Phytochemical Screening of Indian Traditional Medicinal Plants and Evaluate For Pharmacological Activity

A Thesis
Submitted towards the Requirements for the Award of degree of

Doctor of Philosophy

In
Pharmacy
Under The Faculty of Pharmacy
By

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(Enrollment no. 161555817603)

Under the Supervision of
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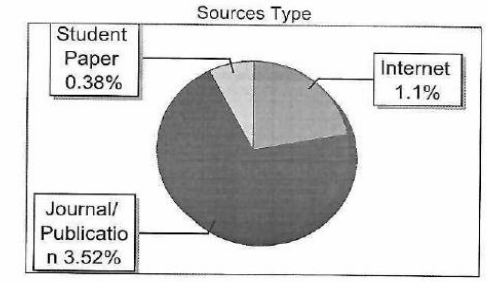
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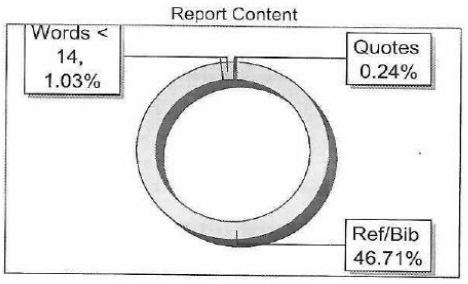
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Date: **(Bharat Goswami)**

Place:

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List of Abbreviations

Abbreviations	Indication
ALP	Alkaline Phosphatase
ALT	Alanine Amino Transferase
ANOVA	Analysis of Variance
AST	Aspartate Amino Transferase
b.w.	Body Weight
CK	Cork
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
Cu	Cuticle
CX	Cortex
DS	Dose (2000 mg/kg body weight)
ED_{50}	Median Effective Dose
Epi	Epidermis
GI_{50}	Drug concentration that inhibits cell proliferation by 50%
IAEC	Institutional Animal Ethics Committee
IL-10	Interleukin 10
IL-6	Interleukin 6
LC_{50}	Drug concentration that kills 50% of the cells
LD_{50}	Median Lethal Dose
M-1	Mouse 1
M-2	Mouse 2
M-3	Mouse 3
M-4	Mouse 4
M-5	Mouse 5
MEAC	Methanolic Extract of Argyreia cymosa
MEC	Methanolic Extract of Argyreia cymosa
MECB	Methanolic Extract of Capparis brevispina
MELR	Methanolic Extract of Limnophila repens
mg/kg	Milligram per Kilogram
MR	Medullary Rays
MX	Meta Xylem
N	Normal
N.F.	Not Found
NE	Non-Evaluable Data

OECD	Organisation for Economic Co-operation and Development
p.o.	Per Oral
Par	Parenchyma Cells
PI	Pith
PP	Palisade Parenchyma
PX	Proto Xylem
S	Stomata
SC	Stone Cells
SEM	Standard Error of Mean
SRB	Sulforhodamine B
Sub	Subsidiary Cells
TGI	Total Growth Inhibition
TLC	Thin Layer Chromatography
TNF-α	Tumor Necrosis Factor Alpha
Tri	Trichomes
VC	Vehicle Control
V^d	Change in Paw Volume (Drug-treated Group)
V^c	Change in Paw Volume (Vehicle Control Group)
V_0	Baseline Paw Volume
WHO	World Health Organization
XP	Xylem Parenchyma
XV	Xylem Vessels
Xy	Xylem
µg/mL	Microgram per Millilitre

ABSTRACT

Plants have always played an essential role in human life, and some plants have become deeply ingrained in human culture and traditional practices. In India and several other parts of the world, plants have been used as medicines since ancient times. A detailed literature survey revealed that *Argyreia cymosa* (Roxb.) Sweet, *Limnophila repens* (Benth.) Benth., and *Capparis brevispina* DC. are good sources of therapeutically active plant compounds used for the treatment of various diseases. In view of this, the present research was designed to evaluate the pharmacognostical, phytochemical, and pharmacological properties of selected Indian traditional medicinal plants. The selected plants, namely *Argyreia cymosa* (Roxb.) Sweet, *Limnophila repens* (Benth.) Benth., and *Capparis brevispina* DC., were studied for their ethnomedicinal, phytochemical, and pharmacological properties. These plants play important roles in folkloric medicine among tribal communities near the Tirumala Hills forest in Chittoor district, India.

Pharmacognostical evaluation, including morphological, organoleptic, microscopic, quantitative microscopic, and powder microscopic studies, was performed separately for the dried leaves, stems, and roots, and the findings were documented. This information describes the similarities and differences among the selected plants and helps in the identification and authentication of the species. The powdered plant materials were standardized using physicochemical parameters according to WHO guidelines. Parameters such as loss on drying, total ash, water-soluble ash, acid-insoluble ash, and fluorescence analysis were found to be within the prescribed limits.

The dried powdered materials were subjected to successive extraction using different solvents in increasing order of polarity. Preliminary phytochemical screening and TLC studies revealed the presence of secondary metabolites such as sterols, flavonoids, alkaloids, and phenolic compounds. Different metabolites were isolated using column chromatography, and their chemical characterization was carried out using spectroscopic techniques such as IR, ^{1}H-NMR, and mass spectroscopy. Compound 1, stigmasterol, and compound 2, 3,5,4′,5′-tetrahydroxy-6-C-(2″,6″-dihydroxy-4-methoxybenzyl) flavan-3-ol, were isolated from *Argyreia cymosa*. Similarly, compound 3, 5,8-dihydroxy-7-methoxyflavone, and compound 4, 5,8,4′-trihydroxyflavone-7-carboxaldehyde, were isolated from *Limnophila repens*. In addition, compound 5, quercetin, and compound 6, bergenin hydrate, were isolated from *Capparis brevispina*.

Furthermore, the crude methanolic extracts of the selected medicinal plants were evaluated for pharmacological activities. Acute toxicity studies showed that the LD_{50} values of the methanolic extracts of the selected medicinal plants were greater than 2000 mg/kg body weight and were therefore considered generally safe. Hence, methanolic extracts at doses of 200 and 400 mg/kg body weight were selected for further pharmacological investigations.

The antinociceptive activity was evaluated using the hot plate, tail immersion, and acetic acid-induced writhing methods. The results indicated that the methanolic extracts of the selected plants possessed significant dose-dependent antinociceptive activity through both central and peripheral analgesic mechanisms. The carrageenan-induced paw edema method was used to evaluate anti-inflammatory activity, and the results showed significant inhibition of inflammation. The Brewer's yeast-induced pyrexia model demonstrated significant antipyretic activity. Hepatoprotective activity was evaluated using a paracetamol-induced hepatic damage model in rats, and the results were compared with the standard drug silymarin. Biochemical parameters such as AST, ALT, ALP, and bilirubin revealed the potential hepatoprotective activity of the selected plant extracts.

The anticancer activity of the selected medicinal plants was evaluated using an in vitro SRB assay on Ishikawa and SCC-29B cell lines at different concentrations of the methanolic extracts. The results demonstrated that the methanolic extracts of the selected plants possess potential anticancer activity against these cell lines.

In conclusion, the selected Indian traditional medicinal plants were found to be authentic and of good quality, as demonstrated by pharmacognostical evaluation, including macroscopic, microscopic, and powder microscopic studies, as well as physicochemical and fluorescence analyses. The acute toxicity study and subsequent pharmacological evaluation indicated that the methanolic extracts were safe at higher concentrations and exhibited significant analgesic, anti-inflammatory, antipyretic, hepatoprotective, and anticancer activities. Therefore, the methanolic extracts of *Argyreia cymosa*, *Limnophila repens*, and *Capparis brevispina* may provide important leads for the development of novel therapeutic agents. Further research is warranted to identify and validate the active constituents responsible for these pharmacological effects.

CHAPTER-1
Introduction

1.1Remedial floraes

Millennia of cross-cultural healing practice have established botanical resources as foundational constituents of formalized therapeutic frameworks, wherein preparations derived from florae matter have addressed both the management of active pathological states and the prevention of disease onset across human and animal populations alike (Lulekal et al., 2008). Within economies where access to industrially manufactured pharmaceuticals remains constrained by cost or supply-chain limitations, phytomedicine-based interventions have emerged as a pharmacologically credible, financially sustainable, and physiologically tolerable modality of primary healthcare delivery (Hoareau & DaSilva, 1999). The taxonomic breadth of species enlisted within these systems is notable: beyond spontaneously regenerating wild vegetation, deliberately cultivated spice-bearing floraes with documented secondary metabolite profiles are routinely incorporated into indigenous Materia medica (Gahukar, 2011). Concurrently, the accelerating adoption of precision-controlled horticultural infrastructure — encompassing glasshouse, greenhouse, and polyhouse facilities — has not supported the ecological and remedial significance of florae communities occupying forested tracts, agrarian margins, and successional landscapes, where the conservation of phytodiversity remains intrinsically linked to pharmacognostic resource availability (Abbasi et al., 2011). The intellectual architecture underpinning current ethnopharmacological science draws substantially from classical systems of healing — most notably Ayurveda, Unani, and Siddha — whose sustained scholarly traditions have, over successive generations, systematically catalogued florae-organism interactions and codified therapeutic applications that continue to inform contemporary drug discovery pipelines (Fabricant & Farnsworth, 2001). Quantitative assessments of sub continental India's remedial flora estimate the engagement of upwards of 2,500 vascular florae taxa within traditional healing contexts, of which a functionally significant subset of approximately 100 species contributes disproportionately to the active pharmacopoeia of everyday clinical practice in indigenous medicine (Sheng, 2001). At the intersection of natural product chemistry and global pharmaceutical economics, it is now well-established that molecules of biological origin underlie the

therapeutic action of nearly half of all clinically authorized drugs; since the majority of the architecturally complex phytochemicals responsible for these effects remain inaccessible to scalable total synthesis, the global procurement demand for authenticated remedial florae material and standardized botanical extracts is anticipated to undergo pronounced and sustained expansion in the decades ahead (Verma & Singh, 2008).

Systematic phytochemical profiling of diverse florae taxa has become a standard investigative approach in natural product research, aimed at characterizing the spectrum of biologically active constituents residing within anatomically distinct organs — encompassing cortical tissues, foliar structures, reproductive organs, root systems, and propagative units such as seeds and fruits (Sani et al., 2014; Patel et al., 2010). These constituents, collectively designated as bioactive compounds, represent a chemically heterogeneous class of secondary metabolites whose interactions with biological systems — whether manifesting as beneficial pharmacological modulation or as cytotoxic and xenobiotic hazards — define their therapeutic or toxicological relevance in both human and veterinary medicine. From an evolutionary biochemistry standpoint, the biosynthetic origins of many such phytochemicals appear to reflect non-directional metabolic divergence rather than adaptive selection pressure; however, attributing universal irrelevance to this apparent stochasticity would be scientifically untenable, as mounting empirical evidence confirms that numerous secondary metabolites fulfil discrete and indispensable physiological functions within florae organisms themselves (Azmir et al., 2013; Singh, 2019). Flavonoid compounds, for instance, constitute a well-characterized antioxidant defense network capable of neutralizing reactive oxygen species generated as by-products of photosynthetic electron transport, thereby mitigating oxidative injury to cellular macromolecules (Wadood et al., 2013). Terpenoid derivatives, by contrast, operate at the interface of florae-environment signalling, functioning as chemical attractants that facilitate mutualistic interactions with pollinators and frugivorous seed-dispersing fauna, while simultaneously exerting allelopathic effects that suppress the establishment and growth of competing vegetation. Alkaloid biosynthesis — encompassing specialized compounds such as phytoalexins — represents yet another dimension of the florae's constitutive and inducible chemical defense repertoire, deployed against herbivorous arthropods and browsing mammals (Zhao et al., 2005). Beyond these well-delineated roles, an expanding body of literature

has revealed that additional classes of secondary metabolites participate in intercellular signalling cascades, acting as molecular messengers that coordinate developmental and stress-response programs across tissue types. The prevailing scientific consensus has accordingly shifted away from treating bioactive compound accumulation as an incidental biochemical anomaly; rather, secondary metabolite production is now recognized as an evolutionarily conserved and functionally integral dimension of florae biology (Karabín et al., 2016). Notably, the capacity for bioactive compound biosynthesis is not confined to taxa conventionally classified as remedial — edible and fodder crops also harbor such molecules, though typically at concentrations substantially lower than those encountered in dedicated remedial or toxicologically significant florae species (Weiss & Meuss, 2001; Wynn & Fougere, 2007). The structural and mechanistic novelty inherent to florae-derived secondary metabolites confers upon them an unparalleled utility in pharmacological screening programs, where the chemical space they occupy frequently exceeds the combinatorial diversity achievable through synthetic chemistry alone (Cos, 2006). This principle finds compelling historical substantiation in the early nineteenth-century isolation of morphine — a structurally complex opioid analgesic — from the latex of Papaver somniferum, an event that not only inaugurated the era of alkaloid-based pharmacotherapy but catalyzed the broader paradigm of systematically mining florae biochemistry for clinically transformative lead compounds.

1.2 Herbal medicine

Within the pharmacognostic literature, the term "herb" carries disciplinary specificity that transcends its colloquial usage; in a clinical and therapeutics context, it designates an unprocessed drug substance of botanical provenance administered primarily for the management of chronic pathological conditions or for the attainment and maintenance of an optimized state of physiological wellbeing (Israel & Youngkin, 1997). Preparations derived from biologically active constituents distributed across morphologically diverse florae organs — whether foliar, floral, cortical, rhizomal, or seed-bearing — are collectively subsumed under the terminological designations of phytomedicine and phytopharmaceuticals, both of which encompass a broad continuum of pharmaceutical delivery systems. These range from solid oral formulations such as compressed tablets and encapsulated powders, to liquid preparations including fluid extracts, alcoholic tinctures, and elixirs, as well as semi-solid topical applications and,

in certain instances, parenterally administered botanical preparations; the therapeutic tradition from which many of these formulations derive extends into antiquity (Srujana, 2012). A definitional boundary of considerable clinical importance, however, distinguishes authentic herbal remedial products from isolated phytogenic compounds: mono-constituent preparations such as digoxin and reserpine — despite originating from florae sources — are explicitly excluded from the herbal medicine category by virtue of their pharmacologically refined and chemically singular active ingredient profiles (Gilani, 2005). An equally critical taxonomic distinction separates phytomedicine from homoeopathy, a therapeutically distinct discipline formalized in the eighteenth century by the German physician Samuel Hahnemann, whose theoretical framework rests upon the doctrinal principles of similitude and progressive dilutional potentization — conceptual foundations irreconcilable with the phytochemical mechanistic basis of herbal pharmacotherapy (Yang, 2017). Within this landscape, the dispensing pharmacist occupies a professionally strategic position as the most accessible and appropriately trained healthcare practitioner to counsel patients on the comparative safety profiles, pharmacodynamic properties, and evidence-based efficacy of botanical remedial products — a role that demands continuous engagement with contemporary peer-reviewed literature and clinical research outputs (Zheng et al., 2000; Ventola, 2010). As a regulatory and classificatory principle, any botanical substance deployed with therapeutic intent qualifies for designation under the phytomedicine framework, irrespective of the degree of processing to which it has been subjected (Tyler, 1995). The continued ascendancy of florae-derived therapeutics within integrative and primary healthcare systems is underpinned by a convergence of attributes that collectively distinguish them from synthetic allopathic pharmacological agents: a well-documented long-term safety record accumulated through centuries of empirical use, a relatively favorable adverse-event profile in comparison to many conventional drugs, logistical accessibility across geographically and economically diverse populations, cost-effectiveness at both individual and systemic healthcare levels, and a temporal record of sustained therapeutic relevance that validates their continued incorporation into modern clinical practice (Clement et al., 2005; Frass et al., 2012; Evans, 2008).

a. Rising health care costs.

b. Because they come from natural sources, they don't have any negative impacts.

c. Identifies and removes the etiology in order to prevent the condition from recurring.

d. The cure for many incurable diseases.

e. Simple access to medications via natural resources.

1.3 Pharmacognosy

Within the broader framework of natural product drug development, pharmacognostic investigation constitutes the foundational analytical tier upon which the systematic quality standardization of crude botanical materials is constructed (Mangathayaru, 2013). The inherent complexity of this standardization enterprise derives from the pronounced physicochemical and compositional heterogeneity characteristic of botanically sourced raw materials, which may be presented for pharmaceutical processing in morphologically distinct configurations — encompassing intact whole-florae specimens, anatomically selective florae fractions such as leaves, roots, bark, or seeds, and processed botanical extracts whose phytochemical profiles are further modulated by extraction methodology and solvent polarity. Consequently, the establishment of rigorous, reproducible quality benchmarks across these varied material forms demands a multi-parametric analytical strategy that accommodates intrinsic biological variability while maintaining regulatory compliance (Mangathayaru, 2013). Central to the realization of consistent product quality in herbal pharmaceutical manufacturing is the implementation of systematic raw material governance protocols, wherein the integrity of the supply chain — from point of botanical collection through post-harvest processing — is subject to documented quality oversight. Preceding all downstream quality control operations, botanical authentication represents the indispensable primary step through which the identity, purity, and genetic fidelity of the starting florae material are unambiguously confirmed, thereby precluding the compounding consequences of misidentification or adulteration that may propagate irreversibly through subsequent manufacturing stages (Evans, 2009; Wallis, 1946). Against this scientific backdrop, the global research community has witnessed a marked and accelerating intensification of standardization efforts directed toward a widening portfolio of therapeutically promising remedial florae species, reflecting both the escalating commercial interest in phytopharmaceuticals product development and the regulatory imperative for evidence-based quality assurance in herbal medicine

(Mukherjee, 2019).

1.4 Pharmacology

As a formally constituted scientific discipline, pharmacology underwent its decisive crystallization during the nineteenth century, when the application of rigorous empirical methodologies — analogous in epistemological orientation to those concurrently transforming experimental biology — enabled the systematic interrogation of drug-organism interactions within clinically relevant frameworks, centuries of empirically intuited but mechanistically uncharacterized therapeutic practice (Boobis et al., 2016). The disciplinary landscape has since undergone a profound and ongoing metamorphosis: contemporary pharmacological inquiry operates at the convergence of genomics, molecular biology, structural biochemistry, and computational chemistry, deploying an expanding arsenal of high-resolution investigative platforms to dissect the mechanistic underpinnings of drug-target engagement at atomic and sub cellular resolution. This multi-disciplinary integration has fundamentally reoriented the translational trajectory of pharmacological science — shifting the field from broad-spectrum empirical treatment toward the rational engineering of molecularly precise interventions that selectively modulate dysregulated biological pathways implicated in the etiopathogenesis of specific disease states, genetic disorders, and infectious agents of microbial or viral origin (Bennett, 2008; Jain, 2009). Beyond curative pharmacotherapy, this convergence of technological capability has catalyzed the development of pharmacologically grounded diagnostic modalities, chemo preventive strategies, and — most consequentially for the future of clinical medicine — individualized therapeutic regimens calibrated to the unique genomic, proteomic, and metabolomic signatures of each patient, heralding the practical realization of precision medicine as an operational healthcare paradigm (Bennett, 2008; Jain, 2009).

1.5 Analgesic action

Occupying the distinction of being among the most venerable codified systems of healing on the Bhartiye subcontinent, Ayurveda attained its intellectual and literary zenith during the Vedic era, with foundational canonical treatises — most notably the Charaka Samhita and Sushruta Samhita — composed and systematically elaborated over the period approximating 1000 BCE, a chronological attribution corroborated by extant historical and archaeological documentation (Somvanshi et al., 2006). The therapeutic

botanical repertoire documented within Ayurvedic Materia Medica encompasses upwards of 600 remedial florae species, among which aromatic and culinary taxa of broad cultural familiarity — including Ocimum tenuiflorum (holy basil), Trigonella foenum-graecum (fenugreek), Zingiber officinale (ginger), and Curcuma longa (turmeric) — constitute recurrently deployed ingredients in classical polyherbal and mono-herbal compounding traditions (Singh, 2011). Ayurvedic formulations are accordingly classified along a structural continuum ranging from preparations incorporating a solitary florae constituent to complex multi-herb composites whose synergistic phytochemical interactions underpin their collective therapeutic efficacy. Phytochemical investigation of indigenous Ayurvedic florae taxa has yielded a series of structurally and pharmacologically significant secondary metabolites. Among these, clausenol — a carbazole-class alkaloid isolated from the aerial tissues of Clausena anisata — has demonstrated substantive antimicrobial activity, with in vitro assessments confirming inhibitory potency against both fungal and bacterial pathogens (Dahanukar, 2000). Independently, cerpegin — a furopyridine alkaloid characterized from Ceropegia juncea — has exhibited antinociceptive activity in acetic acid-induced writhing assays conducted in murine models, establishing its analgesic pharmacodynamic profile under controlled experimental conditions (Rashid et al., 2020). The pharmacological characterization of sarcosine, derived from Sarcocapraeformis, remains an open investigative frontier, with its therapeutic potential yet to be subjected to systematic preclinical or clinical scrutiny. Tylphorine — a phenanthroindolizidine alkaloid whose structural architecture confers pronounced multi-target biological activity — has demonstrated robust anti-inflammatory, anti-anaphylactic, and antispasmodic efficacy across multiple established animal model paradigms (Singh et al., 2008). Complementary investigations employing Tylophora asthmatica extracts further documented suppression of bovine serum albumin- and Freund's adjuvant-induced bronchospasm in rat models, alongside documented myorelaxant activity, antagonism of neuromuscular junction stimulants, and immunomodulatory suppression spanning multiple experimental species (Singh A.P., 2005). Despite substantive advances in analgesic pharmacotherapy over recent decades, the clinical management of pain — particularly in the context of chronic, refractory, or neuropathic pain syndromes — continues to impose an unmet therapeutic burden that existing drug classes inadequately address, necessitating the development of novel, mechanistically differentiated, and clinically safer pain-modulating agents (Dworkin et al., 2003; Vadivelu et al., 2010;

Cohen et al., 2021). Current first-line analgesic modalities carry well-characterized and clinically limiting liability profiles: non-steroidal anti-inflammatory agents are associated with gastric mucosal injury, ulcerogenesis, and renal impairment with prolonged administration, while opioid analgesics precipitate tolerance development, physical dependence, and high propensity for substance use disorder — collectively constraining their long-term applicability in chronic pain management (Hanson et al., 2009; Tabatabai et al., 2014). Botanically derived therapeutics, frequently characterized in the pharmacognostic literature as biosynthetically sophisticated phytochemical repositories, elaborate a structurally diverse array of biologically active constituents — encompassing volatile and fixed lipidic fractions, resinous exudates, oleoresinous composites, sesquiterpenoid scaffolds, cardenolide and steroidal glycosides, nitrogenous alkaloids, and saponin-class amphiphilic compounds — whose mechanistic versatility positions them as a pharmacologically rich domain for the identification of novel analgesic lead structures (Fan et al., 2014). The expanding catalogue of pharmacologically characterized florae-derived bioactive compounds has correspondingly intensified scientific interest in harnessing phytoremedial frameworks for pain modulation; within this context, herbal therapeutic strategies have emerged as a particularly compelling investigative avenue for addressing the dual clinical challenge of opioid addiction management and attenuation of withdrawal-associated symptomatology (Doosti et al., 2013).

1.6 Activity Against inflammation

The immune system's reaction to damage or stress is inflammation. The procedure is initiated to protect the body from infections or irritants. Inflammation may sometimes have negative effects even though it normally protects and cures the human body (Medzhitov, 2010). They exhibit inflammatory diseases such multiple sclerosis, osteoarthritis, rheumatoid arthritis, and retinitis. As a result, formulations of ibuprofen, diclofenac, and other inflammatory drugs were developed. They are referred to as NSAIDs, or nonsteroidal anti-inflammatory drugs. They are inhibitors of COX. Floraes have been shown to generate phytochemicals with anti-inflammatory properties. (Hostettmann and Marston, 2002; Karou et al., 2011). The World Health Organization estimates that 80% of pharmaceuticals made from floraes are used in medical settings. Remedial floraes are used in Ayurveda, Siddha, and Unani to treat a range of human conditions (Kumar et al., 2017). They claim that they have identified over 20,000

different species of therapeutic herbs. Aegle marmelos, Bryophyllumpinnatum, Moringa olifera, Cassia fistula, Hibiscus rosa, and other floraes demonstrated this effect in a study on the anti-inflammatory properties of floraes (Vishal et al., 2014). Because of their availability, viability, and accessibility, floraes continue to seem to be a significant source of medicine. To pinpoint the precise processes of phytochemicals, further investigation is needed. To provide more effective treatments, nanoparticle research on florae and seed extracts is being conducted (Gheena and Ezhilarasan, 2019; Karthiga et al., 2018; Lakshmi et al., 2015; Menon et al., 2018).

1.7 Antipyretic action

Normal human body temperature varies depending on the site of measurement, with the oral temperature averaging 36.8 ± 0.4 °C (98.6 °F). Rectal temperature is typically slightly higher, approximately 37.5 °C, whereas axillary temperature is usually lower, around 36.5 °C. Physiological and environmental factors such as dietary intake, physical exertion, circadian rhythm, infections, neoplastic conditions, connective tissue disorders, and certain unknown influences can lead to fluctuations in body temperature. Fever is generally regarded as a regulated physiological response that plays an important role in host defense mechanisms against infection and tissue injury. From a clinical perspective, fever is defined as an elevation of body temperature above the normal circadian range, commonly exceeding 100.4 °F. However, careful monitoring is necessary in susceptible populations, including individuals with compromised cardiovascular, respiratory, or neurological function, pregnant women, and pediatric patients prone to febrile seizures. Extremely elevated body temperature, commonly referred to as hyperpyrexia, represents a severe pathological condition requiring prompt intervention (Guyton & Hall, 2020).

