum*), and PV737879 (*Citrus latipes*).

BLAST analysis was performed to identify the nearest genetic relatives of the query species (*G. simonsii, V. odoratissimum var. odoratissimum, and C. latipes*) in the NCBI nucleotide database. The results of the BLAST analysis for the query species are presented in Table 4.19 - 4.21. The phylogenetic tree derived from the aligned nucleotide sequences based on the BLAST results offers a visual depiction of the evolutionary relationships among the examined species. The trees were generated using the Maximum Likelihood method in MEGA 12 with the Tamura-Nei model. The constructed phylogenetic trees of the query species are shown in Fig. 4.10 - 4.12.

The BLAST analysis result of *G. simonsii* (Table 4.19) reveals that *G. cheliensis*, *G. griffithii*, and *Goniothalamus sp. HTHP-2020* showed 96 % identity and identical alignment scores of 529, with E-values of 1e-145. These results showed a genetic similarity within the *Goniothalamus* genus and highlight its internal genetic conservation (Joshi & Xu, 2007).

The phylogenetic tree shown in Fig. 4.10, demonstrates a well-resolved topology, clustering the *G. simonsii* closely with members of the genus *Goniothalamus*. The *G. simonsii* forms a distinct clade with *G. cheliensis*, *G. griffithii*, *G. donnaiensis*, and *Goniothalamus sp. HTHP-2020* suggesting a close evolutionary relationship. This clade is characterized by strong bootstrap value of 100 and 0.02 level of divergence, suggesting high confidence in the genetic relationship among the species (Smith et al., 2009). Species from the genera *Asimina*,

Table 4.19. Result of the BLAST Analysis of *G. simonsii* ITS2 sequence showing genetic similarity with other species available in NCBI database

S. No.	Descriptions	Accession No.	Alignment score	Query coverage	E-value	Max. Identity
1	*Goniothalamus simonsii*	PV688311	601	100%	7e-167	100%
2	*Goniothalamus cheliensis*	KR532200	529	100%	1e-145	96%
3	*Goniothalamus griffithii*	KR532201	529	100%	1e-145	96%
4	*Goniothalamus sp.* HTHP-2020	MT777703	529	100%	1e-145	96%
5	*Goniothalamus donnaiensis*	OR199655	518	96%	2e-142	96%
6	*Asimina reticulata*	GQ139699	383	100%	9e-102	88%
7	*Asimina tetramera*	GQ139678	383	100%	9e-102	88%
8	*Disepalum pulchrum*	MT275651	368	100%	3e-97	87%
9	*Annona stenophylla*	MN257805	344	100%	4e-90	86%
10	*Annonaceae sp.* JM-2017	MG548815	272	79%	2e-68	86%

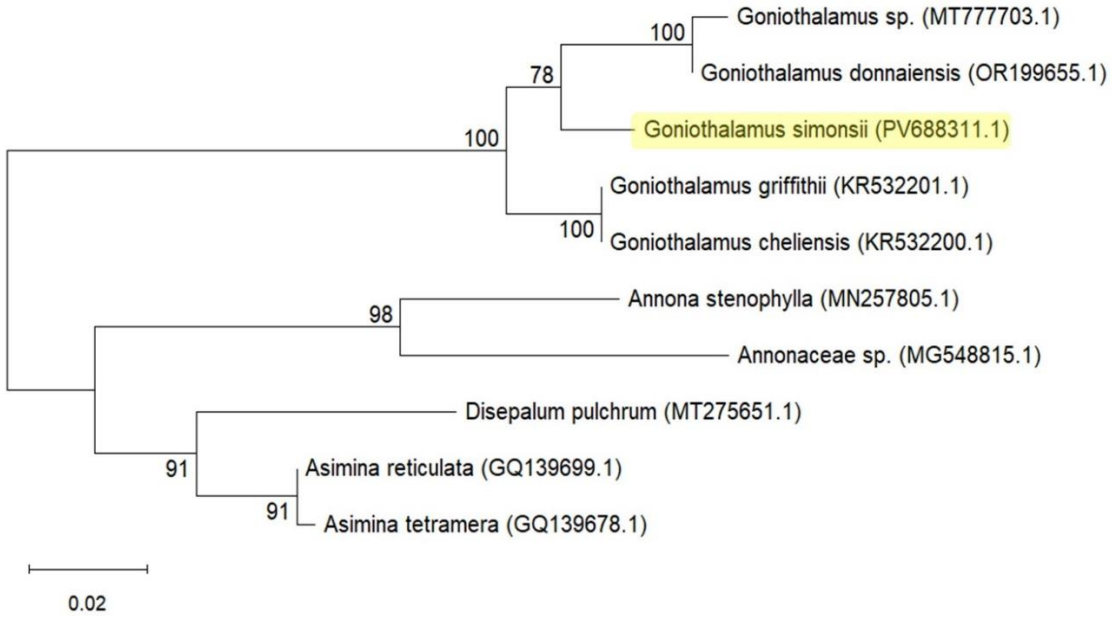

Fig. 4.10. Dendogram of *G. simonsii* (highlighted in yellow) by Maximum Likelihood method using ITS2

Disepalum, and *Annona* form separate clades, indicating more distant evolutionary relationships. These taxa are placed as outgroups or in basal positions relative to the *Goniothalamus* cluster, which further supports the conclusion that the query sequence belongs to *Goniothalamus.*

Similarly, BLAST result presented in Table 4.20, demonstrated that *V. odoratissimum var. odoratissimum* showed genetic similarity with *Viburnum* species such as *V. erubescens, V. brachybotyrum, V. odoratissimum* and *V. grandiflorum*. Among them *V. erubescens* and *V. brachybotryum* showed 99 % identity and an alignment score of 592 - 597, suggesting a maximum similarity to the query species (Smith et al., 2009). Further, the phylogenetic analysis furnished in Fig. 4.11 shows that *V. odoratissimum var. odoratissimum* formed a distinct and supported clade, indicating its close relationship with *V. grandiflorum,* while showing significant divergence from other members such as *V. farreri* and *V. oliganthum*. A bootstrap value of 68 was observed. Their placement in a common clade suggests that these taxa may share a recent common ancestor and possibly similar ecological or morphological characteristics (Smith et al., 2009). The grouping of *V. erubescens, V. brachybotryum,* and *V. oliganthum* into a separate clade suggests a shared lineage distinct from the *V. odoratissimum* cluster, which could be attributed to divergence in genetic, geographical, or ecological factors (Winkworth & Donoghue, 2005).

The BLAST analysis result of *C. latipes* (Table 4.21) reveals that *C. hassaku, C. maxima, C. aurantifolia* and *C. macroptera* showed maximum genetic similarity. The identity of 99 % with alignment score of 1125 - 1146 among these species further suggest their best match and similarity. The phylogenetic tree constructed for *C. latipes* is depicted in Fig. 4.12. *C. latipes* formed a clade with *C. hassaku* with a bootstrap value of 40. The branch length of *C. latipes* is longer than the *C. hassaku* suggesting genetic divergence among them with a level of 0.002 (Hynniewta et al., 2014). The phylogenetic analysis supports the BLAST results by placing the query species firmly within the respective genus. This combination of molecular evidence provides robust support for the taxonomic identification and evolutionary placement of the query species.

Table 4.20. Result of the BLAST Analysis of *V. odoratissimum var. odoratissimum* ITS2 sequence showing genetic similarity with other species available in NCBI database

S. No.	Descriptions	Accession No.	Alignment score	Query coverage	E-value	Max. Identity
1	*Viburnum odoratissimum var. odoratissimum*	PV749115	614	100%	1e-170	100%
2	*Viburnum erubescens*	MH808383	597	100%	3e-166	99%
3	*Viburnum brachybotryum*	OR199793	592	100%	1e-164	99%
4	*Viburnum oliganthum*	MH808385	588	100%	2e-163	98%
5	*Viburnum farreri*	MT227655	586	100%	7e-163	98%
6	*Viburnum awabuki*	LC600954	580	100%	3e-161	98%
7	*Viburnum foetens*	KF019813	579	97%	1e-160	99%
8	*Viburnum grandiflorum*	KF019814	573	97%	5e-159	99%
9	*Viburnum odoratissimum*	KP092591	564	100%	3e-156	97%
10	*Viburnum amplifolium*	MN952543	545	97%	1e-150	97%

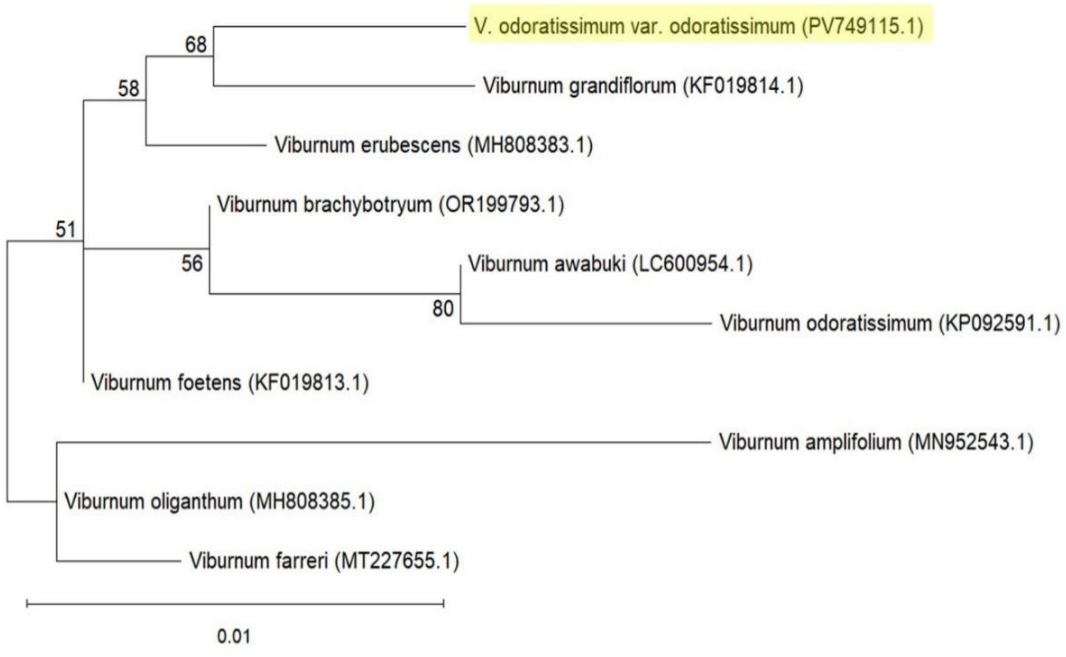

Fig. 4.11. Dendogram of *V. odoratissimum var. odoratissimum* (highlighted in yellow) by Maximum Likelihood method using ITS2

Table 4.21. Result of the BLAST Analysis of *C. latipes* ITS sequence showing genetic similarity with other species available in NCBI database

S. No.	Descriptions	Accession No.	Alignment score	Query coverage	E-value	Max. Identity
1	*Citrus latipes*	PV737879	1170	100%	0.0	100%
2	*Citrus hassaku*	JQ990166	1146	100%	0.0	99%
3	*Citrus maxima*	ON479682	1136	100%	0.0	99%
4	*Citrus aurantiifolia*	FJ641955	1131	100%	0.0	99%
5	*Citrus macroptera*	MW512545	1125	100%	0.0	99%
6	*Citrus hystrix*	FJ641961	1125	100%	0.0	99%
7	*Citrus hassaku*	AB456122	1123	100%	0.0	99%
8	*Citrus reticulata*	MF155029	1118	100%	0.0	99%
9	*Citrus sinensis*	AB456127	1118	100%	0.0	99%
10	*Citrus montana*	AB456057	1107	100%	0.0	98%

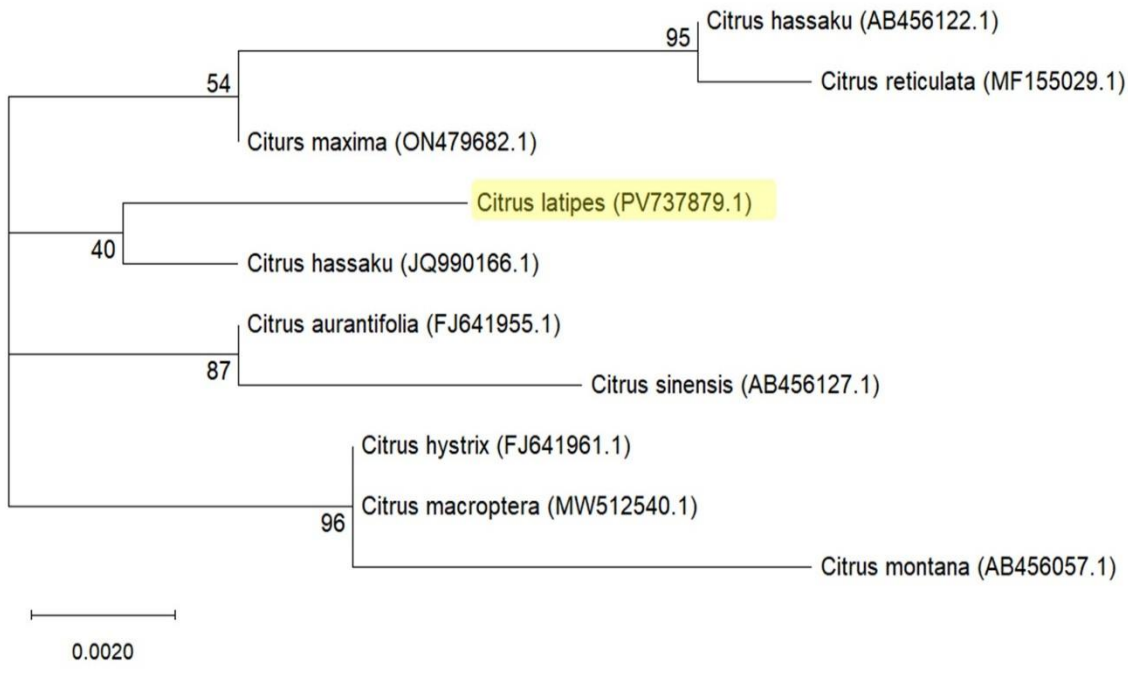

Fig. 4.12. Dendogram of *C. latipes* (highlighted in yellow) by Maximum Likelihood method using ITS2

4.4. Bioactivity Assessment

4.4.1. Total Phenolic and Flavonoid Content

Quantitative investigations of key phytocompounds such as phenols, flavonoids, alkaloids, and terpenoids are vital to ascertain the pharmacological significance of ethnomedicinal plants. In addition, phenols and flavonoids exhibit a significant association with antioxidant activity. The antioxidant properties of phenolic compounds may be mediated through the following mechanisms: (1) scavenging radical species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS); (2) inhibiting the formation of ROS/RNS by obstructing specific enzymes or chelating trace metals involved in free radical production; (3) enhancing or safeguarding antioxidant defences (Dai et al., 2010). In the present study, the Folin Ciocalteu and aluminum chloride colourimetric methods were employed to quantify the total phenolic (TPC) and flavonoid contents (TFC) of *G. simonsii, V. odoratissimum var. odoratissimum* and *C. latipes*, respectively. The details of the phenolic and flavonoid contents are presented in Table 4.22.

The leaf extract of *G. simonsii* exhibited highest TPC with 154.76 ± 7.98 mg GAE/g, whereas, fruit and bark extract showed 92.02 ± 2.28 and 61.50 ± 9.96 mg GAE/g, respectively. Similarly, the leaf extract displayed higher TFC with 55.91 ± 3.05 mg QE/g than the fruit (44.49 ± 2.30 mg QE/g) and bark extract (35.82 ± 0.76 mg QE/g). The current findings are consistent with previous studies on related species within the genus *Goniothalamus* (Abdelwahab et al., 2010; Iqbal et al., 2015; Palani et al., 2020). For instance, TPC and TFC of *G. velutinus* bark and leaf extracts were in the range of 68.33 - 77.74 mg GAE/g and 42.84 - 72.16 mg QE/g, respectively (Iqbal et al., 2015). Likewise, *G. wightii* leaf extract also showed phenolic and flavonoid content in the similar range (67.50 mg GAE/g, 76.80 mg QE/g) as of *G. simonsii* extracts (Palani et al., 2020). However, the ethanolic leaf extract of *G. umbrosus* showed a TPC value of 340 mg GAE/g, which was higher than *Goniothalamus simonsii* (Abdelwahab et al., 2010). These variations within the

same genus can be ascribed to the substantial influence of genetic and environmental factors on the phytochemical synthesis (Mohammadi et al., 2021).

Table 4.22. Results of the Phenolic and Flavonoid content assay

		TPC (mg GAE/g)	TFC (mg QE/g)
G. simonsii	Leaf	154.76±7.98[a]	55.91±3.05[a]
	Fruit	92.02±2.28[b]	44.49±2.30[b]
	Bark	61.50±9.96[c]	35.82±0.76[c]
V. odoratissimum var. odoratissimum	Leaf	350.60±5.10[a]	44.87±2.99[a]
	Fruit	208.88±6.05[c]	22.55±1.99[b]
	Bark	356.71±4.27[a]	4.19±1.02[c]
	Root	303.23±8.45[b]	6.54±0.71[c]
C. latipes	Leaf	70.51±1.95[c]	32.84±2.47[a]
	Fruit	75.55±2.04[c]	24.90±1.13[c]
	Bark	149.36±4.62[a]	37.24±1.26[b]
	Root	132.49±3.38[b]	35.16±0.99[b]

Values are expressed as the mean±SD (n=3). [a-d] Different letters within the same column indicate significant differences between mean values ($p < 0.05$)

In *V. odoratissimum var. odoratissimum*, the bark extract showed highest TPC with 356.71 ± 4.27 mg GAE/g, and the fruit extract lowest with 208.88 ± 6.05 mg GAE/g. Leaf and root extract had 350.60 ± 5.10 mg GAE/g and 303.23 ± 8.45 mg GAE/g, respectively. Regarding TFC, leaf extract showed the highest content with 44.87 ± 2.99 mg QE/g, and the bark extract the lowest content with 4.19 ± 1.02 mg QE/g. The current findings correspond with the findings of previous investigations on the related species from the genus *Viburnum*. The TPC value of *V. odoratissimum var. odoratissimum* fruit extract (208.88 ± 6.05 mg GAE/g) were higher than the fruit extract of *V. opulus* (131.99 mg GAE/g) (Sagdic et al., 2006), *V. mullaha* (12.57 mg GAE/g) (Singh et al., 2017) and *V. coriaceum* (29.75 mg GAE/g)

(Vijaytha et al., 2020). Alongside, the TFC of *V. odoratissimum var. odoratissimum* leaf extract (44.87 ± 2.99 mg QE/g) was higher than the *V. mullaha* (35.03 mg QE/g) (Singh et al., 2017).

In *C. latipes*, the extracts displayed TPC in the range of 70.5 –149.36 mg GAE/g. Similarly, TFC was in the range of 24.90 - 37.24 mg QE/g. The bark and root extract demonstrated significant levels of phenolic and flavonoid contents. The leaf and fruit showed a lower content of phenolics and flavonoids. The highest TPC and TFC were recorded in the bark extract, measuring 149.36 ± 4.62 mg GAE/g and 37.24 ± 1.26 mg QE/g respectively. In contrast, leaf extract showed the lowest TPC (70.15 ± 1.95 mg GAE/g) and TFC (22.84 ± 2.47 mg QE/g). From the Table 4.22 it can be observed that the TPC and TFC of *C. latipes* were marginally elevated compared to other citrus plants. For example, the leaves of *C. sinensis* and *C. aurantium* exhibited a phenolic content ranging from 19 - 45 mg/g and a flavonoid content ranging from 1.0 - 3.5 mg/g (Lagha-Benamrouche et al., 2013). Similarly, the fruit pulp of *C. limetta* and *C. reticulata* exhibited phenolic content of 23.09 mg/g and 39.97 mg/g, and flavonoid content of 1.69 mg/g and 1.93 mg/g respectively (Damián-Reyna et al., 2017). Nevertheless, the findings of the present investigation are in close proximity with the results reported by Loizzo et al. (2012) regarding the phenolic and flavonoid content of the leaf extract of *C. aurantifolia* (79.6 mg/g and 59.6 mg/g).

All the three species (*G. simonsii, V. odoratissimum var. odoratissimum* and *C. latipes*) under investigation displayed significant phenolic as well as flavonoid contents. The findings suggest that the different parts of these three species may have various biological activities including antioxidant, antimicrobial, anticancer, anti-inflammatory, etc., (Kruk et al., 2022).

4.4.2. Antioxidant Activity

Assessing the free radical scavenging activity of medicinal plants is essential to validate their antioxidant potential. Free radicals encompass numerous chemical entities; hence, it is very important to employ multiple approaches to

demonstrate the capacity of plant extracts to neutralise free radicals (Dai et al., 2010). For a comprehensive assessment of free radical scavenging, DPPH, FRAP and ABTS methods were used in the present study. The results of the DPPH (IC_{50} value), FRAP and ABTS assay of the *G. simonsii*, *V. odoratissimum var. odoratissimum* and *C. latipes* are furnished in Table 4.23. Further, Fig. 4.13 - 4.15, illustrates the comparative radical scavenging activities of the standard antioxidant compound (ascorbic acid) and extracts, indicating the percentage of scavenging activities at different concentrations.

In *G. simonsii*, the lowest IC_{50} value was recorded for leaf extract (437.38 ± 13.11 µg/ml), indicating its superior antioxidant activity relative to fruit (659.27 ± 15.66 µg/ml) and bark extract (859.38 ± 11.33 µg/ml). Furthermore, in the FRAP and ABTS assays, leaf extract exhibited values of 536.55 ± 10.65 mM AAE/g and 439.42 ± 22.14 mM AAE/g, respectively. Consistent with the DPPH assay, fruit [489.23 ± 11.09 mM AAE/g (FRAP), 258.81 ± 13.29 mM AAE/g(ABTS)] and bark extract [433.76 ± 7.84 mM AAE/g (FRAP), 244.65 ± 11.76 mM AAE/g(ABTS)] exhibited reduced antioxidant content. The results of the previous studies on the related genus are in close proximity with our findings. For example, FRAP and ABTS value of *G. velutinus* were in the range of 316 - 424 mM/g (Iqbal et al., 2015). The bark and leaves of *G. velutinus* exhibited IC_{50} values of 204 µg/ml and 155 µg/ml in the DPPH assay, which are markedly lower than the values observed in the current study, indicating enhanced antioxidant activity (Iqbal et al., 2015). Nonetheless, the leaf extract exhibited more potential antioxidant activity compared to *G. sesquipedalis* (601.2 µg/ml) (Nawar et al., 2019).