The pathogenesis of fever involves complex interactions between immune mediators and thermoregulatory centers of the central nervous system. The initial phase typically occurs when tissue injury or microbial invasion results in the release of exogenous pyrogens, including bacterial endotoxins, viral particles, fungal metabolites, immune complexes, and certain pharmacological agents. These substances stimulate immune cells to produce endogenous pyrogens, primarily cytokines such as interleukins and tumor necrosis factors. These mediators act on the hypothalamic thermoregulatory center, altering the thermal set point and leading to an increase in body temperature. This process represents a coordinated response aimed at enhancing immune efficiency

and limiting microbial proliferation (Saper & Breder, 1994).

The therapeutic management of fever and inflammation has evolved considerably over time. Historical evidence indicates that florae-derived remedies were used in ancient Greek and Roman medicine to alleviate fever and pain. In particular, preparations derived from willow bark, which contains salicylate-like compounds, were widely recognized for their remedial properties and were documented in early medical writings such as the Corpus Hippocraticum. Subsequent scientific advancements during the nineteenth and twentieth centuries led to the discovery and development of non-steroidal anti-inflammatory drugs (NSAIDs), marking a major milestone in modern pharmacotherapy. Despite structural diversity among these compounds, NSAIDs share common pharmacological properties, including analgesic, antipyretic, and anti-inflammatory actions, along with related adverse effect profiles. Recent developments in molecular pharmacology have provided valuable insights into the biochemical pathways responsible for the therapeutic effects of NSAIDs, particularly their influence on inflammatory mediators and temperature regulation (Vane & Botting, 2003).

1.8 Hepatoprotective action

Among the parenchymal organs constituting the human visceral architecture, the liver occupies an unparalleled position of functional centrality, orchestrating an extraordinarily broad spectrum of biochemical processes indispensable to the preservation of systemic metabolic equilibrium and whole-body homeostatic integrity. Its hepatocellular machinery assumes primary responsibility for the biotransformation of both endogenously synthesized metabolic intermediates and exogenously introduced chemical entities — encompassing pharmaceutical compounds, environmental toxicants, and xenobiotic substances of diverse structural classes — facilitating their conversion through phase I oxidative and phase II conjugative enzymatic reactions into water-soluble derivatives amenable to biliary or renal excretion. This hepatic detoxification capacity is operationally mediated by a sophisticated network of microsomal enzyme systems, prominently including cytochrome P450 isoenzymes, glucuronosyltransferases, and sulfotransferases, whose coordinated catalytic activity constitutes the principal mechanism by which potentially injurious chemical species are rendered biologically inert. Concurrently, the liver fulfills indispensable anabolic and storage functions, maintaining regulated repositories of glycogen as a mobilizable gluconeogenic substrate, alongside fat-soluble vitamins, trace minerals, and coagulation

factor precursor that collectively underpin hematopoietic, immune, and nutritional homeostasis. Beyond these metabolic and storage roles, hepatic tissue harbors a substantial population of Kupffer cells — resident macrophages of mononuclear phagocyte lineage — that perform continuous immunosurveillance by sequestering and degrading circulating microbial organisms, endotoxins, and other pathogen-associated molecular patterns, thereby constituting a critical immunological gatekeeping function at the interface between portal circulation and systemic physiology (Rajesh & Latha, 2004; Vivek, 1994).

The unremitting exposure of hepatic tissue to portal blood — which delivers the cumulative chemical burden absorbed from the gastrointestinal tract prior to systemic distribution — renders the liver constitutively vulnerable to a heterogeneous array of injurious stimuli. These encompass hepatotropic viral pathogens, halogenated and aromatic environmental contaminants, organophosphate and organochlorine-class agrochemicals, occupationally encountered industrial solvents and heavy metal species, chronic ethanol overconsumption, and indiscriminate or prolonged administration of hepatotoxic pharmaceutical agents. Superimposed upon these direct chemical insults, the intracellular accumulation of reactive oxygen species — including superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide (H_2O_2) — imposes a state of sustained oxidative stress within hepatocytes, overwhelming endogenous antioxidant defense systems comprising superoxide dismutase, catalase, and glutathione peroxidase. A particularly consequential downstream manifestation of this oxidative milieu is the peroxidative degradation of polyunsaturated fatty acid residues embedded within hepatocellular membrane phospholipid bilayers — a pathological cascade designated lipid peroxidation — which has been mechanistically implicated as a cardinal mediator of ethanol-associated hepatic parenchymal injury, culminating in progressive membrane disruption, cytosolic enzyme leakage, and functional hepatocellular compromise (Babu, 2001).

The liver thus embodies a dual physiological identity: simultaneously an indispensable biosynthetic and metabolic hub and a frontline biological sentinel against chemically and microbiologically derived systemic threats. Notwithstanding its extraordinary intrinsic regenerative competence — mediated through hepatocyte proliferation, stellate cell activation, and growth factor-driven tissue remodeling — the organ's sustained exposure to cumulative hepatotoxic insults progressively erodes its

functional reserve, rendering it susceptible to irreversible parenchymal damage under conditions of protracted or severe injury. Hepatic pathology in its various clinical manifestations — spanning acute hepatitis, chronic fibrosis, cirrhotic architectural distortion, and fulminant hepatic failure — constitutes a disease burden of substantial global magnitude, with mortality attributable to liver-related disorders representing a disproportionate and largely preventable fraction of worldwide annual fatalities. The epidemiological imperative this imposes upon biomedical research is unambiguous: the identification and clinical translation of efficacious hepatoprotective and hepatorestorative interventions represents a priority of considerable urgency within contemporary hepatology and pharmaceutical sciences (Sharma & Mohammed, 2000; Venkateswaran et al., 1997).

1.9 Anticancer properties

Cancer represents a major global health challenge and comprises more than 100 distinct disease types characterized by uncontrolled cell proliferation and the ability to invade surrounding tissues. Despite advancements in diagnosis and treatment, cancer remains one of the leading causes of mortality worldwide. The limitations associated with currently available therapeutic strategies, including the high cost of chemotherapeutic drugs, limited treatment efficacy in advanced stages, and the occurrence of severe adverse effects, contribute significantly to cancer-related mortality. Among the various mechanisms regulating cell survival, apoptosis, or programmed cell death, is recognized as one of the most important pathways involved in eliminating damaged or abnormal cells (George et al., 2010; Sengupta et al., 2004).

Unlike necrosis, which is typically associated with inflammation and extensive tissue injury due to the uncontrolled release of intracellular components, apoptosis is a highly regulated process that minimizes damage to surrounding tissues. This controlled form of cell death involves characteristic biochemical and morphological changes, including chromatin condensation, DNA fragmentation, and membrane blebbing (Earnshaw, 1995; Fierer et al., 2004). Mitochondria play a central role in the intrinsic pathway of apoptosis. Alterations in mitochondrial membrane integrity result in the release of pro-apoptotic factors, particularly cytochrome c, into the cytoplasm. Released cytochrome c interacts with apoptotic protease activating factor-1 (Apaf-1) and procaspase-9 to form a multiprotein complex known as the apoptosome, which subsequently activates caspase-9. This activation initiates a cascade leading to the

activation of caspase-3, an executioner caspase responsible for the cleavage of cellular proteins such as poly (ADP-ribose) polymerase (PARP) and various endonucleases, ultimately resulting in DNA fragmentation and apoptotic cell death (Cai et al., 1998; Green & Kroemer, 1998).

Natural products have attracted considerable attention as potential anticancer agents due to their ability to selectively induce apoptosis in malignant cells while exerting minimal effects on normal tissues. Several florae-derived compounds have demonstrated significant cytotoxic and pro-apoptotic activities against a wide range of cancer cell lines (Chiao et al., 1995; Hirano et al., 1995; Jiang et al., 1996; Shaikh et al., 2011). Many conventional anticancer therapies, including ionizing radiation and chemotherapeutic agents such as cisplatin and camptothecin, exert their therapeutic effects primarily through the induction of apoptosis (Radford et al., 1994). Camptothecin, a quinoline-based alkaloid originally isolated from the Chinese tree Camptotheca acuminata, has demonstrated broad-spectrum antitumor activity and has been used in the treatment of several malignancies. Owing to the limitations of synthetic anticancer drugs, there is growing interest in identifying natural compounds with potent anticancer potential. It has been estimated that more than half of currently available anticancer drugs are either derived directly from floraes or developed based on florae-derived molecules (Newman et al., 2003; Nipun et al., 2011).

Furthermore, modern experimental approaches such as cell viability assays and apoptosis detection techniques enable researchers to evaluate cytotoxic mechanisms and characterize pathways of programmed cell death. The combination of these analytical methods in multiplex studies provides valuable insights into the molecular events associated with anticancer activity (Dive et al., 1992).

1.10 Reasons why herbal remedies are becoming more and more popular

The increasing global acceptance of herbal and alternative therapies can be attributed to several contributing factors. One major reason is the desire of patients to take a more active role in managing their own health. Individuals suffering from chronic or life-threatening illnesses often experience a sense of loss of control over their treatment decisions, which encourages them to explore complementary and alternative medical systems that allow greater participation in healthcare choices (Canter & Ernst,

2004). This sense of involvement is believed to enhance patient confidence and satisfaction with treatment outcomes.

Another important factor supporting the use of alternative therapies is the widespread belief that many traditional healing systems emphasize the integration of mind–body interactions. Such systems are thought to address not only the physical manifestations of disease but also psychological and emotional well-being. This holistic approach to healthcare has contributed to the popularity of herbal medicine and complementary therapies, particularly among individuals seeking treatments that align with natural healing principles (Loya et al., 2009).

In addition to these factors, herbal remedies are often perceived as safer and more affordable alternatives to synthetic pharmaceuticals. Many patients believe that florae-based medicines produce fewer adverse effects compared to conventional drugs, especially during long-term therapy. Furthermore, the accessibility of herbal products, particularly in developing countries, has played a crucial role in their widespread use. Cultural traditions, historical acceptance of remedial floraes, and the availability of locally sourced herbal materials have further strengthened public confidence in herbal-based treatments. Consequently, the continued interest in herbal medicine has stimulated extensive scientific research aimed at validating the therapeutic potential and safety of florae-derived compounds.

1.11 The need of researching different floraes for their medical value

India is recognized as one of the world's major centers of biological diversity and is included among the twelve megadiverse countries, possessing an exceptionally rich variety of florae species. The country is estimated to harbor approximately 45,000 florae species, of which nearly 15,000–18,000 are flowering floraes. A significant proportion of India's geographical area comprises diverse ecological regions that support a wide range of flora and fauna with potential remedial value. Traditional systems of medicine such as Ayurveda, Siddha, and Unani have extensively utilized florae-based materials for therapeutic purposes over several centuries. Historical literature and classical remedial texts document nearly 1,500 florae species with recognized remedial importance, while approximately 800 species continue to be widely used in various traditional healthcare practices (Rawat et al., 2015).

Despite the extensive traditional use of remedialfloraes, a large number of these

species remain scientifically underexplored. Many floraes with documented ethnoremedial applications have not yet undergone systematic phytochemical, pharmacological, and toxicological investigations to validate their therapeutic potential. Scientific evaluation is necessary to identify bioactive constituents, isolate active compounds, and develop novel therapeutic agents or lead molecules for modern drug discovery. In recent decades, there has been increasing interest in natural product research due to the growing demand for safer and more effective therapeutic alternatives. Natural products derived from remedialfloraes have played a crucial role in the development of numerous modern pharmaceuticals, emphasizing the importance of ethnopharmacological knowledge in drug discovery programs (Carter, 2011; Newman & Cragg, 2012).

Therefore, the present study focuses on the investigation of selected remedialfloraes with the objective of scientifically validating their traditional uses and exploring their potential as sources of biologically active compounds. Such research contributes to bridging the gap between traditional knowledge and modern pharmacological applications, thereby supporting the development of novel therapeutic agents derived from natural sources.

CHAPTER-2

LITERATURE REVIEW

2.1 Introduction

Numerous labs worldwide are screening floraes from a pharmacological and toxicological standpoint. The following floraes are selected for in-depth examination and research of their phytochemical and pharmacological characteristics in light of the previously provided information. The author provides a completed and documented literature review in this chapter.

2.2 Literature review and florae profile of the selected florae

A. C. (Roxb.) Sweet

2.2A Literature review of *Argyreiacymosa*

To gather pharmacological, phytochemical, and ethnomedical data on *A. C.* (Roxb.), a thorough study of the literature was conducted. Sweet and related species. The gathered information from this survey is shown below.

Argyreia species' ethnopharmacological profile

Argyreia traditional indigenous medical systems, a number of species of the genus Argyreia have been used to cure a variety of illnesses. Among the ethnopharmacological applications that have been identified are:

- Traditionally, ocular drops made from the fresh juice taken from the roots have been used (Quattrocchi, 2016).
- Urinary symptoms, such as painful or profuse micturition, have been treated using root decoctions made with milk (Quattrocchi, 2016).
- Crushed leaves have been administered topically to wounds and cuts to promote healing (Quattrocchi, 2016).
- Traditional medicine has advised powdered roots combined with milk to treat sexually transmitted infections (Khare, 2004).
- According to Khare (2004), pulp powder has been suggested as a therapy for filarial infections.

• Root decoctions have long been used to treat diarrhoea and for purification, while young vines mixed with ginger rhizome have been administered topically to lower fever (Gurib-Fakim et al., 1993).

A. C. pharmacological investigations

According to the literature, *A. C.* has only been the subject of a small number of pharmacological studies. On the other hand, a number of additional species in the genus have received scientific validation for a range of pharmacological properties. Table 2.1 provides a summary of these results.

Table2.1Pharmacological information of *A. C. and other* species

Plant	Plant Part	Solvent	Uses	References
Argyreia cymosa	Leaves	Water	Antibacterial activity	Packialakshmi and Beevi,2014
	Bark	Petroleum ether Ethylacetate	Antioxidant activity	Badami,et al.,2008
Argyreia speciosa	Leaf,roots, and flower	Ethanol	Aphrodisiac activity	Subramoniam, et al.,2007
	Root	Ethanol	Immunomodulatory activity	Gokhale, et al.,2003
	Root	Ethanol and ethyl acetate	Hepatoprotective activity	Habbu, et.,2008
	Root	N-hexane, chloroform, ethyl acetate, andwater	Central nervous system activity	Galani and Patel,2009
	Stem	Methanol	Hypoglycemic	Latha,etal.,2008
	Whole Plant	Water	Nootropic	Hanumanthachar, et al.,2007
	Root	Ethanol	Anti-inflammatory activity	Srivastava, et.,1972
	Root	Methanol	Analgesic activity	Bachhavetal.,2009
	Leaves	Ethanol	Antibacterial activity	Mishra and Chaturvedi,1978
	Leaves	Water and ethanol	Nematicidal activity	Praveen,et al.,1990

Phyto constituents Information of *Argyreia cymosa*

As per literature survey, till date, no phytochemical has been isolated from *A. C.,* but many chemical constituents are widely isolated from the other species of *Argyreia* were illustrated in Table 2.2

Table2.2 List of isolated phyto constituents from various species of *Argyreia*

Plant	Compound Name	Part of the Plant	Reference
Argyreia speciosa	Tricontanol, Epifriedelinolacetate, Epifriedelinol and β-sitosterol	Leaves	Sahu and Chakravarti,1971
	Quercetin and Kaempferol, Kaempferol-3-o-l-rhamnopyranoside	Leaves	Sohrabetal.,1992
	Tetradecanypalmitatete,5,8-oxidotetracosan-10-onestigmasterolyl, Hydroxycinnamide hydroxycinnamicylyl	Root	Rani and Shukla, 1997; Srivastava and Shukla.,1998
	Ergometrine, ethyl caffeate, glutamic acid, glycine, isoleucine, leucine, lysine, phenylalanine, pyrosine, praline, and α-aminobutyric acid	Seeds	Agrawal and Rastogi,1974;Nair,1987; Jaiswal et al,1984
	N-triacontanol, βsitosterol, hydroxycinnamic, loctadecanolate,and caffeic acid	Fruits	Purushothaman,1982
Argyreia nervosa	Pennidavine, Caffeic Acid,Ergoline, Other Alkaloids,chanoclavine-I, Chanoclavine-II,Festuclavine, Lysergine, Lysergol,Isolysergol,Penniclavine, Steoclavine, Isosetoclavine,Lysergic acid, and Alphahydroxyethylamide	Seeds	Kala,2005.

Florae profile of *A. C.*(Roxb.)Sweet



Figure 2.1: *Argyreia cymosa*(Roxb.) Sweet

2.2B Florae profile of *A. C.*(Roxb.) Sweet

Argyreia cymosa (Roxb.) Sweet

Vernacular Names: The florae is available in different places in India and with different names, as mentioned below

Telugu:	Madditige, Gantapala
Tamil:	Nilanarai
Malayalam:	Nilanarakam
English:	ClusteredWoodrose

Scientific classification: According to the taxonomy of these florae, the scientific classification is given below

Kingdom	Floraeae
Sub-kingdom	Angiosperms
Class	Eudicots
Order	Solanales
Family	Convolvulaceae
Tribe	Ipomoeeae
Genus	Argyreia

Species *cymosa*

Botanical description

Argyreia cymosa is a member of the family Convolvulaceae. The Convolvulaceae family has over 1650 exquisitely attractive species. A. cymosa, one of the more than 135 species in the class Argyreia, is a stray in thickets; its stem is terete, pubescent, and woody; its leaves are oblong to cordiform, 6-8 X 4-6 cm, chartaceous, with minor pubescence on different sides; its blooms are pinkish in axillary, corymbose cymes; its natural product is globose and glabrous, measuring 1.7 x 1.4 cm. (Pullaiah, 2000; Matthew, 1995).

Various species of the genus Argyreia: Some of the most well-known species of Argyreia are found around the world in a variety of climate zones.

Argyreia capitiformis	*Argyreia mollis*
Argyreia cheliensis	*Argyreia monglaensis*
Argyreia eriocephala	*Argyreia monosperma*
Argyreia formosana	*Argyreia nervosa*
Argyreia fulvocymosa	*Argyreia osyrensis*
Argyreia fulvovillosa	*Argyreia pierreana*
Argyreia henryi	*Argyreia roxburghii*
Argyreia lineariloba	*Argyreia splendens*
Argyreia marlipoensis	*Argyreia strigillosa*
Argyreia mastersii	*Argyreia velutina*
Argyreia maymyo	*Argyreia wallichii*

2.3 Literature review and florae profile of the selected florae,*L. R.*

2.3A Literature review and florae profile of the selected florae Limnophila repens

The following details are from a thorough review of the literature on the phytochemical, pharmacological, and ethnomedical components of Liminophila repens and other species.

Limnophila repens' ethanomedical development

For many years, various species of the genus Limnophila have been used in traditional indigenous medicine to treat a wide range of illnesses. Below is a summary of several species' documented ethnopharmacological usage.

• The leaves of Limnophila erecta have historically been used to cleanse wounds and to make a decoction that is used to treat fever (Quattrocchi, 2016).

• The whole florae and cooked leaves of Limnophila indica have been used as a brain tonic. The whole florae has been used to cure pustular skin eruptions, liniments have been used to treat elephantiasis, and the expressed juice has been administered topically to treat high fevers. Furthermore, leaf infusions are said to have antibacterial and carminative qualities and have been used to treat skin conditions, diarrhoea, and indigestion (Quattrocchi, 2016).

• Traditionally, the liquid made from boiled Limnophila rugosa leaves has been used to treat hemorrhagic ulcers and as a laxative and stomachic (Quattrocchi, 2016).

• Traditionally, people have rubbed Limnophila rugosa's aerial portions on their feet. Steam baths and leaf decoctions have been used to lower fever and ease irritated eyes. Additionally, impotence and moderate gonorrhoea have historically been treated using a decoction made from Limnophila rugosa and Ocimum basilicum L. (Quattrocchi, 2016).

• It has been found that Limnophila sessiliflora leaf extracts exhibit antipyretic properties (Quattrocchi, 2016).

• The whole florae has been employed as an aperient in inflammatory circumstances, and the leaves of Limnophila villosa have been utilised as febrifuges (Quattrocchi, 2016).

Details on the pharmacology of Limnophila species

According to the review of the literature, no pharmacological research on *L. R.* has been published. Nonetheless, a number of additional species in the genus have been scientifically assessed and shown to exhibit a variety of pharmacological properties; they are included in Table 2.3.

Table2.3 Pharmacological activities of different species of *Limnophila*

Plant	Plant Part	Solvent	Uses	References
Limnophila conferata	Aerial	Ethanol	Anthelmintic activity	Reddy,et.,1991
			Antimicrobial activity	
			Anti-inflammatory activity	
			Wound Healing Activity	
			Anti-tubercular activity	
			Cytotoxicity activity	
Limnophila aromatica	Leaves	Methanol	Antimicrobial activity, antioxidant activity	Nanasombat and Teckchuen, 2009
	Whole Plant	Water	Vascular protective activity	Kukongviriyapan, etal.,2007
Limnophila heterophylla	Whole Plant	Ethanol	Antibacterial and Antifungal activity	Padiya,etal.,2013
	Roots	Methanol	Antioxidant activity	Sundararajan and Koduru,2016
Limnophila geoffrayi	Aerial	Water	Antimicrobial,antifungal ,and insecticidal activities	Thongdon and Inprakhon,2009

Details on the phytochemical composition of Limnophila species

According to the literature review, several phytochemical compounds were extensively extracted from the other Liminophila species, as shown in Table 2.4, but none were obtained from Liminophila repens.

Table2.4 List of isolated phyto constituents from various species of *Liminophila*

Plant name	Compound Name	Part of the Plant	References
Limnophila rugosa	5-Hydroxy-6,7,4'-trimethoxyflavone, 5-Hydroxy-7,2',4'-trimethoxyflavone, 5-Hydroxy-7,8,2',4'-tetramethoxyflavone, p-Methoxybenzoic acid, Anisaldehyde, Valeric acid, Methylchavicol, Betulin, Betulinic acid, Linalool, Ursolic acid	Aerial parts and roots	Brahmachari, 2008
Limnophila indica	5,2'-Dihydroxy-8,3',4'-trimethoxyflavone, 5,7,2',5'-Tetramethoxyflavone	Aerial parts and roots	Brahmachari, 2008
Limnophila heterophylla	Ursolic acid, α-Pinene, 5-Hydroxy-7,8,2',4'-tetramethoxyflavone	Aerial parts and roots	Jash & Brahmachari, 2014
Limnophila conferta	Thymol	Essential oil (from whole plant)	Reddy et al., 1991

2.3B Florae profile of *Limnophila repens*



Figure2.2 *Limnophila repens* (Benth.)Benth

Botanical name

L. R. (Benth.) Benth., an aquatic and semi-aquatic herbaceous species assignable to the family Floraeaginaceae (previously circumscribed within Scrophulariaceae prior to molecular phylogenetic reclassification), constitutes a botanically and ethnopharmacologically significant taxon distributed across the wetland ecosystems, marshy margins, and flooded agricultural terrains of the Bhartiye subcontinent. The species is recognized under a plurality of regional vernacular designations reflective of its broad phytogeographic presence across linguistically diverse Bhartiye states: it is referred to as Burada Bakkena in Telugu-speaking communities of Andhra Pradesh and Telangana, as Amaragandhi within the Kannada-speaking regions of Karnataka, and as Mangannari in the Malayalam-speaking populace of Kerala, while classical Sanskrit botanical literature records it under the designation Malayalamagandha; the accepted English vernacular denomination is Marsh Woodwort. Across these geographically and culturally distinct regions, the florae have been incorporated into indigenous healing traditions and occupies a documented position within the ethnoremedial pharmacopoeia of traditional practitioners, warranting systematic phytochemical and pharmacological investigation of its constituent secondary metabolites and their associated bioactivities.

Scientific classification: The taxonomic positioning of *L. R.* (Benth.) Benth. within the hierarchical classification framework of the florae kingdom reflects its phylogenetic affinities as determined by both classical morphological criteria and contemporary molecular systematic analyses. Assignable to the kingdom Floraeae and the division Tracheophyta — encompassing all vascular land floraes characterized by the possession of specialized water- and nutrient-conducting xylem and phloem tissues — the species belongs to the subdivision Angiospermae, denoting its status as a flowering flora whose seeds are enclosed within a mature ovary wall. Within the angiosperm radiation, L. repens is classified under the class Magnoliopsida, a taxon broadly corresponding to the eudicotyledonous lineage distinguished by trinucleate pollen, tricolpate apertures, and characteristic net-veined foliar architecture. At the ordinal level, the species is accommodated within Lamiales — a speciose and morphologically diverse clade comprising economically and remedially significant families including Lamiaceae, Oleaceae, and Floraeaginaceae. Its family assignment to Floraeaginaceae, as recognized under the Angiosperm Phylogeny Group IV (APG IV) classification, supersedes the earlier circumscription within Scrophulariaceae, which

molecular phylogenetic investigations demonstrated to be polyphyletic; the genus Limnophila R. Br. — encompassing aquatic and semi-aquatic herbaceous taxa distributed primarily across tropical and subtropical Asia — accommodates the species epithet repens, with the complete and correctly formatted binomial designation being *L. R.* (Benth.) Benth., wherein the parenthetical citation acknowledges the original basionym authority and the unparenthesized citation records the author responsible for the subsequent combinatorial transfer.

Botanical description

L. R. (Benth.) Benth. — recognized in the synonymic literature under the alternate designation Limnophila conferta Benth. — is an aromatic herbaceous species exhibiting a growth habit ranging from erect to procumbent, with all vegetative organs permeated by a characteristically pungent volatile fragrance attributable to the presence of essential oil-secreting glandular structures distributed across the florae's surface. The foliage is composed of elliptic-ovate leaves measuring 1–2 cm in length and 0.4–0.9 cm in breadth, characterized by an acute to shortly acuminate apical terminus, a crenate-serrate marginal dentition, and a cuneate to attenuate basal configuration; the laminar texture is chartaceous, the venation pattern is pinninerved, the adaxial and abaxial surfaces are glabrous, and the petiolar development is markedly abbreviated, rendering the leaves subsessile in their attachment to the stem. The inflorescence architecture consists of axillary, solitary or condensed racemose floral arrangements of limited elongation. The calyx is lanceolate in outline, externally hirsute, and prominently striate, bearing five lobes of approximately 4 mm in length. The corolla, which may present as violet-pink or, less frequently, as yellow, comprises a pubescent tubular structure measuring approximately 5 mm in diameter and 6–8 mm in total length, with the external surface of the tube and the corolla throat adorned with longitudinal purple-pigmented striations that serve as putative pollinator guidance markings. The androecium consists of four stamens arranged in a didynamous configuration — two of greater and two of lesser length — a floral character consistent with the general stamineal organization of Floraeaginaceae. The gynoecium bears an ellipsoidal ovary measuring 3–4 mm in length. Seeds are minute, numerous, and characterized by a reticulate surface ornamentation. Phenologically, the species expresses reproductive activity — encompassing both anthesis and fruiting — across the period extending from July through December. The genus Limnophila accommodates approximately fifteen

documented species, the taxonomic circumscription and synonymic relationships of which have been comprehensively surveyed in the botanical literature (Chetty et al., 2008; Quattrocchi, 2016).

Different species of the genus Limnophila: Species belonging to the genus Limnophila are extensively distributed in diverse environmental conditions throughout several regions of the world. Members of this genus are mainly encountered in warm and humid habitats and are recognized for their biological and therapeutic significance. Important representatives include Limnophila aromatica, Limnophila indica, Limnophila rugosa, Limnophila sessiliflora, and *L. R.*

Limnophila dasyantha

Limnophila erecta

Limnophila heterophylla

Limnophila indica

Limnophila rugosa

Limnophila sessiliflora

Limnophila tenera

Limnophila aromatic

Limnophila bangweolensis

Limnophila barteri

Limnophila borealis

Limnophila ceratophyl

Limnophila connate

2.4 Literature review and florae profile of *C. B. DC.*

2.4A Literature review of *C. B. DC.*

An in-depth evaluation of available scientific literature was undertaken to gather relevant information concerning the ethnopharmacological relevance, phytochemical composition, and reported biological activities of *C. B.* DC., as well as other associated species of the genus. The summarized findings are presented in the following section.

Ethanomedical information of *C. B. DC.*

Except for *C. B.*, species within the genus Capparis have been extensively utilized in traditional medical systems for the treatment of diverse pathological conditions over several millennia. Ethnohistorical evidence from Bhartiye remedial literature suggests that the therapeutic use of Capparis species dates back to ancient

civilizations, with the earliest recorded application attributed to the Sumerians around 2000 BC (Romeo et al., 2007). Various florae parts, including fruits, flowers, roots, and root bark, have been traditionally employed and are reported to possess a wide range of bioactivities, such as anti-atherosclerotic, antihypertensive, antibacterial, anti-inflammatory, analgesic, anti-asthmatic, hypolipidemic, and antifungal properties (Chahlia, 2009; Duman et al., 2013). In addition to their remedial significance, extracts obtained from caper buds and fully matured fruits are widely used as flavoring agents in the culinary industry, underscoring their nutraceutical importance (Farrel, 1998). Certain Capparis species have also been incorporated into homeopathic formulations, particularly through the use of bark preparations (Hassan and Mohammed, 2010). Moreover, phytochemical and nutritional evaluations indicate that floral buds and fruits of Capparis species are rich in essential minerals and macronutrients, including proteins, carbohydrates, lipids, and vitamins, which supports their widespread consumption as vegetables and in pickled forms (Ozcan, 2005). Traditional medical practices in India and Pakistan further recognize caper fruits for their antidiabetic potential and mild laxative effects, leading to their routine inclusion in dietary preparations such as curries and pickles (Vaidya, 1995; Yadav et al., 1997).

Pharmacological information of *C. B. and other species*

The literature survey indicates that only limited pharmacological activities have been reported for *L. R*. In contrast, multiple pharmacological activities have been scientifically validated for other species within the genus, as summarized in

Table 2.5.**Table2.5 Pharmacological information of *C. B.* DC.**

Plant	**Plant Part**	**Solvent**	**Use**	**References**
Capparis brevispina	Stem bark	Ethanol	Hepatotoxicity in Wistar rats	Anju et al., 2017
Capparis brevispina	Leaves	Hexane	Antioxidant and cytotoxic	Subramanian and Ramani,2020
Capparis spinosa	Fruits	Water	Anti-inflammatory activity	Azhary etal.,2017
Capparis spinosa	Aerial	Water	hypolipidemic	Eddouks et al., 2005
Capparis spinosa	Bud	Water	Anti oxidant	Mansour et al., 2006
Capparis decidua	Leaf	Powder	Anti diabetic	Yadav, 1997
Capparis decidua	Stems	Aqueous and Methanol	Hepato protective	Ali, 2009
Capparis decidua	Stems	Acetone	Antibacterial potential	Gull et al., 2015
Capparis zeylanica Linn.	Leaves	Methanol	Immuno stimulant effect	Ghule et al., 2006

Phytochemical constituents' information of the *Capparis species*

According to a review of the literature, only a few phytochemicals have been recovered from Capparis brevespina so far, although numerous phytochemical elements have been extensively isolated from the other Capparis species, as shown in Table 2.6.

Table2.6List of Phyto chemical constituents of *Capparis species*.

Florae name	Compound	Part of florae	References
Capparis spinosa	Rutin, quercetin3-o-glucoside	Aerial	Sharafet al.,2000
	Quercetin3-O-glucoside-7-O-rhamnoside		
	Quercetin3-O-[6‴-α-l-rhamnosyl-6″-β-d-glucosyl] -β-d-glucoside		
Capparis species	Kaempferol7-rhamnosideandkaempferol3-rutinoside,	Aerial	Tagnaoutetal.,2016
	Kaempferol3,7-dirhamnoside		
	Kaempferol3-glucoside-7-rhamnoside		
	Kaempferol3-rhamnoside-7-glucoside		
	Quercetin7-rhamnosideandquercetin3-rutinoside		
	Quercetin7-rutinosideandquercetin3,7-dirhamnoside		
	Quercetin3-glucoside-7-rhamnoside		
	Isorhamnetin3-rutinoside, Isorhamnetin3,7-dirhamnoside		
	Apigenin6,8-di-c-glucoside		
Capparis spinosa	6s-Hydroxy-3-oxo-α-ionolglucosides	Fruits	Ihsanetal.,2002
	Corchoionosidec((6s,9s)-roseoside)		
	Prenylglucoside		
Capparis spinosa	G–Sitosterolglucoside-6'-octadecanoic	Aerial	Khanfaretal.,2002
	3-Methyl-2-butenyl-g–glucoside		
Capparis moonii	L-Stachydrineand rutin	Fruits	Kanthamanietal.,1960
Capparis decidua	Fatty acids and ecospinois	Leaf	Nazaretal.,2020
Capparis decidua	Capparisinine	Leaf	Ahmad,1987

2.4B Florae profile of the *C.B.* DC.



Figure2.3 *C. B.*DC.

The florae names, localities, and dates of collection are furnished below

Vernacular Names: The floraes available in different places India and with different names as mentioned below.

Telugu	Arudonda
Tamil	Adaanda, Kattukanji
Malayalam	Chedimukanthi
English	Bhartiye Caper

Scientific classification: According to taxonomy of these florae scientific classification was given below.

Kingdom	Planate
Sub-kingdom	Viridifloraeae
Class	Magnoliopsida
Order	Brassicales
Family	Cappareceae
Genus	*Capparis*
Species	*brevispina*

Botanical description

The florae have two unevenly branching stems and is a shrub. The leaves are

thick, coriaceous, and vary in form from elliptical to elongated, with tiny stipular spines. Cataphylls stand upright. Flowers are axillary, solitary, and have a diameter of 4–5 cm. They are carried on pedicels that are about 4 cm long. Two whorls of sepals make up the calyx. Flowers have four round, white petals. The gynandrophore is either the same length as the filaments or somewhat longer. There are several appressed stamens with dorsifixed anthers. The ovary has an elliptical shape with a diameter of around 6 mm.

Different species of the genus *Capparis*: Different species of Capparis were found around the world in different climatic zones, including some of the most well-known spices.

Capparis rotundifolia	*Capparis floribunda*
Capparis decidua	*Capparis stylosa*
Capparis pedunculosa	*Capparis grandis*
Capparis sepiaria	*Capparis zeylanica*
Capparis divaricata	*Capparis bisperma*
Capparis incanescens	*Capparis horrida*

The comprehensive review of the literature shows that there are still few scientific studies on the aforementioned floraes. However, rural and tribal cultures have been using these species for ages to cure a variety of illnesses. Due to their relative safety and reduced frequency of side effects, herbal medications and associated items are becoming more and more popular in modern times. The current work concentrated on a thorough pharmacognostic, phytochemical, and pharmacological assessment of *A. C.*, *L. R.*, and *C. B.* in order to close this gap and provide empirical support.

2.5 Collection, identification and authentication

In November 2022, fresh, disease-free entire floraes of Argyreia cymosa, *L. R.*, and *C. B.*in were collected at the Botanical Garden's Sector 37 in the Noida region of Uttar Pradesh, India. The DOB and Florae Taxonomy at Department of Botany at GLA University, Mathura (U.P.) assisted in identifying the florae.

CHAPER – 3

AIM AND OBJECTIVE

3.1 AIM

The aim of the research was pharmacognostical study, phytochemical exploration, and pharmacological testing of some Bhartiye remedialfloraes with the following objectives

3.2 OBJECTIVE

The study was designed to investigate three selected Bhartiye remedialfloraes—*A. C.* (Roxb.) Sweet, *L. R.* (Benth.) Benth, and *C. B.* DC—based on their ethnoremedial relevance. The specific objectives of the research included:

1. Selection of the remedialfloraes grounded on documented ethnopharmacological evidence.
2. Compilation of a comprehensive botanical description and literature review for *A. C.*, *L. R.*, and *C. B.*.
3. Pharmacognostical profiling of the selected florae species to evaluate their morphological, anatomical, and microscopical characteristics.
4. Standardization of the selected floraes to ensure quality, purity, and consistency.
5. Preliminary phytochemical screening to identify the major classes of bioactive constituents present in each flora.
6. Isolation and structural characterization of phytochemical constituents using advanced spectroscopic techniques, including IR, NMR, and mass spectrometry.
7. Evaluation of the anti-nociceptive activity of *A. C.*, *L. R.*, and *C. B.*.
8. Assessment of anti-inflammatory potential in the selected floraes.
9. Investigation of anti-pyretic effects.
10. Examination of hepatoprotective activity.
11. Exploration of anti-cancer potential in vitro/in vivo models.

This systematic approach was aimed at providing a scientific basis for the traditional uses of these floraes while contributing to the development of potential herbal therapeutics.

CHAPTER – 4

PHARMACOGNOSTICAL EVALUATION

4.1 Introduction

Organoleptic evaluation refers to the assessment of herbal materials using sensory perception through the human sense organs. This method involves the examination of characteristics such as colour, odour, taste, size, shape, surface texture, and other distinctive physical features. These observable attributes provide preliminary information regarding the identity, purity, and quality of florae materials or their extracts. In many cases, remedialfloraes or crude drug samples can be initially recognized based on their characteristic external appearance. When visual features alone are insufficient for identification, additional sensory parameters such as characteristic aroma or taste may aid in distinguishing specific florae materials. Although organoleptic evaluation is considered one of the simplest and most traditional methods of analysis, it remains an essential preliminary step in pharmacognostical investigations (Shah, 2009).

In addition to organoleptic analysis, detailed macroscopic and microscopic examinations play a critical role in the authentication and standardization of remedialfloraes. Comprehensive pharmacognostical evaluation includes the study of morphological characteristics such as leaf arrangement, size, venation pattern, and surface features, along with anatomical characteristics observed through microscopic techniques. Powder microscopy further supports florae identification by revealing diagnostic features such as trichomes, stomata, fibers, vessels, and calcium oxalate crystals. Since limited systematic information is available regarding the detailed macro- and micro-morphological characteristics of certain remedialfloraes, the present investigation was designed to evaluate the pharmacognostical parameters of selected Bhartiye remedial species, namely *A. C.*, *L. R.*, and *C. B.*. Such evaluation provides essential baseline data for the correct identification, authentication, and quality control of these remedialfloraes, thereby supporting their safe and effective therapeutic use.

4.2 Materials and method

4.2.1 Florae material

In this investigation, the dried leaves, stems, and roots of certain remedial floraes of Argyreia cymosa (Roxb.) Sweet, *L. R.* (Benth.) Benth, and *C. B.* DC were assessed

separately.

4.2.2 Chemicals and requirements

Phloroglucinol, concentrated hydrochloric acid (HCl), chloral hydrate solution, and iodine were used as the primary chemicals and reagents in the present investigation. Morphological and microscopical evaluations were performed using precision instruments, including a screw gauge, a calibrated scale, and a sharp blade. Microscopic observations and image documentation were carried out using a binocular Olympus CX21i microscope equipped with a CMOF analogue camera and an AV digitaliser.

4.3 Methods

4.3.1 Macroscopic evaluation

Organoleptic assessment of the florae materials was carried out to record their sensory characteristics using human sense organs. During this evaluation, various physical parameters such as colour, odour, taste, texture, and approximate weight of the leaves were carefully observed and documented. The observations were performed under standard laboratory conditions to ensure consistency in recording the sensory attributes of the florae materials. These characteristics provided preliminary information useful for the identification and quality evaluation of the crude drug samples.

The organoleptic study was conducted following standard pharmacognostical procedures described in established literature. The method involves the systematic examination of florae materials based on sensory perception and has been widely applied for the preliminary evaluation of herbal drugs. The same standardized procedure was applied uniformly to all selected florae materials to maintain accuracy and reproducibility of observations (Khandelwal, 2008; Kokate, 1986).

4.3.2 Microscopic evaluation of selected floraes

Microscopic examination was performed as an essential step for the preliminary identification of florae materials and for detecting possible adulterants or foreign matter present in crude or powdered herbal samples. This technique is particularly useful for identifying small fragments of florae tissues that cannot be distinguished through macroscopic observation alone. Microscopy enables detailed analysis of internal anatomical features, including tissue arrangement, cellular structure, and the nature of cell walls, which serve as important diagnostic characteristics for florae authentication.

For microscopic analysis, thin transverse sections of the selected florae materials were prepared and properly mounted on clean glass slides using suitable staining agents and reagents. Stains such as safranin and fast green were used to enhance the contrast of cellular components, thereby facilitating clear visualization of different tissue types. The prepared slides were examined under a compound microscope, and characteristic features such as epidermal layers, vascular bundles, fibers, trichomes, and other cellular elements were carefully observed and recorded. The same standardized staining and mounting procedures were applied uniformly to all florae samples to ensure consistency and reproducibility of results. The methodology adopted for microscopic examination was based on standard pharmacognostical procedures described in established literature (Khandelwal, 2008; Kokate, 1986).

4.3.2.1 Anatomical studies

Fresh florae materials were carefully excised and immediately fixed in FAA (Formalin–Acetic Acid–Alcohol) solution, consisting of 5 mL formalin, 5 mL glacial acetic acid, and 90 mL of 70% ethanol, to preserve cellular structure and prevent tissue degradation. The specimens were kept in the fixative solution for approximately 48 hours. After fixation, the samples were removed from the preservative, thoroughly washed with distilled water to eliminate excess fixative, and prepared for sectioning according to standard pharmacognostical procedures (Khandelwal, 2008; Kokate, 1986).

Thin transverse sections of the selected florae materials were prepared manually. Small portions of florae tissues were held firmly between the thumb and fingers of the left hand, and fine sections were obtained by carefully moving a sharp blade across the sample. The prepared sections were transferred into a watch glass containing water to prevent drying. The sections were then treated with chloral hydrate solution and gently heated to remove colouring substances and enhance transparency. After clarification, the sections were stained using phloroglucinol and hydrochloric acid (1:1) to detect lignified tissues. The stained sections were mounted in glycerin on clean glass slides and examined under a compound microscope at low and high magnifications.

Representative microscopic features were observed and documented to identify important tissues such as epidermis, vascular bundles, fibers, and other cellular structures. For detailed documentation, photomicrographs of significant tissues and

diagnostic cellular components were captured at different magnifications using an Olympus CX21i binocular microscope equipped with an analogue CMOS camera and AV-digitalizer. Images were recorded using compatible imaging software (Grand VCD 2000+) under bright-field illumination conditions suitable for histological observation. The captured images were stored digitally, and hard copies were prepared for further analysis and documentation. The same standardized procedure was followed for all florae samples to ensure consistency and reliability of the microscopic evaluation.

4.3.2.2 Powder microscopy of selected florae

Shade-dried leaves, stems, and roots of the selected florae materials were finely powdered using a mechanical grinder and passed through sieve No. 60 to obtain a uniform particle size. The sieved powder samples were subjected to detailed powder microscopic analysis to identify characteristic diagnostic features. A small quantity of each powdered sample was placed on a clean glass slide, and a few drops of chloral hydrate solution were added. The mixture was gently heated for 1–2 minutes to clear and render the tissues transparent, as chloral hydrate acts as an effective clearing agent that improves the visibility of cellular structures.

After clearing, the powdered material was treated with phloroglucinol and hydrochloric acid (1:1) to detect the presence of lignified tissues, which appeared as a characteristic pink or reddish coloration under microscopic observation. The cleared powder was then mounted in glycerin and examined under a compound microscope to observe structural elements such as fibers, vessels, trichomes, and other cellular components. For the identification of calcium oxalate crystals, unstained powder samples were mounted in water and examined directly under the microscope. Similarly, starch grains were detected by treating the powdered material with dilute iodine solution, which produced a characteristic blue or bluish-black coloration in the presence of starch.

The same standardized procedure was followed for all powdered florae materials to ensure consistency in observations and reproducibility of results. The methodology adopted for powder microscopy was based on standard pharmacognostical techniques described in established references (Khandelwal, 2008; Kokate, 1986).

4.3.3 Quantitative microscopy of selected floraes

The following characteristics were identified using the usual approach and quantitative microscopical analysis of certain florae material leaves (Evans et al, 2009; Khandelwal,

2008).

4.3.3.1 Determination of stomata number

Stomatal density was determined as the average number of stomata present per square millimetre (mm^2) of the leaf epidermis. Fresh leaf samples were initially cleaned to remove adhering impurities and were subjected to clarification by boiling in chlorinated soda solution or chloral hydrate solution until the tissues became sufficiently transparent. Following clarification, the upper and lower epidermal layers were carefully separated using fine forceps. The isolated epidermal peels were then mounted on clean microscope slides using glycerin water as the mounting medium.

For quantitative analysis, a camera lucida attached to the compound microscope was used in combination with a drawing board to prepare scaled outlines of the microscopic field. A stage micrometer was employed to calibrate the field of view and to accurately delineate an area equivalent to 1 mm^2. The mounted epidermal peel was placed on the microscope stage, and both epidermal cells and stomata were clearly visualized and examined. All stomata located within the defined 1 mm^2 area were counted, including those with at least half of their structure lying within the boundary of the marked region.

The counting procedure was repeated for ten different microscopic fields for each florae sample to ensure reliability of observations. The average number of stomata obtained from these readings was calculated and expressed as stomatal density in terms of number of stomata per mm^2. It is recognized that stomatal density may vary depending on several factors, including leaf maturity, florae age, leaf size, and prevailing environmental conditions. Therefore, uniform sampling conditions were maintained as far as possible throughout the analysis.

4.3.3.2 Determination of stomatal index

The stomatal index represents the proportion of stomata in relation to the total number of epidermal cells present within a defined area of the leaf surface, with each stoma considered as a single epidermal unit. This parameter is widely used in pharmacognostical studies as a reliable diagnostic feature, since it remains relatively constant for a given species and is less affected by external factors compared to stomatal density.

The stomatal index was calculated using the following formula:

$$S.I = \frac{S}{E+S} x\ 100$$

Where;

SI= Stomatal index, S= No. of stomata per unit area

E= No. of epidermal cells in the same unit area.

For the determination of stomatal index, the central portion of fresh leaves was selected and cleared by boiling in chloral hydrate solution or treated with chlorinated soda solution until the tissues became transparent. The upper and lower epidermal layers were carefully separated using fine forceps. The isolated epidermal peels were mounted on clean glass slides using glycerin water as the mounting medium.

A camera lucida attached to the microscope and a drawing board were used to prepare accurate scaled drawings of the microscopic field. A stage micrometer was employed to delineate a square area equivalent to 1 mm². The prepared slide containing the cleaned epidermal peel was placed on the microscope stage, and both stomata and epidermal cells were clearly identified. The number of stomata present within the defined 1 mm² area was counted, along with the number of surrounding epidermal cells in the same field.

The stomatal index was calculated using the above formula for each field of observation. Separate determinations were carried out for both the upper (adaxial) and lower (abaxial) epidermal surfaces. The counting procedure was repeated for multiple microscopic fields, and the mean value was calculated to obtain reliable results. The same standardized method was applied uniformly to all selected florae samples. Unlike stomatal density, the stomatal index is considered relatively stable and less influenced by environmental conditions such as florae age, leaf size, or climatic factors.

4.3.3.3 Determination of vein-islets number

The epidermal layers were separated using forceps after cleaning the leaf tissue with chlorine soda or an equivalent clearing solution. The top and lower epidermis were then placed individually on microscope slides using glycerin water as the mounting solution. To achieve precise scaling, a camera lucida and an illustration table were

utilised, and a stage micrometer was used to outline a square area of 1 mm². The prepared slide containing the cleaned epidermal peel was put on the microscope stage, and both epidermal cells and stomata were recognised and studied.

In each microscopic field, the numbers of epidermal cells and stomata inside the prescribed 1 mm² region were counted. The stomatal index was determined using the previously described method. Measurements for the top and lower epidermis were recorded separately, and the same approach was systematically applied to all florae samples. Unlike stomatal number, the stomatal index is regarded to be essentially stable and is not considerably changed by variables such as florae age, leaf size, or climatic circumstances.

4.3.3.3 Determination of veinlet termination number

The vein termination number is defined as the average number of veinlet terminations present per square millimetre (mm²) of the leaf surface. A veinlet termination refers to the ultimate free end of a small veinlet within the leaf lamina. This parameter was determined from the region located midway between the midrib and the margin of the leaf, where venation patterns are clearly distinguishable.

For the determination of vein termination number, the procedure followed was similar to that used for evaluating the vein islet number, with minor modifications. Selected leaf samples were cleared using chloral hydrate or chlorinated soda solution until the tissues became sufficiently transparent. The cleared leaf segments were then mounted on clean glass slides using glycerin as the mounting medium. The prepared slide was placed on the microscope stage, and a defined area of 1 mm² was marked with the help of a camera lucida and stage micrometer.

Within the marked area, the number of veinlet terminations was carefully counted under appropriate magnification. Only clearly visible terminal vein endings within the defined square were included in the count. The observations were repeated for several microscopic fields to ensure accuracy, and the average number of veinlet terminations per mm² was calculated for each florae sample. The same standardized procedure was applied uniformly to all leaf samples to maintain consistency and reproducibility of results.

4.4 Results

4.4.1 Pharmacognostic study of *Argyreia cymosa* lleaf

Macroscopical and microscopic evaluation performed for *Argyreia cymosa* as per the above standard procedures and the results as follows.

4.4.1.1 Macroscopy of *Argyreia cymosa* leaf

*A. C.*is a straggling climber with a woody, terete, and glabrous stem that grows on tree trunks. The leaves have a coriaceous to somewhat cretaceous texture, are deltoid to cordiform in shape, and have scant pubescence on both sides. They measure around 6-8 × 4-6 cm. With bases truncate to cordate and apices acute to obtuse, the leaf margins are complete. The fruits are globose, and the flowers are pink and placed in axillary cymes.



Figure4.1 ***3 Argyreia cymosa*** **leaf**

4.4.1.2 Organoleptic characters of *Argyreia cymosa* leaf

Organoleptic evaluation of rudimentary preparation *A. C.* leaf was carried out as per standard procedures and results were represented Table 4.1.