V. odoratissimum var. odoratissimum extracts demonstrated dose-dependent free radical scavenging activities. Table 4.23 reveals that the leaf extract exhibited the lowest IC_{50} value of 32.25 ± 1.40 µg/ml, alongside the highest FRAP (827.25 ± 11.78 mM AAE/g) and ABTS value (763.14 ± 16.38 mM AAE/g), signifying its superior antioxidant efficacy compared to other parts (fruit, bark, and root) of the species. Nonetheless, the bark extract exhibited an analogous IC_{50} value (36.86 ± 1.74 µg/ml), FRAP (618.50 ± 9.18 mM AAE/g), and ABTS value (656.79 ±

9.38 mM AAE/g) to that of the leaf extract. The fruit extract showed reduced antioxidant potency with IC_{50} value of 85.49 ± 3.72 µg/ml and FRAP value of 564.41 ± 8.39 mM AAE/g compared to other parts of the species. Considering the findings of the previous studies on the related species from the genus *Viburnum*, the results from the presently studied species (*V. odoratissimum var. odoratissimum*) were significantly more substantial. For instance, Česonienė et al. (2012) and Polka et al. (2019) established that *V. opulus* fruit exhibits considerable antioxidant activity, with a FRAP value of 190 mM AAE/g, which is significantly lower than that of the species under investigation. Furthermore, Levent et al. (2008) reported that *V. lantana* exhibits DPPH radical scavenging activity (IC_{50} value 85 µg/ml), which is lower than that observed for *V. odoratissimum var. odoratissimum*. Vijaytha et al. (2020) reported a low antioxidant activity of *V. coriaceum* (IC_{50} 1500 µg/ml). Nonetheless, in light of the standard antioxidant property, the antioxidant values of the *V. odoratissimum var. odoratissimum* extracts indicate strong free radical scavenging activity.

C. latipes extracts displayed concentration-dependent free radical scavenging activity. Bark and root extract showed a significant IC_{50} value with 314.47 ± 5.75 µg/ml and 340.05 ± 4.18 µg/ml, respectively. Among the tested extracts, bark extract had the highest FRAP and ABTS values with 563.79 ± 11.6 mM AAE/g and 1044.29 ± 7.85 mM AAE/g, respectively, followed by root extract with 473.6 ± 9.44 mM AAE/g and 856.97 ± 13.56 mM AAE/g. Meanwhile, the FRAP value of leaf and fruit extract were in the range of 139 - 160 mM AAE/g. The enhanced antioxidant activity of the bark and root extract relative to the other extracts can be correlated with the higher concentration of the pharmacologically active phytocompounds (Table 4.16, 4.17). Similar studies have been carried out previously using other species within the same genus (*Citrus*). For instance, the fruit extract of *C. sinensis* and *C. aurantium* displayed IC_{50} values of 0.9 mg/ml and 1.8 mg/ml, respectively in the DPPH assay (Lagha-Benamrouche et al., 2013; Asjad et al., 2013). Whereas, peel and leave extract of *C. aurantifolia* showed IC_{50} values of

93.8 µg/ml and 89.7 µg/ml, and FRAP values of 146.0 µm/g and 122.7 µm/g respectively (Loizzo et al., 2012).

Table 4.23. Results of DPPH, FRAP and ABTS assay

		DPPH (IC_{50} µg/ml)	FRAP value (µM AAE/g)	ABTS value (µM AAE/g)
G. simonsii	Leaf	437.38±13.11[c]	536.55±10.65[a]	439.42±22.14[a]
	Fruit	659.27±15.66[b]	489.23±11.09[b]	258.81±13.29[b]
	Bark	859.38±11.33[a]	433.76±7.84[c]	244.65±11.76[b]
V. odoratissimum var odoratissimum	Leaf	32.25±1.40[c]	827.25±11.78[a]	763.14±16.38[a]
	Fruit	85.49±3.72[a]	564.41±8.39[c]	613.30±11.01[c]
	Bark	36.86±1.74[c]	618.50±9.18[b]	656.79±9.38[b]
	Root	50.97±1.61[b]	612.21±4.78[b]	464.53±19.33[d]
C. latipes	Leaf	1896.09±12.90[a]	160.78±7.87[c]	386.68±9.09[c]
	Fruit	1497.70±5.17[b]	139.22±3.84[c]	410.28±6.92[c]
	Bark	305.5±4.18[d]	563.79±11.6[a]	644.29±7.85[a]
	Root	346.37±5.75[c]	473.6±9.44[b]	856.97±13.56[b]

Values are expressed as the mean±SD (n=3). [a-d] Different letters within the same column indicate significant differences between mean values ($p < 0.05$)

The findings from the DPPH, FRAP, and ABTS assays indicate that *G. simonsii*, *V. odoratissimum var. odoratissimum* and *C. latipes* extracts possessed considerable antioxidant activity and offers strong candidature for the development of antioxidant drugs.

The strength, direction, and statistical significance of the linear relationships among TPC, TFC, DPPH, FRAP, and ABTS were evaluated using Pearson's correlation analysis. The graphical representation of the correlation results is presented in Fig. 4.16.

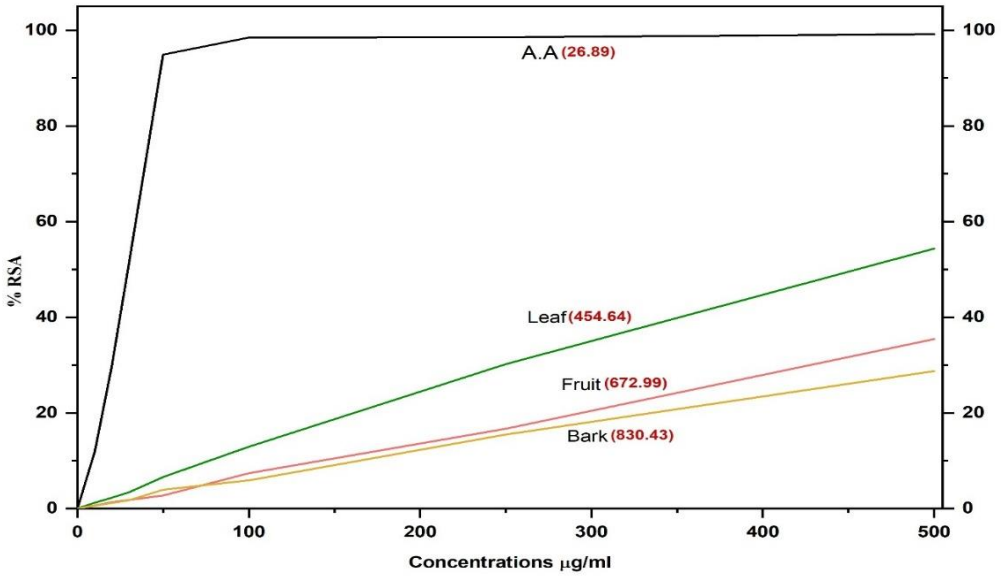

Fig. 4.13. Graph demonstrating comparative DPPH radical scavenging activities of the *G. simonsii* extracts and a standard antioxidant (AA - ascorbic acid)

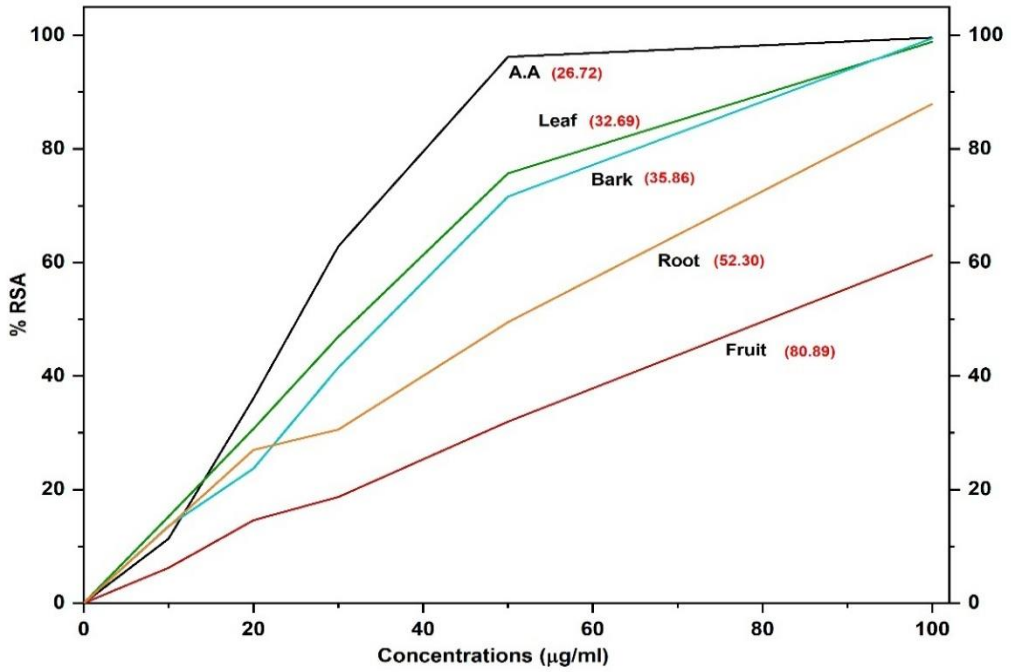

Fig. 4.14. Graph demonstrating comparative DPPH radical scavenging activities of the *V. odoratissimum var. odoratissimum* extracts and a standard antioxidant (AA)

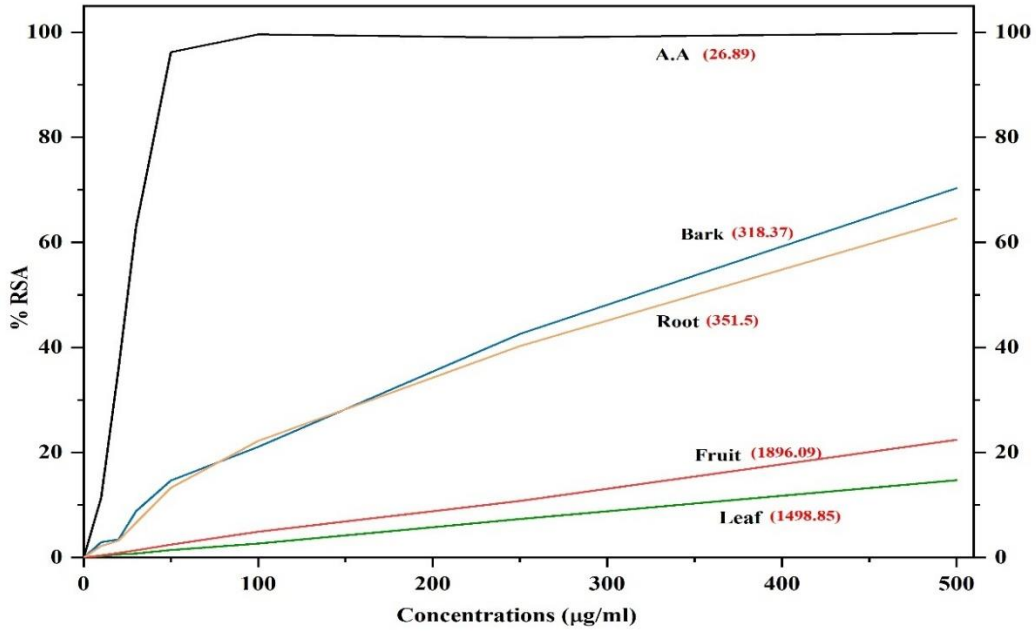

Fig. 4.15. Graph demonstrating comparative DPPH radical scavenging activities of the *C. latipes* extracts and a standard antioxidant (AA)

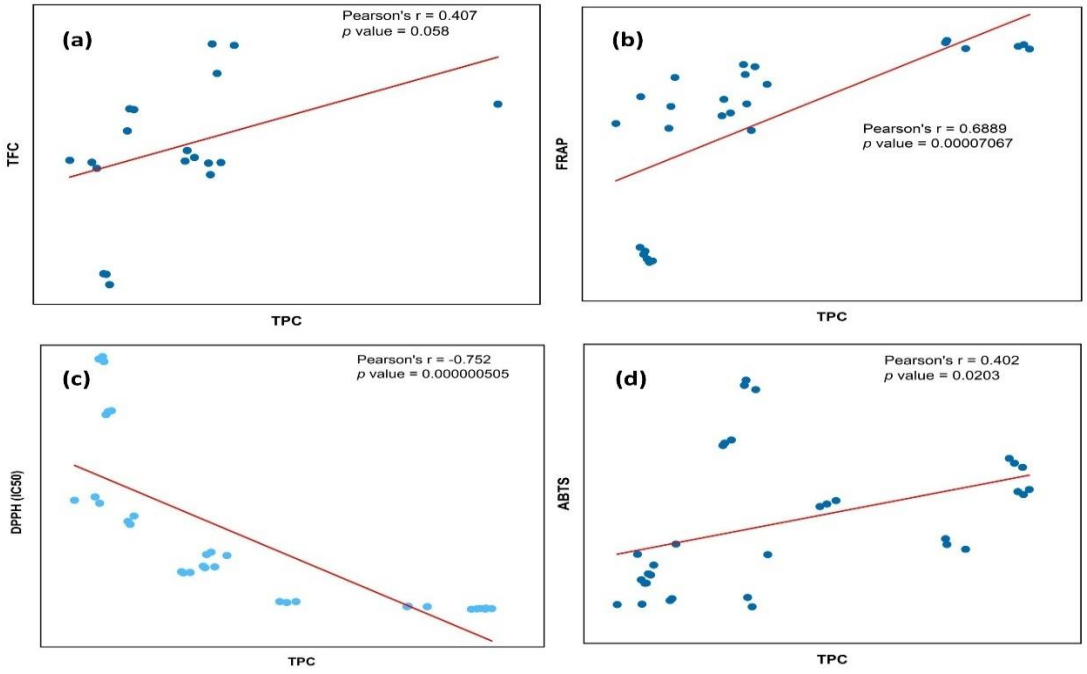

Fig. 4.16. Pearson's correlation analysis illustrating the linear relationship among (a) TFC and TPC, (b) FRAP and TPC, (c) DPPH and TPC and (d) ABTS and TPC

A positive linear relationship was observed between TPC and TFC, FRAP, and ABTS, with Pearson's *r* values of 0.407, 0.688, and 0.402, respectively. The corresponding *p*-values (< 0.05) indicate that these correlations are statistically significant. Conversely, a strong negative linear relationship was observed between DPPH and TPC, with a Pearson's *r* value of −0.752 and a *p*-value of 0.0000005 (< 0.05), confirming the statistical significance of this inverse association.

4.4.3. Antimicrobial Activity

Antibiotic resistance remains a significant challenge for the healthcare sector globally. The rise and expansion of multidrug-resistant pathogens have significantly jeopardized contemporary antibacterial treatment (Romero et al., 2005; Boucher et al., 2009). This has prompted the exploration of alternative sources of antimicrobial agents, particularly plants, which generate a diverse array of bioactive compounds with established medicinal uses (Dubale et al., 2023). Extracts derived from medicinal plants have been reported to exhibit various biological activities, including antimicrobial, anti-inflammatory, and antioxidant effects. Antimicrobial compounds derived from medicinal plants may impede the proliferation of bacteria, fungi, viruses, and protozoa through mechanisms distinct from those of current antimicrobials, potentially offering substantial clinical value in addressing resistant microbial strains (Vaou et al., 2021). Certain active compounds exhibit intrinsic antibacterial properties and possess antibiotic resistance-modifying capabilities; additionally, some compounds, although ineffective as standalone antibiotics, can enhance the efficacy of antibiotics when used in combination, thereby aiding in the mitigation of bacterial antibiotic resistance (Palani et al., 2020). Chemically intricate compounds possess significant therapeutic potential due to their reduced side effects relative to synthetic drugs and a lower likelihood of resistance development (Lewis & Ausubel, 2006; Ruddaraju et al., 2020).

Phytochemical screening identified the presence of various classes of secondary metabolites, including alkaloids, polyphenols, flavonoids, glycosides, saponins, tannins, triterpenes, and steroids. These metabolites exhibit activity against

pathogenic microorganisms (Erfan & Marouf, 2019; Hemeg et al., 2020). The presence of certain metabolites in the investigated plant extracts may provide a preliminary justification for their antimicrobial properties. Considering these facts, the current study additionally conducted an evaluation of the antimicrobial activity of the extracts derived from *G. simonsii*, *V. odoratissimum var. odoratissimum* and *C. latipes* using agar-well diffusion and micro-broth dilution (Minimum Inhibitory Concentration) methods.

The results of the agar-well diffusion assay of *G. simonsii* extracts are presented in Table 4.24. Fig. 4.17 illustrates the antimicrobial efficacy of the different parts (leaf, fruit and bark) of the species, revealing a clear zone of inhibition (ZOI) against the tested microbes. The ZOI (in mm) in descending order is as follows:

Leaf extract: *Bacillus cereus* (22.63 ± 1.9) >*Escherichia coli* (22.48 ± 1.29) >*Staphylococcus aureus* (21.48 ± 1.01) >*Candida albicans* (19.81 ± 2.48) >*Salmonella enterica* (14.38 ± 0.36) >*Yersinia pestis* (13.21 ± 0.29).

Fruit extract: *Bacillus cereus* (21.53 ± 1.21) >*Staphylococcus aureus* (16.53 ± 0.16) >*Candida albicans* (16.49 ± 1.70) >*Escherichia coli* (16.46 ± 1.55) >*Yersinia pestis* (13.83 ± 0.40) >*Salmonella enterica* (11.96 ± 0.81).

Bark extract: *Candida albicans* (21.45 ± 1.56) >*Bacillus cereus* (18.14 ± 0.56) >*Staphylococcus aureus* (15.72 ± 3.07) >*Escherichia coli* (14.34 ± 2.57) >*Yersinia pestis* (10.39 ± 0.67) >*Salmonella enterica* (8.56 ± 0.47).

The most significant ZOI was recorded for leaf extract against *Bacillus cereus* (22.63 ± 1.9 mm), while the least was noted for bark extract against *Salmonella enterica* (8.56 ± 0.47 mm). The leaf extract demonstrated equivalent efficacy against both gram-positive (21-22 mm) and gram-negative bacteria (13-22 mm), and yeast (19.81 mm). The fruit extract showed appreciable efficacy against gram-positive bacteria (16-21 mm) in contrast to gram-negative bacteria (11-16 mm). Similarly, the bark extract displayed significant efficacy against gram-positive bacteria (15-18 mm), and

lesser activity against gram-negative bacteria (10-14 mm). All the extracts were equally effective against *Candida albicans* (Fungus) (16-21 mm). The findings indicate that the extracts demonstrated reduced effectiveness against gram-negative bacteria. The *G. simonsii* extracts exhibited greater antimicrobial efficacy relative to certain previously studied species of the genus *Goniothalamus*. For example, *G. sesquipedalis* exhibited ZOI measuring 10.03 mm against *Escherichia coli* (Konsam et al., 2015). Likewise, *G. wightii* demonstrated a 6 mm ZOI against *Staphylococcus aureus* and *Candida albicans* (Palani et al., 2020). *G. cordiopetalus* exhibited comparable efficacy to the currently examined plant species, demonstrating ZOI of 20 mm against *Staphylococcus aureus*, 16.5 mm against *Bacillus cereus*, and 15.0 mm against *Escherichia coli* (Hisham et al., 2006).

The MIC values of the *G. simonsii* extracts and standards against a broad-spectrum of bacteria and fungus are presented in Table 4.25. Fig. 4.18 depicts the 96-wells plates of MIC results. The observed MIC values for the extracts were in the range of ≥1000 - 58.59 µg/ml, whereas for the standards (Positive control), they ranged 25 – 3.125 µg/ml. Among the extracts, leaf extract exhibited the lowest MIC against *Bacillus cereus* with 58.59 µg/ml (lower the MIC, greater the efficacy), while the highest MIC was observed for fruit and bark extract against *Salmonella enterica* and *Yersinia pestis* (>1000 µg/ml).

Nevertheless, the majority of the extracts displayed significant MIC values against *Staphylococcus aureus* (468.75 µg/ml), *Bacillus cereus* (58.59 – 468.75 µg/ml), *Escherichia coli* (117.19 – 468.75 µg/ml) and *Candida albicans* (117.19 - 468.75 µg/ml). These results are in close proximity with the findings on *G. tortilipetalus* conducted by Anatachodwanit et al. (2024). *G. tortilipetalus* demonstrated MIC values of 640 µg/ml for *Staphylococcus aureus* and 1240 µg/ml for *Escherichia coli*.

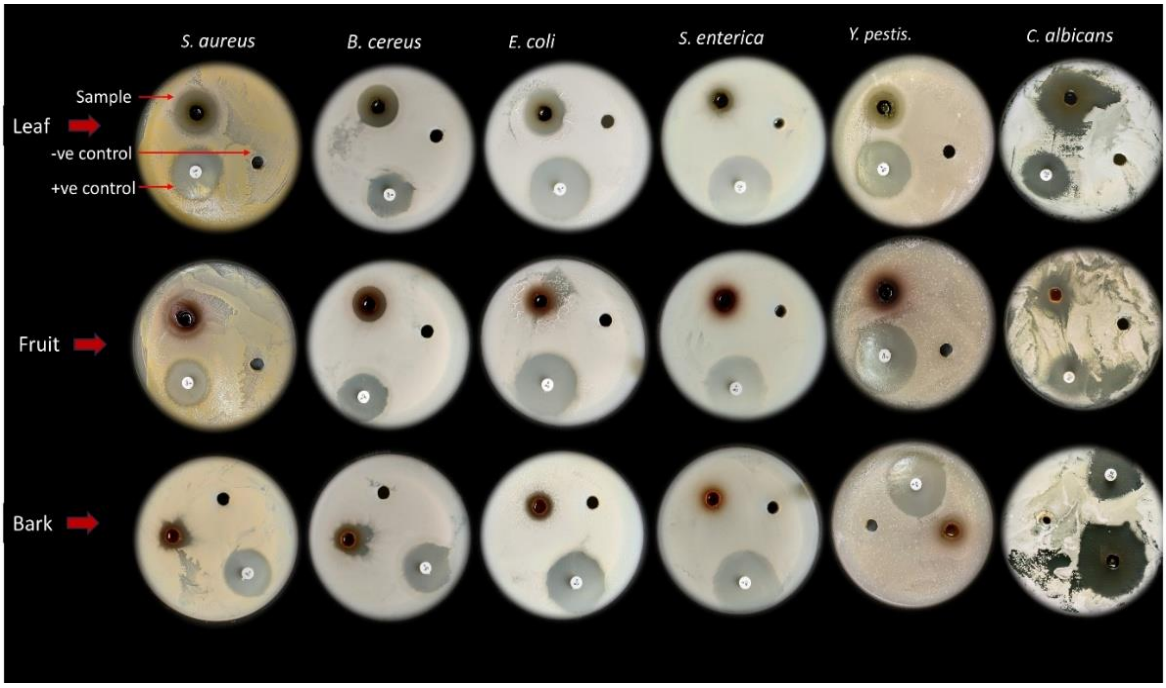

Fig. 4.17. Antimicrobial activities of the *G. simonsii* extracts showing zone of inhibition

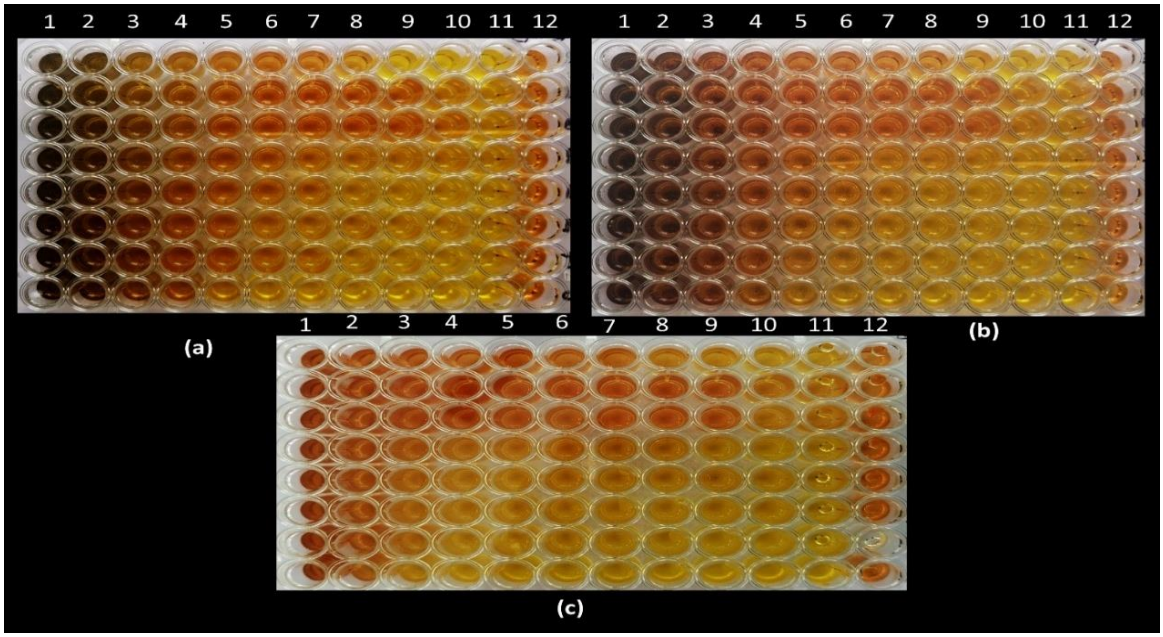

Fig. 4.18. 96-well plates of MIC assessment of *G. simonsii* extracts (a) leaf, (b) fruit and (c) bark. Column 1-10 contains test drug, bacteria and media, column 11 serves as positive control (bacteria without drug sample), column 12 serve as negative control (only media)

Table 4.24. Results of the antimicrobial activity assay of *G. simonsii* extracts

	Diameter of zone of inhibition (mm)					
	S. aureus	***B. cereus***	***E. coli***	***S. enterica***	***Y. pestis***	***C. albicans***
Leaf	21.48±1.01*	22.63±1.9	22.48±1.29	14.38±0.36	13.21±0.29	19.81±2.48
Fruit	16.53±0.16	21.53±1.21	16.46±1.55	11.96±0.81	13.83±0.40	16.49±1.70
Bark	15.72±3.07	18.14±0.56	14.34±2.57	8.56±0.47	10.39±0.67	21.45±1.56
Positive control	28.37±0.59	25.36±0.37	33.6±1.03	34.06±1.26	30.65±0.43	24.41±2.62
Negative control	NI**	NI	NI	NI	NI	NI

*mean±SD, **NI is No inhibition zone

Table 4.25. Minimum inhibitory concentrations (MIC) of the *G. simonsii* extracts against tested microorganisms

	MIC (µg/ml)					
	S. aureus	***B. cereus***	***E. coli***	***S. enterica***	***Y. pestis***	***C. albicans***
Leaf	468.75	58.59	117.19	937.5	468.75	117.19
Fruit	468.75	117.19	468.75	>1000	937.5	468.75
Bark	937.5	468.75	468.75	>1000	>1000	117.19
Positive control	6.25	6.25	3.125	6.25	3.125	25

The extracts derived from *V. odoratissimum var. odoratissimum* showed varying degree of antimicrobial activities towards gram-positive and gram-negative bacteria. The agar-well diffusion test results are furnished in Table 4.26. Fig. 4.19 illustrates the antimicrobial efficacy of the different parts of *V. odoratissimum var. odoratissimum*, revealing a distinct ZOI against the tested microbes. ZOI values (mm) of *V. odoratissimum var. odoratissimum* in descending order is as follows:

Leaf extract: *Bacillus cereus* (17.46 ± 3.06) >*Escherichia coli* (17.16 ± 0.94) >*Staphylococcus aureus* (16.69 ± 2.08) > *Yersinia pestis* (14.06 ± 0.71).

Fruit extract: *Escherichia coli* (10.60 ± 0.55) >*Bacillus cereus* (10.13 ± 1.41) >*Staphylococcus aureus* (9.71 ± 3.12).

Bark extract: *Bacillus cereus* (18.16 ± 1.86) >*Staphylococcus aureus* (17.78 ± 0.29) >*Candida albicans* (16.49 ± 1.8) >*Escherichia coli* (16.36 ± 2.92) >*Yersinia pestis* (14.09 ± 2.88).

Root extract: Escherichia *coli* (16.37 ± 1.14) >*Staphylococcus aureus* (15.45 ± 1.28) >*Bacillus cereus* (14.87 ± 0.63) >*Candida albicans* (14.26 ± 1.01) >*Yersinia pestis* (13.23 ± 1.54).

Bark extract showed highest ZOI against *Bacillus cereus* (18.16 ± 1.86 mm), while lowest was observed for fruit extract against *Staphylococcus aureus* (9.71 ± 3.12 mm). Nevertheless, all the extracts showed considerable antimicrobial efficacy displaying ZOIs ranged from 10-18 mm. Among the extracts, bark and leaf extract stands out best with their ZOI ranged from 16-18 mm. However, the extracts showed no activity towards *Salmonella enterica.* The MIC values are presented in Table 4.27 and 96-well plates are displayed in Fig. 4.20. Leaf extract showed the lowest MIC value with 156.25 µg/ml against *Bacillus cereus*. The MIC values of all the extracts were in the range of >1000 – 100 µg/ml. The findings of the present study are in agreement with those reported by Wintola & Afolayan (2015), which demonstrated that anti-dysenteric plant extracts produced a 12-15 mm ZOI against the same bacteria used in this study. Sagdic et al. (2006) also demonstrated that methanolic extracts of *V. opulus* exhibited a 19-22 mm ZOI against *Bacillus cereus* and *Staphylococcus aureus*. Roy et al. (2017) indicated that methanolic fractions of *V. foetidum* exhibited ZOI of 18-20 mm against *Bacillus cereus* and *Staphylococcus aureus* bacteria.

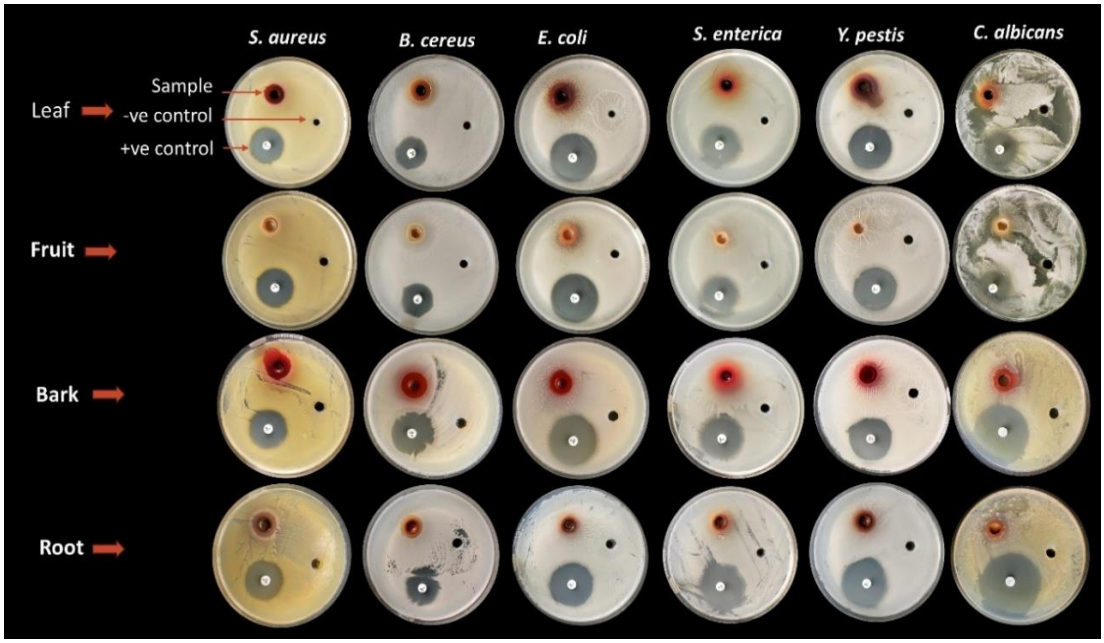

Fig. 4.19. Antimicrobial activities of the *V. odoratissimum var. odoratissimum* extracts showing zone of inhibition

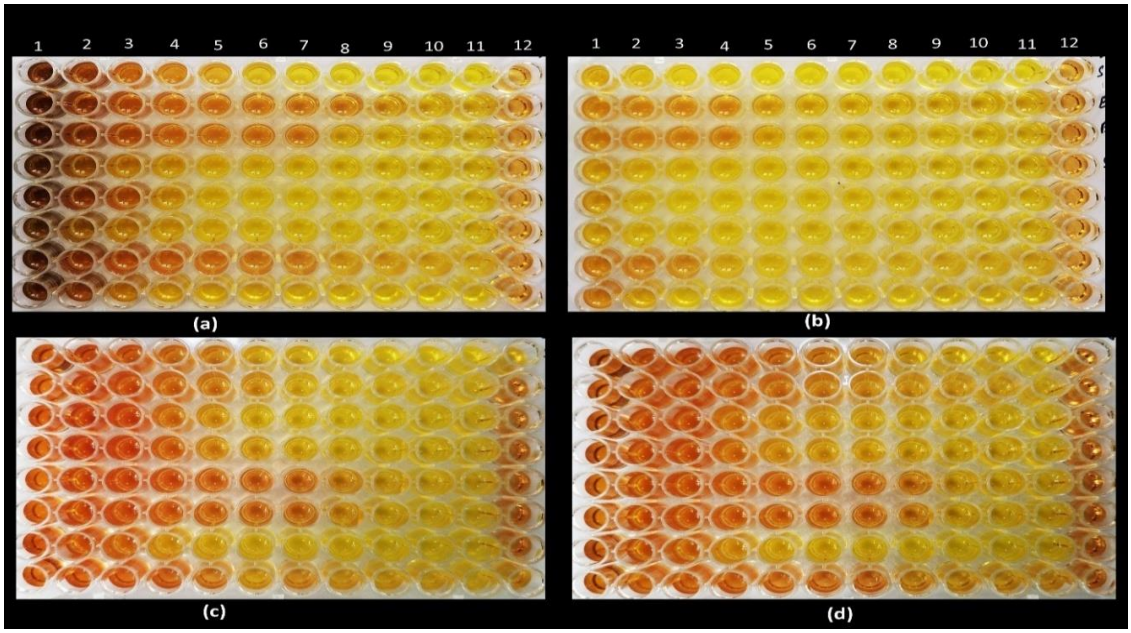

Fig. 4.20. 96-well plates of MIC assessment of *V. odoratissimum var odoratissimum* extracts (a) leaf, (b) fruit, (c) bark and (d) root. Column 1-10 contains test drug, bacteria and media, column 11 serves as positive control (bacteria without drug sample), column 12 serve as negative control (only media)

Table 4.26. Results of the antimicrobial activity assay of *V. odoratissimum var. odoratissimum* extracts

	Diameter of zone of inhibition (mm)					
	S. aureus	***B. cereus***	***E. coli***	***S. enterica***	***Y. pestis***	***C. albicans***
Leaf	16.69±2.08*	17.46±3.06	17.16±0.94	NI	14.06±0.71	NI
Fruit	9.71±3.12	10.13±1.41	10.60±0.55	NI	NI	NI
Bark	17.78±0.29	18.16±1.86	16.36±2.92	NI	14.09±2.88	16.49±1.8
Root	15.45±1.28	14.87±0.63	16.37±1.14	NI	13.23±1.54	14.26±1.01
Positive control	28.37±0.59	25.36±0.37	33.6±1.03	34.06±1.26	30.65±0.43	24.41±2.62
Negative control	NI**	NI	NI	NI	NI	NI

*mean±SD, **NI is No inhibition zone

Table 4.27. Minimum inhibitory concentrations (MIC) of the *V. odoratissimum var. odoratissimum* extracts against tested microorganisms

	MIC (µg/ml)					
	S. aureus	***B. cereus***	***E. coli***	***S. enterica***	***Y. pestis***	***C. albicans***
Leaf	625	156.25	332.03	ND*	234.38	ND
Fruit	>1000	625	>1000	ND	ND	ND
Bark	625	234.37	937.5	ND	625	>1000
Root	468.75	234.37	703.12	ND	625	>1000
Positive control	6.25	6.25	3.125	6.25	3.125	25

ND* Not Detected

Iqbal et al. (2022) isolated three alkaloid compounds (Viburnoate A, B, C) from *V. grandiflorum* and demonstrated that these compounds exhibited greater inhibition against gram-positive bacteria compared to gram-negative bacteria.

The *C. latipes* extracts displayed significant inhibitory activities against the tested microbes. The antimicrobial activities of the extracts are presented in Table 4.28. Fig. 4.21 illustrates the antimicrobial efficacy of the different parts of *C. latipes.* The ZOI in descending order is as follows:

Leaf extract: *Bacillus cereus* (12.62 ± 2.47) >*Escherichia coli* (11.89 ± 2.62) >*Staphylococcus aureus* (11.43 ± 2.01).

Fruit extract: *Bacillus cereus* (15.96 ± 2.84) >*Escherichia coli* (14.64 ± 0.44) >*Staphylococcus aureus* (13.33 ± 1.69) >*Candida albicans* (14.73 ± 0.86) >*Yersinia pestis* (11.95 ± 1.08).

Bark extract: *Escherichia coli* (12.69 ± 1.63) >*Bacillus cereus* (11.5 ± 2.27) >*Staphylococcus aureus* (10.15 ± 1.46) >*Yersinia pestis* (9.75 ± 0.48).

Root extract: *Staphylococcus aureus* (13.31 ± 3.35) >*Bacillus cereus* (11.22 ± 1.43) >*Escherichia coli* (11.15 ± 0.69) >*Yersinia pestis* (10.11 ± 0.43).

The highest antibacterial activity was observed in the fruit extract against *Bacillus cereus* (15.96 ± 2.01 mm) and the lowest activity was shown by root extract against *Escherichia coli* (11.12 ± 0.49 mm). However, most of the extracts showed no inhibitory activity against *Salmonella enterica*. Except for leaf extract, all the extracts exhibited considerable inhibitory activity against *Candida albicans* (12.0 - 14.33 mm). Fruit extract exhibited strong antimicrobial activities against broad-spectrum bacteria and fungi. The MIC values of all the extracts (leaf, fruit, bark and root) are furnished in Table 4.29. Fig. 4.22 represent the 96-well plates of MIC test result. The extracts displayed MIC values in the range of ≥1000 – 625 µg/ml. The majority of the extracts had similar MIC values of 1250 µg/ml, whereas root and fruit extract demonstrated significant MIC values of 625 µg/ml against *Escherichia coli* and *Bacillus cereus*, respectively. All the *C. latipes* extracts showed significant antimicrobial activities against the tested bacteria and fungi. The strong antimicrobial activities shown by the fruit extract against broad-spectrum bacteria and fungi can be attributed to the elevated concentrations of cis-Vaccenic acid (51.74 %), n-

Hexadecanoic acid (9.12 %), and pyrazol-phenol (14.13 %) in the extract (Sun et al., 2021). The results of antimicrobial activity of *C. latipes* are consistent with previous findings on the same genus (Sah et al., 2011; Haraoui et al., 2019). For example, *C. latipes* extracts showed inhibition ranging from 10-15 mm, equivalent to the ZOI reported in the extracts from *C. medica* Linn (10 - 13 mm) (Sah et al., 2011). Similarly, the antibacterial efficacy of *C. latipes* extracts is comparable to that exhibited by *C. aurantium* and *C. maxima* (11 - 12 mm) (Haraoui et al., 2019). The *C. latipes* extract exhibited antifungal activity (12 - 14 mm) against the *Candida albicans*. However, as outlined in previous investigations, no similar activities were detected in the related species, including *C. medica* Linn, *C. sinensii*, and *C. aurantium* (Haraoui et al., 2019). The extracts showed a MIC values in the range ≥1250 – 625 µg/ml. These results are in close proximity with the results of *C. medica* Linn extracts as reported in one of the previous investigations (Sah et al., 2011). For instance, root, leaf, bark, and fruit extract of *C. medica* Linn exhibited MIC values of 500 µg/ml and 10000 µg/ml, respectively.

The extracts derived from these rare medicinal plants display varying degree of antibacterial efficacy. Majority of the extracts showed negligible efficacy towards gram-negative bacteria. The lesser efficacy against gram-negative bacteria can be ascribed to their greater tolerance to specific antibiotics, which is due to the existence of an outer membrane that functions as a barrier to permeability (Rhetso et al., 2020). These variations may also arise due to differences in chemical composition and the mechanisms of action associated with their bioactive components (Barbieri et al., 2017). Moreover, the efficacy of the extracts is dependent not only upon the presence of high-value bioactive compounds but also on their concentration and potential interactions with cellular components (Dzotam et al., 2015).

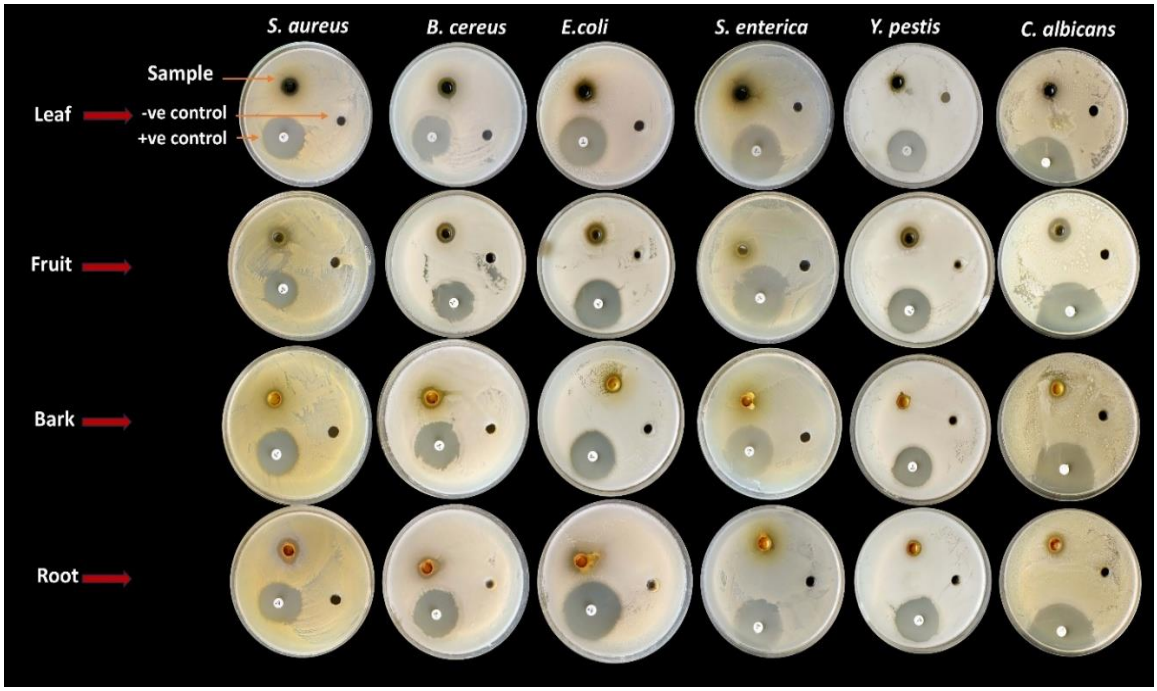

Fig. 4.21. Antimicrobial activities of the *C. latipes* extracts showing zone of inhibition

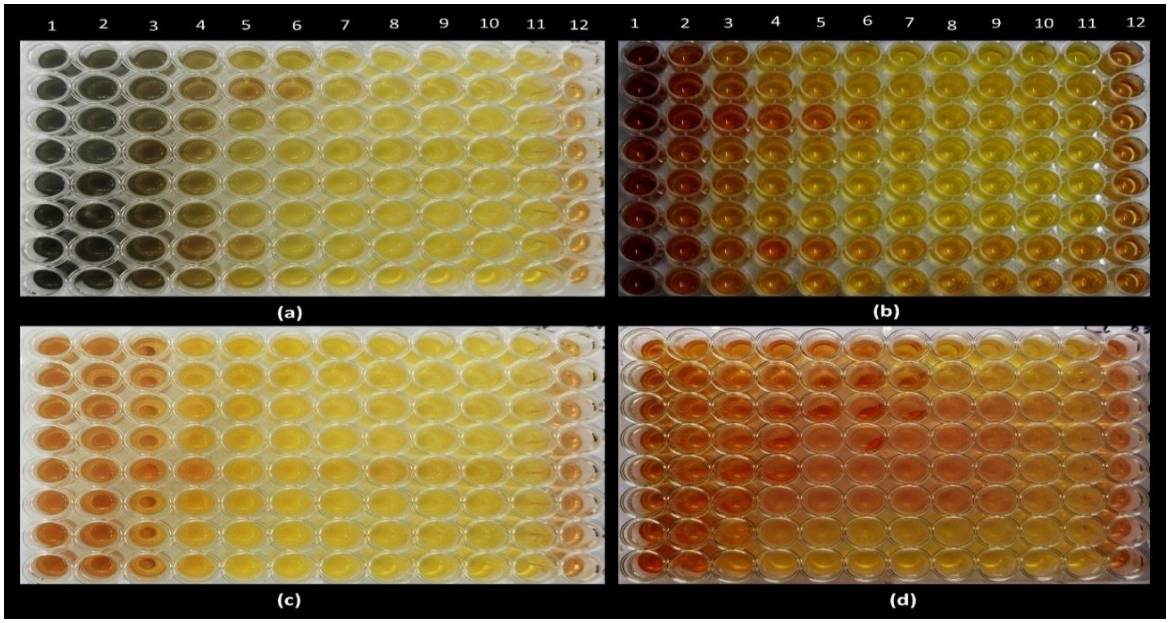

Fig. 4.22. 96-well plates of MIC assessment of *C. latipes* extracts (a) leaf, (b) fruit, (c) bark and (d) root. Column 1-10 contains test drug, bacteria and media, column 11 serves as positive control (bacteria without drug sample), column 12 serve as negative control (only media)

Table 4.28. Results of the antimicrobial activity assay of *C. latipes* extracts

	Diameter of the zone of inhibition (mm)					
	S. aureus	***B. cereus***	***E. coli***	***S. enterica***	***Y. pestis***	***C. albicans***
Leaf	11.43±2.01*	12.62±2.47	11.89±2.62	NI**	NI	NI
Fruit	13.33±1.69	15.96±2.84	14.64±0.44	11.31±1.56	11.95±1.08	14.73±0.86
Bark	10.15±1.46	11.5±2.27	12.69±1.63	NI**	9.75±0.48	12.36±1.28
Root	13.31±3.35	11.22±1.43	11.15±0.69	NI**	10.11±0.43	12.72±1.91
Positive control	26.98±0.81	25.06±2.41	29.73±0.01	31.05±2.35	32.62±2.74	34.29±6.11
Negative Control	NI	NI	NI	NI	NI	NI

*mean±SD, **NI is No inhibition zone

Table 4.29. Minimum inhibitory concentrations (MIC) of the *C. latipes* extracts against tested microorganisms

	MIC (µg/ ml)					
	S. aureus	***E. coli***	***B. cereus***	***S. enterica***	***Y. pestis***	***C. albicans***
Leaf	> 1250	> 1250	> 1250	ND*	ND	ND
Fruit	1250	1250	625	> 1250	>1250	1250
Bark	1250	1250	1250	ND	>1250	> 1250
Root	1250	625	1250	ND	1250	1250
Positive control	6.25	3.125	6.25	6.25	3.125	25

ND* is Not Detected

For instance, the antibacterial effect of tannins is due to their capacity to interact with proteins present in the bacterial cell wall, resulting in the formation of insoluble complexes that impair bacterial function. These interactions can denature and precipitate proteins, disrupt cellular metabolism, and damage cell membranes (Maisetta et al., 2019). Saponins, owing to their detergent-like characteristics, can induce the leakage of proteins and specific enzymes from cells

(Maisetta et al., 2019). The antibacterial efficacy of alkaloids arises from their capacity to interchelate with the DNA of both gram-positive and gram-negative bacteria, thereby disrupting cell division (Yan et al., 2021). In contrast, the activity of flavonoids is attributed to their ability to bind with intracellular and soluble proteins, as well as with bacterial cell walls. Steroids exhibit antibacterial activity by interacting with membrane lipids, leading to membrane leakage (Górniak et al., 2019). The issue of antimicrobial resistance is escalating over time, and the future efficacy of antimicrobial agents such as chemical antibiotics is uncertain. Consequently, measures must be implemented to address this issue and to promote the incorporation of medicinal plants as an alternative strategy to manage the menace of antimicrobial resistance.