Table 4.1: Organoleptic characters of *A. C.* leaf

Organoleptic characters	**Observation**
Colour	Green
Odour	Characteristic
Taste	Characteristic
Texture	Smooth
Fracture	Fibrous

4.4.1.3 Microscopy of *A. C.* leaf

The transverse section of the leaf passing through the midrib region showed a well-defined, single-layered epidermis covered with a thick cuticle. The epidermal cells were rectangular to slightly barrel-shaped, with both the upper and lower surfaces appearing convex. The cells of the upper epidermis were comparatively larger than those of the lower epidermis. Numerous long, multicellular, uniseriate trichomes with pointed apices were observed arising from the epidermal layer, serving as characteristic diagnostic features.

In the midrib region, the transverse section exhibited four to six layers of collenchyma located beneath the upper epidermis and two to three layers beneath the lower epidermis. The collenchyma cells were oval to polygonal in shape and possessed thickened cell walls, providing mechanical support. Beneath the collenchyma, a well-developed parenchymatous ground tissue was present, consisting of loosely arranged cells with small intercellular spaces. The ground tissue cells were generally round, oval, or polygonal in outline and exhibited distinct intercellular spaces.

Several vascular bundles, ranging from eight to twelve in number, were observed embedded within the ground tissue. These vascular bundles were arranged in a continuous arc-like pattern and were of the conjoint, collateral type. The phloem consisted of sieve tubes, companion cells, and phloem parenchyma, whereas the xylem was composed of vessels showing annular thickening, along with tracheids, fibers, and xylem parenchyma.

The laminar region of the leaf showed a typical dorsiventral arrangement. Beneath the upper epidermis, two to three layers of palisade parenchyma were present, consisting of elongated and closely packed cells. Towards the lower epidermis, two to four layers of spongy parenchyma were observed, characterized by loosely arranged cells with prominent intercellular spaces that facilitate gaseous exchange. These anatomical features were clearly visible in the transverse section of the leaf lamina (Fig. 4.2–4.7).

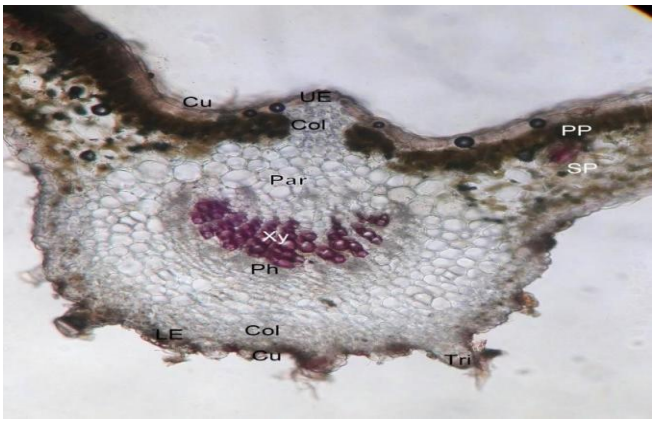


Figure 4.2 T S of midrib section of Argyreiacymosa leaf Cu: Cuticle; UE: Upper epidermis, Col: Collenchyma cells, PP: Palisade Parenchyma, SP: Spongy Parenchyma, Xy: Xylem, Ph: Phloem, Par: Parenchyma cells, Tri: Trichomes, LE: Lower epidermis.

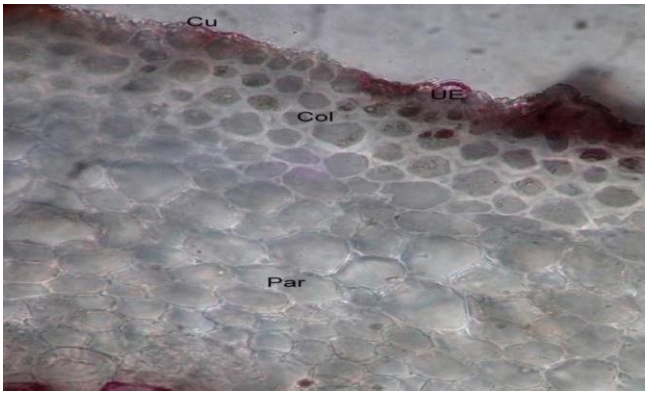


Figure 4.3: In detailed T S of the midrib of the leaf showed upper epidermis, collenchymas and parenchymatous cells Cu:Cuticle, UE:Upper Epidermis, Col: Collenchyma cells, Par: Parenchyma.

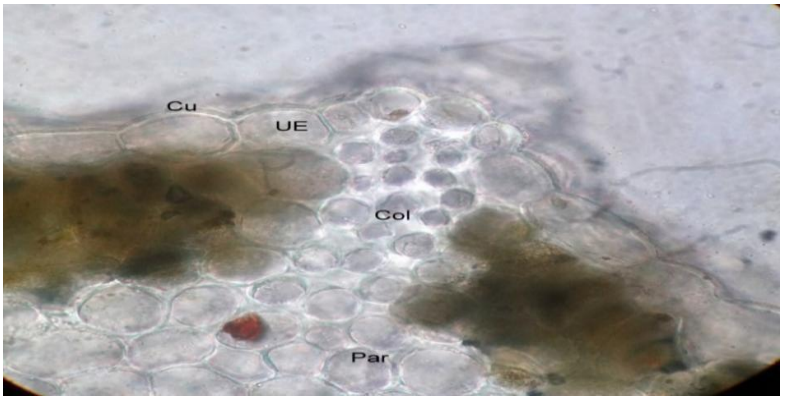


Figure 4.4 Detailed T S of the midrib of the leaf showed upper epidermis, collenchymas and parenchymatous cells. Cu:Cuticle, UE:Upper Epidermis, Col: Collenchyma cells; Par: Parenchyma.

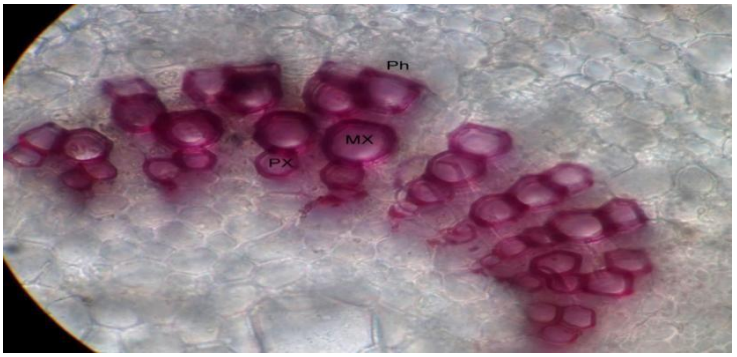


Figure4.5 TS of midrib portion of *A. C.* leaf showed vascular bundles Ph: Phloem, MX: Metaxylem, PX: Protoxylem.

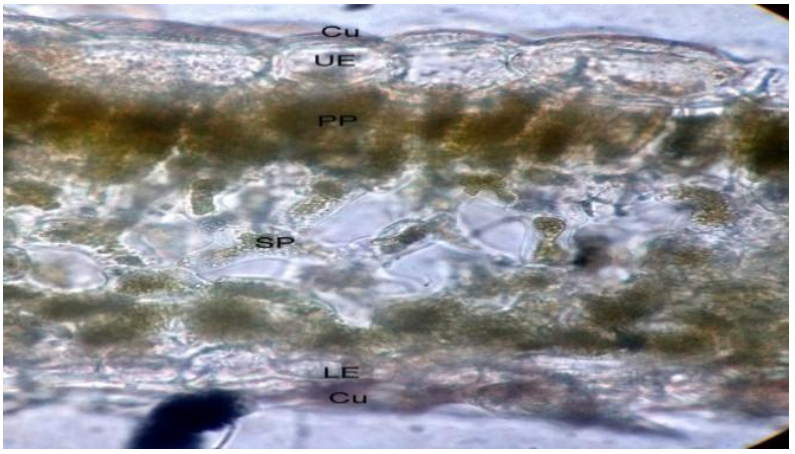


Figure 4.6 T S of the lamina of *A. C.* leaf Cu: Cuticle, UP: Upper Epidermis, PP:Palisade Parenchyma cells, SP:Spongy Parenchyma cells, LE: Lower epidermis.

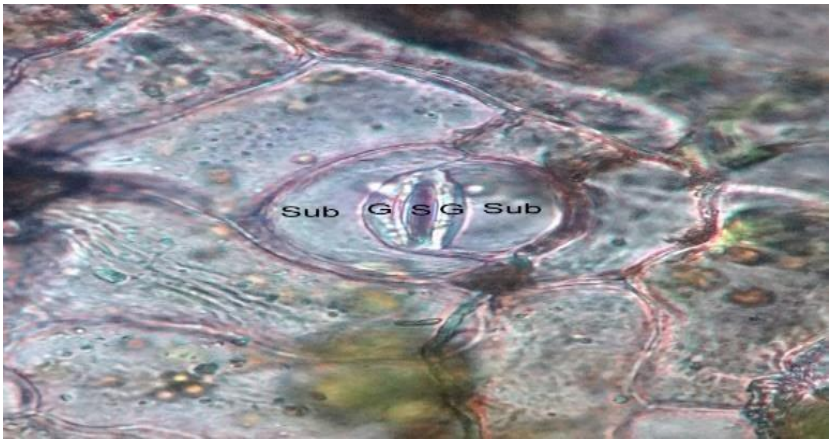


Figure 4.7 Epidermal cells contain paracytic stomata. G:Guard cells, S: Stomata, Sub:Subsidiary cells.

4.4.1. Quantitative microscopy of *A. C.* leaf

Quantitative microscopy of crude drug *A. C.* was carried out as per procedures

and results were represented in the table 4.2.

Table 4.2 Quantitative microscopy of *A. C.* leaf

S.No.	Parameter	Value (per mm²)
1	Stomatal Number of Upper Surface	16.52 ± 2.85
2	Stomatal Number Lower Surface	25.22 ± 4.88
3	Stomatal Index Upper Epidermis	33.23 ± 3.68
4	Stomatal Index — Lower Epidermis	46.78 ± 4.36
5	Vein Islet Number	15 ± 3.0
6	Vein Termination Number	21 ± 3.2

4.4.1.5 Powder microscopy of *A. C.* leaf

Upon macroscopic preliminary assessment, the finely comminuted botanical powder displayed a characteristic greenish hue, attributable to the persistence of chloroplast-derived pigmentary constituents within the structurally degraded cellular matrix following desiccation processing. Systematic powder microscopy conducted under brightfield illumination yielded a morphologically diverse spectrum of cellular and tissue-derived fragments, each carrying distinct taxonomic and pharmacognostic interpretive value. Among the identifiable components, remnants of thin-walled, isodiametric parenchymatous cells were encountered with notable frequency, their preserved cytoplasmic boundaries serving as architectural indicators of the parent organ's ground tissue system. Interspersed within the powder matrix, secondarily thickened vascular elements of xylem origin — distinguished by annular, spiral, and reticulate wall patterning consistent with lignin deposition — provided unambiguous evidence of vascular tissue differentiation within the source florae. Elongated, columnar palisade mesophyll cells, recognizable by their axially oriented arrangement and densely granular cytoplasmic contents, were likewise encountered, pointing toward foliar tissue as a probable anatomical origin of the specimen. Scattered fragments of the adaxial and abaxial epidermal layers were further resolved, characterized by their flattened periclinal walls, defined anticlinal boundaries, and partially preserved cuticular overlays. Commanding particular diagnostic prominence among all observed features was the recurrent identification of paracytic-type stomatal complexes — a

configuration in which bilateral subsidiary cells assume a positional relationship parallel to the longitudinal axis of the paired guard cells — a stomatal arrangement extensively documented as a dependable anatomical marker in the pharmacognostic characterization and botanical authentication of herbal source materials. Composite photomicrographic documentation of all aforementioned microscopic elements is systematically presented in Figure 4.8.

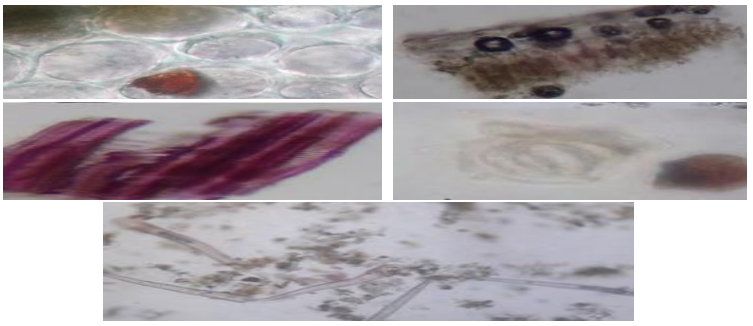


Figure4.8 Powder microscopy of the *A. C.*leaf showed the presence of
(A)Parenchyma cells (B)Palisade Parenchyma (C)Lignified XylemVessels (D) Stomata and (E) Covering Trichomes.

4.4.2 Pharmacognostical evaluation of *A. C.*stem

4.4.2.1 Macroscopy of *A. C.*stem

The stem was woody, terete, and pubescent in nature; the stem has a characteristic odour and taste. (Fig 4.9 and Table 4.3)



Figure4.9*Argyreia cymosa* stem

4.4.2.2 Organoleptic evaluation of *A. C.*stem

Table 4.3 Organoleptic characters of *A. C.*stem

Organoleptic characters	Observation
Colour	Green
Odour	Characteristic
Taste	Characteristic
Texture	Smooth
Fracture	Fibrous

4.4.2.2 Microscopy *A. C.* stem

A cross-over section of the stem showed barrel-shaped cells. There were no intercellular gaps in the loosely arranged, trichome-covered epidermis. The hypodermis was composed of four to five layers of collenchyma cells, which offered extra protection and support. It was followed by sclerenchymatous cells arranged in one or two layers. The vascular system was composed of open, insurance, and joint groups. Xylem was found in the persistent ring, which is composed of filaments, vessels, and xylem parenchyma. Parenchyma, strands, friend cells, and strainer cells make up phloem. There are unseriate medullary beams accessible. Parenchymatous cells are active in the focal area, as seen in the figures.4.13–4.14.

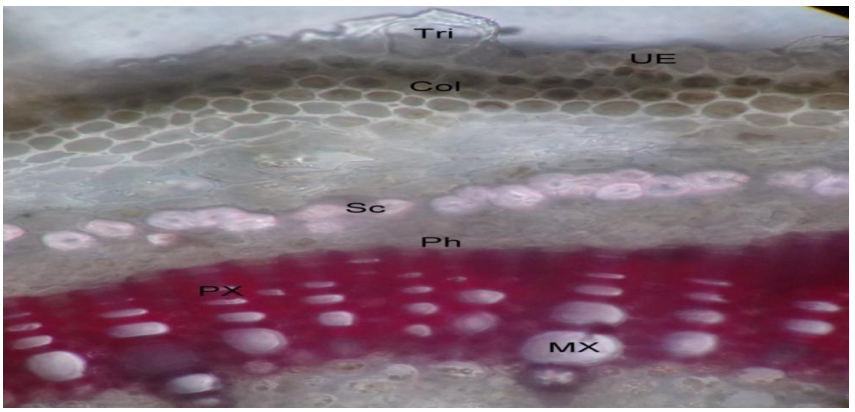


Figure 4.10 T. S of the stem of *A. cymosa.* Tri: trichome, UE:Upper epidermis, Col:collenchyma, Sc:scleroidal cells, Xy:xylem, Ph:phloem, PX:ProtoXylem and MX: MetaXylem.

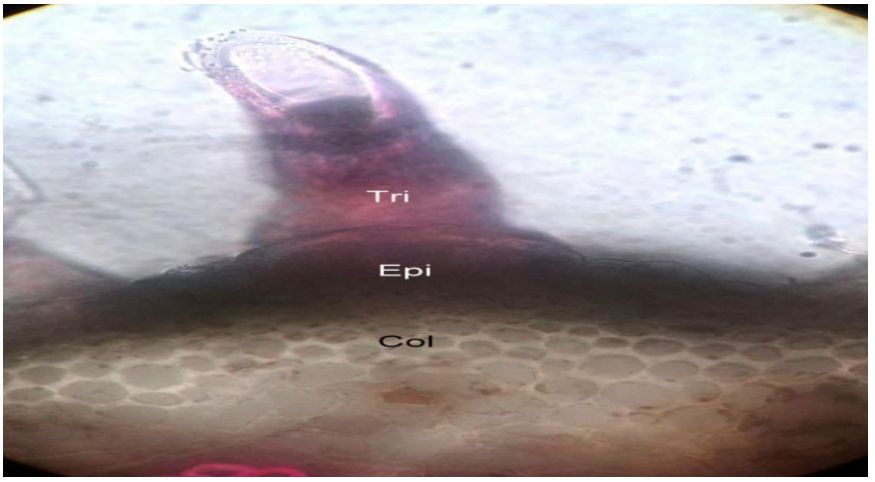


Figure4.11T.S of stem of *A.cymosa* showed Trichomes, epidermis and collenchyma.Tri: trichome, Epi: Epidermis and Col: collenchyma.

4.4.2.4Powder microscopy *Argyreia cymosa* stem

The powdered stem of *A. C.* exhibited a pale buff coloration and showed several characteristic diagnostic features under microscopic examination. The powder revealed the presence of sclereids, xylem fibers, xylem vessels, parenchymatous cells, phloem fibers, and medullary ray fragments, which are typical anatomical elements of the stem tissue. These distinguishing microscopic features are illustrated in Figure 4.12.

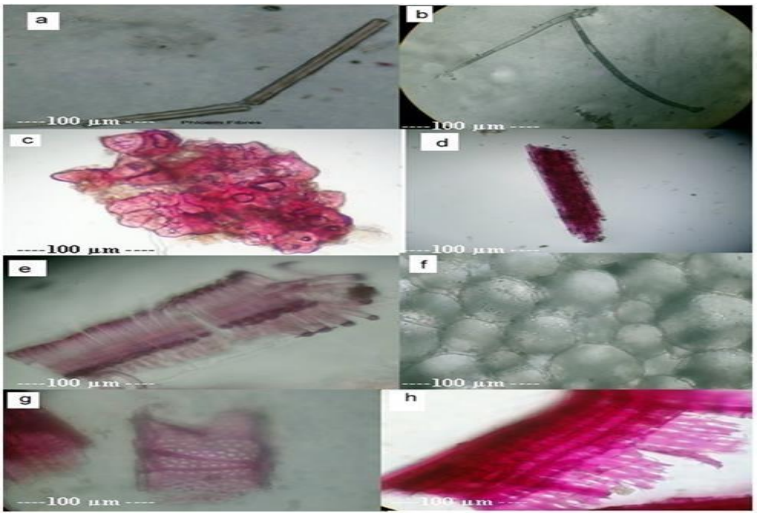


Figure 4.12: Powder microscopy of the stem of *A. C.*: (a) phloem fibers, (b) multicellular uniseriate trichomes, (c) sclereids, (d) lignified xylem fibers, (e) xylem vessels, (f) parenchyma cells, (g) xylem vessels, and (h) xylem vessels with medullary rays.

4.4.3 Pharmacognostic evaluation of *Argyreia cymosa* root

4.4.3.1 Macroscopy of *Argyreia cymosa Root*

Figure4.13 *Argyreia cymosa* root



4.4.3.2 Organoleptic characters of *Argyreia cymosa* root

Roots were in buff colour, without characteristic odour and characteristic taste shown in table 4.4.

Table4.4Organolepticcharactersof *Argyreia cymosa* root

Organoleptic characters	Observation
Colour of the root	Buff
The odour of the root	No characteristic
Taste of the root	No taste
Size of the root	30 to 35 cm
The texture of the root	Rough
Fracture of the root	Fibrous

4.4.3.3 Microscopical evaluation of *Argyreia cymosa* root

The transverse section of the root revealed a well-developed cork layer, consisting of approximately six to seven layers of dilated, thick-walled cells, forming the outer protective tissue. Beneath the cork, lignified sclereids (stone cells) were observed, providing additional mechanical support. The cortex was composed of thin-walled parenchymatous cells with small intercellular spaces, indicating a loosely arranged cellular structure.

Towards the inner region, distinct phloem and xylem tissues were observed extending up to the endodermis. Within this region, the xylem tissue occupied a comparatively larger area than the phloem, indicating a well-developed vascular system. The phloem strands were located between the xylem elements and were associated with phloem parenchyma, which consisted of thin-walled, non-lignified cells.

The vascular region also showed the presence of medullary rays, which were

predominantly parenchymatous and mostly uniseriate in structure. These medullary rays were arranged between the xylem elements and extended radially across the vascular tissue. The xylem region, occupying the central portion of the root, was composed of characteristic vascular elements, including spirally or annular thickened xylem vessels, xylem fibers, and xylem parenchyma. These anatomical features were clearly observed in the transverse sections illustrated in Figures 4.14–4.18.

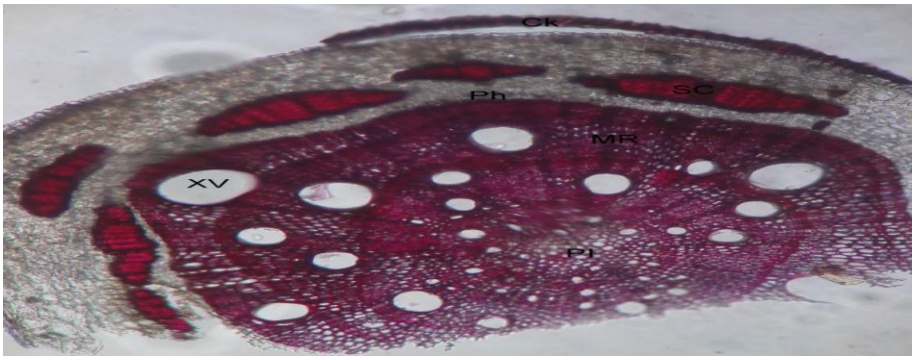


Figure4.14 Transverse section of *Argyreia cymosa* root. CK: Cork, SC:Stone Cells; Ph: Phloem; MR: Medullary rays; XV: Xylem vessels; PI: Pith.

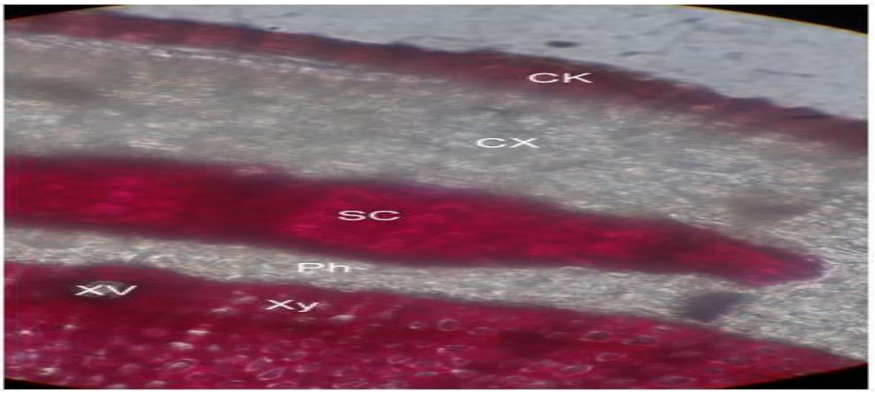


Figure4.15TSsection of *Argyreia cymosa* root CK: Cork; CX:Cortex; SC:Stone Cells; Ph: Phloem; Xy: Xylem and XV: Xylem Vessels.

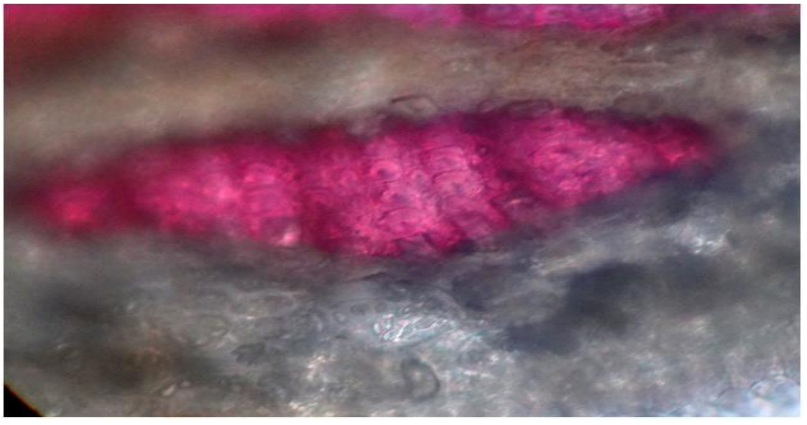


Figure4.16 *Argyreia cymosa* of stone cells magnification at 40

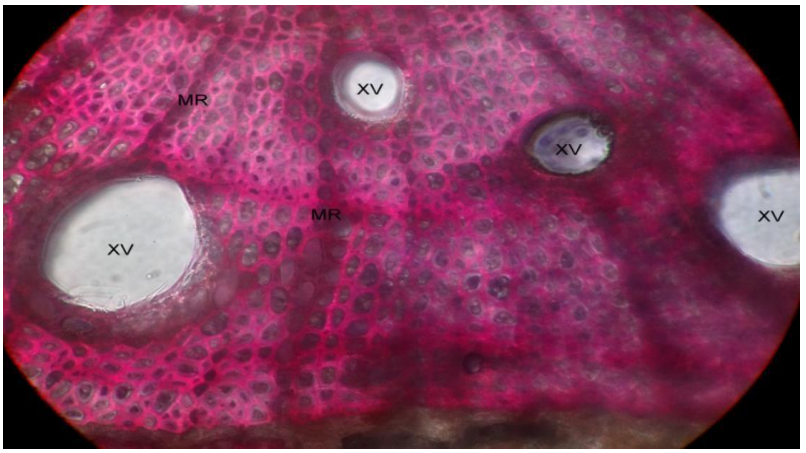


Figure4.17TS of *Argyreia cymosa* roots showed xylem vessels and medullary rays. MR:Medullary rays; XV:XylemVessels.