4.4.4. Cytotoxic Activity on Colon Cancer Cell line

The prevalence of colon cancer has been steadily rising due to recent lifestyle modifications, including a diet low in vegetables and fruits, insufficient physical activity, excessive alcohol consumption, and being exposed to hazardous chemicals (Haggar & Boushey, 2009). Despite regular examinations and early detection reducing mortality rates, colon cancer continues to claim a significant number of lives worldwide every year. Consequently, it is imperative to identify novel therapeutics or potential drugs that selectively target cancer cells while sparing normal cells (Ogbole et al., 2017). Therefore, the present study additionally investigates the anticancer efficacy of *G. simonsii, V. odoratissimum var. odoratissimum,* and *C. latipes* against the human colon cancer cell line HT-29. The antiproliferative activity of the selected plant extracts were assessed using the MTT assay method. It is to be mentioned here in specifically that these plant species have been used by the indigenous Garo populace of Meghalaya for treatment of major gastrointestinal complications (even cancer) for centuries. This furnishes the clear-cut rationale for anticancer bioassay using these plant extracts.

The different parts of *G. simonsii* plant, including leaf, fruit, and bark, exhibit substantial antioxidant properties. The leaf extract with a low IC_{50} value

(DPPH) of 437.38 ± 13.11 µg/ml exhibited the highest free radical scavenging activity. Considering the fact that, elevated free radical scavenging activity is associated with enhanced toxicity to cancer cells, leaf extract was consequently chosen for evaluation (Al-Rimawi et al., 2024). The antiproliferative activity of leaf extract at different concentrations is depicted in Fig. 4.23. The results indicated that leaf extract inhibited colon cancer cells' viability (HT-29) in a dose-dependent manner. A significant inhibitory effect was seen at dosages ranging 10-250 µg/ml with an IC_{50} value of 8.82 µg/ml. The antiproliferative activity of leaf extract on HT-29 can be attributed to the presence of potential phytocompounds, including hexadecenoic acid, cis-vaccenic acid, β-sitosterol, Spathulenol, Methoxy-4-vinylphenol, stigmasterol, Cholest-4-en-3-one, etc., (Table 4.7) (do Nascimento et al., 2018; Luo et al., 2021). Previous studies on the related species also endorse the antiproliferative efficacy against the HT-29 cell line. For example, *G. giganteus*, *G. undulatus*, and *G. amuyon* showed considerable toxicity against HT-29 cell lines (Choo et al., 2014). In this regard, our experimental findings correspond with previous studies on related species of the genus *Goniothalamus* (Choo et al., 2014; Luo et al., 2021).

The leaf extract of *V. odoratissimum var. odoratissimum*, demonstrated substantial phenolic and flavonoid content. Furthermore, it exhibited remarkable free radical scavenging activities in comparison to other plant parts of the species. Consequently, leaf extract was chosen for the antiproliferative activity assessment. The results of the antiproliferative activity against the HT-29 cell line is illustrated in Fig. 4.24. The extract exhibited dose-dependent antiproliferative activity, with an IC_{50} value of approximately 214.85 µg/ml. The cytotoxic activity of leaf extract against the colon cancer cell line (HT-29) is attributable to the presence of various phytocompounds, including β-sitosterol (Nandi et al., 2024), neophytadiene (Gonzalez-Rivera et al., 2023), Ergosta-5,22-dien-3-ol (Hazra et al., 2023), ß-Amyrin (Alam et al., 2023), and Lup-20(29)-en-3-ol (Liu et al., 2021). The findings of this study align with the results of previous studies on related species within the same genus (*Viburnum*). For example, the ethanol extract of *V. opulus*

berries reduces the proliferation of HT-29 cell lines at an IC_{50} of 390 µg/ml (Dienaite et al., 2020). Similarly, the phenolic compounds extracted from *V. opulus* berries exhibited antiproliferative activity against the HT-29 cell line at a concentration of 400-500 µg/ml (Chojnacka et al., 2019).

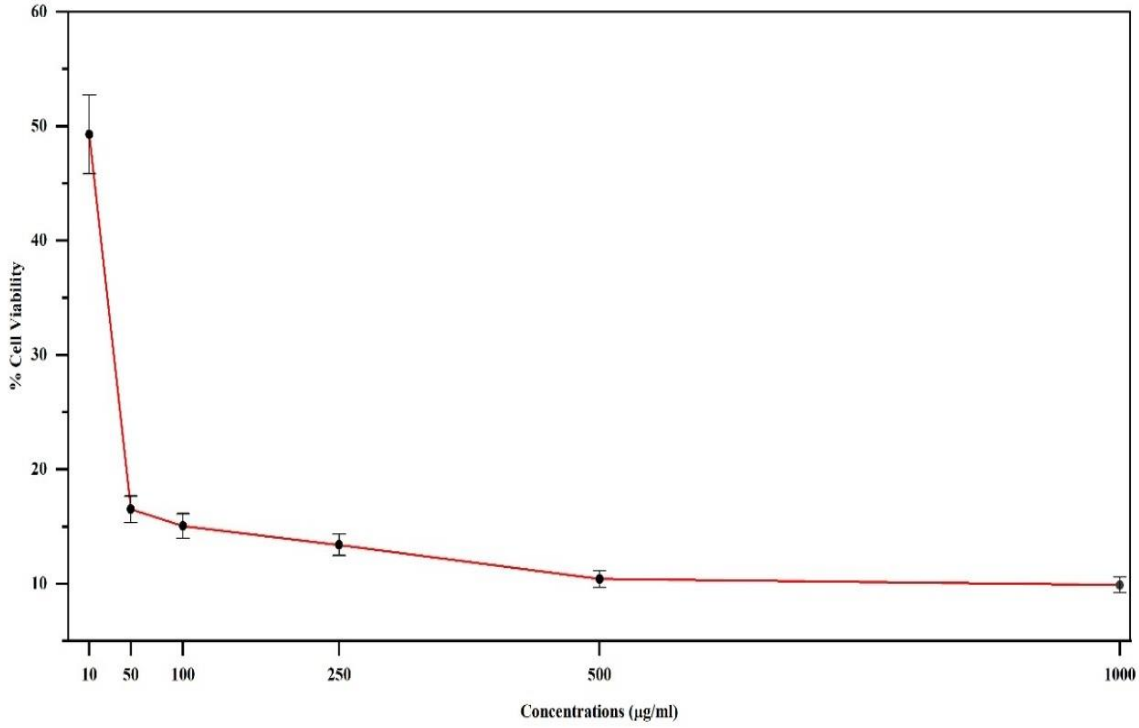

Fig. 4.23. Antiproliferative activity of *G. simonsii* leaf extract on the HT-29 cell line

The various parts of *C. latipes* exhibited varying levels of phytoconstituents and antioxidant activities. However, the bark extract demonstrated significant phytocompounds with considerable concentrations of phenolics and flavonoids in comparison to the other parts (Table 4.22). Additionally, it also demonstrated considerable antioxidant activity (Table 4.23). Consequently, bark extract was selected for antiproliferative activity assay on the HT-29 cell line. The results revealed that bark extract suppressed the proliferation of colon cancer cell (HT-29) in a dose-dependent manner (Fig. 4.25). A notable inhibitory impact was detected at doses from 50 - 1000 µg/ml, with an IC_{50} value of 52.39 µg/ml. The antiproliferative activity of *C. latipes* extract on HT-29 can be correlated with the

presence of potent phytocompounds, including hexadecenoic acid, cis-vaccenic acid and β-sitosterol in significant quantities (Awad et al., 1996). The findings of the present investigation correspond to the findings of previous studies on related species. For instance, Rossi et al. (2020) revealed that *C. deliciosa* oils significantly inhibit HT-29 cells, with an IC_{50} value of 110 µg/ml. Weimer et al. (2021) similarly observed that *C. aurantifolia* decreased the viability of HT-29 cells by 50 % at a dosage of 57.9 µg/ml.

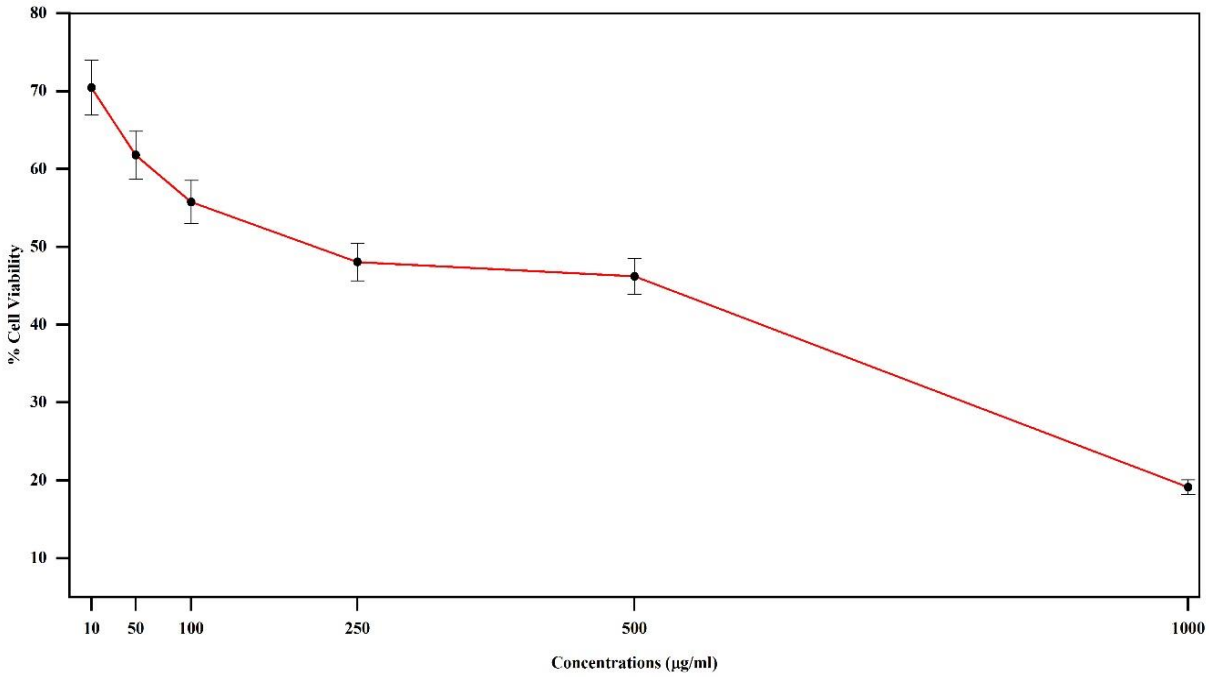

Fig. 4.24. Antiproliferative activity of *V. odoratissimum var. odoratissimum* leaf extract on the HT-29 cell line

These observations represent pre-clinical data and require subsequent clinical studies. The antioxidant activity is advantageous in cancer therapies by aiding in the stabilization of free radicals produced by the related inflammatory processes.

The present study did not provide a comprehensive explanation of the mechanism inducing antiproliferative activity on the colon cancer cell line; however, this activity can be associated with the following mechanism as discussed below.

Botanical derivatives have demonstrated effects on numerous oncological pathways that control apoptosis, cell cycle progression, proliferation, angiogenesis, metabolism, and the epigenetic regulation of gene expression (Akhtar et al., 2020). Apoptosis is a precisely regulated mechanism of programmed cell death that eliminates damaged or abnormal cells (Evan and Vousden, 2001). The prevalent apoptotic pathways identified in literature encompass: p53, caspase, and MAPK-modulated pathways, cell cycle arrest, reprogrammed metabolism, epigenetics (histone and DNA modifications), and angiogenesis inhibition.

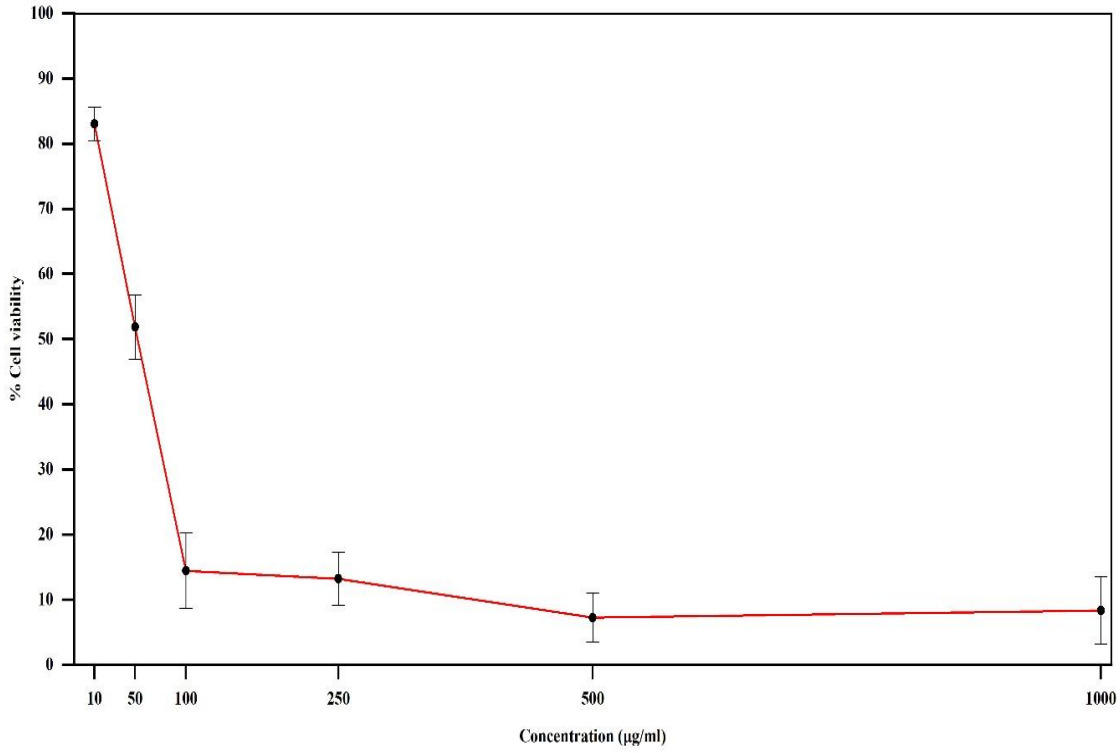

Fig. 4.25. Antiproliferative activity of *C. latipes* bark extract on the HT-29 cell line

Chemical compounds obtained from medicinal plants, including xanthorrhizol, quercetin, formononetin, decursin, n-butylidenephthalide, and baicalein, have been shown to induce apoptosis through the modulation of signaling pathways in cancer cells (Banerjee et al., 2023). For example, Xanthorrhizol, a sesquiterpenoid compound from the rhizome of *Curcuma xanthorrhiza*, has been reported to induce apoptosis in liver cancer (HepG2) (Handayani et al., 2007),

cervical cancer (HeLa), breast cancer cells (MCF-7), and other cancer cells via pathways involving upregulated p53, the pro-apoptotic protein Bax, and the mitochondrial release of cytochrome c (Oon et al., 2015). Quercetin inhibited HeLa cell proliferation by arresting cell cycle progression and inducing apoptosis related to p53 and BCL-2 (Priyadarsini et al., 2010). Moreover, it elevated the activity of caspase-3 and caspase-9, resulting in caspase-dependent extrinsic apoptosis (Calgarotto et al., 2018). A deeper comprehension of the molecular mechanisms by which plant derivatives modulate signaling cascades in cancer cells will facilitate the discovery of novel cancer therapeutics and enhance targeted therapies.

4.5. Product Synthesis and their Characterizations

4.5.1. Characterizations of the Microcapsules

The results of particle characterization are presented in Table. 4.30. The moisture content of the microcapsule was 5.05 ± 0.21 %. The moisture content values are similar to those reported in previous studies, including the microencapsulation of oregano essential oil through spray drying with gum arabic, maltodextrin, and modified starch (1.30 – 3.65 %) (Alvarenga Botrel et al., 2012) and the microencapsulation of fingered citron extract that use maltodextrin via spray drying (4.50 – 5.70 %) (Mahdi et al., 2020). The relationship among the wall materials and the core material profoundly influenced the moisture content of the samples. The optimal stability of instant tea powder is achieved when the moisture content is maintained within a range of 3 % to 5 % (Vardin & Yasar, 2012).

The wettability of microcapsules is a critical physical property pertinent to the reconstitution of powder (Bae & Lee, 2008), and it is affected directly by the molecular interactions within the two phases (Flores-Mancha et al., 2020). In the current study, the duration required for the powder to achieve complete wetting was 236 ± 4.58 s (Table 4.30). The choice of wall material substantially influenced this property (the wettability of the microencapsulated powder under investigation was also in close proximity with the findings from previous studies). For instance, the wettability of rosemary essential oil microencapsulated with

maltodextrin and gum arabic ranged 84 s - 307 s (de Barros Fernandes et al., 2014). Mahdi et al. (2020) also demonstrated that microcapsules derived from fingered citron extract, combined with maltodextrin, modified starch, and gum arabic, required 12.76 - 14.44 min to get saturated. Nonetheless, the reduced wetting time of the powder under investigation can be attributed to the rapid dissolution of maltodextrin in water, generally occurring within seconds (Barthold et al., 2019).

Solubility is the final step in particle dissolution and is a critical determinant of the quality of powder often used as a core ingredient in the food industry. Insoluble powder can lead to processing challenges and financial losses (de Barros Fernandes et al., 2014). The solubility value (90.61 ± 0.73 %) of the polyherbal extract microcapsule is presented in Table 4.30. The findings of the present study are consistent with those of previous studies. For example, microcapsules derived from fingered citron extract combined with maltodextrin, modified starch, and gum arabic exhibited solubility ranging from 71.67 - 91.26 % (Mahdi et al., 2020). Similarly, soybean oil extract maltodextrin-based microcapsules and *Crotalaria longirostrata* leaf extract maltodextrin-based microcapsules exhibited solubility levels ranging from 80.32 - 90.90 % and from 82.15 - 91.98 %, respectively (Navarro-Flores et al., 2020; Zhu et al., 2022).

Efficiency is a crucial factor in enhancing an encapsulation procedure, irrespective of the processes and materials involved (de Souza et al., 2018). The encapsulation efficiency of the polyherbal extract microcapsule is 88.18 ± 2.06 %, as shown in the Table. 4.30. Similar findings were observed in previous studies regarding microcapsules containing *Crotalaria longirostrata* leaf extract (78.28 - 89.83 %) (Navarro-Flores et al., 2020) and soybean oil (80.32 - 90.90 %) (Zhu et al., 2022).

Table 4.30. Moisture content, wettability, solubility and encapsulations of microcapsules

Moisture content (% w/w)	Wettability (s)	Solubility (WSI %)	Encapsulation efficiency (% w/w)
5.05±0.21	236±4.58	90.61±0.73	88.18±2.06

4.5.2. Morphology of the microcapsules

The microstructures of the microcapsules derived from the polyherbal extract are illustrated by SEM micrographs as shown in Fig. 4.26. The samples were analyzed using SEM to identify fractures, cracks, or other potential defects that could compromise the integrity of the encapsulated material, as any fracture may result in degradation and oxidation (Mahdi et al., 2020). The SEM micrographs demonstrated that the microcapsules displayed spherical and semi-spherical forms, although the conventional morphology of spray-dried particles was spherical, with an average size of 10 – 100 µm (Fang & Bhandari, 2010). Additionally, the SEM micrograph showed indentations, uneven and creased surfaces, along with minor agglomeration. No fractures were observed in the synthesized microcapsule powder.

The average area of microcapsule powder was 6.92 µm², with a minimum area of 0.0019 µm² and a maximum area of 29.586 µm². The average length was 2.77 µm, with a minimum of 0.423 µm and a maximum of 6.217 µm. Microcapsules exhibited varying sizes, apparently attributable to an increase in inlet temperature within the spray dryer, which expedited the drying rate of the droplets (Álvarez-Henao et al., 2010). The SEM analysis results in this study are in agreement with the findings from the previous studies, which indicated irregularly spherical particles exhibiting numerous shrinkages and surface dents (de Barros Fernandes et al., 2014; Chew et al., 2018; Navarro-Flores et al., 2020; Mahdi et al., 2020). Diverse morphologies and irregular surfaces may be associated with variations in feeding ratio, droplet size, and temperature during the drying process. The contraction of the

particle, coupled with a nascent expansion, may result in alterations in particle size. Furthermore, the surface irregularity of the microcapsules may be advantageous for improved dispersibility and rehydration of the powder (Chew et al., 2018).

4.5.3. Thermogravimetric analysis (TGA)

The thermal stability of the maltodextrin microcapsules obtained from polyherbal extracts was assessed using TG-DTG, with the corresponding plots displayed in Fig. 4.27. The microcapsules exhibited thermal decomposition in three phases. Approximately, 9.16 % of the total mass was lost below 200 °C, which can be attributed to the loss of residual moisture on the microcapsule surfaces and the vaporization of associated molecular water (Jiang et al., 2021). The second stage occurred within 200 and 350 °C, during which the microcapsules demonstrated a mass reduction of 60.76 %. This can be due to the fact that microcapsule structure suffers severe damage during the mass-loss phase as the wall material decomposes, the shell fractures, and bioactive compounds degrade (Yingngam et al., 2019). The DTG curve indicates that the peak degradation of the microcapsules occurred precisely at 313 °C. The third stage occurred between 350 and 575 °C, exhibiting a mass loss of 30.22 %, which can be attributed to the continued decomposition of the wall material, the rapid loss of residual bioactive compounds, and the carbonization of remaining materials (Li et al., 2020). The findings validated that maltodextrin-based microcapsules containing core bioactive compounds from polyherbal extracts exhibited thermal stability below 200 °C, indicating their potential utility in herbal tea or other nutraceutical product formulation.

4.5.4. FTIR analysis

The FTIR spectrum is displayed in Fig. 4.28. The peaks along with their corresponding functional groups of the microcapsules derived from polyherbal extract are provided in Table 4.31. Nine (9) peaks were observed at the wavenumbers of 3406 cm^{-1}, 2932 cm^{-1}, 2101 cm^{-1}, 1641 cm^{-1}, 1414 cm^{-1}, 1152 cm^{-1}, 1022 cm^{-1}, 930 cm^{-1}, and 577 cm^{-1}. Characteristic hydroxyl peaks (O-H stretching and O-H bending) were detected at approximately 3406 cm^{-1} and 1414 cm^{-1}.

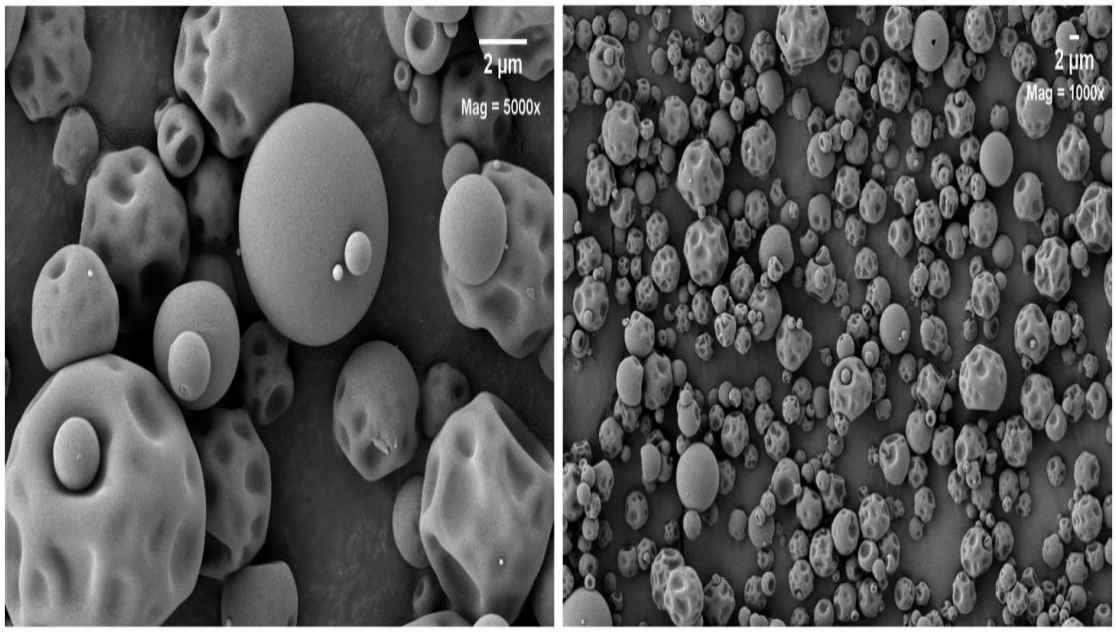

Fig. 4.26. SEM micrographs of the microcapsules derived from polyherbal extracts. Scale and magnification are provided in the micrographs

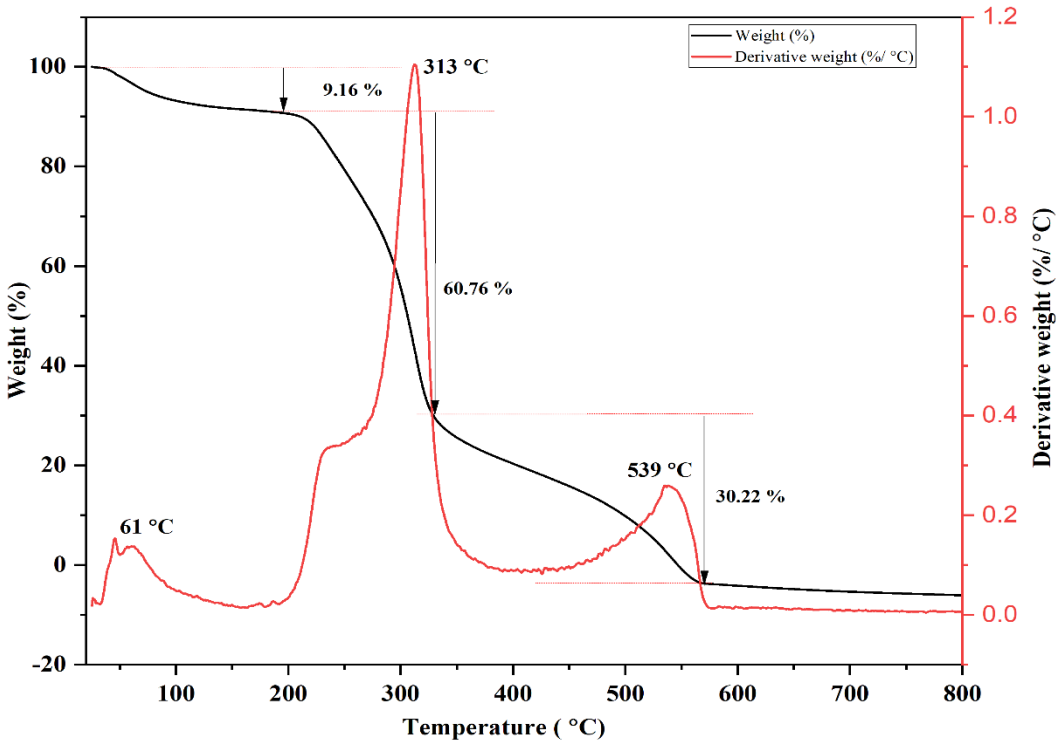

Fig. 4.27. TGA and DTG curves of microcapsules derived from polyherbal extract

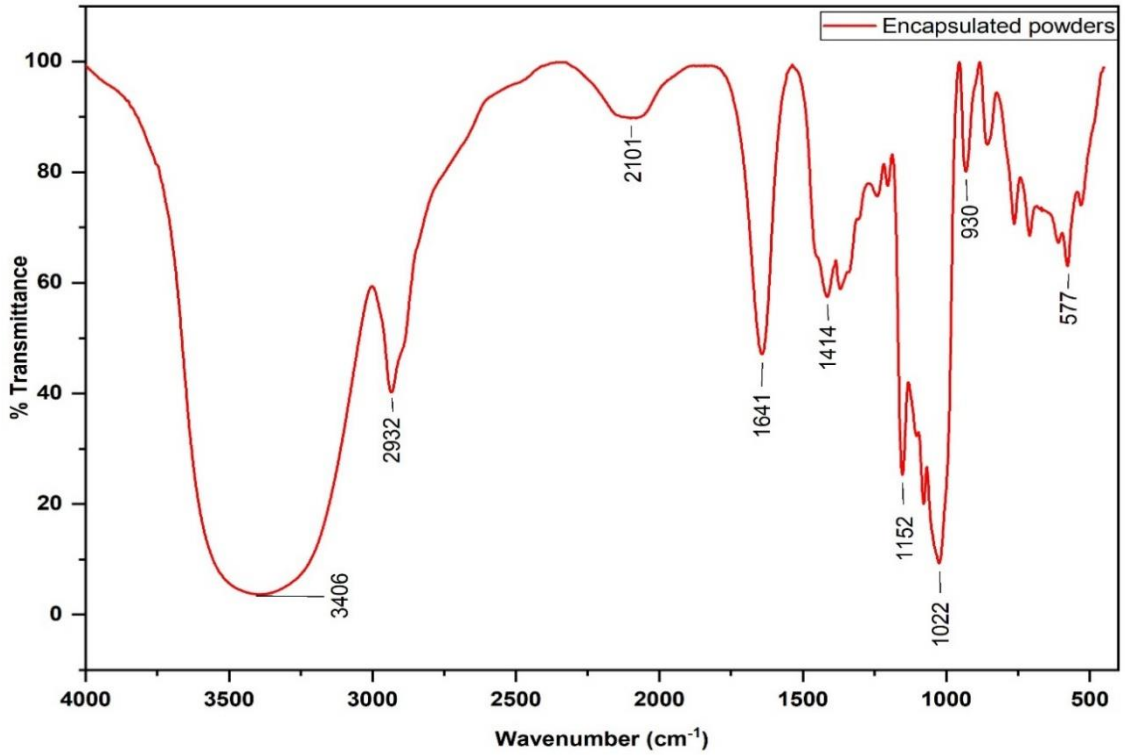

Fig. 4.28. FTIR spectrum of the microcapsule derived from polyherbal extract

Table 4.31. FTIR spectral values and the corresponding functional groups of the microcapsules

Wavenumber (cm^{-1})	Appearance	Group	Compound class
3406	Stretch	O-H	Alcohol
2932	Stretch	C-H	Alkane
2101	Stretch	C≡C	Alkyne
1641	Bending	N-H	Amine
1414	Bending	O-H	Alcohol
1152	Stretch	C-O	Ether (Aliphatic)
1022	Stretch	C-O-H	Alcohol
930	Bending	=C-H	Alkene
577	Stretch	R-Br	Halo compound

Peaks related to the C-H stretching of the carboxylic group were stretching, subsequently indicating the presence of carbonyl or amide group. The peaks at 1152 cm^{-1} and 1022 cm^{-1} correspond to C-O stretching and C-O-H bending, respectively. A peak at 930 cm^{-1} and 577 cm^{-1} corresponds to =C-H bending and R-X stretching, respectively. These results were consistent with the previous investigations (Kang et al., 2019; Mahdi et al., 2020).

4.5.5. GC-MS analysis

A total of 15 compounds were identified in microcapsules derived from the polyherbal extract by GC-MS analysis. The details of identified compounds with their retention time and peak area (%) are furnished in the Table. 4.32. The TIC is depicted in Fig. 4.29. The compound 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl was detected with the highest peak area of 25.92 %, followed by n-Hexadecanoic acid (17.43 %). Other detected compounds include Ergosta-5, 22-dien-3-ol (5.55 %), Melibiose (2.45 %), Imidazole, 2-amino-5-[(2-carboxy) vinyl] (1.20 %), Desulphosinigrin (0.13 %), Caffeine (3.03 %) and Pregn-4-ene-3,20-dione, 17,21-dihydroxy-, bis (O-methyl oxime) (0.27 %). These compounds demonstrated numerous bioactivities, including antioxidant, antimicrobial, anticancer, anti-inflammatory, antiproliferative, neuroprotective, etc. (Aparna et al., 2012; Chen et al., 2021; Krishnappa et al., 2024).

4.5.6. *In vitro* release profile

The *in vitro* digestion of spray-dried polyherbal extract microcapsules was conducted to assess the effectiveness of wall material solids in safeguarding bioactive compounds (core material) from severe gastric conditions (pH 2.2) while simultaneously facilitating the release of contents in the intestine (pH 7.5), thereby ensuring the bio-accessibility of the bioactive compounds. A mixture of non-capsulated polyherbal extracts was utilized as a control to evaluate the digestion profile of micro-capsulated polyherbal extracts. The results are illustrated in Fig. 4.30a and Fig. 4.30b.

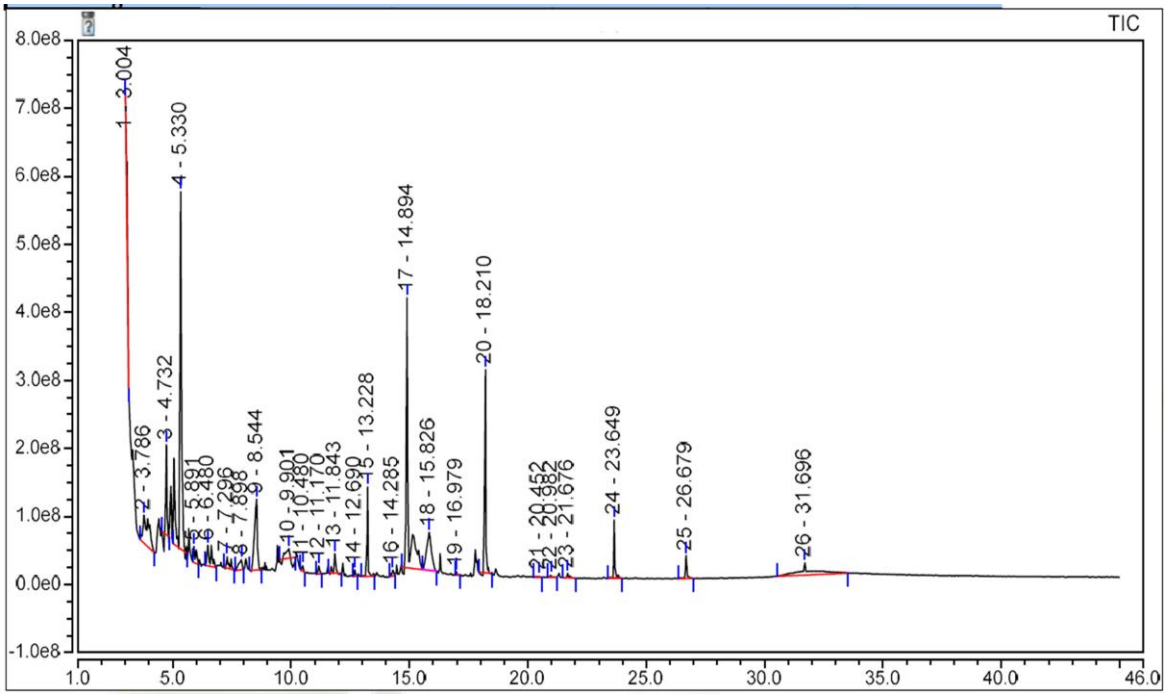

Fig. 4.29. TIC of the microcapsule derived from polyherbal extracts

Table 4.32. Chemical constituents of the microcapsules

RT (min)	Compounds	Peak Area (%)
3.789	Methyl 6-oxoheptanoate	6.39
4.732	Cyclopentane, 1-acetyl-1,2-epoxy	3.13
5.330	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	25.92
6.480	Melibiose	2.45
7.898	Imidazole, 2-amino-5-[(2-carboxy) vinyl]	1.20
8.544	D-Allose	7.60
10.480	Desulphosinigrin	0.13
11.843	Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro	1.15
13.228	Caffeine	3.03
14.894	n-Hexadecanoic acid	17.43
15.826	Lactose	6.74
16.979	Tetraacetyl-d-xylonic nitrile	0.10
18.210	Octadecanoic acid	8.64
21.676	Pregn-4-ene-3,20-dione, 17,21-dihydroxy-, bis(O-methyl oxime)	0.27
31.696	Ergosta-5,22-dien-3-ol, acetate	5.55

In the gastric digestion phase, non-encapsulated extracts exhibited elevated phenolic content (Fig. 4.30a) and DPPH scavenging activity (Fig. 4.30b).

In contrast, microencapsulated powder demonstrated minimal phenolic content and DPPH scavenging activity. This suggests that non-capsulated extracts release the highest levels of bioactive compounds. In contrast, the capsulated powder release minimal bioactive compounds from their core, indicating that the core material is protected from gastric juices. The resistance of microcapsules to gastric digestion may be ascribed to maltodextrin, which forms a thick, insoluble layer that protects the encapsulates from gastric degradation (Yadav et al., 2020). The minimal DPPH scavenging activity observed during the gastric digestion phase of encapsulated powders can be attributed to the highly acidic conditions (Sari et al., 2015). In the intestinal phase, non-capsulated extracts exhibited diminished phenolic content and DPPH scavenging activity, signifying minimal retention of bioactive compounds from the gastric phase. The encapsulated powder demonstrates increased phenolic content (Fig. 4.30a) and DPPH scavenging activity (Fig. 4.30b), signifying optimal retention of bioactive compounds during the gastric phase. The maximum phenolic content and DPPH scavenging activity exhibited by the microcapsules indicate that most bioactive compounds were released during the intestinal phase. The above condition may be ascribed to the action of bile salts and pancreatin in the intestinal fluid, which causes the disintegration of solid wall materials and the release of core substances (Sarkar et al., 2010). Our findings are consistent with those of Meena et al. (2021), who indicated that exposure to the intestinal conditions destabilized the curcumin emulsion, leading to the release of approximately 88 % of curcumin from the encapsulates. Similarly, Zorzenon et al. (2020) formulated *Stevia* extract microcapsules with maltodextrin with highest release of phenolic compounds and antioxidant activity occurring under intestinal conditions.

4.5.7. Storage stability of the microcapsules

The results of variations in phenolic contents encapsulated microparticles and non-capsulated polyherbal extracts (as control) during storage at different temperatures (40 ºC and 20 ºC) for 45 days are illustrated in Fig. 4.31.

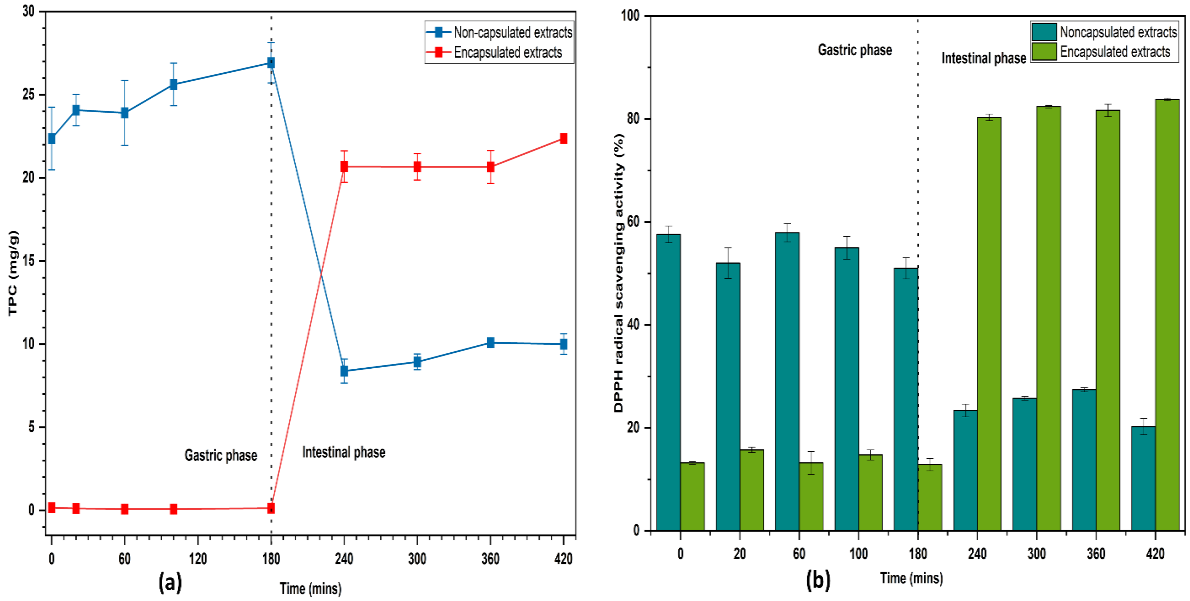

Fig. 4.30. Graph illustrating the release of non-capsulated extract powder and core material of microcapsules at gastric and intestinal phase (a) phenolic content, (b) DPPH radical scavenging activity

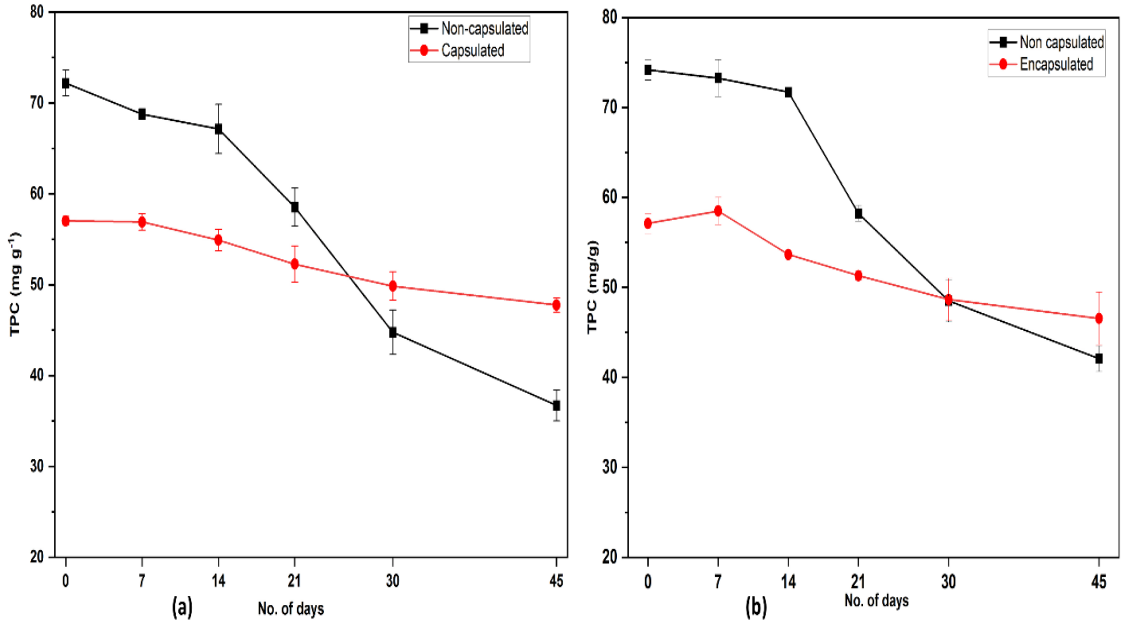

Fig. 4.31. Graph demonstrating the storage stability of non-capsulated extract powder and microcapsules at (a) 45 ºC, (b) 20 ºC

Fig. 4.31a demonstrated that after 45 days of storage at 40 ºC, the phenolic content depletion was 62.99 % for the control and 28.50 % for the microencapsulated powders. This indicates that the non-encapsulated extract powder demonstrates greater degradation than the micro-encapsulated powders. Likewise, Fig. 4.31b indicated that after 45 days of storage at 20 ºC, the control exhibited a 43.26 % reduction in phenolic content, whereas the micro-encapsulated powder demonstrated a 16.43 % reduction. Previous investigation demonstrated a reduction in polyphenols during storage stability assessments at 40 ºC, 25 ºC, and 4 ºC (Zokti et al., 2016). The reduced loss of phenolic compounds in microcapsules relative to the control can be ascribed to the strong protective shell generated by maltodextrin (Zhu et al., 2022). The thermal property studies conducted in the present investigation confirmed that maltodextrin begins to degrade typically between 140 - 180 ºC, with maximum decomposition occurring around 300 ºC.

4.5.8. pH and Total Soluble Solid of the Fortified Herbal Green Tea

The fortified herbal green tea was assessed for pH and total soluble solids content. The pH value of the formulated green tea was 6.37, which is within the standard pH range for green tea (5 - 7) (Tan et al., 2023). A total soluble solid of 0.9 ± 0.14 °Brix was observed for the fortified herbal green tea. This soluble solid content falls within the standard soluble solid contents of green tea (07 - 0.97 °Brix) as reported in a previous study made by Tan et al. (2023). These findings indicate that the synthesized microcapsules do not influence the quality of green tea in terms of pH and soluble solid content.