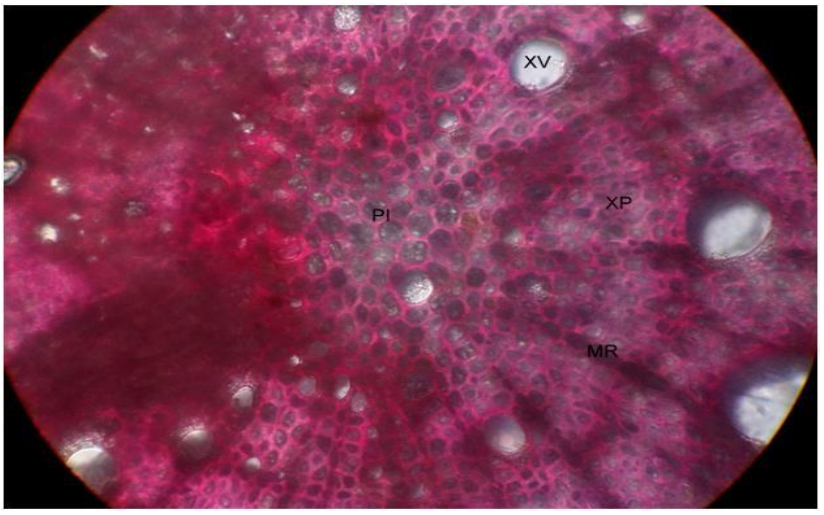


Figure 4.18 Central part of the *Argyreia cymosa* root XV:Xylem Vessels; XP:Xylem Parenchyma; MR: Medullary rays and PI: Pith.

4.4.3.4 Powder microscopy of *Argyreia cymosa* root

Microscopic interrogation of the pulverized root material, following standard powder mounting procedures, unveiled a diagnostically informative assemblage of histological elements whose collective identity provided compelling anatomical evidence for the botanical origin and tissue composition of the specimen under examination. The brownish coloration characteristic of the powdered root preparation was primarily attributable to the abundance of cork cells — polyhedral to tabular, thin- to moderately-walled suberized elements originating from the periderm layer — whose suberized secondary walls confer the distinctive brownish pigmentation observed macroscopically in the powder matrix, simultaneously functioning as a protective barrier

against desiccation and microbial infiltration in the intact root organ. Complementing the dermal components, xylem fibres were identified as elongated, fusiform, heavily lignified parenchymatous cells with acutely tapering terminations and distinctly narrowed lumina, their robust secondary wall architecture reflecting their primary role in structural reinforcement of the vascular cylinder. Concurrent with these fibrous elements, xylem vessels (channels) — presenting as isolated vessel members or fragmented vessel sequences with characteristic bordered pitting, annular, spiral, or reticulate secondary wall elaborations — were consistently encountered, serving as unambiguous indicators of well-differentiated primary and secondary vascular tissue within the root axis. The ground tissue contribution to the powder profile was represented by parenchyma cells, discernible as thin-walled, isodiametric to polygonal cells with intact primary walls and centrally positioned nuclei in some instances, their occurrence reflecting the storage and metabolic functional parenchyma of the cortical and medullary regions. In parallel, phloem fibres — morphologically distinguishable from xylem fibres by their relatively less heavily lignified, more uniform, and characteristically grouped arrangement — were observed as elongated sclerenchymatous elements constituting the mechanical component of the phloem tissue complex of considerable diagnostic and taxonomic significance, sclerides — including brachysclerides (stone cells) and/or macrosclerides — were documented as isolated or small-clustered, isodiametric to irregularly shaped, thick-walled elements with conspicuous simple or branched pits traversing the lamellated secondary wall, their pronounced lignification rendering them highly birefringent under polarized illumination. Furthermore, medullary rays were identified as uniseriate to multiseriate radially oriented files of parenchymatous cells, their structural continuity across the vascular tissue plane indicative of their physiological role in lateral translocation of photosynthates and mineral solutes between the vascular cylinder and peripheral cortical zones. The complete spectrum of aforementioned histological constituents, as systematically catalogued in Table 4.19, collectively authenticates the pharmacognostic profile of the powdered root and supports its reliable botanical identification.

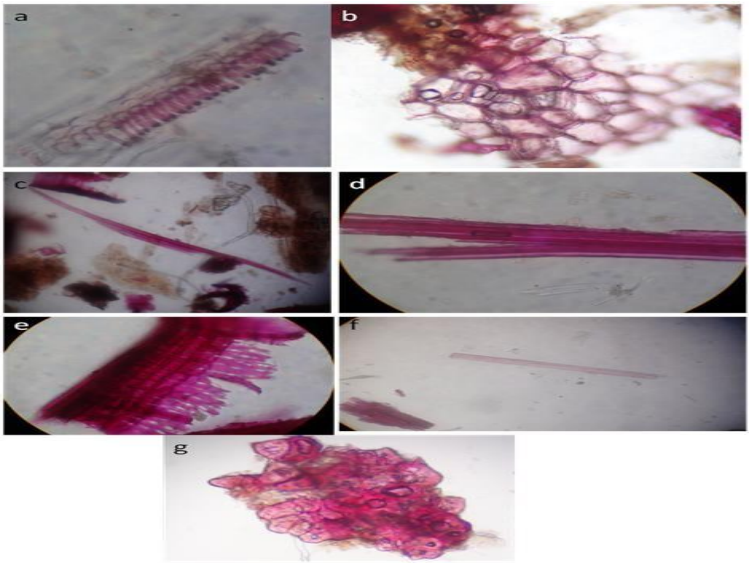


Figure 4.19 Powder microscopic nature of the root of the *Argyreiacymosa*. a) Lignified xylem vessels, b) Cork cells in surface view, c) Xylem fibre with tapered ends, d) Xylem fibres with non-tapered ends, e) Medullary rays f) Phloem fibres and g) Stone cells.

4.4.4 Pharmacognostic study of *L. R.*leaf

4.4.4.1 Morphology of *L. R.*leaf

*L. R.*may have either homophyllous or heterophyllous opposite or whorled leaves. The leaves are lanceolate to ovate with serrated edges when they are homophyllous. The submerged leaves of heterophyllous forms are laciniate to compound-pinnate, progressively moving upward into fleshy, whole to deeply pinnatifid emersed leaves (Figure 4.20).



Figure 4.20 *Limnophila repens* leaf

4.4.4.2 Organoleptic characters of *L. R.*leaf

Table 4.5 Organoleptic characters of *Limnophila repens* leaf

Leaf	Characteristics
Colour	Green
Shape	Elliptic – oblong
Size	1-2.5x0.4-1.2 cm
Base	Narrowed
Margin	Serrate
Apex	Sub acuminate

4.4.4.3 Microscopic investigation of *L. R.*leaf: Systematic microscopic investigation of the *L. R.* leaf, encompassing both epidermal surface analysis and transverse sectional examination, disclosed a structurally stratified and histologically elaborate laminar organization characterized by a constellation of diagnostically significant anatomical constituents. The adaxial and abaxial epidermal surfaces were constituted by sparsely distributed, rectangular to sub-rectangular cells demarcated by comparatively straightened anticlinal wall margins, among which stomatal complexes of the anomocytic type were consistently encountered — each guard cell pair encircled by four to five morphologically undifferentiated subsidiary cells disposed in a geometrically irregular, non-concentric arrangement — a stomatal configuration representing a reliable pharmacognostic authentication marker for the species. The epidermal surface additionally revealed diagnostically informative vascular imprints manifesting as veins, vein islets, and vein terminations, constituting quantifiable anatomical constants applicable to species-level differentiation, as comprehensively documented in Figure 4.21. In transverse sectional profile, the outermost laminar boundary was delineated by a continuous, moderately thickened cuticle of lipid-impregnated extracellular composition investing both epidermal surfaces, beneath which the upper epidermis comprised a uniseriate layer of compactly arranged, tangentially flattened rectangular cells, further distinguished by the projection of multicellular uniseriate trichomes from the outer periclinal wall surface alongside anomocytic stomatal complexes. The subjacent mesophyll exhibited a dorsiventral differentiation pattern, wherein the adaxially positioned palisade parenchyma — composed of vertically elongated, compactly juxtaposed columnar cells of elevated chloroplast density arranged

perpendicularly to the epidermal plane — transitioned abaxially into a spongy parenchyma of irregularly shaped, loosely interconnected cells permeated by pronounced intercellular lacunar spaces facilitating gaseous diffusion and internal CO_2 redistribution toward the photosynthetically active palisade domain. At the sub-epidermal interfacial zones beneath both adaxial and abaxial surfaces, discrete strands of angular collenchyma were resolved, constituted by living, polyhedral cells bearing unevenly deposited, cellulose-enriched primary wall thickenings concentrated at angular cell junctions, conferring peripheral mechanical resilience and flexible structural integrity to the laminar framework. Embedded centrally within the mesophyll continuum, collateral vascular bundles were prominently identified, displaying characteristic spatial polarity wherein adaxially positioned, lignified xylem elements — comprising vessel members, tracheids, and fibres with secondarily thickened walls — were juxtaposed against abaxially disposed, non-lignified phloem constituents of sieve tube members and companion cell complexes responsible for photo assimilate translocation. The lower epidermis, structurally recapitulating the adaxial epidermal organization through its rectangular cellular profile and distributed stomatal complement, collectively affirmed the amphistomatic foliar character of this species, with the entirety of the aforementioned histological profile systematically illustrated in Figures 4.21–4.24.

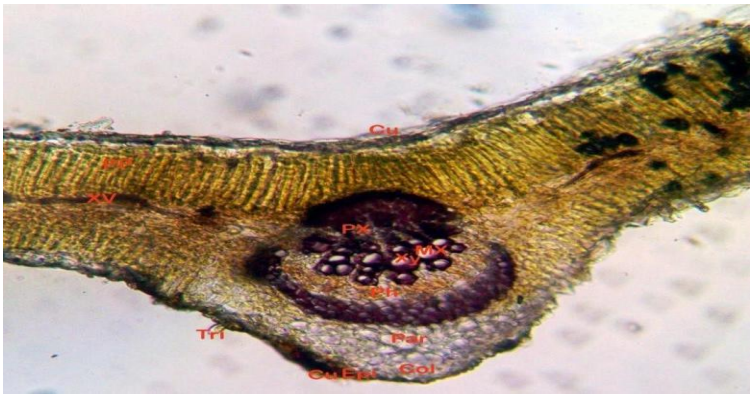


Figure 4.21 T S of *L. R.*leaf midrib portion. Cu: Cuticle, Epi: Epidermis, Col:Collenchyma cells, PP:Palisade Parenchyma, Xy:Xylem, PX: Proto Xylem, MX: Meta xylem, Ph: Phloem, Par: Parenchyma cells and Tri: Trichomes.

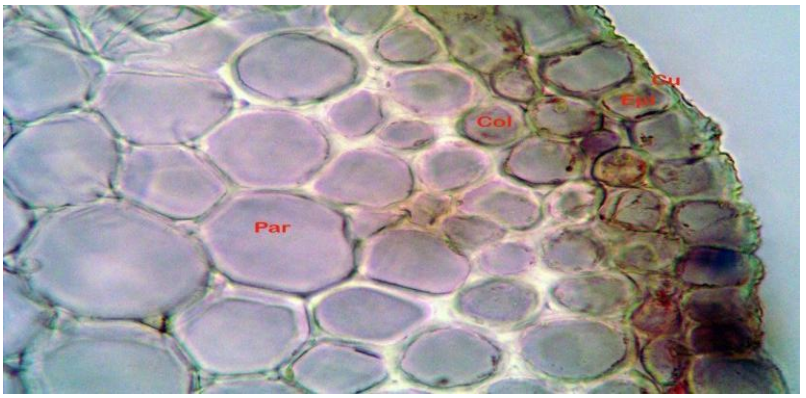


Figure4.22Detailed TS of the midrib of the leaf *Lminophila repens* Cu: Cuticle, Epi: Epidermis, Col: Collenchyma cells, Par: Parenchyma.

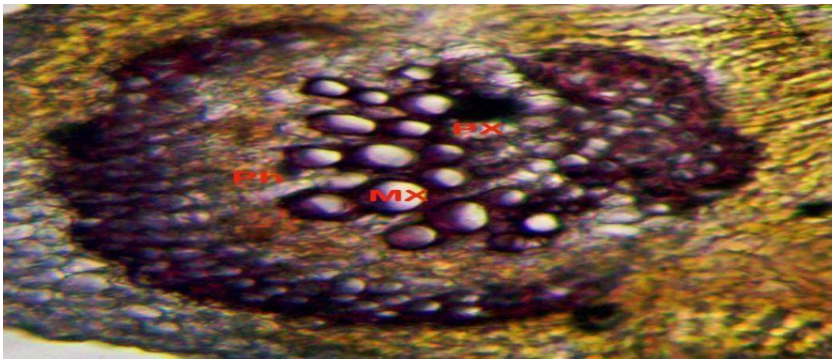


Figure4.23 TS of midrib portion showing vascular bundles *Limnophila repens* Ph: Phloem, MX: Metaxylem, PX: Protoxylem.

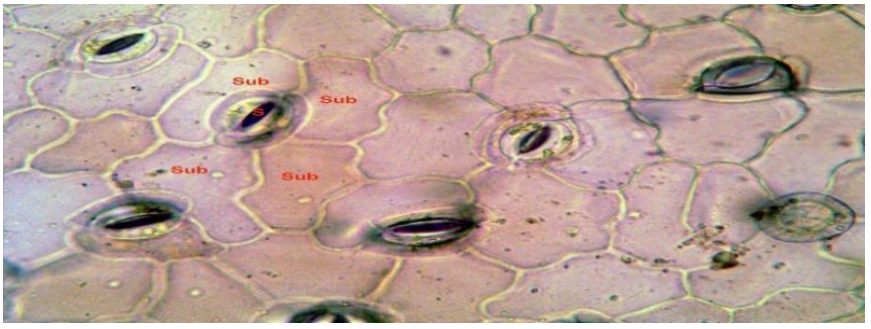


Figure4.24 *Liminophila repens* epidermal cells showed Anomocytic stomata.
S: Stomata; Sub: Subsidiary cells.

4.4.4.4 Quantitative microscopy of *L. R.*leaf

Table 4.6: Quantitative microscopy of Limnophila repens leaf

S.No.	Parameter	Value (per mm²)	Tissue Location	Diagnostic Interpretation
1	Stomatal Number — Upper Epidermal Surface	7.52 ± 2.56	Adaxial epidermis	Lower stomatal density with comparatively heterogeneous spatial distribution; indicative of partial adaxial stomatal representation
2	Stomatal Number — Lower Epidermal Surface	10.55 ± 1.52	Abaxial epidermis	Elevated stomatal density with uniform distribution; confirms abaxial stomatal predominance consistent with hypostomatic foliar tendency
3	Stomatal Index — Upper Epidermis	12.56 ± 3.55	Adaxial epidermis	Higher standard deviation reflects intercellular distributional heterogeneity across the adaxial epidermal domain
4	Stomatal Index — Lower Epidermis	15.58 ± 1.25	Abaxial epidermis	Lower standard deviation denotes uniform stomatal frequency relative to total epidermal cell density; reliable pharmacognostic constant
5	Vein Islet Number	5.88 ± 2.25	Mesophyll venation	Reflects degree of vascular reticulation and minor vein network complexity within the laminar tissue
6	Vein Termination Number	8.32 ± 1.22	Mesophyll venation	Higher frequency relative to vein islets indicates elaborately branched terminal vein ramifications; species-specific authentication marker

Quantitative micro morphometric analysis of the foliar epidermal and venation architecture of *L. R.*, as systematically enumerated and presented in the corresponding table, yielded a comprehensive set of diagnostic anatomical constants of considerable pharmacognostic and taxonomic interpretive value. Stomatal enumeration conducted per unit epidermal area (mm²) disclosed a pronounced abaxial predominance in stomatal density, wherein the lower epidermal surface recorded a stomatal number of 10.55 ± 1.52 per mm², substantially exceeding the upper epidermal stomatal complement of 7.52 ± 2.56 per mm² — a differential distribution pattern suggestive of hypostomatic tendency with partial adaxial stomatal representation, functionally correlated with the optimization of gaseous exchange while concurrently minimizing transpirational water deficit under prevailing ecological conditions. This abaxial stomatal dominance was further substantiated through stomatal index computation — a parameter that normalizes stomatal frequency relative to total epidermal cell density, thereby providing

a more biologically reliable comparative metric than absolute stomatal counts alone. The stomatal index of the lower epidermis was quantified at 15.58 ± 1.25, markedly surpassing the upper epidermal stomatal index of 12.56 ± 3.55, with the relatively elevated standard deviation observed in the upper surface values indicative of greater intercellular distributional heterogeneity across the adaxial epidermal domain. Complementing the stomatal quantitative profile, foliar venation architectural parameters were simultaneously assessed, yielding a vein islet number of 5.88 ± 2.25 per mm² and a vein termination number of 8.32 ± 1.22 per mm² — both constituting stable, species-specific anatomical constants that reflect the degree of vascular reticulation and minor vein ramification complexity within the mesophyll tissue. The relatively higher vein termination frequency in relation to vein islet density signifies an elaborately branched minor vein terminal network, a venation trait with well-established utility as a reproducible pharmacognostic fingerprint for botanical authentication and adulterant discrimination of herbal source materials at the species level.

4.4.4.5 Powder microscopy of *L. R.*leaf

As shown in figure 4.25, the greenish powdered florae debris contains fragments of parenchyma, lignified arteries, epidermal cells, and anomocytic stomata.

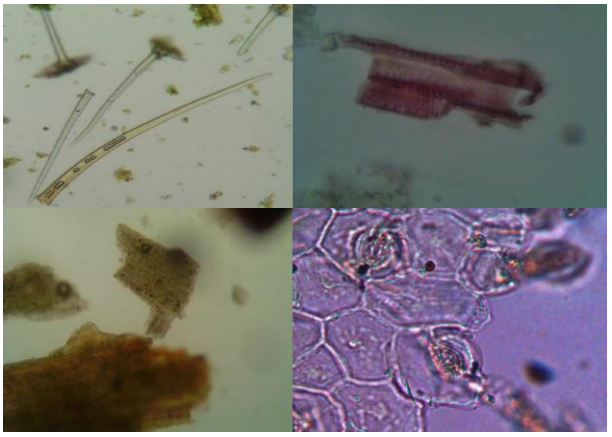


Figure 4.25 Powder microscopic characters of the *Limnophila repens* leaf A) Covering Trichomes, B) Lignified xylem vessels, C) Parenchyma cells, D) Stomata.

4.4.5 Pharmacognostical study of *L. R.*stem

4.4.5.1 Macroscopy *L. R.*stem

The stems are round to quadrangular, erect or creeping in the air or flexuous in the water.



Figure2.26*Limnophila repens* stem

4.4.5.2 Organoleptic evaluation of *L. R.*stem

Table 4.7: Organoleptic characters of *L. R.*stem

Leaf	**Characteristics**
Colour	Green
Taste	Characteristics
Odour	Odourless
Branching	Much Branched
Position	Vertical
Surface	Glabrous
Texture	Smooth
Shape	Cylindrical

4.4.5.3 Microscopy *L. R.*stem

Transverse sectional investigation of the *L. R.* stem disclosed a comprehensively organized anatomical framework consistent with the canonical tissue arrangement characteristic of dicotyledonous florae axes, wherein the spatial disposition of constituent tissue systems adhered to the centripetal organizational hierarchy of dermal, ground, and vascular systems. The peripheral boundary of the stem was demarcated by a single-layered epidermis constituted by tangentially elongated, compactly appressed cells bearing conspicuously thickened outer periclinal walls — a structural reinforcement interpreted as an adaptive cuticular fortification mechanism conferring resistance against mechanical abrasion, desiccation stress, and pathogenic incursion at the stem surface interface. Subtending the epidermal boundary, the cortical zone was resolved as a continuous belt of thin-walled, isodiametric to polygonal parenchymatous cells with moderately developed intercellular lacunae, constituting a functionally versatile ground tissue continuum involved in transient nutrient storage, lateral

metabolite translocation, and peripheral structural reinforcement of the stem axis. The vascular architecture displayed the hallmark dicotyledonous configuration of collateral vascular bundles arranged in a discrete annular ring, collectively encircling the central pith — an organizational pattern reflecting the ontogenetic activity of the procambial strand and serving as an unambiguous morpho-anatomical indicator of the dicotyledonous systematic affinity of the taxon. Within each vascular bundle, the phloem component was constituted by compactly arranged, thin-walled sieve tube members, companion cells, and phloem parenchyma with minimal intercellular separation, positioned at the abaxial pole of the bundle and functionally dedicated to the bidirectional translocation of photo assimilates and osmotically active solutes. The adaxially positioned xylem tissue comprised a well-differentiated assemblage of heavily lignified vascular elements — including wide-lumened vessel members with bordered pit ornamentation, narrow-lumened tracheids, and thick-walled xylem fibres bearing pronounced secondary wall thickenings — collectively executing the dual physiological mandates of unidirectional apoplastic water and mineral ion conduction and rigid biomechanical skeletal support of the stem framework. The central-most region of the stem was occupied by a prominent pith, constituted predominantly by lignified parenchymatous cells of enlarged isodiametric to sub-angular morphology, whose secondary wall lignification distinguishes this pith configuration from the unlignified medullary parenchyma encountered in more mesophytic herbaceous taxa, suggesting a functional contribution toward axial rigidity and structural load distribution within the stem. The entirety of the aforementioned histological organization, reflecting the well-differentiated and taxonomically congruent vascular anatomy of L. repens, is systematically illustrated and annotated Showing Figure 4.27.

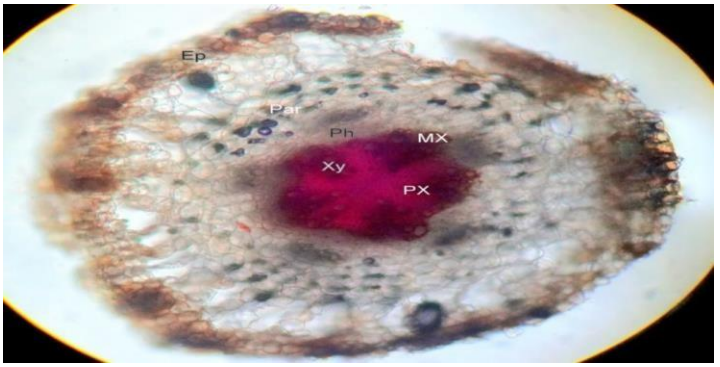


Figure4.27 T.S of the stem of Limnophila repens. Col:collenchyma Ep:Epidermis, Xy: xylem, Ph: phloem, PX: Proto Xylem and MX: Meta Xylem.

4.4.5.4 Powder microscopy of *L. R.*stem

Upon macroscopic preliminary evaluation, the finely comminuted stem-derived powder of *L. R.* manifested a distinctive light buff chromatic signature — a pallid ochre pigmentary expression mechanistically attributable to the predominant liberation of suberized periderm constituents and partially lignified ground tissue elements during the mechanical disintegration process, collectively imparting a coloration profile perceptibly lighter than the deeper brownish tones characteristically associated with heavily suberized root-derived powder matrices of comparable botanical origin. Rigorous brightfield powder microscopic interrogation of the mounted preparation subsequently unveiled a structurally heterogeneous and pharmacognostically interpretable constellation of cellular and ergastic constituents, each independently contributing to the diagnostic fingerprint profile of the stem material. Polyhedral to tabular cork cells of periderm derivation — delineated by moderately thickened, suberin lamellae-invested secondary walls and characteristically flattened periclinal profiles — were recurrently encountered across the powder field, their suberized wall chemistry and geometric regularity serving as unambiguous indicators of secondary dermal tissue contribution to the powder assembly. Interspersed throughout, isodiametric to sub-angular parenchymatous cells bounded by delicate, intact primary cellulosic walls and retaining residual cytoplasmic constituents were abundantly resolved, their morphological diversity reflecting simultaneous cortical, medullary, and vascular ray parenchyma origins within the intact stem architecture. Elongated fusiform phloem fibres — distinguishable by their acutely tapering apical terminations, characteristically narrowed and occluded lumina, moderately lignified lamellated secondary walls, and obliquely oriented simple pitting — were discretely identified as sclerenchymatous mechanical constituents of the phloem tissue complex, their comparative wall chemistry and pitting geometry enabling reliable differentiation from the more heavily lignified xylem fibre counterparts. Discretely distributed pigment-laden cells — enclosing intracellular deposits of yellowish-brown to deep amber amorphous secondary metabolite complexes, tentatively assignable to condensed tannin aggregates, polyphenolic flavonoid derivatives, or oxidized phenylpropanoid biosynthetic products — were encountered as histochemically significant powder constituents, their occurrence serving as indirect anatomical surrogates for the secondary metabolite accumulation capacity of the species. Additionally, starch grains — manifesting as

simple, compound, and semi-compound amyloplast-derived granules exhibiting species-characteristic size distributions, centrally to eccentrically positioned hilar configurations, and faintly discernible concentric lamellation striae — were identified as diagnostically informative ergastic cellular inclusions, their micro morphometric parameters constituting reproducible quantitative constants applicable to pharmacognostic authentication, species differentiation, and adulteration screening of L. repens stem-derived herbal material in accordance with established quality control frameworks. The comprehensive assemblage of aforementioned histological and ergastic diagnostic constituents is systematically documented and annotated in Figure 4.28.

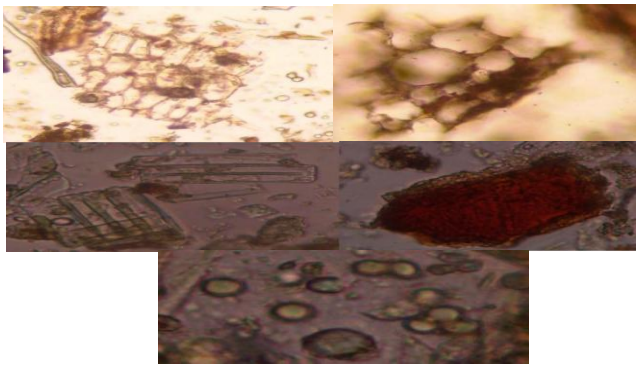


Figure4.28 Powder microscopy of the stem of *Limnophila repens*; a: corkcells; b: Parenchyma cells; c: Phloem fibres; d: Pigment cells; e: Starch grains.

4.4.6 Pharmacognostic evaluation of *L. R.*root

4.4.6.1 Macroscopical evalution of *L. R.*root



Figure4.29*Limnophila repens* root

4.4.6.2 Organoleptic characteristics of *L. R.*root

Table 4.8: Organoleptic characters of *L. R.*root

Buff	Pallid ochre / light fulvous
Conical	Acuminate to coniform / basally broadened, apically attenuated
Characteristic (odour)	Distinctive organoleptic olfactory profile
Characteristic (taste)	Species-specific gustatory sensory profile
Downwards	Pendulous to decurrent spatial orientation
Smooth	Glabrous to laevigate surface topography
Present	Consistently encountered / documented
Colour	Chromatic expression
Shape	Laminar morphology / geometric configuration
Odour	Organoleptic olfactory emanation
Taste	Gustatory sensory profile
Texture	Surface topographic character
Rootlets	Adventitious rhizoidal appendages

4.4.6.3 Microscopical evaluation *L. R.*root

The transverse section of the root of *L. R.* revealed a well-defined outer periderm, consisting of two to three layers of narrow, elongated cork cells containing dark brown granular matter. Beneath the periderm, the cortical region was composed predominantly of thin-walled parenchymatous cells with small intercellular spaces, indicating a compact arrangement of cortical tissues.

The endodermis was distinct and marked the boundary between the cortex and the vascular region. Within the vascular cylinder, xylem and phloem tissues were clearly differentiated. The phloem was distributed between the xylem elements and was associated with non-lignified phloem fibers and phloem parenchyma. The vascular tissue was arranged in a radial pattern, typical of dicot roots.

The medullary rays were composed of parenchymatous cells and were observed to be uniseriate to triseriate in width, extending radially between the vascular elements. The xylem region occupied a major portion of the central area and was surrounded by medullary rays. The xylem tissue consisted of spirally or annular thickened vessels, xylem fibers, and xylem parenchyma, which contributed to conduction and mechanical support. These anatomical characteristics were clearly observed in the transverse section illustrated in Figure 4.30.



Figure4.30 Transverse section of *Limnophila repens* root.

4.4.6.4 Powder microscopy of *L. R.*root

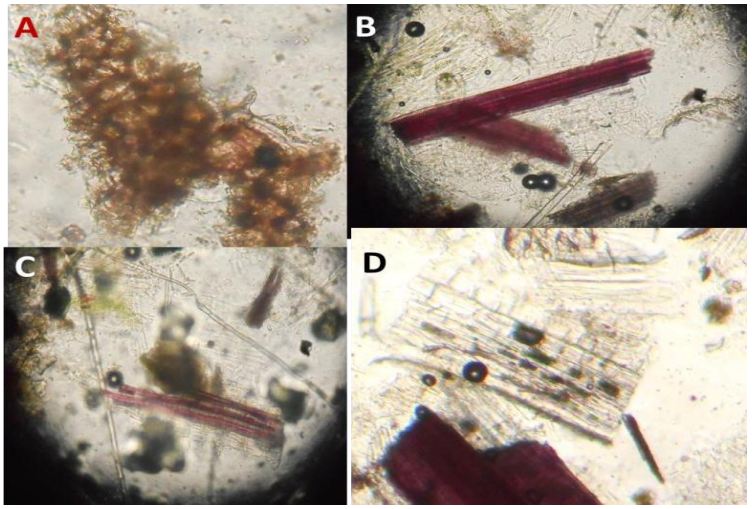


Figure2.31 Powder microscopic characters of *Limnophila repens* root A)Cork B)Xylem Fibres C)Xylem vessels D)Phloem fibres.

4.4.7 Pharmacognostical evaluation of *C. B.* leaf

4.4.7.1 Macroscopy of *C. B.*leaf

C. B. is a shrub with hairy branchlets that grows to a height of around 2-4 meters. The coriaceous, elliptic to oblong leaves are around 5-7 x 2-4 cm in size. Stipular spines are tiny, and cataphylls are straight. Flowers are axillary, solitary, and have a diameter of 4–5 cm. They are carried on pedicels that are about 4 cm long. Four sepals grouped in two whorls make up the calyx. There are four white petals with earthy-colored patches that range in length from oblong to elongated. There are many exserted stamens, and the gynandrophore is as long as or longer than the filaments. The ovary is oval, about 6 mm long, and the anthers are dorsifixed (Figure 4.32).



Figure4.32 *Capparis brevispina* leaf

4.4.7.2 Organoleptic evaluation of *C. B.*leaf

Table 4.9: Organoleptic characters of *C. B.* leaf

Leaf	**Characteristics**
Colour	Buff
Taste	Characteristic
Odour	Characteristic
Rootlets	Present
Position	Downwards
Texture	Smooth
Shape	Conical

4.4.7.3 Microscopy of *C. B.*leaf

Outside of the midrib, the epidermal layer is less visible and wavy. The ground tissue is made up of small, uniform parenchymatous cells. The vascular thread is distinct, unmolded, and single. It is made up of vessels and strands with thick walls that have been reduced to thick spiral sections. The xylem strand is encased in the broad phloem. The width of the vascular strand is 400 µm. In sectional view, the horizontal vein and planoconvox are also visible. Figures 4.33 to 4.34 show that it contains a broad circular segment of vascular strand with long, thick spiral sections of insurance groups, a confined epidermal layer with thick walls, and a minimised, thick-walled ground parenchyma.

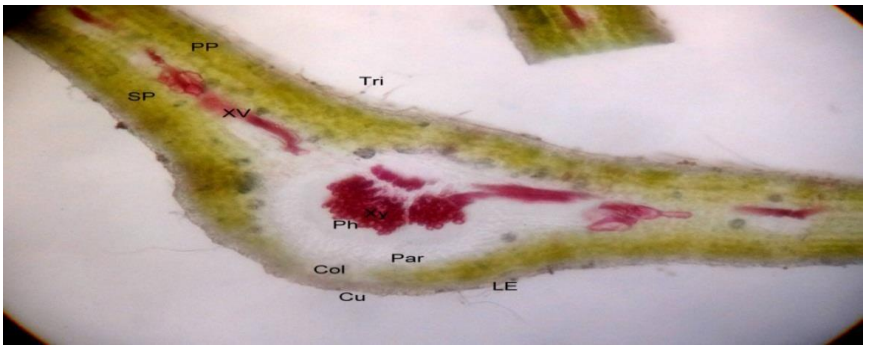


Figure4.33 Transverse section of midrib portion of *Capparis brevispina.* Cu: Cuticle, UE: Upper epidermis, Col: Collenchyma cells, PP: Palisade Parenchyma, SP: Spongy Parenchyma, X: Xylem; Ph: Phloem, Par: Parenchyma cells, LE: Lower epidermis and Tri: Trichomes.

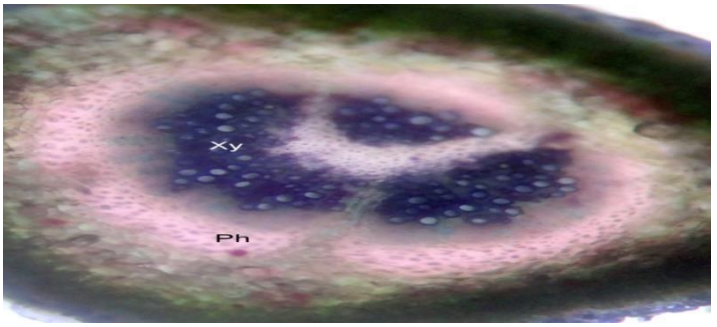


Figure4.34 Transverse section of the midrib portion of *Capparis brevispina* Ph: Phloem andXy: Xylem.

4.476.4 Quantitative microscopy of *C. B.*leaf

Table 4.10: Quantitative microscopy of *Capparis brevispina* leaf

S.No.	Parameter	Value (per mm²)
1	Stomatal Number of Upper Surface	76.12 ± 12.85
2	Stomatal Number Lower Surface	204.52 ± 28.46
3	Stomatal Index Upper Epidermis	12.52 ± 2.32
4	Stomatal Index — Lower Epidermis	36.94 ± 6.52
5	Vein Islet Number	77.42 ± 6.25

4.4.7.5 Powder microscopy of *C. B.*leaf

The powder florae material is greenish, shows pieces of lignified xylem vessels, stomata, and covering trichomes showed in Figure 4.35

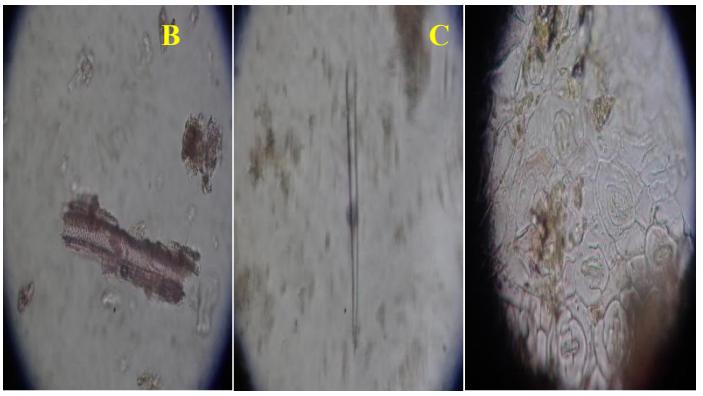


Figure4.35 Powder microcopy of *Capparis brevispina* leaf (A)lignified xylem vessels (B)covering trichomes (C)Stomata.

4.4.8 Pharmacognostic study of *C. B.*stem

4.4.8.1 Macroscopy of *C. B.*stem



Figure 4.36 Macroscopic study of *Capparis brevispina* stem

4.4.8.2 Organoleptic evaluation of *C. B.*stem

Table 4.11: Organoleptic characters of *C. B.*stem

Stem	Characteristics
Colour	Buff
Shape	Cylindrical
Odor	Odorless
Taste	Characteristic
Fracture	Fibrous
Surface	Rough

4.4.8.3 Microscopy of *C. B.*stem.

A part of the stem of *C. B.*is sliced crosswise reveals a huge number of spread and stellate trichomes adhering to a significant area of skin. Cicatrices, or stumps from broken trichomes, are found on parts of stems with more experience. There was a single epidermal layer cell, isodiametric parenchyma cells, and a substance containing various

starch grains and precious calcium oxalate stones. Figure 4.37 illustrates the lignified arteries and external phloem of the vascular pack.

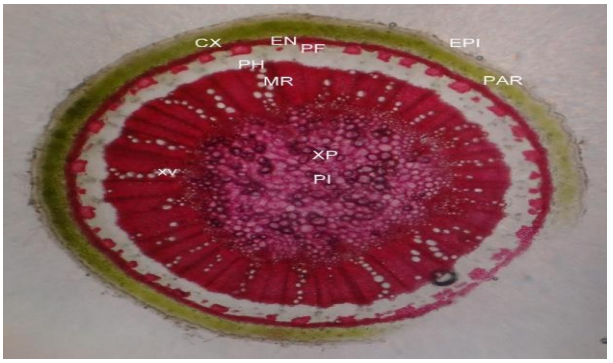


Figure 4.37 T. S of stem of *C. B.*.EPI: Epidermis; PAR: Palisade parenchyma; C X: Collenchyma; EN: Endodermis; PH:Phloem; XP:Xylem parenchyma; MR:Medullary rays and XV:XylemVessels

4.4.8.4 Powder microscopy *C. B.*stem.

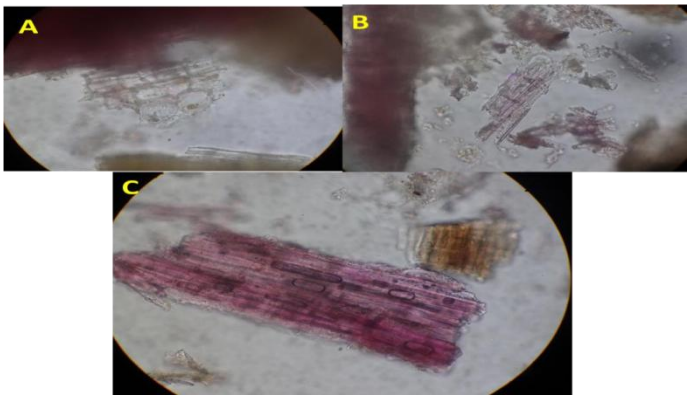


Figure4.38 Powder microscopy of *Capparis brevispina* stem A:Parenchyma cells; B: Phloem Fibers; C: Xylem Fibers.

4.4.9 Pharmacognostic evaluation of *C. B.*root

4.4.9.1 Macroscopy of *C. B* .root



Figure4.39*Capparis brevispina* root

4.4.9.2 Organoleptic evaluation of *C. B.*root

Table 4.12: Organoleptic characters of *C. B.*root

Root	Characteristics
Colour	Buff
Shape	Cylindrical
Odor	Odorless
Taste	Characteristic
Fracture	Fibrous
Surface	Rough

4.4.9.3 Microscopy of *C. B.*root

The root's transverse slice reveals a cortex made up of four to seven layers of collenchymatous tissue, followed by one to two layers of epidermal cells. There is an exterior layer of cork (phellem) that is trichome-free. The cortical area is made up of isodiametric parenchymatous cells with sporadic stone cells and a significant number of exceptionally big starch grains. The medullary area is well-developed, with a well-defined vascular system and 15–25 radial rows of parenchyma cells scattered with lignified medullary rays. There is a noticeable pith in the middle. As seen in Figure 4.40, oil globules are dispersed throughout the section's cells, ranging from the epidermis to the inner pith.

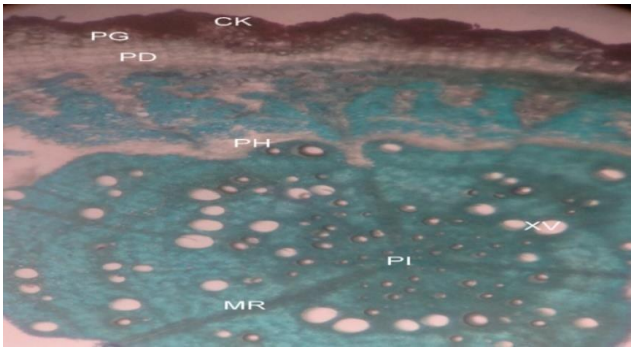


Figure: 4.40 Transverse section of *C. B.* root
CK: Cork cells, PG:Pellogen, PD:periderm, PH:Phloem, XV:xylem, MR:medully rays and PI:pith

4.4.8.4 Powder microscopy of *C. B.*root

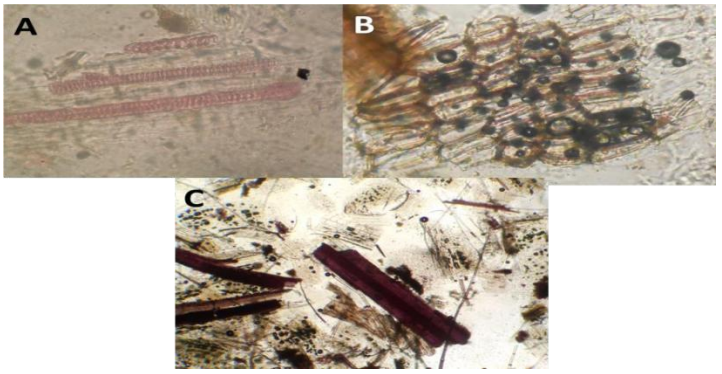


Figure4.41Powder microscopy of root of *Capparis brevispina* A:epidermalcells with cork B: xylem vessel and C: xylem fibers

4.5 Discussion

4.5.1 Pharmacognostical study

A detailed examination of morphological traits, including organoleptic qualities, anatomical features, powder microscopy, and physicochemical parameters, was carried out for *A. C.*, *L. R.*, and *C. B.*. This study was attempted to develop thorough macroscopic and microscopic profiles, together with powder microscopic and physicochemical features, therefore giving valid qualitative and quantitative criteria for the identification and authenticity of these remedialfloraes. The observed features of the above-mentioned species are provided as follows.

Organoleptic evaluation

In order to ensure pureness, correctness, and identity, characteristics including colour, aroma, taste, structure, and form were assessed in the organoleptic assessment. Consistency confirmation for the organoleptic examination of Argyreiacymosa, *L. R.*, and *C. B.* entire floraes was given in this section, which is helpful for identifying adulterants. Large, terete, pubescent, cordiform deltoid leaves, round, acute or obtuse, chartaceous, 6-8 X 4-6 cm, sparsely on both sides, pubescent; pinkish flowers, corymbose cymes; globose, 1.7x1.4 cm, glabrous fruit with 2, 3, or 4 flat, ovate to elliptical seeds. *L. R.* has opposing or whorled leaves that are either homophyllous, lanceolate, ovate, and serrated, or heterophyllous with submersed leaves that are laciniate to compound pinnate and grading transitionally upward to fleshy, whole, and

deeply pinnatifid. *C. B.*, shrubs with rough branchlets that grow to a height of two to four meters. The coral, elliptic or oblong, toxic leaves are 5-7 x 2-4 cm and have smooth cataphylls and small stipular spines.

4.5.3 Microscopic analysis

Microscopic analysis Axilla, by itself, 4 to 5 cm in diameter around the flowers; pedicel, 4 cm; sepals, 4 in 2 whorls; petals, 4 oblong, obovate, white with dark spots; multiple stammers, exerts, gynandrophorus, dorsifixed anthers as long as or longer than the threaded; 6 mm ovaries; ellipsoid, few to many ovules

A drug's complete analysis is made possible by microscopic inspection, which may be combined with histological characteristics to aid in identification. With the use of histological characteristics, the identity of Argyreiacymosa, *L. R.*, and *C. B.* complete floraes is confirmed. One-layered, thick-walled, cuticle-covered epidermal cells are visible in the cross section of the Argyreia cymosic leaf via the midrib. The top and bottom margins of the convex-shaped epidermal cells were triangular. The cells in the upper epidermis were smaller than those in the lower epidermis. The epidermis was covered in long, multicellular, uniserated trichomes with a sharp tip. The cross-section of the top epidermis in the midrib region shows four to six rows of collenchyma and two to three layers of the lower epidermis. Collenchyma cells were round, strongly walled, and had few air gaps. They were encircled by broad parenchymatic soil tissue. The cells in the field tissue were intercellular, circular, oval, and polygonal. The field tissues comprised eight to twelve vascular bundles. Closed collateral. The xylem is composed of spiral-ring thickened channels, trachrezh, and xylem parenchyma, whereas the phloem is composed of similar cells and sieve tubes. The leaf's laminar TS shows two to three layers of palisade cells under the upper epidermis and two to four layers of spongy parenchyma beneath the lower epidermis.

The greenish florae material of Argyreia cymosa leaf exhibits paracytic stomata, palisade cells, lignified arteries, and portions of parenchyma and epidermal cells. The transverse slice of the Argyreia cymosa stem showed a barrel-shaped epidermis loaded with multi-cell uniseriate trichomes and compactly constructed without intercellular gaps. Four to five bound collenchyma cells make up the hypodermis, which provides extra support and defence. Two to three layers of sclerenchyma cells were seen. Transparent, longitudinal, and joint vascular bundles. Xylem included the continuous

network of loops, fibres, and xylem parenchyma. Branches, seven cells, supporting cells, and parenchyma phloem make up phloem. Medullary rays were underappreciated. The centre is made up of parenchyma cells. Argyreia cymosa had sclerides, xylem fibres, xylem tubes, parenchymal cells, phloem fibres, and medullary rays.

The Argyreia cymosa root transverse section, which consists of around 6-7 tangentially dispersed, thick wall cells, has been reported to contain cork. There are lignified stone cells below. The cortex was composed of parenchymal cells with narrow walls and little intercellular space. The endodermis demonstrates how well-described phloem and xylem are. Compared to phloem endodermis, xylem takes up a lot more room. The surge is behind the medullary rays. Additionally, the medullary rays continue to be connected to parenchyma. Phloem revealed the existence of non-linear phloem fibres. Additionally, the presence of phloem parenchyma was found. Medullary rays were densely packed in the centre of the xylem. Spherical parenchyma xylem, xylem, and xylem make up the xylem tissue. As demonstrated in Figure 4.22, the grey root powder of Argyreiacymosa displayed medullary rays, cork cells, Xylem tubes, parenchyma cells, phloem fibres, and sclerides.

The cell-covered stomatoes with the epidermis layer are represented by the leaf surface of Liminophila repens. Epidermal cells are rectangular, thin, and flat. Four to five parental cells have anomocytic or sporadic stomata. Examining the leaf's surface reveals that the veins, vein islet, and vein terminus are all visible. The cuticle layer, vascular cords, and epidermal membrane are visible in the Liminophila repens cross section. Rectangular cells make form the outer wall of the top epidermis, which is composed of trichomes. The trichomes are multicellular and connected. The mesophyll is defined by palisade and spongy parenchyma. Typically, there are two to three layers of closely spaced sponge parenchyma. Strips of collenchyma follow the upper and lower epidermal membranes. The collenchyma has strong cellulose cell walls. The cells that make up collenchyma tissue are spherical and have thick walls. Xylem is lignified, but phloem is not. The top and lower epidermis are similar.

Liminophila repens leaf powder is a greenish florae substance containing anomocytic stomata, lignified arteries, and portions of parenchyma and epidermal cells. The Liminophila repens stem florae exhibited the typical traits of a dicot florae. In the microscopic examination of the florae, vascular bundles and a wide pith were grouped in a ring. Tangentially elongated cells with a thick wall make up the top epidermal cork.

The evolution of thin-walled parenchyma with restricted intracellular cortical regions is seen below. Phloem is a tiny, isodiametric, intercellular parenchyma. The pith contains important cells. Starch grains, pigment cells, phloem fibres, cork cells, and parenchyma cells were all visible in the light buff-colored powdered stem of *L. R.*.

The cross-sectional form of *L. R.*. Repens discovered that the cortex was made up of parenchymatic cells with thin walls and minimal intercellular space. On the cork, there were a few tiny, tangentially expanded cells of dark brown granular material that suggested peridermic involvement. One or two covered rows of elongated cells with thin walls make up phelloderm. The endodermis contains phloem and xylem. The medullary rays are separated by the phloem. The structure of vascular bundles was very ordered, and the phloem had well-formed, non-lignified phloem fibres. Additionally, the existence of phloem parenchyma was shown. Since the xylem area was a component of the phloem region, it was surrounded by single medullary rays. Circular xylem tubes, xylem fibres, and xylem parenchyma comprise the xylem tissue. The powdered root of *L. R.* revealed cork cells, phloem fibres, xylem fibres, and xylem vessels.

A *C. B.*leaf's transverse slice showed a wavy midrib surface and a thin, hardly perceptible epidermal layer. The ground tissue is homogenous and parenchymatous, with small, compact cells. The vascular thread has an urn-like depth, is large, and is solitary. It is made up of thick radial segments of dense, compact, thick-walled fibres and vessels. The xylem strand is encased in the broad phloem. The width of the vascular strand is 400 µm. In sectional view, the lateral vein is likewise noticeable and planoconvox. It has a broad arc of vascular strand made up of long, thick radial segments of collateral bundles, a compact, thick-walled ground parenchyma, and a thin, thick-walled epidermal layer.

The greenish leaf powder florae material of *C. B.* displays fragments of covering trichomes, stomata, and lignified xylem arteries. Several ramified, stillate trichomes that are affixed to a thick layer of cuticle may be seen in a cross-section of C. brevispina. Scarring (stumps of weaker trichomes) is seen in older stem sections. There was an isodiametric parenchyma, a pith with several starch grains and prismatic calcium oxalate crystals, and a single layer of epidermal cells. The outer phloem and lignified arteries make up the vascular bundle.