4.5.9. Cytotoxicity of the Fortified Herbal Green Tea

The current investigation also employed cytotoxicity assessment of the formulated herbal tea using haemolysis activity assay and MTT assay to ascertain their biocompatibility with human red blood cells (RBCs) or prospective toxicity to cancer cells. The results, depicted in graphs (Fig. 4.32), show the percentage of haemolysis induced by different sample concentrations (5, 10, 25, 50 and 100 %) of the sample in comparison to the positive control (Triton-X 100).

Triton-X 100 induced 100 % haemolysis, serving as the positive control for complete haemolysis. The formulated herbal tea displayed haemolysis of 2.17 %, 2.86 %, 4.40 %, 5.11 % and 5.82 % at the concentrations of 5 %, 10 %, 25 %, 50 % and 100 %, showing that the tea sample exhibits minimal haemolysis even at high concentration. The haemolysis percentage remained below 6 % for all the concentrations, indicating its excellent haemocompatibility and endorsing its suitability for human consumption.

The antiproliferative activity of formulated herbal tea on the human colon cancer cell line (HT-29) was assessed using the MTT test. The graph illustrating the antiproliferative activity of formulated tea at various concentrations is provided in Fig. 4.33. The results indicated that herbal tea inhibited the growth of colon cancer cells (HT-29) in a dose-dependent manner. A significant inhibitory effect was seen at dosages ranging 0.5 – 8.0 mg/ml. An approximate 57 % reduction of the viability of the colon cancer cell line was observed at a concentration of 2 mg/ml. Maltodextrin, which is used as a carrier, does not exhibit any antiproliferative activity (Garcia-Lazaro et al., 2020). Hence, the antiproliferative activity of herbal tea on HT-29 can be attributed to the presence of potential phytocompounds, including hexadecenoic acid, Ergosta-5,22-dien-3-ol, Desulphosinigrin, Imidazole, 2-amino-5-[(2-carboxy) vinyl] and Pregn-4-ene-3,20-dione, 17,21-dihydroxy-, bis(O-methyl oxime) in the core material and the presence of epigallocatechin in the green tea (Aparna et al., 2012; Bruce et al., 2021; Ray & Paul, 2022). Our experimental findings are consistent with a previous study conducted by Garcia-Lazaro et al. (2020). They demonstrated that micro-capsulated Yerba mate extract demonstrates antiproliferative effects on murine colon cancer and human colon adenocarcinoma cells.

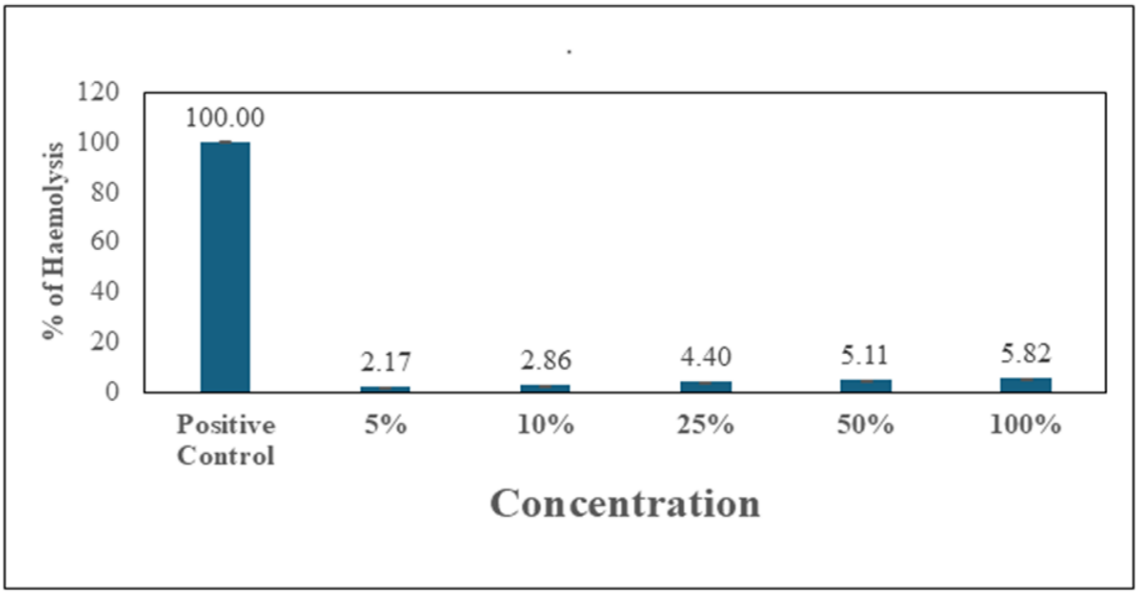

Fig. 4.32. Graph illustrating the haemolytic activities of the fortified herbal green tea at various concentrations

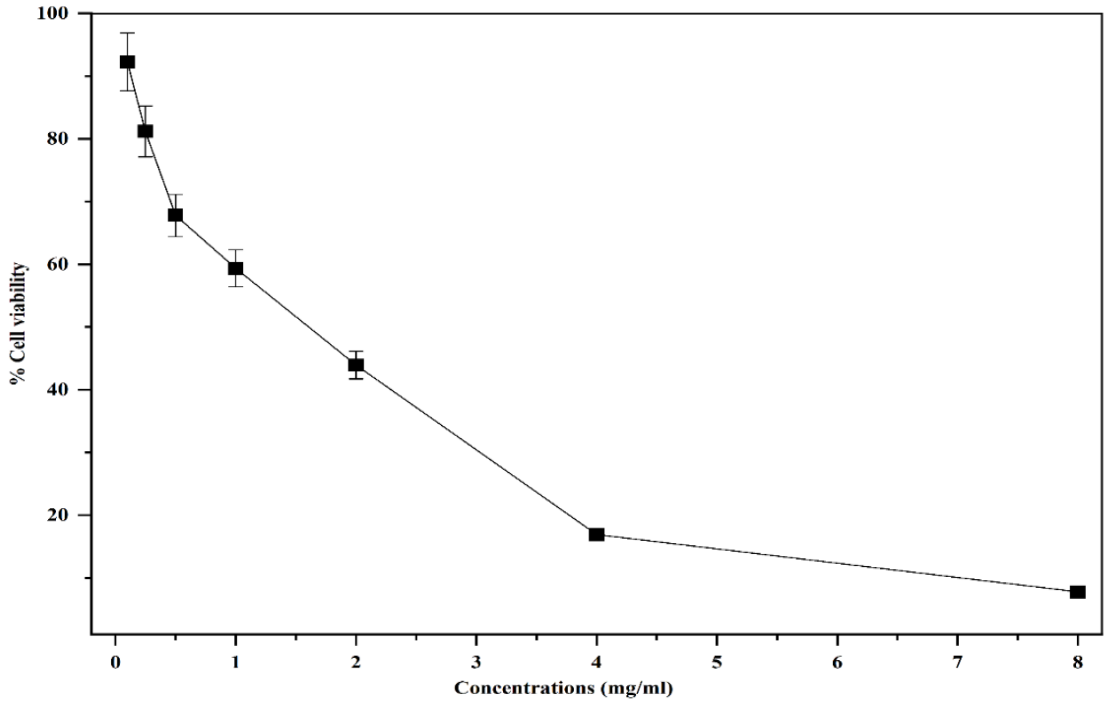

Fig. 4.33. Antiproliferative effect of the fortified herbal green tea on HT-29 colon cancer cell line

4.5.10. Sensory evaluation of the Fortified Herbal Green Tea

The sensory profiles of the formulated herbal tea, based on the mean scores of 12 panellists for all quality attributes (colour, appearance, taste, texture, flavour, and overall acceptability), are depicted in Fig. 4.34. The average scores for the quality attributes were 7.08 for colour, 6.92 for appearance, 6.92 for texture, 8.17 for taste, 5.92 for flavour, and 8.08 for overall acceptability. The prepared herbal tea achieved an overall acceptability score of 8.08, indicating it resides at the favourable end of the scale, implying a significant degree of preference and an excellent chance of consumer acceptance (Everitt, 2009).

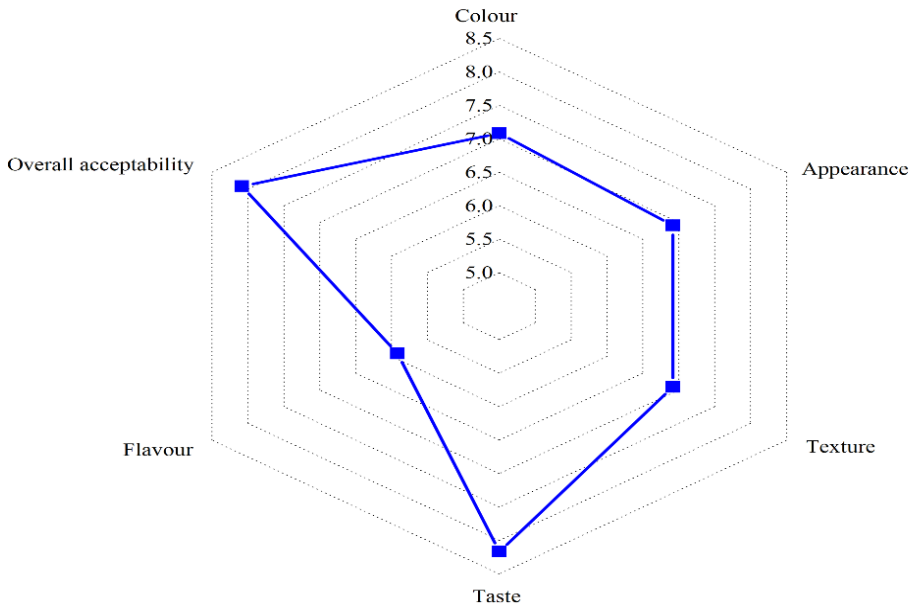

Fig. 4.34. Radar chart displaying sensory profiles of the fortified herbal green tea

4.5.11. Physicochemical Analysis of the Ready-to-Consume (RTC) Juice

The physicochemical analysis of ready-to-consume (RTC) juice is essential for assessing its quality, nutritional value, and authenticity. It aids in evaluating parameters such as total soluble solids, acidity, moisture, pH, and the presence of significant compounds like phenols and sugars, which directly influence

flavour, shelf stability, and health advantages. This analysis is essential for guaranteeing consumer safety and satisfaction, as well as for preserving the authenticity of juice products in the marketplace (Kaddumukasa et al., 2017). Consequently, the RTC juice formulated in the present study was evaluated for pH, acidity, soluble solid contents, moisture and ash content.

The results of the physico-chemical analysis of the formulated pomelo based RTC juice enriched with microencapsulated polyherbal extracts is furnished in Table 4.33. The juice exhibited a pH value of 3.03 ± 0.05, which falls within the optimal range of 2.5 to 4.0 as reported by Reddy et al. (2016). This acidic pH is favorable for inhibiting the growth of spoilage microorganisms, thereby enhancing the microbial stability and shelf life of the product. Titratable acidity, an important determinant of taste, preservation, and overall stability (Sami et al., 2021), was recorded at 0.34 ± 0.13 % (w/v). This value is congruent with the optimal range of 0.3 - 1.4 %, which ensures a balance between tartness and palatability while contributing to product longevity (Hariharan & Mahendran, 2016). Total soluble solids (TSS), measured at 10.26 ± 1.14 °Brix, serves as a key indicator of sweetness and flavor profile. According to Salehi (2020), higher TSS levels correlate with increased sugar content, influencing both the mouthfeel and overall sensory appeal of the juice. The detected value corresponds to a moderately sweet taste, in line with Food and Agriculture Organization standards (FAO, 2025). Furthermore, the moisture content of the juice was 84 ± 2.09 %, which is within the ideal range of 80 – 90 % recommended for RTC juices. This moisture level contributes to desirable textural attributes and ensures product stability and acceptability (FAO, 2025).

All the physico-chemical parameters of the formulated pomelo-based RTC juice enriched with microencapsulated polyherbal extracts are within the recommended ranges, ensuring desirable taste, microbial stability, and extended shelf life. These findings confirm the suitability of the formulation for safe consumption and commercial viability.

Table 4.33. Results of the physicochemical analysis of the formulated RTC juice

Physicochemical properties	Result
pH	3.03±0.05
Tithable acidity (% w/v)	0.34±0.13
Total soluble solid (°Brix)	10.26±1.14
Moisture content (% w/v)	84±2.09
Ash content (% w/v)	0.49±0.07

4.5.12. Nutritional Profiles of the RTC Juice

Nutritional profiling of formulated RTC juices plays a crucial role in assessing the quality, safety, and health benefits of the product. With increasing consumer awareness and demand for healthier food options, it is crucial for manufacturers to ensure that their products are nutritionally balanced, safe, and comply with regulatory standards. Nutritional profiling provides detailed information about the composition of essential nutrients, such as carbohydrates, proteins, fats, vitamins, minerals, and bioactive compounds like antioxidants, making it an indispensable aspect of product development and quality assurance (Maillot et al., 2008).

The results of the nutritional profiling of the formulated RTC juice are presented in Table 4.34. The energy content of the formulated juice was 64.2 Kcal/100g, indicating a relatively low caloric density. Foods with lower energy density are generally regarded as more favourable from a nutritional standpoint, as they allow for greater consumption volume with reduced caloric intake (FAO, 2025). Such products are particularly beneficial in promoting satiety while supporting dietary interventions aimed at weight management and overall health. The low-calorie profile of the juice enhances its appeal as a functional beverage, aligning with consumer preferences for nutrient-rich formulations (Large et al., 2020). The nutrient content of the formulated juice is as follows: carbohydrates (31.5 mg/100g), proteins (10.4 mg/100g), free fatty acids (14.2 mg/100g), and vitamin C (2.1 mg/100g). The

vitamin C content is relatively low compared to the recommended daily intake of 75 – 90 mg for adults (López-Pastor et al., 2020). The juice also comprises of trace amounts of key minerals, such as calcium (0.87 mg/100g), potassium (0.35 mg/100g), and sodium (0.01 mg/100g). While the levels of calcium and potassium is below the recommended dietary allowance (RDA), the notably low sodium content is a favourable attribute, particularly for individuals seeking to limit sodium consumption due to its association with hypertension and related health risks (Schaffer et al., 2022).

Table 4.34. Nutritional profiles of the formulated RTC juice

Test Parameters	Unit	Result
Calorie	Kcal/100g	64.2
Carbohydrate	mg/100g	31.5
Protein	mg/100g	10.4
Free fatty acids	mg/100g	14.2
Calcium	mg/100g	0.87
Sodium	mg/100g	0.01
Potassium	mg/100g	0.35
Vitamin C	mg/100g	2.1

4.5.13. GC-MS analysis of the RTC Juice

GC-MS analysis of the formulated pomelo-based RTC juice enriched with microencapsulated polyherbal extracts revealed the presence of 15 bioactive compounds. The identified compounds with their retention time and peak area (%) are furnished in the Table 4.35. The TIC is depicted in Fig. 4.35. The major constituent was 5-Hydroxymethylfurfural (50.96 %), followed by Hydrazinecarboxamide, 2-(2-methylcyclohexylidene) (14.75 %) and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (8.90 %). Other key compounds that are detected includes D-Allose (3.23 %), 2,5-Furandione, dihydro-3-methylene (4.03 %), Cyclopentane, 1-acetyl-1,2-epoxy (7.51 %), n-Hexadecanoic acid (0.41 %), caffeine (0.39 %), and Ergosta-5,22-dien-3-ol, acetate, (3ß,22E) (0.10 %).

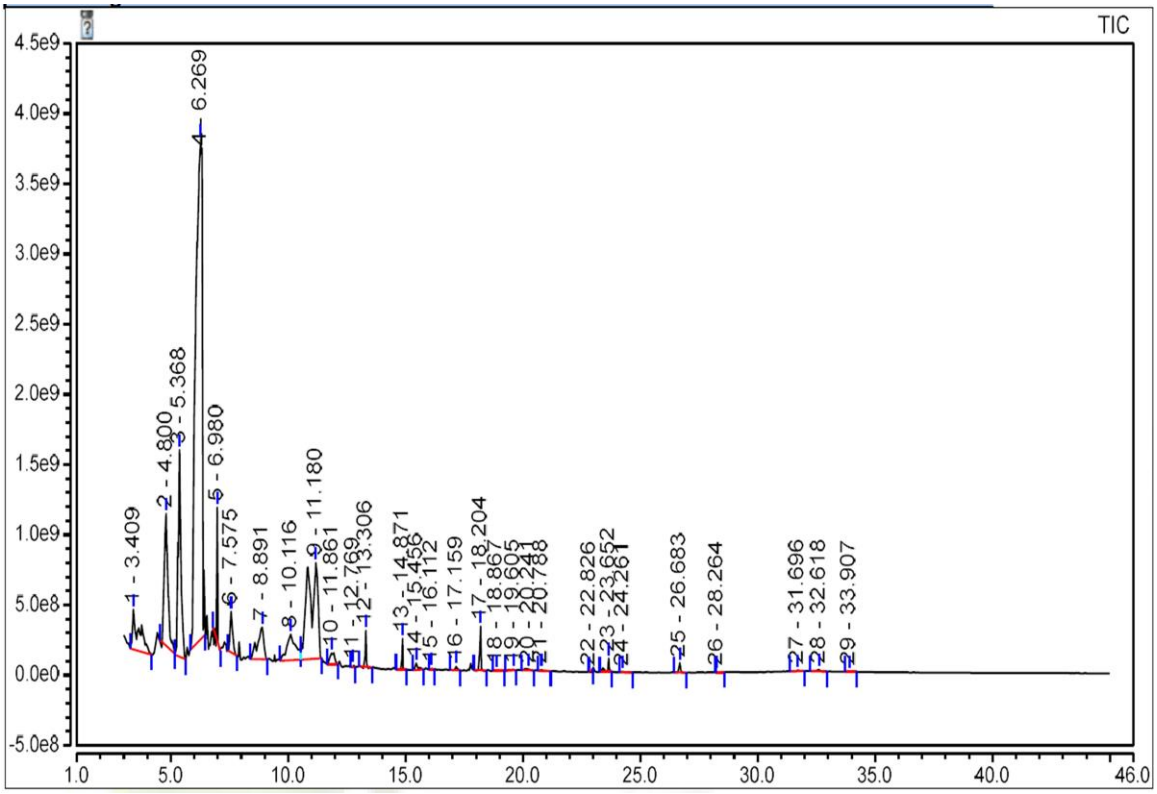

Fig. 4.35. TIC of the formulated RTC juice enriched with microencapsulated polyherbal extracts

Table 4.35. Chemical constituents of the formulated RTC juice

R.T. (min)	Name of the compound	Peak area (%)
3.409	2,5-Furandione, dihydro-3-methylene	4.03
4.800	Cyclopentane, 1-acetyl-1,2-epoxy	7.51
5.368	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	8.90
6.269	5-Hydroxymethylfurfural	50.96
8.891	D-Allose	3.23
10.116	d-Glycero-d-ido-heptose	3.60
11.180	Hydrazinecarboxamide, 2-(2-methylcyclohexylidene)	14.75
11.861	7-Methyl-Z-tetradecen-1-ol acetate	0.81
13.306	Caffeine	0.39

14.871	n-Hexadecanoic acid	0.41
16.112	12-Methyl-E,E-2,13-octadecadien-1-ol	0.50
17.159	1-Heptatriacotanol	0.12
26.683	Octadecanoic acid	0.14
28.264	Ethyl iso-allocholate	0.2
32.618	Ergosta-5,22-dien-3-ol, acetate, (3ß,22E)	0.10

Several identified compounds such as 5-Hydroxymethylfurfural, D-Allose, n-Hexadecanoic acid, 1-Heptatriacotanol and Ethyl iso-allocholate are known for diverse pharmacological activities such as antioxidant, anti-inflammatory, anticancer, neuroprotective effects, etc. For instance, 5-Hydroxymethylfurfural was reported to have antioxidant, anti-inflammatory, anti-hypoxic, and anti-sickling activities. It is potentially effective in treating conditions like sickle cell disease, and cognitive disorders (Pagare et al., 2024). Similarly, D-Allose, a rare sugar and C-3 epimer of D-glucose, exhibits a range of pharmacological properties (anti-cancer, anti-inflammatory, and anti-oxidative). It is extensively researched for its potential in treating conditions like hypertension and ischemia-reperfusion injury (Akumwami et al., 2024). 1-Heptatriacotanol is a long-chain fatty alcohol with several reported pharmacological properties. It exhibits antioxidant, antimicrobial, and wound healing effects. Additionally, it is also investigated for potential anti-hypercholesterolemic, anticancer, and neuroactive properties (Hadi et al., 2016).

These findings highlight the potential therapeutic value of the formulated RTC juice, reinforcing its functional and health-promoting properties.

4.5.14. Antioxidant activity of the RTC Juice

Antioxidants play a pivotal role in fruit juices, contributing not only to their nutritional value but also enhancing their sensory qualities and stability during storage. Oxygen exposure during processing and storage often leads to the oxidation of pigments, vitamins, and flavour compounds. The presence of antioxidants helps mitigate this degradation, thereby preserving the colour, aroma, and overall taste of the juice. Overall, antioxidants significantly enhance product quality, support

consumer health, and increase commercial viability (Alim et al., 2023). In light of this, the formulated ready-to-consume (RTC) juice was assessed for its antioxidant potential, focusing on total phenolic content (TPC) and DPPH free radical scavenging capacity. The results are presented in Table 4.36.

The formulated juice exhibited a TPC of 90.36 ± 1.71 mg/100 ml and demonstrated strong antioxidant activity with an IC_{50} value of 18.46 ± 2.51 µl/ml. These findings are consistent with previous studies on fruit-based RTC juices (Beh et al., 2012; Kim et al., 2017). For instance, Stella et al. (2011) reported TPC values for RTC orange juices ranging from 18.7 to 54.2 mg/100 ml, while Kim et al. (2017) observed TPC values between 47.1 and 326.8 mg/100 ml and IC_{50} values ranging from 0.27 to 3.81 mg/ml in RTC grape juices.

Table 4.36. Phenolic content and free radical scavenging activity of formulated RTC juice

Sample	TPC (mg GAE/ 100ml)	DPPH (IC_{50} - µl/ml)
RTC Juice	90.36 ± 1.71	18.46 ± 2.51

4.5.15. Cytotoxicity of the RTC Juice

The assessment of cytotoxicity is a fundamental step in evaluating the safety and biocompatibility of any food or nutraceutical product, including ready-to-consume (RTC) juices enriched with bioactive compounds. Cytotoxicity tests provide critical insights into the potential toxic effects of the juice or its constituents on living cells, thereby ensuring its safe consumption for human use (da Silva et al., 2017).

The cytotoxicity assessment of the formulated RTC juice was carried out using haemolysis activity assay and MTT assay to ascertain their biocompatibility with human red blood cells (RBCs) or prospective toxicity to cancer cells. The results, depicted in the graphs (Fig. 4.36), show the percentage of haemolysis induced by different sample concentrations (5, 10, 25, 50 and 100 %) of

the sample in comparison to the positive control (Triton-X 100). Triton-X 100 induced 100 % haemolysis, serving as the positive control for complete haemolysis. The formulated RTC juice displayed haemolysis activity below 10 % at all the concentrations, showing that the juice sample exhibits minimal haemolysis even at high concentration. The haemolysis percentage remained below 10 % for all the concentrations, indicating its excellent haemocompatibility and endorsing its suitability for human consumption (Guo et al., 2021).