Fragments of parenchyma, phloem fibres, and lignified xylem fibres may be

found in the light buff stem florae material of *C. B.*. One to two layers of epidermal cells are covered by four to seven layers of cortical collenchyma cells and a cork devoid of trichomes in a cross-section of the *C. B.*root. Large starch grains and stone cells are widely scattered throughout the isodiametric parenchyma cells. 15–25 layers of lignified medullary rays composed of parenchymal cells with collateral vascular distribution. In the centre is a pith. The two cells in this area were oil reserves that stretched from the epidermis to the pith. The powdered root of *C. B.*in revealed the presence of cork cells, phloem fibres, xylem vessels, and xylem fibres due to its light brown hue.

4.6 Conclusion

Pharmacognostic analysis of the aforementioned remedialfloraes reveals that these floraes have certain morphological, microscopical, and powder microscopical characteristics related to colour, odour, taste, size, stomatal number, vein islet and termination number, xylem, and phloem. These characteristics are useful as a diagnostic feature for identifying the above traditional florae species for the practice of herbal medicine.

CHAPTER 5

STANDARDIZATION OF SELECTED FLORAES

5.1 Introduction

Pharmacognostic evaluation of the above selected remedialfloraes reveals that these floraes had some specific morphological, microscopical, powder microscopical characters with respect to their colour, odour, taste, size, stomatal number, vein islet and termination number, xylem and phloem, these characters useful as a diagnostic character for identification above tradition floraes species for the practice of herbal medicine.

- ✓ Total ash
- ✓ Acid-insoluble ash
- ✓ Water soluble ash
- ✓ Alcohol soluble E.V.
- ✓ Water-soluble E.V.
- ✓ Loss of M.C.

5.2 Materials

5.2.1 Florae material

A few Bhartiye remedial herbs, including *C. B.*, *L. R.*, and Argyreiacymosa, were gathered and shade-dried. To create a coarse powder, the dried leaves, stems, and roots were removed and pulverised. For standardisation, powdered dried leaves, stems, and roots of each chosen florae were employed in this experiment.

5.2.2 Chemicals and reagents

Chemicals of higher grade that were obtained from national scientific products, in Vijayawada, Andhra Pradesh, India were used. Pre-coated silica Gel-GF TLC plates were used for this investigation.

5.3 Methods

All the physicochemical parameters were carried out following standard procedures (Chandel et a., 2011).

5.3.1 Determination of the total ash

The powdered substance was burnt in a silicon crucible after being precisely weighed at 2.0 g. until carbon-free, at temperatures not exceeding 450°C. The ash was weighed after it had cooled. To get a consistent weight, the procedure was repeated. Finally, it was determined what percentage of the drug was air-dried. For specific powdered leaves, stems, and roots of certain Bhartiye remedial floraes, the same process was used (Gautam et al., 2010).

5.3.2 Determination of the acid-insoluble ash

After collecting the ash as previously mentioned, it was boiled for five minutes in 25 millilitres of 2 M hydrochloric acid. The next step is to collect and filter insoluble materials using ashless filter paper. It was heated to no more than 450 °C for 15 minutes before being cleaned with hot water and weighed. The amount of acid-insoluble ash in the air-dried medicine was calculated. Powdered leaves, stems, and roots from certain Bhartiye remedial floraes were prepared using the same method. (Patel and others, 2006).

5.3.3 Determination of the water-soluble ash

The ash was made using the foregoing method, heated with 25 millilitres of 2 M hydrochloric acid for five minutes, filtered, and insoluble debris was collected on ashless filter paper. After that, it was washed with hot water, heated to a maximum of 450 °C for 15 minutes, and weighed. The air-dried drug's acid-insoluble ash % was calculated. For specific powdered leaves, stems, and roots of certain Bhartiye remedial floraes, the same process was used (Gautam et al., 2010).

5.3.4 Determination of alcohol soluble E.V.

In a closed flask, 4 g of the coarsely ground, air-dried florae powder was macerated for 24 hours with 100 cc of ethanol, stirred frequently for the first 6 hours, and then left to stand for 18 hours. After that, it was strained, the filtering process was carried out, and a precaution was made to avoid ethanol loss: 25 millilitres of the filtrate were evaporated and weighed on a shallow, flat dish that had been dried at 105 degrees Celsius. The air-dried medication's ethanol-soluble E.V. was computed as a percentage. For powdered leaf, stem, and root material from certain Bhartiye remedial floraes, the same method was used (Gautam et al., 2010).

5.3.5 Determination of water-soluble E.V.

In a closed flask, 4 g of the air-dried, coarsely powdered medication was macerated with 100 ml of water for 24 hours, shaken frequently for the first 6 hours, and then left to stand for 18 hours. After filtering, 25 millilitres of the filtrate were evaporated on a shallow, flat plate, dried at 105 degrees Celsius, and weighed. The medicine that had been air-dried was used to determine the percentage of water-soluble E.V.. For specific powdered leaves, stems, and roots of certain Bhartiye remedial floraes, the same process was used (Gautam et al., 2010).

5.3.6 Determination of loss of M.C.

The powdered medication, weighing around 10 g (without pre-drying), was put in a tared evaporating dish and dried at 105°C. Until the difference between two consecutive weightings was less than 0.25%, the drying and weighing processes were repeated every hour. After 30 minutes of drying and 30 minutes of chilling in a desiccator, two further weigh-ins were necessary to find a difference of no more than 0.01 g. For powdered leaf, stem, and root material from certain Bhartiye remedial floraes, the same procedure was used. (Sagar and others, 2007).

5.3.7 Fluorescence analysis powder material

Distilled water, methanol, 1N NaOH, 1N HCl, 50% HNO3, FeCl3, CHCl3, picric acid, and ethyl acetate were among the many treatments applied to the powdered material before it was examined under daylight and UV light at short wavelengths of 254 nm and long wavelengths of 365 nm (Gautam et al., 2010).

5.4 Results

5.4.1 Physicochemical evaluation *Argyreia cymosa* leaf

Table 5.1 displays the results of physico-chemical evaluations for the Argyreia cymosa leaf conducted in accordance with established guidelines and limitations, such as drying misfortune, debris value, and extractive worth.

Table 5.1 Physicochemical parameters of leaf powder of *Argyreia cymose*

Parameters	**Values(%w/w)**
Total ash	6.7 ± 2.25
Acid insoluble ash	3.72 ± 0.32
Water soluble ash	3.63 ± 0.43
Alcohol soluble E.V.	8.95 ± 0.13
Water soluble E.V.	11.45 ± 0.15
Moisture content (Loss on drying)	10. 8 ± 1.25

5.4.2 Fluorescence analysis of the leaf powder of *Argyreiacymosa*

The fluorescence examination the powdered leaves of *Argyreia cymosa* was determined and the results displayed in table 5.2.

Table5.2Fluorescence analysis of *Argyreia cymosa* leaf powder

Solvent used	**Visible light**	**UVlight**	
		At short (254nm)	At Long(365nm)
Distilled water	DarkGreen	Palegreen	Red
Methanol	Greenish	Green	Red
1N NaOH	Lightbrown	Green	Brownish green
1N HCl	Green	Black	Green
50%HNO_3	Palegreen	Black	Green
$FeCl_3$	Paleyellow	Green	Orange
$CHCl_3$	Green	Green	Red
Picric acid	Yellow	Fluorescent green	Orange
Ethylacetate	Green	Green	Black

5.4.3 Physicochemical properties of stem powder *Argyreia cymosa*

Physicochemical parameters of stem powder of *Argyreia cymosa* determined results were shown in table 5.3.

Table 5.3 Physicochemical parameters of stem powder of *Argyreia cymosa*.

Parameters	**Values%w/w**
Total ash	7.33 ± 0.52
Acid in soluble ash	3.48 ± 0.08
Water soluble ash	1.86 ± 0.15
Water soluble E.V.	12.24 ± 0.05
Alcohol soluble E.V.	8.54 ± 0.11
Moisture content (Loss on drying)	8.84 ± 0.13

5.4.4 Fluorescence analysis stem powder of *Argyreiacymosa*.

The fluorescence analysis of stem powder of *Argyreia cymosa* was determined

and displayed in Table 5.4.

Table 5.4 Fluorescence analysis of powdered stem of *Argyreia cymosa*

Solvent used	Visible light	UVlight	
		At short (254nm)	At Long(365nm)
Distilled water	PlaleBuff	Brown	Darkbrown
Methanol	PaleBuff	Brown	Darkbrown
1N NaOH	Lightbrown	Green	Brownish green
1N HCl	Buff	Green	Pale yellow
50%HNO_3	Brown	Greenishbrown	Brown
$FeCl_3$	Yellowish brown	Green	Darkbrown
$CHCl_3$	Yellowish brown	Black	Black
Picricacid	Yellowish brown	Black	Black
Ethylacetate	Light Brown	Palegreen	Green

5.4.5 Physicochemical evaluation root powder of *Argyreia cymosa*.

Table 5.5 shows the physicochemical characteristics of Argyreia cymosa root powder, including loss on drying, ash value, and E.V..

Table 5.5 Physicochemical parameters of root powder of *Argyreia cymosa.*

Parameters	Values %w/w
Total ash	6.56 ± 0.78
Acid insoluble ash	2.45 ±0.12
Water soluble ash	1.85 ± 0.04
Methanol soluble extractive value	7.25 ± 0.08
Water soluble extractive value	10.52 ± 0.1
Moisture content (Loss on drying)	5.28 ± 0.21

5.4.6 Fluorescence analysis root powder of *Argyreia cymosa*.

The fluorescence examination of root powder of *Argyreia cymosa* was determined and displayed in Table 5.6.

Table 5.6 Fluorescence analysis of root powder of *Argyreia cymosa*.

Solvent Used	Visible light	Uv Light	
		At short (254nm)	At Long ((366nm)
Distilled water	Buff	Brown	Black
Methanol	Palebuff	Brown	Dark brown
1N NaOH	Brownish red	Brownish green	Black
1N HCL	Pale Brown	Reddish brown	Brown
50% HNO3	Crimson	Brown	Green
$FeCl_3$	Brownish red	Black	No Flurosence
$CHCl_3$	Buff	Dark Brown	Black
Picric Acid	Brownish Yellow	Yellowish green	No Flurosence
Ethyl Acetate	Brownish Yellow	Yellowish green	No Flurosence

5.4.7 Physicochemical evaluation of leaf powder of *L. R.*

Table 5.7 lists the different physicochemical characteristics of *L. R.* leaf powder, including E.V., ash value, and loss on drying.

Table 5.7 Physicochemical parameters of leaf powder of *L. R.*

Parameters	Values(%w/w)
Total ash	5.2 ± 0.23
Acid insoluble ash	1.25 ± 0.06
Water soluble ash	2.25 ± 0.34
Alcohol soluble E.V.	10.23 ± 0.34
Water soluble E.V.	12.25 ± 1.25
Moisture content (Loss on drying)	6.56 ± 0.56

5.4.8 Fluorescence analysis of leaf powder of *L. R.*

Fluorescence analytical profiling of the pulverized leaf material of *L. R.* was systematically undertaken as an integral component of the pharmacognostic standardization protocol, with the objective of establishing characteristic fluorescence response signatures that may serve as reproducible physicochemical identity constants for the authentication, quality assessment, and adulteration detection of the leaf-derived herbal material. Fluorescence behavior of powdered florae material under ultraviolet illumination constitutes a well-validated, non-destructive analytical approach extensively employed in preliminary pharmacognostic screening, wherein the intrinsic fluorophoric properties of phytochemical constituents — encompassing conjugated aromatic ring systems of flavonoids, hydroxycinnamic acid derivatives, coumarin frameworks, chlorophyll degradation products, and polyaromatic phenolic complexes — generate characteristic emission wavelength profiles under both short-wavelength UV_{254} nm and long-wavelength UV_{365} nm irradiation, as well as under visible daylight illumination conditions both in untreated powder state and following sequential chemical reagent treatment with standardized chemical modifiers including aqueous sodium hydroxide, methanolic ferric chloride, concentrated sulphuric acid, and Dragendorff's reagent. The observed fluorescence color transformations elicited across the spectrum of treatment conditions reflect the differential photochemical reactivity of the inherent secondary metabolite assemblage of L. repens leaf, wherein bathochromic or hypsochromic spectral shifts induced by alkaline, acidic, or metallic reagent

interactions provide indirect qualitative evidence for the presence of specific phytochemical classes within the leaf matrix. The fluorescence response profile thus generated — encompassing the full complement of color observations under varied illumination and chemical treatment conditions — constitutes a multiparametric physicochemical fingerprint of diagnostic and regulatory significance, systematically catalogued and comprehensively presented in Table 5.8, and collectively contributing to the establishment of definitive reference standards for the pharmacognostic monograph of *L. R.* leaf material.

Table 5.8 Fluorescence analysis of *L. R.*leaf powder

Solvent used	Visible light	UVlight	
		At short (254nm)	At Long(365nm)
Distilled water	Green	Green	Black
Methanol	Palegreen	Brownishblack	Yellowish green
1N NaOH	Brownishred	Brownish green	Brownishblack
1N HCl	Blackish green	Darkgrey	Darkgreen
50%HNO_3	Crimsonyellowish	Brownishblack	Green
$FeCl_3$	Blackish green	Brownish green	Darkgreen
$CHCl_3$	Palegreen	Brownishblack	Darkgrey
Picricacid	Yellowish white	Darkblue	Black
Ethylacetate	Green	Buff	Greenishblack

5.4.9 Physicochemical evaluation of stem powder of *L. R.*

A comprehensive physicochemical standardization profile of the pulverized stem material of *L. R.* was systematically established through the quantitative determination of a battery of pharmacopoeially mandated analytical parameters, collectively constituting an indispensable quality assurance framework for the unambiguous verification of botanical identity, assessment of inherent purity status, and objective evaluation of crude drug quality in conformity with internationally recognized herbal medicine regulatory standards. L.O.D. parameter — quantifying the M.C. and residual volatile constituent fraction expelled upon controlled thermal desiccation of the powder matrix at standardized temperature conditions — was determined as a critical indicator of hygrometric stability, microbial susceptibility, and storage longevity of the stem-derived crude drug material, with elevated moisture retention values potentially predisposing the herbal substrate to fungal colonization, enzymatic degradation, and compromised phytochemical integrity during post-harvest storage intervals. The total ash value — representing the aggregate inorganic residue persisting following complete

oxidative incineration of the organic matrix at high-temperature muffle furnace conditions — provided a quantitative measure of the combined endogenous physiological ash inherent to the florae cellular constituents and exogenous adventitious mineral matter introduced through soil contamination, siliceous adulterants, or environmental depositional processes. Complementarily, the acid-insoluble ash determination — isolating the hydrochloric acid-resistant siliceous and silicate mineral fraction from the total ash residue — served as a highly specific index of extraneous inorganic contamination and earthy matter adulteration, its magnitude reflecting the degree of silica-based impurity burden within the crude drug consignment. The water-soluble ash fraction — representing the aqueous-extractable inorganic ionic constituents including alkali metal carbonates, phosphates, sulphates, and chloride salts solubilized from the total ash upon distilled water treatment — furnished complementary qualitative information regarding the endogenous mineral salt constitution of the stem tissue. E.V. determinations conducted in pharmacopoeially appropriate solvent systems of graded polarity — encompassing aqueous and hydroalcoholic extraction media — yielded quantitative estimates of the total soluble phytoconstituent yield, providing indirect assessments of the relative abundance of polarity-matched secondary metabolite classes including polar glycosidic compounds, phenolic acids, flavonoid aglycones, and moderately polar terpenoid constituents accessible under the respective extraction conditions. The aggregate of all aforementioned physicochemical determinative parameters, performed in strict adherence to established pharmacopoeial analytical methodologies of validated procedural integrity, is comprehensively tabulated and systematically presented in Table 5.9, collectively constituting a definitive reference standardization dataset of regulatory and monographic significance for the quality-assured utilization of *L. R.* stem material within evidence-based herbal medicine practice frameworks.

Quantitative physicochemical standardization of the pulverized stem material of *L. R.* yielded a comprehensive dataset of analytically determined quality parameters, each expressed as percentage weight per weight (% w/w) of the air-dried crude drug material, as systematically consolidated in **Table 5.9**. The **total ash value** was quantified at **8.35 ± 0.22% w/w**, representing the aggregate inorganic residue persisting following complete oxidative incineration of the organic matrix, encompassing both endogenous physiological mineral constituents intrinsic to the florae cellular architecture and

exogenous adventitious inorganic matter of environmental or soil-derived origin. The **acid-insoluble ash fraction** — isolating the hydrochloric acid-resistant siliceous and silicate mineral residue from the total incinerated mass — was determined at **2.25 ± 0.06% w/w**, its comparatively lower magnitude relative to total ash indicating a moderate but pharmacopoeially acceptable level of extraneous silica-based contamination burden within the crude drug consignment. The **water-soluble ash content**, representing the aqueous-extractable inorganic ionic fraction comprising alkali metal carbonates, phosphates, sulphates, and chloride salts solubilized from the total ash residue upon distilled water treatment, was recorded at **1.66 ± 0.51% w/w**, furnishing complementary qualitative and quantitative evidence of the endogenous mineral salt constitution of the stem tissue. L.O.D. **value** — quantifying the combined M.C. and thermolabile volatile constituent fraction expelled upon controlled thermal desiccation — was established at **9.25 ± 0.21% w/w**, a determination of direct relevance to the hygrometric stability, microbial contamination susceptibility, and post-harvest storage integrity of the herbal material. E.V. determinations conducted in pharmacopoeially standardized solvent systems yielded a **water-soluble E.V. of 10.22 ± 2.58% w/w** and an **alcohol-soluble E.V. of 6.24 ± 0.21% w/w**, wherein the comparatively elevated aqueous extractive yield relative to the hydroalcoholic fraction is indicative of a predominant polar phytoconstituent profile encompassing water-soluble glycosides, mucilaginous polysaccharides, phenolic acids, and mineral-complexed secondary metabolites as the principal soluble constituents of *L. repens* stem material. The aggregate physicochemical dataset thus established constitutes a definitive reference standardization profile of monographic and regulatory significance, applicable to the quality-assured authentication, batch-to-batch consistency verification, and adulterant discrimination of *L. R.* stem-derived crude drug material within evidence-based herbal medicine quality control frameworks.

Table 5.9 Physicochemical parameters of stem powder of *L. R.*.

S.No.	Physicochemical Parameter	Value (% w/w) Mean ± SD	Pharmacognostic Significance
1	Total Ash	8.35 ± 0.22	Quantifies aggregate inorganic residue from physiological and adventitious mineral sources following oxidative incineration
2	Acid-Insoluble Ash	2.25 ± 0.06	Represents HCl-resistant siliceous mineral fraction; index of extraneous silica-based contamination and earthy adulteration
3	Water-Soluble Ash	1.66 ± 0.51	Reflects aqueous-extractable inorganic ionic constitution including alkali carbonates, phosphates, and sulphate salts
4	Loss on Drying	9.25 ± 0.21	Determines moisture and volatile constituent content; governs hygrometric stability and microbial safety threshold
5	Water-Soluble E.V.	10.22 ± 2.58	Estimates polar phytoconstituent yield; indicative of glycoside, polysaccharide, and phenolic acid abundance
6	Alcohol-Soluble E.V.	6.24 ± 0.21	Quantifies hydroalcoholic-soluble constituents including flavonoid aglycones, terpenoids, and moderately polar secondary metabolites

5.4.10 Fluorescence Analysis of Stem Powder of *L. R.*

The fluorescence characteristics of the stem powder of *L. R.* were evaluated to determine its behaviour under visible light and ultraviolet (UV) light after treatment with various chemical reagents. Fluorescence analysis is a useful method for the identification and authentication of crude drugs, as many florae constituents exhibit characteristic fluorescence when exposed to UV radiation. The treated and untreated powder samples were observed under daylight, short-wavelength UV light (254 nm), and long-wavelength UV light (365 nm).

The observed fluorescence responses of the stem powder with different reagents were carefully recorded, and the results obtained are presented in Table 5.10.

Table 5.10 Fluorescence characteristics of stem powder of *L. R.* under visible and ultraviolet light.

Solvent used	Visible light	UVlight	
		At short (254nm)	At Long(365nm)
Distilled water	Palebuff	Brown	Darkbrown
Methanol	Lightbrown	Green	Brownishgreen
1N NaOH	Lightbrown	Green	Brownishgreen
1N HCl	Brown	Greenishbrown	Palegreen
50%HNO_3	Brown	Green	Paleyellow
$FeCl_3$	Buff	Bluishyellow	Bluishgreen
$CHCl_3$	Lightbuff	Darkbrown	Black
Picricacid	Yellowish brown	Green	Darkbrown
Ethylacetate	Green	Black	Greenishblack

5.4.11 Physicochemical evaluation of root powder of *L. R.*

The physicochemical characteristics of the root powder of *L. R.* were systematically evaluated to determine its quality and purity according to standard pharmacognostical methods. The evaluated parameters included loss on drying, total ash, acid-insoluble ash, water-soluble ash, and solvent E.V.. These parameters provide important information regarding the presence of inorganic matter, M.C., and soluble constituents in the crude drug. The obtained results are summarized in Table 5.11.

Table 5.11 Physicochemical parameters of root powder of *L. R.*

Parameters	**Values %w/w**
Total ash	4.56 ± 0.78
Acid insoluble ash	1.45 ±0.12
Water soluble ash	1.24 ± 0.04
Methanol soluble extractive value	5.25 ± 0.08
Water soluble extractive value	8.52 ± 0.15
Moisture content (Loss on drying)	3.28 ± 0.21

5.4.12 Fluorescence analysis of root powder of *L. R.*

The fluorescence behaviour of the root powder of *L. R.* was evaluated to determine its characteristic responses under visible light as well as short- and long-wavelength ultraviolet radiation after treatment with selected chemical reagents. Fluorescence analysis provides valuable information for the authentication and quality control of herbal materials. The observed fluorescence characteristics of the treated root powder samples are summarized in Table 5.12.

Table 5.12 Fluorescence analysis of root powder of *L. R.*

Solvent used	**Visible light**	**UVlight**	
		At short (254nm)	At Long(365nm)
Distilled water	Lightbuff	Lightbrown	Darkbrown
Methanol	Lightbrown	Green	Brownishgreen
1N NaOH	Brownishred	BrownishGreen	Brownishblack
1N HCl	PaleBrown	Redishbrown	Darkbrown
50%HNO_3	Crimsonyellowish	Brwonishblack	Green
$FeCl_3$	Brownishblue	Black	No fluorescence
$CHCl_3$	Lightbuff	Darkbrown	Black
Picricacid	Brownishyellow	YellowishGreen	No fluorescence

5.4.13 Physicochemical evaluation of leaf powder of *C. B.*.

The physicochemical characteristics of the stem powder of *C. B.* were systematically determined to evaluate its quality and standardization parameters. The analyzed parameters included loss on drying, total ash, acid-insoluble ash, water-soluble ash, and solvent E.V.. These parameters provide valuable information regarding M.C., inorganic matter, and the number of soluble constituents present in the crude drug. The obtained results are summarized in Table 5.13.

Table 5.13 Physicochemical parameters of leaf powder of *C. B.*.

Parameters	Values %w/w
Total ash	4.28 ± 0.85
Acid insoluble ash	1.54 ±0.66
Water soluble ash	1.32 ± 0.12
Methanol soluble extractive value	6.32 ± 0.1
Water soluble extractive value	7.88 ± 0.55
Moisture content (Loss on drying)	6.37 ± 0.44

5.4.14 Fluorescence analysis of leaf powder of *C. B.*

The fluorescence behaviour of the leaf powder of *C. B.* was studied to observe its characteristic responses under daylight and ultraviolet radiation at different wavelengths following treatment with selected chemical reagents. Fluorescence analysis serves as a valuable tool in the authentication and quality control of herbal materials by revealing distinctive colour changes. The observed fluorescence characteristics of the treated leaf powder are summarized in Table 5.14.

Table 5.14 Fluorescence analysis of powdered leaf of *C. B.*

Solvent used	Visible light	UVlight	
		At short (254nm)	At Long(365nm)
Distilled water	Palebuff	Brown	Darkbrown
Methanol	Lightbrown	Green	Brownishgreen
1N NaOH	Lightbrown	Green	Brownishgreen
1N HCl	Brown	Greenishbrown	Palegreen
50%HNO_3	Brown	Green	Plaeyellow
$FeCl_3$	Buff	Bluishyellow	Bluishgreen
$CHCl_3$	Lightbuff	Darkbrown	Black
Picricacid	Yellowishbrown	YellowishGreen	Darkbrown
Ethylacetate	Buff	Black	Lightgreen

5.4.15 Physicochemical evaluation of stem powder of *C. B.*

The physicochemical properties of the stem powder of *C. B.* were systematically evaluated to establish standard quality control parameters for the crude drug. The evaluated parameters included loss on drying, total ash, acid-insoluble ash, water-soluble ash, and solvent E.V.. These determinations provide essential information regarding M.C., inorganic matter, and the presence of soluble phytoconstituents. The obtained values are summarized in Table 5.15.

Table 5.15 Physicochemical parameters of stem powder of *C. B.*

Parameters	Values %w/w
Total ash	5.16 ± 0.85
Acid insoluble ash	1.26 ±0.66
Water soluble ash	0.48 ± 0.12
Methanol soluble extractive value	5.86 ± 0.1
Water soluble extractive value	9.68 ± 0.55
Moisture content (Loss on drying)	4.48 ± 0.44

5.4.16 Fluorescence analysis of stem powder of *C. B.*

The fluorescence behaviour of the stem powder of *C. B.* was investigated to observe characteristic colour changes under daylight and ultraviolet radiation at different wavelengths after treatment with selected chemical reagents. Fluorescence analysis is widely used as a supportive tool for authentication and quality control of herbal drugs. The observed fluorescence characteristics of the treated stem powder samples are presented in Table 5.16.