The primary ingredients of the microcapsules used to enrich the formulated ready-to-consume (RTC) juice were the plant samples investigated in the present study, viz; *G. simonsii* (leaf), *V. odoratissimum var. odoratissimum* (leaf and bark), and *C. latipes* (bark). These plant extracts have demonstrated antiproliferative activity against colon cancer cell line (HT-29). Consequently, to assess the potential anticancer efficacy of the enriched RTC formulation, its antiproliferative activity was evaluated against the human colon cancer cell line (HT-29) using the MTT assay. The results, as illustrated in Fig. 4.37, demonstrated that the juice exerted a dose-dependent inhibitory effect on the proliferation of HT-29 cells. Notably, significant cytotoxic activity was observed at concentrations ranging from 1.0 to 4.0 mg/ml, with an IC_{50} value of 2.25 mg/ml, indicating effective suppression of cancer cell growth at moderate dosages.

The observed antiproliferative effect can be attributed to the presence of bioactive phytochemicals encapsulated within the formulation. These include compounds such as hexadecenoic acid, Ergosta-5,22-dien-3-ol, 5-Hydroxymethylfurfural, D-Allose, Ergosta-5,22-dien-3-ol acetate (3β,22E), and 1-Heptatriacotanol, all of which have been previously reported to exhibit anticancer properties (Aparna et al., 2012; Bruce et al., 2021; Ray & Paul, 2022).

Collectively, the results suggest that the formulated RTC juice possesses promising antiproliferative activity, likely due to the synergistic action of its phytoconstituents. This endorses its candidature as a functional beverage with supplementary health benefits.

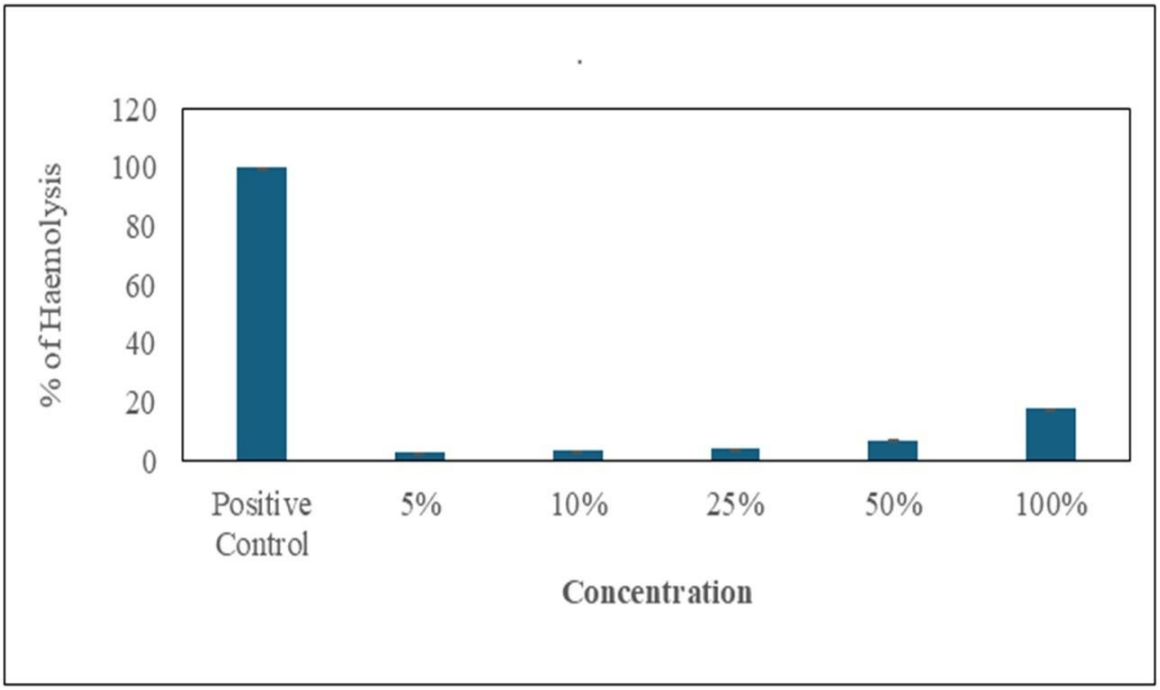

Fig. 4.36. Graph illustrating the haemolytic activities of the formulated RTC juice at various concentrations

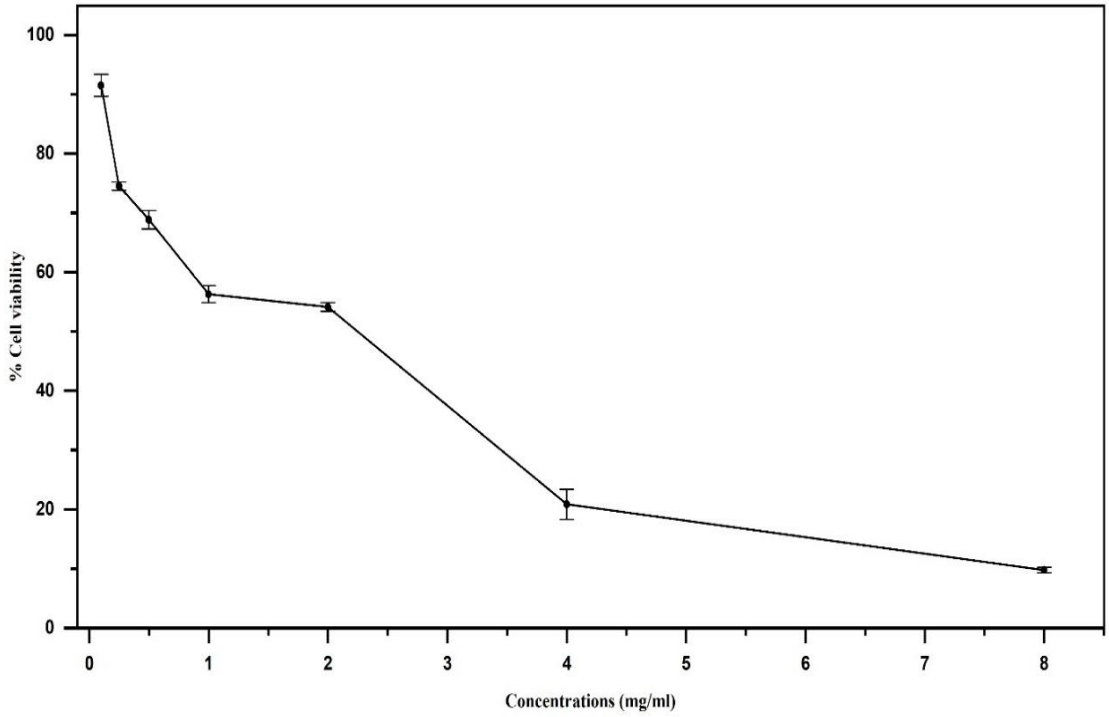

Fig. 4.37. Antiproliferative effect of the formulated RTC juice on HT-29 colon cancer cell line

4.5.16. Sensory evaluation of the RTC Juice

Sensory evaluation is a critical component in product development, providing insight into consumer perception and acceptability based on key organoleptic attributes such as colour, appearance, texture, taste, flavour, and overall acceptability. The ratings are generally based on a 9-point hedonic scale, where higher scores reflect greater acceptability (Santos et al., 2017).

The sensory profiles of the formulated RTC juice, based on the mean scores of 12 panellists for all quality attributes (colour, appearance, taste, texture, flavour, and overall acceptability), are displayed in Fig. 4.38. The sensory analysis revealed that the juice received a mean score of 7.08 for colour, indicating a generally favourable visual appeal. Appearance was rated at 6.83, reflecting moderate to high acceptance in terms of clarity and visual presentation. The texture of the juice received a relatively high mean score of 7.83, suggesting that the product exhibited a desirable mouthfeel and consistency. Taste, a critical determinant of consumer preference, was also well-received, with a mean score of 7.67, implying a good balance of sweetness, acidity, and palatability. The flavour, which encompasses both taste and aroma, was rated at 7.20, indicating overall positive feedback, although slightly lower than taste and texture. Notably, the juice achieved a high score of 8.5 for overall acceptability, reflecting strong consumer satisfaction when all sensory attributes were considered collectively (Pereira et al., 2019). These results suggest that the formulated RTC juice was generally well-accepted by the panellists and possessed favourable organoleptic properties suitable for commercial development.

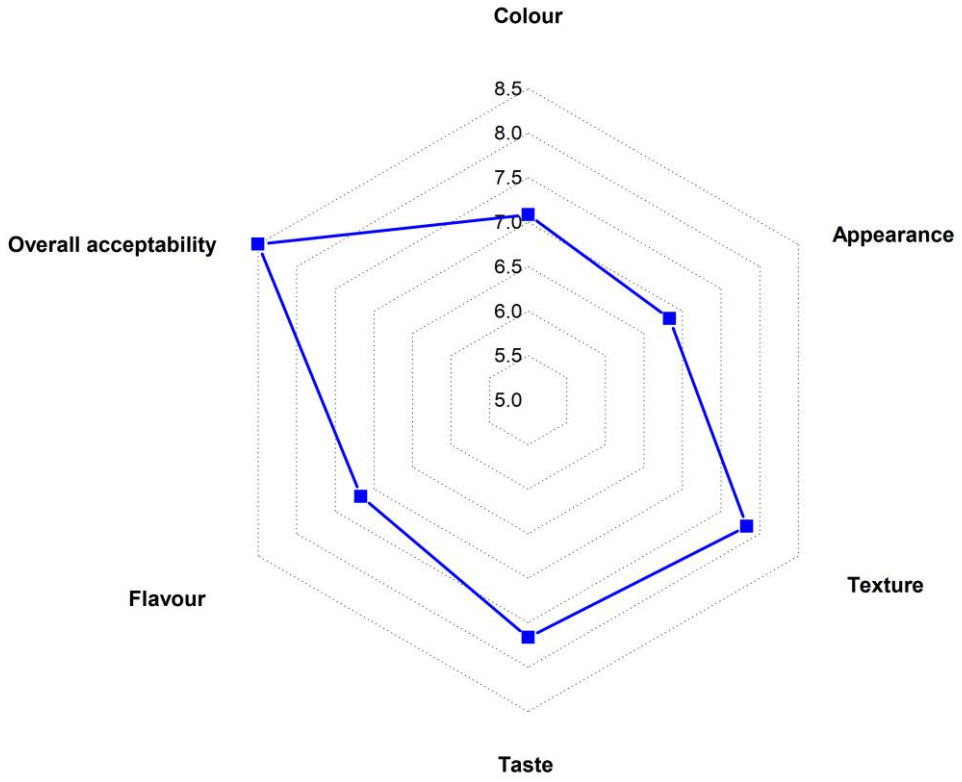

Fig. 4.38. Radar chart showing sensory profiles of the formulated RTC juice

CHAPTER 5

CONCLUSION

Chapter 5: Conclusion

Meghalaya, nestled in the northeastern region of India, boasts a rich mosaic of topographical features that significantly contribute to its status as a hotspot of medicinal plant diversity. The state is known for its dynamic landscape marked by high rainfall, steep hills, deep gorges, and lush forests. This diverse landscape plays a pivotal role in nurturing a multitude of ecological niches that supports the luxuriant growth of a plethora of medicinal plants. Many of these plants are endemic, rare and traditionally significant. The rich floral diversity, coupled with the deep-rooted traditional knowledge of indigenous communities, makes the region a living repository of ethnomedicinal wisdom. For centuries many of these plant species have been used in traditional healing practices to address ailments, including common infections, as well as more intricate conditions such as diabetes, cancer, gastrointestinal complications and reproductive disorders. However, a significant portion of these plants remains unexplored by the scientific community. Hence, ethnopharmacological studies of medicinal plants in the state holds profound significance, both scientifically and culturally. Ethnopharmacological studies can significantly contribute to the preservation of indigenous knowledge while also establishing a scientific foundation for the efficacy and safety of traditionally utilized medicinal plants.

Moreover, ethnopharmacological research promotes biodiversity conservation. By emphasizing the therapeutic significance of particular species, such studies can guide conservation priorities and sustainable harvesting practices. In Meghalaya, where excessive harvesting and habitat degradation threatens numerous medicinal plants, scientific validation may facilitate their integration into conservation programs and cultivation initiatives. Based on the aforementioned information, this study was conducted to scientifically validate the unexplored ethnomedicinal plants of the state of Meghalaya. This study explores three rare and threatened ethnomedicinal plants (*G. simonsii, V. odoratissimum var. odoratissimum,* and *C. latipes*) of the state, focusing on their spatial distribution, genetic identity,

biochemical characteristics, and bioactive properties. The study additionally encompasses the feasibility of herbal product formulations.

The following conclusions were drawn from the present study:

1. GIS-based spatial distribution mapping reveals *G. simonsii* to be confined to the low-elevation subtropical forests in the western part of the state, particularly Garo Hills region.
2. *V. odoratissimum var. odoratissimum* grows in the mid to high elevation moist forests of the Khasi Hills region.
3. *C. latipes* shows broad adaptability, thriving in the humid evergreen forests.
4. The spatial distribution study highlights the significance of microhabitat requirements for the survival of these rare floral species of the state.
5. Biochemical characterization showed high carbohydrate, protein, and lipid content in the fruits of all three species. Additionally, all the three species tested positive for alkaloids, flavonoids, phenols, steroids, glycosides, tannins, terpenoids, and saponins suggesting broad-spectrum bioactivity.
6. FTIR analysis identified key functional groups like phenols, amines, alkanes, alkenes, ethers, ester, and ketones, confirming the chemical richness of the extracts.
7. GC-MS profiling detected 24 compounds in *G. simonsii*, 21 in *V. odoratissimum*, and 22 in *C. latipes*. Detected key compounds are cis-Vaccenic acid, Spathulenol, β-sitosterol, osthole, n-Hexadecanoic acid, Stigmasterol, Neophytadiene, ß-Amyrin, Ergosta-5,22-dien-3-ol, Aziridine, Ricinoleic acid and Lup-20(29)-en-3-ol. These compounds exhibit various biological activities including antioxidant, antimicrobial, anticancer, anti-inflammatory, immunomodulatory, cardioprotective, etc.
8. DNA barcoding using rbcL and ITS2 confirmed the accurate taxonomic identification. Corresponding GenBank accession numbers were generated, viz, PV688311 (G. *simonsii*), PV749115 (*V. odoratissimum*

var. *odoratissimum*), and PV737879 (*C. latipes*). Phylogenetic analyses further revealed close genetic affiliations of each species within their respective genera.

9. Substantial phenolic and flavonoid contents were detected in the different parts of all three species. The leaf of *V. odoratissimum var. odoratissimum* exhibited the highest phenolic content (350.60 mg GAE/g), whereas leaf of *G. simonsii* recorded the highest flavonoid content (55.91 mg QE/g).
10. All three species exhibited significant free radical scavenging activity in the DPPH, FRAP, and ABTS assays. The leaf of *V. odoratissimum var. odoratissimum* exhibited the lowest IC_{50} value (32.25 µg/ml), the highest FRAP value (827.25 µM AAE/g), and ABTS value (763.14 µM AAE/g), signifying excellent antioxidant activity.
11. Antimicrobial activity assay revealed that, the extracts of all three species were effective against gram-positive bacteria (*Staphylococcus aureus* MTCC 11949 and *Bacillus cereus* MTCC 8361), gram-negative bacteria (*Escherichia coli* MTCC 593, *Salmonella enterica* MTCC 1166, and *Yersinia pestis*), and a fungus (*Candida albicans* MTCC 13013). Among all the 3 tested plant species (samples), *G. simonsii* leaf extract displayed the highest ZOI with 22.63 mm (against *Bacillus cereus*).
12. Minimum inhibitory concentration analysis confirmed that the values were ranged from 58.59 µg/ml to >1000 µg/ml. The lowest MIC value was observed for *G. simonsii* leaf extract (58.59 µg/ml).
13. All the selected extracts of the three species exhibited dose-dependent antiproliferative activity against HT-29 colon cancer cells. *G. simonsii* leaf had highest potency (IC_{50} = 8.82 µg/ml). *C. latipes* bark (IC_{50} = 52.39 µg/ml) and *V. odoratissimum var. odoratissimum* leaf (IC_{50} = 214.85 µg/ml) also showed efficacy.
14. The antiproliferative activity against the HT-29 cell line is attributed to the substantial abundance of phytocompounds, including hexadecenoic

acid, cis-vaccenic acid, Cholest-4-en-3-one, ß-Amyrin, β-sitosterol, neophytadiene, Spathulenol, Methoxy-4-vinylphenol, and stigmasterol.

15. The developed polyherbal extract microcapsules using maltodextrin displayed high solubility (90.6 %), acceptable encapsulation efficiency (~88 %), and good thermal stability up to 200 °C.
16. GC-MS analysis of microcapsules confirmed presence of bioactive compounds (E.g., pyranones, ergosterol, caffeine etc.).
17. A significant protection of encapsulant (polyherbal extract) of the microcapsules in gastric phase and release in intestinal phase was confirmed in *in vitro* release study.
18. Microcapsule enriched fortified green tea and ready-to-consume juice exhibited low hemolysis (<6 %) thereby indicating its excellent hemocompatibility.
19. Nutritional profiling of the formulated juice was as follows: 64.2 kcal/100g calorie, 0.87 mg/100g calcium, 2.1 mg/100g vitamin C, 0.1 mg/100g sodium, 0.10 mg/ml protein, and 1.42 % free fatty acids.
20. Sensory evaluation of the formulated products achieved high acceptability score (~ 8.08/9), suggesting strong consumer appeal.

These findings highlight the pharmacological potential of *G. simonsii*, *V. odoratissimum*, and *C. latipes* as sources of high value bioactive compounds for futuristic drug discovery. Their antioxidant, antimicrobial, and anticancer properties, endorsed by biochemical, molecular, and product formulation data, projects strong candidature for pharmacological research and drug discovery.

FUTURE PROSPECTS

For a better comprehensive understanding of the ethnopharmacological relevance, greater investigative capacities as mentioned below can be undertaken as a part of planned future research activity.

1. Population study of the species using key parameters including density, abundance and frequency.
2. Pure compound isolation and structure elucidation using spectroscopic techniques such as NMR, HPLC and X-ray crystallography.
3. A comprehensive *in silico* study employing molecular docking and dynamics simulations to understand the therapeutic mechanisms of bioactive compounds derived from the species at the molecular level.
4. Pharmacological validation using *in vivo* approaches to understand compound behaviour within a multicellular, dynamic environment, effective dose and potential organ specific damage.
5. Clinical trials for confirming therapeutic claims under controlled, reproducible conditions and to ascertain adverse effects and drug interactions.
6. Sustainable harvesting emphasizing both *in situ* and *ex situ* conservation approaches for the preservation and propagation of the rare medicinal plants.
7. Integrating traditional ethnomedicinal knowledge in herbal product formulations for the development of safer, more effective, and environmentally sustainable therapeutic agents.

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APPENDIX

Appendix I

Description the Species

***Goniothalamus simonsii* Hook.f. & Thomson**

Goniothalamus simonsii Hook. f. Thomson represents one of the thirteen species of *Goniothalamus* recorded in India. This species is a small tree characterised by 2-3 m tall, narrowly elliptic or obovate-oblong leaves and axillary, solitary, triangular, cream-coloured flowers. Flowering occurs between May and June, while fruiting takes place between August and September. The plant primarily grows at an altitude of 2-3000 ft along shaded streams in the forest. It is exclusively endemic to Meghalaya, one of the Northeastern states of India. The species is classified as 'Endangered' by the IUCN under criteria B1+2c. The species has been used by the indigenous people of Meghalaya, India, for the treatment of gastrointestinal complications [diarrhea, irritable bowel syndrome (IBS), peptic ulcer, etc.], throat irritation, typhoid fever, and malaria. However, the species has failed to gain the attention of the scientific community. Currently, there is no scientific information available on the phytochemical composition and bioactivity of the species, which warrants proper scientific highlighting and dedicated research endeavours.

Different parts of *G. simonsii*, (a) leaf, (b) flower and (c) fruit

Viburnum odoratissimum var. odoratissimum

Viburnum odoratissimum var. odoratissimum (Synonym *Viburnum simonsii* Hook. f & Thoms.) is a small tree with a height of up to 40 ft, thin greyish bark; leaves are oppositely arranged, elliptic, distantly cuspidate, dentate and glabrous with lateral nerves of 5 - 8. The flowers are white and sweet-scented. Fruits are 0.4 - 0.8 cm in diameter with a bright red colour. Flowering time occurs between March and June, whereas fruiting time takes place from July to October. It is endemic to the Eastern Himalayan range. In India, the distribution is currently limited to the state of Meghalaya. The plant is locally called *Soh-lang-eit-ksew.* Traditionally, the fruits of this species are used as tonic and anti-spasms. Despite its use in traditional medicine for centuries, this plant species remains unexplored scientifically, and thus, its pharmaceutical values are still unknown. Additionally, the declining population of the species may be attributed to a lack of information concerning its economic and therapeutic values.

Different parts of *Viburnum odoratissimum var. odoratissimum*, (a) Tree, (b) Flower and (c) Fruit and leaf

Citrus latipes (Swingle) Tanaka

Citrus latipes (Swingle) Yu. Tanaka is an evergreen tree species growing up to 15 m in height. The species produces bisexual, sweet-scented, white-coloured flowers and green fruit, which turn yellow upon full maturity. It primarily grows in evergreen tropical and subtropical forests at altitudes of 500–1200 m above sea level. The flowering and fruiting seasons are from March to May and from October to December. It is commonly called *Khasi papeda* and is endemic to Northeast India. The species has a restricted population. There are no global population estimates. Recently, the International Union for Conservation of Nature (IUCN) has placed the species under the 'Near Threatened' category B2b. No specific conservation action has been taken for the species, and it is not well represented in *ex-situ* collections. The species is locally called '*Soh Kymphor Shrieh*' and is classified as 'Rare' in Meghalaya, India. Traditionally, different parts of this plant have been used to treat various ailments. For example, leaves are used to treat colds, headaches, and body aches. The decoction of fruit is given for stomach disorders. Fruit juice is applied directly to cure skin diseases and as an antidote for food poisoning. However, due to the unpleasant taste of the fruit (bitter sour), the plant is unpopular. The species has been neglected among the local population of Meghalaya, as reflected in its declining population. Additionally, despite the extensive use of *C. latipes* in traditional medicine, there is no scientific report on its phytochemical constituents and bioactive properties.

Different parts of *C. latipes* (a) Tree, (b) Leaf (c) Flower and (d) Fruit

APPENDIX II

Certificate of species identification and authentication

भारत सरकार/GOVERNMENT OF INDIA
पर्यावरण वन एवंजलवायु परिवर्तन मंत्रालय/MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण/BOTANICAL SURVEY OF INDIA
प्रभारी वैज्ञानिक का कार्यालय/OFFICE OF THE SCIENTIST IN-CHARGE
पर्वी क्षेत्रीय केंद्र/EASTERN REGIONAL CENTRE

दूरभाष/Telephone: 0364- 2223971, 2223618 ई-मेल/e-mail- bsibsishll@yahoo.co.in Telefax: 0364- 2224119

संख्या/No.: BSI/ERC/ Tech/2023-24/ 1847 दिनांक/Dated: 26/02/2024

सेवा में/To,

Samson Rosly Sangma
Ph.D Scholar
Dept. of Forestry
Nagaland University
Nagaland-798627

विषय/Sub.: Identification of plant specimens.

महोदय/Sir,

With reference to your letter regarding the subject cited above, I am to inform you that your plant specimens have been identified and confirmed as below-

Sl. No.	Name of Specimen	Family	Collection Number
1.	*Viburnum odoratissimum* var. *odoratissimum*	Caprifoliaceae	NA
2.	*Goniothalamus simonsii* Hook.f. & Thomson	Annonaceae	NA
3.	*Citrus latipes* (Swingle) Tanaka	Rutaceae	NA

धन्यवाद/Thanking You

भवदीय/Yours sincerely

26/2/2024

डॉ. एन. ऑडियो/Dr. N. Odyuo
वैज्ञानिक-ई एवं कार्यालय प्रमुख/ Scientist-E & Ho.O

APPENDIX III

Endangered and threatened medicinal plants used in Meghalaya for various ailments

S. No	Name of the Plant	Family	Uses	Distribution	IUCN Status	References
1	*Acer laevigatum* Wall.	Aceraceae	Leaf paste is used externally in case of sprain.	Indo-Malaya, Himalayas & NEI	DD	Mir et al. (2014); Haridasan et al. (2010)
2	*Goniothalamus simonosii* Hk.f. & Thom.	Annonaceae	Fruit and leaf taken stomach problems	Meghalaya	EN	Mir et al. (2014)
3	*Trachyspermum khasianum* H. Wolff.	Apiaceae	The mashed leaves and stem applied on septic wounds.	Meghalaya	DD	Mir et al. (2014)
4	*Centella asiatica* L.	Apiaceae	Plant mashed and applied to boils and tumors. Extract taken for dysentery, diarrhea and cough along with ginger.	Meghalaya	LC	Rao (1981)
5	*Rauvolfia serpentina* (L.) Benth. ex Kurz.	Apocynaceae	During fever the root juice is taken raw or boiled to bring down the body temperature	East Asia (from India to Indonesia)	EN	Mir et al. (2014)
6	*Alstonia scholaris* (L.) R.Br	Apocynaceae	Juice used for cold, cough and gastrointestinal problems.	Indo- Malaya, throughout India,	LC	Mir et al. (2014); Haridasan et al. (2010)
7	*Wrightia coccinea* (Roxb. Ex Hornem.) Sims.	Apocynaceae	Paste used to keep blood pressure under control.	Indo-Malaya, confined to NEI	LC	Haridasan et al. (2010); Laloo et al. (2000)
8	*Holarrhena antidysenterica* (Roth.) A. DC.	Apocynaceae	The powdered bark to cure dysentery and the root used as an antidote for snake bite.	Indian Subcontinent & Indo-China	DD	Mir et al. (2014)
9	*Ilex khasiana* Purk.	Aquifoliaceae	Decoction used in cold, cough and tuberculosis	Meghalaya	CR	Mir et al. (2014); Laloo et al. (2000)
10	*Ilex embeloides* Hook.f.	Aquifoliaceae	Decoction used in cold,	Meghalaya	TH	Mir et al. (2014)

			cough and tuberculosis			
11	*Acorus calamus* L.	Araceae	Leaf juice is used to treat paralysis, epilepsy and stomach problem. Root juice taken for malaria and snake bites.	India & Sri Lanka	LC	Mir et al. (2014)
12	*Panax pseudoginseng* Wall.	Araliaceae	Used to stop or slow down bleeding. Sometimes taken by people who vomit up or cough up blood, or find blood in their urine or faeces.	Eastern Himalaya, Tibet, Burma & China	CR	Mir et al. (2014); Haridasan et al. (2010)
13	*Calamus floribundus* Griff.	Arecaceae	Extract taken for indigestion, stomach ache and malaria.	NEI	DD	Mir et al. (2014)
14	*Calamus erectus* Roxb.	Arecaceae	Used in indigestion, stomach problems, eczema, wounds and diabetes.	Himalaya & NEI	VU	Mir et al. (2014); Laloo et al. (2000)
15	*Impatiens tripetala Roxb. ex DC.*	Balsaminaceae	Used to promote appetite and as digestive enzyme.	NEI	DD	Mir et al. (2014)
16	*Begonia rubro-venia* Cl.	Begoniaceae	Whole plant is taken to cure diarrhea and dysentery.	Himalayas & NEI	DD	Mir et al. (2014)
17	*Viburnum simonsii* Hk.f.&Th.	Caprifoliaceae	Used as tonic and to prevent Spasms and gastrointestinal problems	Meghalaya	DD	Mir et al. (2014); Haridasan et al. (2010)
18	*Euonymus lawsonii* Cl. & Pr.	Celastraceae	Bark used in syphilis, indigestion and liver disorder. Seed oil used for removing lice.	Meghalaya and Arunachal Pradesh	DD	Mir et al. (2014)
19	*Garcinia pedunculata* G.Don	Clusiaceae	Dysentery and urinary troubles.	Indo-Burma, confined to NEI	DD	Mir et al. (2014); Kayang et al. (2003)
20	*Daphniphyllum himalayense* (Benth.) Muell.-Arg.	Daphniphyllaceae	A paste of the wood is applied as a poultice to boils.	NEI	DD	Mir et al. (2014)
21	*Dipsacus asper* Wall. ex. DC.	Dipsacaceae	For skin diseases	Eastern Himalaya	NT	Haridasan et al. (2010)

22	*Diospyros pilosula* (DC.) Hiern	Ebenaceae	Stomach disorder, piles, kidney stone, diarrhea and dysentery	Burma, NEI & Andaman	DD	Mir et al. (2014); Haridasan et al. (2010)
23	*Beliospermum micranthum* Muell.-Arg	Euphorbiaceae	Juice and paste taken to cure asthma and body ache.	Meghalaya	DD	Mir et al. (2014); Rao (1981)
24	*Saraca asoca* (Roxb.) de Willde.	Fabaceae	As antibacterial, for fever and cold.	Indo-Malaya & throughout India	VU	Mir et al. (2014); Rao (1981)
25	*Sophora acuminata* Baker.	Fabaceae	Purification of blood after pregnancy.	Bangladesh, Burma & Eastern Himalayas	DD	Mir et al. (2014); Rao (1981)
26	*Apios cornea* Benth.	Fabaceae	Along banana leaf paste applied to cure joint pain.	Meghalaya	DD	Mir et al. (2014); Laloo et al. (2000)
27	*Dalhousiea bracteata* (Garh ex Roxb) Wt.	Fabaceae	Paste applied to cure cuts and wounds.	India-Burma & Bangladesh	DD	Mir et al. (2014); Haridasan et al. (2010)
28	*Parkia roxburghii* G.Don	Fabaceae	Infections, stomach disorders and menstruation disorder.	Indo-Malaya & NEI	DD	Rao (1981)
29	*Xylia xylocarpa* (Roxb.) Taub.	Fabaceae	Stem bark used as antidiarrheal. Leaf and root decoction, used to cure fevers.	Indo-Malaya	LC	Mir et al. (2014); Haridasan et al. (2010);
30	*Xylosma longifolium* Clos.	Flacourtiaceae	Paste is externally used for skin diseases. Juice used for stomach ache.	Himalayas	LC	Mir et al. (2014); Laloo et al. (2000)
31	*Chirita hamosa* R.Br.	Gesneriaceae	Decoction taken to treat respiratory disorders.	Indo-Malaya, Meghalaya & western Ghats	NE	Haridasan et al. (2010); Rao (1981)
32	*Lindera latifolia* Hk.f.	Lauraceae	Paste applied topically to treat skin diseases.	Meghalaya	LC	Haridasan et al. (2010); Rao (1981)

33	*Cinnamomum pauciflorum* Nees.	Lauraceae	Bronchitis, asthma, diarrhea and nausea.	NEI	VU	Singh and Borthakur (2011)
34	*Ficus subincisa* Buch.-Ham.	Moraceae	To treat digestive system disorders.	Himalayas to Burma	DD	Mir et al. (2014)
35	*Nepanthes khasiana* Hk.f.	Nepenthaceae	Juice of young flowers mixed with rice beer and taken to cure stomachache, eye sores or urinary troubles.	Meghalaya	EN	Mir et al. (2014); Haridasan et al. (2010); Kayang et al. (2003)
36	*Paphiopedilum insigne* (Wall. ex Lindl.) Pfitzer.	Orchidaceae	Stomach troubles, dysentery and rheumatism.	Meghalaya	EN	Mir et al. (2014)
37	*Morinda umbellata* L.	Rubiaceae	Leaves used as decoction for diarrhea and dysentery.	Burma, Bangladesh & Himalaya	NT	Haridasan et al. (2010)
38	*Paramigyna micrantha* Kurz	Rutaceae	Decoction of the roots is drunk to assuage abdominal, discomfort, and as diuretic.	Meghalaya	DD	Mir et al. (2014); Haridasan et al. (2010)
39	*Zanthoxylum khasianum* Hk.f.	Rutaceae	Alimentary canal disorders, stomachic, anthelmintic.	Meghalaya	DD	Mir et al. (2014); Rao (1981)
40	*Citrus latipes* (Swingle) Tanaka.	Rutaceae	Fruit juice is taken as an appetizer; paste of leaves is applied on joints suffering from gout and rheumatism. Juice of fruit is rubbed on rashes and ringworm	Meghalaya	NT	Mir et al. (2014)
41	*Taxus wallichiana* Zucc.	Taxaceae	Used for the treatment of bronchitis, asthma, epilepsy, snake bites, scorpion stings, lung diseases and diabetes	Himalaya	EN	Mir et al. (2014); Haridasan et al. (2010); Kayang et al. (2003)
42	*Schima khasiana* Dyer.	Theaceae	Skin irritations, anthelmintic and rubefacient	Meghalaya	LC	Mir et al. (2014)

43	*Camellia caduca* Cl. ex Brandis.	Theaceae	Juice taken for digestive and urinary problems	Meghalaya	DD	Haridasan et al. (2010)
44	*Clerodendrum hastatum* (Roxb.) Lindl.	Verbenaceae	Leaves and stem boiled and the water is taken to reduce high blood pressure.	Bangladesh & Meghalaya	DD	Mir et al. (2014); Haridasan et al. (2010)
45	*Calamus floribundus* Griff	Arecaceae	Extract taken for indigestion, stomachache	NEI	DD	Mir et al. (2014); Rao (1981)
46	*Citrus indica* Yu. Tanaka	Rutaceae	Raw fruit juice is taken orally for dysentery.	Meghalaya	EN	Rao (1981)
47	*Agapetes odontocera* (Wight) Hook.f	Vaccinaceae	Dried powder form is used in making pickles, and eat to cure the disease.	Meghalaya	EN	Singh and Borthakur (2011)

Legends NEI = Northeast India, EN = Endangered, TH =Threatened, NT = Near Threatened, VU = Vulnerable, LC = Least Concern, CR = Critically Endangered, DD = Data Deficient

APPENDIX IV

Anticancer drugs derived from plants and their mode of actions

S. No	Anticancer drug	Plant source	Mode of Mechanism	References
1	Vinca alkaloid	*Catharanthus roseus*	Disrupt the microtubule dynamics during cell division, causing a specific block in mitosis that ultimately leads to cell death.	Mukhtar et al., 2014
2	Vinblastine	*Catharanthus roseus*	It acts as a stathmokinetic oncolytic drug, impeding the dynamic movement of microtubules during cell division.	Dhyani et al., 2022
3	Vincristine	*Catharanthus roseus*	It inhibits cell division by blocking the formation of microtubules, which are part of the cytoskeleton and mitotic spindle.	Škubník et al., 2021
4	Vinorelbine	*Catharanthus roseus*	It works by suppressing mitosis during metaphase through tubulin-mediated interaction with microtubular proteins in the mitotic spindle.	Bhambhani et al., 2021
5	Epipodophyllotoxin	*Podophyllum peltatum*	It targets topoisomerase II, an enzyme crucial for DNA replication and repair. By stabilizing the topoisomerase II-DNA complex, it prevents the proper unwinding and replication of DNA, leading to DNA damage and ultimately, cancer cell death.	Ardalani et al., 2017
6	Etoposide	*Podophyllum peltatum & Podophyllum emodi*	It forms a ternary complex with both topoisomerase II and DNA, thereby impeding the re-ligation of DNA strands. As a result, DNA breakage occurs.	Montecucco et al., 2015
7	Teniposide	*Podophyllum peltatum*	It binds to both topoisomerase II and DNA, resulting in the formation of a complex that triggers double-stranded DNA breaks and impedes their repair. As a consequence, cells are unable to complete the mitotic phase, ultimately leading to cell death.	Liscano et al., 2020
8	Paclitaxel	*Taxus brevifolia*	It affects the usual functioning of microtubule growth by binding to the tubulin subunit. It also binds to the apoptosis inhibitor protein Bcl-2, inhibiting its normal activity and promoting apoptosis in the cancer cells.	Leung & Cassimeris, 2019
9	Docetaxel	*Taxus baccata*	It impedes the disassembly of microtubules within cells. This disruption leads to the accumulation of microtubules, ultimately	Mukhtar et al., 2014

			initiating the process of apoptosis.	
10	Camptothecins	*Camptotheca acuminata*	It effects on the DNA enzyme topoisomerase I.	Martino et al., 2017
11	Cephalotaxanes	*Cephalotaxus*	It hinders the synthesis of proteins and targets specific molecular processes.	Lichota & Gwozdzinski, 2018
12	Homoharringtonine	*Cephalotaxus harringtonia*	It prevents the production of specific proteins, especially Mcl-1, which leads to apoptosis.	Lü & Wang, 2014
13	Flavopiridol	*Dysoxylum binectariferum*	It inhibits cyclin-dependent kinases, leading to apoptosis and impedes the division of non-small lung cancer cells.	Cuneo et al., 2019
14	Combretastatin	*Combretum caffrum*	In reference to the previously identified vascular disrupting substance colchicine, combretastatin attaches to the -subunit of tubulin at a location known as the colchicine site.	Karatoprak et al., 2020
15	Colchicine	*Colchicum autumnale*	It promotes apoptosis, stabilize microtubule production, binds permanently to tubulin, and arrests the cell cycle.	Ben-Chetrit, 2019
16	Ellipticine	*Ochrosia elliptica*	It inhibits CDK2 kinase and prevents the phosphorylation of the p53 protein, making it useful in treating human lung and colon cancer.	Mazumder et al., 2022
17	Berberine	*Berberis* *Vulgaris and Tinospora cordifolia*	It promotes apoptosis and cell cycle arrest in the G2/M phase	Rauf et al., 2021
18	Capsaicin	*Genus Capsicum*	It triggered apoptosis for activating the Rac1, ROS pathways.	Sarkar et al., 2015
19	Safranal	*Crocus sativus*	It demonstrates inhibitory effects on iNOS (inducible nitric oxide synthase) and COX-2 (cyclooxygenase-2) enzymes, which are associated with inflammation and cancer promotion.	Ahmed et al., 2020
20	Epigallocatechin	*Camellia sinensis*	It demonstrates ATP-competitive activity against the active site of the PI3K kinase domain in different cancer.	Negri et al., 2018

APPENDIX V

DETAILS OF THE PRIMERS

Primers	Sense	Sequence	Length	Scale	Purification
1R_kim	F	ACCCAGTCCATCTGGAAATCTTGGTTC	27	25 nmole	Desalted
3F_kim	R	CGTACAGTACTTTTGTGTTTACGAG	25	25 nmole	Desalted
rbcLa_F	F	ATGTCACCACAAACAGAGACTAAAGC	26	25 nmole	Desalted
rbcLa_R	R	GTAAAATCAAGTCCACCRCG	20	25 nmole	Desalted
matK-413f-1	F	TAATTTACRATCAATTCATTCAATATTTCC	30	25 nmole	Desalted
matK-1227r-3	R	GARGATCCRCTRTRATAATGAAAAAGATTT	30	25 nmole	Desalted
matK-XF	F	TAATTTACGATCAATTCATTC	21	25 nmole	Desalted
matK-NR1	R	ACAAGAAAGGCGAAGTAT	18	25 nmole	Desalted
rbcL-A	F	ATGTCACCACAAACAGAGACTAAAGC	26	25 nmole	Desalted
rbcL-724	R	TCGCATGTACCTGCAGTAGC	20	25 nmole	Desalted
ITS2	F	ATGCGATACTTGGTGTGAAT	20	25 nmole	Desalted
	R	GACGCTTCTCCAGACTACAAT	21	25 nmole	Desalted
rbcl	F	ATGTCACCACAAACAGAAAC	20	25 nmole	Desalted
	R	TCGCATGTACCTGCAGTAGC	20	25 nmole	Desalted
matK	F	AGAGGTATTTGCTGCTGTGGTG	22	25 nmole	Desalted
	R	GGAAAGAGTAAAGCAAGAACGTGT	24	25 nmole	Desalted
psbA-trnH	F	AGGTATCTGGTTCACTGCTTTAGGT	25	25 nmole	Desalted
	R	GCCTTGATCCACTTGGCTACAT	22	25 nmole	Desalted
ITS2	F	GCGATACTTGGTGTGAAT	18	25 nmole	Desalted
	R	GACGCTTCTCCAGACTACAAT	21	25 nmole	Desalted
matK-413f-5	F	TAATTTACGATCAATTCATTCACTATTTCC	30	25 nmole	Desalted
matK-1227r-1	R	GARGAYCCRCTRTRATAATGAGAAAGATTT	30	25 nmole	Desalted

APPENDIX VI

LIST OF PUBLICATIONS

Published

1. **Sangma, S. R**., Phukan, M. M., Chongloi, V., Verma, D. K., Bora, P., Kumari, S., & Pankaj, P. P. (2023). Phytochemical profiling, antioxidant and antimicrobial investigations on Viburnum simonsii Hook. f. & Thoms, an unexplored ethnomedicinal plant of Meghalaya, India. *Future Journal of Pharmaceutical Sciences*, *9*(1), 114. [**Springer**]
2. Chongloi, V., Gogoi, P. P., **Sangma, S. R**., Sinha, U. B., Bora, P., & Phukan, M. M. (2025). Antioxidant, antimicrobial and in silico investigations on pyrolytic bio-oil from invasive *Stachytarpheta jamaicensis*. *Environmental Science and Pollution Research*, 1-18. [**Springer**]
3. **Sangma, S. R.,** Phukan, M. M., Gogoi, D., Pankaj, P. P., Chongloi, V., Phytochemical Profiling, Bioactivity Evaluation and In Silico Insights Onto *Goniothalamus simonsii* Hook. f. Thoms.: An Endangered Medicinal Plant. *Chemistry & Biodiversity*, https://doi.org/10.1002/cbdv.202501513 [**Wiley**]

Manuscript Communicated

1. **Sangma, S.R**., Phukan, M.M., Sultana, S., Sharma, D., Chowdhury, P., Kumar, R., Saha, R., & Chongloi, V. Phytochemical analysis, antioxidant, antimicrobial and cytotoxicity properties of *Citrus latipes* (Swingle) Yu. Tanaka, a threatened ethnomedicinal plant of Northeast, India. [**Manuscript No: 252800587 – Under Review**] *Journals of Herbs, Spices & Medicinal Plants***. [Taylor & Francis]**
2. **Sangma, S.R**., Sharma, S., Mehmud, S., Phukan, M.M., Saha, R. Green synthesis of ZnO nanoparticles using *Trigonostemon semperflorens* (Roxb.) Müll. Arg. leaves and evaluating their antioxidant and antimicrobial potential. [**Manuscript ID: e6cde318-4258-4135-b18a-62ec424bff25 – Under Review**]. Nanotechnology for Environmental Engineering. **[Springer]**

3. Mehmud, S., **Sangma, S. R**., Sharma, S., Phukan, M. M. and Saha, R. Green synthesis of iron nanoparticles using leaf extract of *Gomphostemma mastersii* and its antioxidant and antimicrobial activity. [Manuscript ID: 257096913]. Journal of Biologically Active Products from Nature. [**Taylor & Francis**]

Book Chapters

1. Phukan, M. M., **Sangma, S. R**., Kalita, D., Bora, P., Das, P. P., Manoj, K., ... & Sundaram, K. M. (2023). Alkaloids and terpenoids: synthesis, classification, isolation and purification, reactions, and applications. In *Handbook of biomolecules* (pp. 177-213). [Elsevier].
2. Phukan, M. M., **Sangma, S. R**., Kalita, D., Pankaj, P. P., Das, P. P., Bora, P., ... & Kataki, R. (2023). Next-generational biosurfactant and their practical application in the food industry. In *Applications of Next Generation Biosurfactants in the Food Sector* (pp. 361-389). [**Elsevier**].
3. Phukan, M. M., Pankaj, P. P., **Sangma, S. R**., Ahmed, R., Manoj, K., Saha, J., ... & Bora, P. (2023). Antimicrobial, anti-inflammatory, and wound-healing activities of medicinal plants. *Phytochemicals in Medicinal Plants: Biodiversity, Bioactivity and Drug Discovery*, 205. [**De Gruyter**]

Paper Presentations

1. Presented paper titled, "Antioxidant properties of fruit extract of *Goniothalamus simonsii* Hook. F. Thoms, an endemic ethnomedicinal plant of Meghalaya" in the **International Conference** on 'Bioresource & Bioeconomy' organized by Dept. of Botany, Nagaland University, Lumami, on September – 2022.
2. Presented paper titled, "Biochemical and antimicrobial perspectives of viburnum simonsii Hook. F. Thoms. A rare flora of Meghalaya, India" in the **National Conference** on 'Reviving Traditional Knowledge for Biodiversity Conservation' organized by Dept. of Zoology, Nagaland University, Lumami, on March – 2023.

3. Presented paper titled, "Evaluation of the Citrus latipes (Swingle) Tanaka for their antioxidant, antimicrobial and phytochemical constituents" in the **National Conference** on 'Recent Breakthroughs and Innovations in Science and Technology' organized by Dept. of Science and Pharmaceutical Science, Assam Downtown University, Guwahati, on March – 2024.
4. Presented paper titled, "Phytochemical profile and bioactivities of *Goniothalamus simonsii* Hook. F. Thoms. An endangered Medicinal plant of Meghalaya" in the **11th International Convention Society for Ethnopharmacology**, India on 'Bioeconomy from Bioresources: Promoting Traditional Resources for NER for Vikshit Bharat' organized by BRIC – Institute of Bioresource and Sustainable Development (IBSD), on November– 2024.
5. Presented paper titled, "Phytochemical and bioactivity analysis of *Goniothalamus simonsii* Hook. F. Thoms. An endangered Medicinal plant of Meghalaya" in the **International Conference** on Medicinal Plants, Biodiversity Conservation, Natural Products for Health Care Needs in Traditional System of AYUSH, organized by Dept. of Forestry, Nagaland University on May– 2025.