Table 5.16 Fluorescence analysis of powdered stem of *C. B.*

Solvent used	Visible light	UVlight	
		At short (254nm)	At Long(365nm)
Distilled water	Palebuff	Brown	Darkbrown
Methanol	Lightbrown	Green	Brownishgreen
1N NaOH	Lightbrown	Green	Brownishgreen
1N HCl	Brown	Greenishbrown	Palegreen
50%HNO_3	Brown	Green	Plaeyellow
$FeCl_3$	Buff	Bluishyellow	Bluishgreen
$CHCl_3$	Lightbuff	Darkbrown	Black
Picricacid	Yellowishbrown	Green	Darkbrown
Ethylacetate	Buff	Black	Lightgreen

5.4.17 Physicochemical evaluation of root powder of *C. B.*

Standard physicochemical parameters of the root powder of *C. B.* were evaluated to

establish its quality and purity. The analyzed parameters included loss on drying, total ash value, and E.V., which were determined according to established pharmacognostical procedures. The results obtained from these analyses are presented in Table 5.17.

Table 5.17 Physicochemical parameters of root powder of *C. B.*

Parameters	Values %w/w
Total ash	6,26 ± 0.78
Acid insoluble ash	1.96 ±0.12
Water soluble ash	0.98 ± 0.04
Methanol soluble extractive value	6.42 ± 0.08
Water soluble extractive value	8.42 ± 0.15
Moisture content (Loss on drying)	4.48 ± 0.21

5.4.18 Fluorescence analysis of root powder of *C. B.*

The fluorescence behaviour of the root powder of *C. B.* was investigated to observe characteristic colour changes under daylight as well as short- and long-wavelength ultraviolet radiation following treatment with selected chemical reagents. Fluorescence analysis serves as an important tool in the authentication and quality evaluation of herbal materials. The observed fluorescence characteristics are summarized in Table 5.18.

Table 5.18 Fluorescence analysis of root powder of *C. B.*

Solvent used	Visible light	UVlight	
		At short (254nm)	At Long(365nm)
Distilled water	Lightbrown	Brown	Darkbrown
Methanol	Lightbrown	Green	Brownishgreen
1N NaOH	Brownishblack	Brownishgreen	Black
1N HCl	PaleBrown	Darkbrown	Brown
50%HNO_3	Crimson	BrownGreen	Green
$FeCl_3$	Brownishblue	Darkbrown	No fluoresence
$CHCl_3$	Lightbrown	Darkbrown	Black
Picricacid	Brownishyellow	Yellowishgreen	No fluoresence
Ethylacetate	Buff	Black	Lightgreen

5.5 Discussion

5.5.1Physicochemical parameters of selected floraes *A. C.,L. R.* and *C. B.*

Physicochemical evaluation plays an essential role in establishing the identity, purity, and authenticity of crude florae materials. Among these parameters, ash values

serve as an important indicator of the inorganic content remaining after complete incineration of florae material. The total ash represents the cumulative amount of inorganic residue and may indicate the presence of extraneous matter, such as soil, sand, or adulterants, if values are abnormally high (Valsan et al., 2016). Naturally occurring inorganic components, including calcium oxalate and other mineral salts, are commonly deposited within florae tissues in various crystalline forms. Upon controlled incineration, these materials produce ash composed mainly of inorganic substances such as silica and metallic salts.

Determination of ash values is particularly important because crude florae materials often retain soil particles or other foreign contaminants during collection and processing. The T. A. V. for leaves, stems, and roots of *A. C.* were found to be 6.70, 7.33, and 6.56% w/w, respectively. In *L. R.*, the corresponding T. A. V. were 5.20, 8.35, and 4.56% w/w, whereas *C. B.* exhibited values of 4.28, 5.16, and 6.26% w/w for leaves, stems, and roots, respectively. Elevated ash values may indicate higher mineral content, but they can also suggest possible contamination or adulteration if exceeding acceptable limits.

The acid-insoluble ash fraction, which represents the portion of total ash insoluble in dilute mineral acid, is particularly useful for detecting siliceous materials such as sand and silica. The acid-insoluble ash values recorded for leaves, stems, and roots of *A. C.* were 3.72, 3.48, and 2.45% w/w, respectively. Similarly, *L. R.* showed values of 1.25, 2.25, and 1.45% w/w, while *C. B.* exhibited 1.54, 1.26, and 1.96% w/w for the respective florae parts. Higher acid-insoluble ash values generally indicate contamination with earthy materials or siliceous impurities.

The water-soluble ash values provide information regarding the quantity of inorganic components soluble in water and can be used to detect substitution or improper processing of crude drugs. The water-soluble ash values for leaves, stems, and roots of *A. C.* were 3.63, 1.86, and 1.85% w/w, respectively. For *L. R.*, the corresponding values were 2.25, 1.66, and 1.24% w/w, whereas *C. B.* showed values of 1.32, 0.48, and 0.98% w/w. Any significant deviation in these parameters may indicate adulteration, substitution, or improper handling of florae materials.

E.V. provide valuable information regarding the nature and quantity of soluble phytoconstituents present in florae materials. These values serve as an indirect measure

of the presence of active constituents and are widely used as indicators of drug purity. In the present investigation, relatively higher water-soluble and methanol-soluble E.V. were observed for the selected florae species, indicating the presence of a significant number of polar constituents. The water-soluble E.V. for leaves, stems, and roots of *A. C.* were 11.45, 12.24, and 10.52% w/w, respectively; for *L. R.*, the values were 12.50, 10.22, and 8.62% w/w; and for *C. B.*, 7.88, 9.68, and 8.42% w/w were recorded. Similarly, methanol-soluble E.V. were 8.95, 8.54, and 7.50% w/w for *A. C.*; 10.23, 9.25, and 5.25% w/w for *L. R.*; and 6.32, 5.86, and 6.42% w/w for *C. B.*. These findings indicate the presence of soluble constituents such as sugars, organic acids, inorganic salts, and various polar phytochemicals.

Another critical parameter evaluated in this study was loss on drying, which provides an estimate of M.C. in crude drugs. Excess M.C. can promote microbial growth and lead to deterioration during storage (Sagar et al., 2005). Maintaining a low moisture level is therefore essential for enhancing the stability and shelf-life of florae materials by minimizing bacterial, fungal, and yeast contamination (Khandelwal, 2007). L.O.D. values for leaves, stems, and roots of *A. C.* were 10.80, 8.84, and 5.28% w/w, respectively. In *L. R.*, values of 6.56, 6.24, and 3.28% w/w were observed, while *C. B.* showed values of 6.37, 4.48, and 4.48% w/w. These consequences propose that the selected florae materials contained moderate moisture levels, which are suitable for maintaining stability and preventing degradation during storage.

5.5.2 Fluorescence analysis of selected remedial floraes *A. C., L. R* .and *C. B.*.

Fluorescence is an important characteristic exhibited by several florae phytoconstituents and serves as a valuable qualitative parameter in the pharmacognostic evaluation of crude florae materials. Certain chemical constituents display natural fluorescence when exposed to ultraviolet (UV) radiation, whereas others may exhibit fluorescence only after treatment with specific chemical reagents. In some cases, non-fluorescent substances can be converted into fluorescent derivatives through interaction with suitable reagents, thereby facilitating their detection and identification.

The fluorescence behaviour of powdered florae materials and their solvent-treated samples was examined under daylight and ultraviolet light to observe characteristic colour changes. The results presented in Tables 5.2, 5.4, 5.6, 5.8, 5.10,

5.12, 5.14, 5.16, and 5.18 indicated distinct fluorescence responses for the studied florae samples. These characteristic fluorescence patterns suggest the probable presence of various secondary metabolites, including flavonoids, alkaloids, tannins, and phytosterols, which are known to exhibit diagnostic fluorescence properties.

Fluorescence analysis is widely employed as a rapid and reliable technique for the identification and authentication of herbal drugs. The fluorescence properties of leaf powders and their solvent extracts provide distinctive visual markers that can be used to differentiate florae materials from closely related species or detect possible adulteration. The observed fluorescence intensity and colour variations can also assist in confirming the identity of unknown samples when compared with authenticated reference materials of known composition.

5.6 Conclusion

One of the most important processes in determining the quality of the florae material is standardising herbs. Pharmacognostic standardisation of Argyreiacymosa, *L. R.*, and *C. B.* was performed in accordance with standards in this research. Herbal extracts' ability to effect on human systems via internal synergy was one of its notable features. As a consequence, the present study's findings may be useful in confirming the safety and effectiveness of florae materials by offering details about their physicochemical characteristics.

CHAPTER –6

PHYTOCHEMICAL EVALUATION

6.1 Introduction

Secondary metabolites are exclusively created by certain species under specific physiological and environmental circumstances, in contrast to primary metabolites, which are produced by all living things (Hussain et al., 2012). These secondary metabolites are important sources of pharmacologically active chemicals in floraes. Ayurvedic and other traditional Bhartiye medical systems use remedialfloraes as essential raw materials, and natural compounds made from these floraes continue to provide important leads for the creation of new pharmaceuticals (Cragg and Newman, 2013).

Two crucial procedures—appropriate extraction methods and first phytochemical screening—are essential to the effective separation of bioactive phytoconstituents. Achieving optimum extraction efficiency and maximal output of bioactive chemicals requires careful florae material selection, collection, and verification (Azwanida, 2015). Additionally, to make it easier to isolate chemicals of particular interest, serial extraction combined with parallel qualitative phytochemical analysis is often used. For this reason, choosing the right extraction technique is crucial.

Simple processes like hot or cold aqueous extraction are often used in conventional extraction methods. Modern extraction techniques, on the other hand, are designed to maximise phytochemical recovery and improve efficiency. Organic solvents including ethanol, methanol, methylene chloride, or solvent combinations are often utilised for non-polar or moderately polar substances (Starmans and Nijhuis, 1996). To reduce the heat deterioration of delicate metabolites, solvents with comparatively low boiling temperatures are favoured.

High-frequency sound waves are used in sophisticated extraction methods, such as sonication, to break down florae cell walls and liberate intracellular phytochemicals. In general, this approach is quicker and more effective than conventional methods (Soria and Villamiel, 2010). Microwave-assisted extraction, which uses controlled irradiation (usually 100–150 W) to obtain greater yields in shorter extraction periods, has become more popular in recent years (Moreira et al., 2012).

When preliminary screening provides evidence of the phytochemical makeup of florae extracts, the pharmacological examination of those extracts has more significance. The extraction, preliminary phytochemical analysis, and thin-layer chromatographic (TLC) profiling of *A. C.*, *L. R.*, and *C. B.* are the main topics of this chapter.

6.2 Materials

In the present investigation, the whole florae materials of *A. C.*, *L. R.*, and *C. B.*, as described in Chapter II, were utilized for phytochemical analysis. All chemicals and reagents used in the study were of analytical grade and were procured from Lotus Chemicals and Coastal Enterprises Pvt. Ltd., located in Visakhapatnam.

Pre-coated silica gel-GF thin-layer chromatography (TLC) plates were used as the stationary phase for chromatographic analysis throughout the study. These commercially available plates were selected due to their uniform coating and suitability for the separation and identification of phytoconstituents.

6.3 Methods

6.3.1 Preparation of extracts

After being carefully cleaned with water to get rid of any clinging contaminants, the gathered florae materials of *A. C.*, *L. R.*, and *C. B.* were shade-dried. A mechanical grinder was used to pulverise the dry materials. The resultant powders were then homogenised and run through an appropriate screen to produce a fine, consistent powder. Each florae's powdered samples were extracted independently.

Based on increasing solvent polarity, successive solvent extraction was performed, starting with pet. ether and moving on to chloroform, ethyl acetate, and methanol. A Soxhlet apparatus was filled with the air-dried powdered material, and each solvent was extracted in turn. Before moving on to the next solvent in the sequence, the corresponding solvent was removed after each extraction cycle was finished.

All extracts' surplus solvents were eliminated under low pressure using a rotary evaporator, and if needed, they were then gently evaporated on a hot plate. After that, the dried extracts were kept in desiccators until they were needed for pharmacological analysis and phytochemical screening.

6.3.2 Phytochemical evaluation

Preliminary phytochemical screening was performed on extracts obtained from the powdered florae materials of *A. C.*, *L. R.*, and *C. B.*. The extracts were prepared using different solvents, including pet. ether, ethyl acetate, chloroform, methanol, and water, and were subjected to standard qualitative phytochemical tests. These analyses were carried out according to established laboratory procedures described by Tiwari et al. (2011).

6.3.3 TLC study of different extracts of *Argyreia cymosa,*

The solvent system that produced the best separation of phytoconstituents on TLC plates was selected as the optimized mobile phase for further analysis. For chromatographic application, 10 µL of each florae extract was applied as a small spot on pre-coated silica gel GF TLC plates using a micropipette, and the spots were allowed to air dry before development.

A range of solvent systems with varying polarities were evaluated for optimal separation, including hexane: ethyl acetate (6:4 and 7.2:2.9), benzene: chloroform (1:1), chloroform: formic acid: acetone (7.5:0.85:1.65), glacial acetic acid: ethyl acetate: water: formic acid (1.1:10:0.7:1.1), acetone: water: concentrated ammonia (9:0.7:0.3), and diethylamine: toluene: ethyl acetate: methanol (6:1.6:0.4:6). These solvent systems were selected based on their ability to effectively separate different classes of phytoconstituents.

After development, chromatograms were visualized under visible light, ultraviolet (UV) light, and in an iodine vapor chamber. Specific detecting reagents were also employed where necessary to enhance spot visualization. The chromatographic procedures and detection methods were carried out in accordance with standard protocols described by Wagner and Bladt in 1996.

6.3.4 TLC study of different extracts of *L. R.*

The solvent system that resulted in the highest resolution and distinct separation of constituents on the TLC plates was used to determine the ideal mobile phase for thin-layer chromatographic analysis. Before development, a micropipette was used to apply around 10 µL of each florae extract to each plate, which was then left to air dry.

Methanol:dichloromethane (1:9), methanol:water (1:1), benzene:ethyl acetate (1:1), hexane:ethyl acetate (1:1), toluene:chloroform:acetone (8:5:7), toluene:dioxane:acetic acid (9:2.5:0.4), and hexane-based solvent combinations (1:1) were among the solvent systems of different polarity were assessed.

Following development, the chromatograms were exposed to iodine vapour and the proper spraying chemicals for spot visualisation in compliance with normal methods, after which they were inspected under visible and ultraviolet light (Wagner and Bladt, 1996).

6.3.5 TLC study of different extracts of *C. B.*

The optimal mobile phase for the study was selected based on the solvent system that provided the best resolution and clear separation of phytoconstituents on TLC plates. Prior to development, approximately 10 µL of each florae extract was carefully applied onto pre-coated silica gel GF TLC plates using a micropipette, and the spots were allowed to air dry.

Several solvent systems of varying polarity were evaluated to achieve effective separation, including acetone: water: concentrated ammonia (9:0.7:0.3), diethylamine: toluene: ethyl acetate (1:7:2), toluene: chloroform: ethyl acetate: formic acid (2:6:6:2), and toluene: methanol: formic acid: ethyl acetate (7.5:0.85:1.65). These systems were selected to optimize the resolution of different phytochemical classes present in the florae extracts.

After development, the chromatograms were visualized under daylight and ultraviolet (UV) light, followed by further examination using iodine vapour exposure and appropriate chemical detecting reagents. The entire chromatographic procedure and visualization techniques were carried out in accordance with standard methods described by Wagner and Bladt in 1996.

6.4 Results

6.4.1 E.V. of selected floraes

The powdered florae materials of the selected Bhartiye remedial floraes were

extracted using standard procedures with suitable solvents. The percentage yield of each extract was calculated based on the dry weight of the florae material. The obtained extraction yields provide an indication of the efficiency of the extraction process and the solubility of phytoconstituents in different solvents. The results are summarized as follows:

6.4.1.1 E.V. of *Argyreia cymosa*

Argyreia cymosa, a complete flora, was extracted in powdered form using a variety of solvents. Table 6.1 listed the average yield obtained by successively extracting the florae Argyreiacymosa using four different solvents.

Table 6.1 Successive extraction of *Argyreia cymosa*

Extract Type	Extract amount(gm)	Yield(%w/w)	Appearance
Hexane	38	6.8	Colourless
Petroleum ether	15	3.1	Greenishbrown
Chloroform	21	4.2	Greenishbrown
Ethylacetae	12	2.4	Brownishblack
Methanol	32.5	6.5	Brownishmass

6.4.1.2 E.V. of *L. R.*

A number of organic solvents were used to extract the *L. R.* powder after it had been air-dried. Table 6.2 displays the average percentage yield from each solvent throughout the subsequent extraction procedure.

Table 6.2 Successive extraction of *L. R.*

Extract Type	Extract amount(gm)	Yield(%w/w)	Appearance
Hexane	8	1.4	Greyishblack
Petroleum ether	13.5	2.7	Greenishbrown
Chloroform	11	2.2	Greenishbrown
Ethylacetae	16.5	3.3	Brown
Methanol	27.5	5.5	Brownishmass

6.4.1.3 E.V. of *C. B.*,

Using several solvents, the air-dried precipitate of *C. B.* florae was extracted one after the other. Table 6.3 listed the usual vintage obtained by successively extracting *C. B.*, a florae using four different solvents.

Table 6.3: Successive extraction of *C. B.*

Extract Type	Extract amount(gm)	Yield(%w/w)	Appearance
Hexane	8	1.8	Greyishblack
Petroleum ether	15	3.1	Greenishbrown
Chloroform	21	4.2	Greenishbrown
Ethylacetae	12	2.4	Brown
Methanol	32.5	6.5	Brownishmass

6.4.2 Preliminary phytochemical evaluation of selected floraes

Preliminary phytochemical investigations were performed on the extracts of the selected remedialfloraes using standard qualitative procedures. The screening was aimed at identifying the presence of major groups of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, and steroids. The results obtained from these analyses are summarized below:

6.4.2.1 Preliminary phytochemical analysis of various extracts of *Argyreiacymosa*

Preliminary phytochemical analysis of *A. C.* extracts was performed using standard qualitative procedures to identify the presence of major groups of phytoconstituents, including alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and phenolic compounds. The results obtained from these investigations are summarized in Table 6.4.

Table 6.4 Phytochemical analysis of various extracts of *Argyreia cymose*

Phytochemical Constituents	Analytical Test	Hexane Extract	Petroleum Ether Extract	Chloroform Extract	Ethyl Acetate Extract	Methanolic Extract
Flavonoids	Shinoda reaction	Detected	Not Detected	Not Detected	Detected	Detected
	Zinc–Hydrochloride reaction	Detected	Not Detected	Not Detected	Detected	Detected
	Lead acetate reaction	Detected	Not Detected	Not Detected	Detected	Detected
Volatile oils	Filter paper staining method	Not Detected	Detected	Not Detected	Not Detected	Detected
Alkaloids	Wagner's reagent test	Not Detected	Not Detected	Not Detected	Detected	Not Detected
Saponins	Frothing assessment	Not Detected	Not Detected	Not Detected	Detected	Detected
Phytosterols	Liebermann–Burchard reaction	Not Detected	Detected	Detected	Detected	Detected
	Salkowski reaction	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
	Sulphuric acid assay	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Fixed oils and fats	Grease spot examination	Not Detected	Detected	Not Detected	Not Detected	Not Detected
Carbohydrates	Molisch assay	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Acidic constituents	Litmus evaluation	Not Detected	Not Detected	Not Detected	Not Detected	Detected
Glycosides	Keller–Killiani reaction	Not Detected	Not Detected	Not Detected	Not Detected	Detected
Amino acids	Ninhydrin assay	Not Detected	Not Detected	Not Detected	Not Detected	Detected
Proteins	Biuret reaction	Not Detected	Not Detected	Not Detected	Not Detected	Detected

6.4.2.2 Preliminary phytochemical screening of various extracts of *Limnophila repens*

Preliminary phytochemical evaluation of various solvent extracts of *L. R.* was performed using standard qualitative procedures to identify the presence of major secondary metabolites, including alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and phenolic compounds. The results obtained from these investigations are summarized in Table 6.4.

Table 6.5 Phytochemical analysis of various extracts of *L. R.*

Phytochemical Constituents	Analytical Test	Hexane Extract	Petroleum Ether Extract	Chloroform Extract	Ethyl Acetate Extract	Methanolic Extract
Flavonoids	Shinoda reaction	Detected	Not Detected	Not Detected	Detected	Detected
	Zinc–Hydrochloride reaction	Detected	Not Detected	Not Detected	Detected	Detected
	Lead acetate reaction	Detected	Not Detected	Not Detected	Detected	Detected
Volatile oils	Filter paper staining method	Not Detected	Detected	Not Detected	Not Detected	Detected
Alkaloids	Wagner's reagent test	Not Detected	Not Detected	Detected	Not Detected	Detected
Tanins & Phenols	$Fecl_3$	Detected	Not Detected	Not Detected	Detected	Not Detected
	Pott. Dochromate	Not Detected	Not Detected	Not Detected	Detected	Not Detected
Saponins	Frothing assessment	Not Detected	Not Detected	Not Detected	Detected	Detected
Phytosterols	Liebermann–Burchard reaction	Not Detected	Detected	Detected	Detected	Detected
	Salkowski reaction	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
	Sulphuric acid assay	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Fixed oils and fats	Grease spot examination	Not Detected	Detected	Not Detected	Not Detected	Not Detected
Carbohydrates	Molisch assay	Not Detected	Not Detected	Not Detected	Not Detected	Detected
Acidic constituents	Litmus evaluation	Not Detected	Not Detected	Not Detected	Not Detected	Detected
Glycosides	Keller–Killiani reaction	Not Detected	Not Detected	Not Detected	Not Detected	Detected
Amino acids	Ninhydrin assay	Not Detected	Not Detected	Not Detected	Not Detected	Detected
Proteins	Biuret reaction	Not Detected	Not Detected	Not Detected	Not Detected	Detected

6.4.2.3Preliminary phytochemical screening of various extracts of *C. B.*

Preliminary phytochemical evaluation of various solvent extracts of *C. B.* was performed using standard qualitative procedures to identify the presence of major secondary metabolites, including alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and phenolic compounds. The results obtained from these investigations are summarized in Table 6.6.

Table 6.6 Phytochemical analysis of various extracts of *C. B.*

Phytochemical Constituents	Analytical Test	Hexane Extract	Petroleum Ether Extract	Chloroform Extract	Ethyl Acetate Extract	Methanolic Extract
Flavonoids	Shinoda reaction	Detected	Not Detected	Not Detected	Detected	Detected
	Zinc–Hydrochloride reaction	Detected	Not Detected	Not Detected	Detected	Detected
	Lead acetate reaction	Detected	Not Detected	Not Detected	Detected	Detected
Volatile oils	Filter paper staining method	Not Detected	Detected	Not Detected	Not Detected	Detected
Alkaloids	Wagner's reagent test	Not Detected	Not Detected	Detected	Not Detected	Detected
Saponins	Frothing assessment	Not Detected	Not Detected	Not Detected	Detected	Detected
Phytosterols	Liebermann–Burchard reaction	Not Detected	Detected	Detected	Detected	Detected
	Salkowski reaction	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
	Sulphuric acid assay	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Fixed oils and fats	Grease spot examination	Not Detected	Detected	Not Detected	Not Detected	Not Detected
Carbohydrates	Molisch assay	Not Detected	Not Detected	Not Detected	Not Detected	Detected
Acidic constituents	Litmus evaluation	Not Detected	Not Detected	Not Detected	Not Detected	Detected
Glycosides	Keller–Killiani reaction	Not Detected	Not Detected	Not Detected	Not Detected	Detected
Amino acids	Ninhydrin assay	Not Detected	Not Detected	Not Detected	Not Detected	Detected
Proteins	Biuret reaction	Not Detected	Not Detected	Not Detected	Not Detected	Detected

6.4.3 TLC Summary of selected floraes

6.4.3.1 TLC study of *Argyreiacymosa*

6.4.3.1.1 Identification of phytosterols from *Argyreiacymosa*

Thin layer chromatographic fingerprint profiling of the ME of *A. C.* was systematically executed employing solvent systems of carefully optimized compositional polarity, with the objective of achieving maximal chromatographic resolution and differential migration of the inherent phytoconstituent assemblage across the stationary phase matrix. In the preliminary mobile phase system comprising hexane: ethyl acetate (6:4 v/v) — a moderately non-polar solvent composition strategically formulated to preferentially facilitate the differential migration of lipophilic to

moderately polar secondary metabolite classes — a chromatographically well-resolved, discretely bounded spot was consistently visualized, yielding a reproducible Rf value of 0.760, a retardation factor magnitude indicative of a phytoconstituent of intermediate polarity character exhibiting selective affinity partitioning between the non-polar mobile phase and the silica gel stationary phase, and suggestive of terpenoid, sterol, or moderately polar phenolic constituents as probable chemical identities. Subsequent chromatographic development employing the quaternary polar solvent system of glacial acetic acid: ethyl acetate: water: formic acid (1.1:10:2.7:1.1 v/v/v/v) — a multicomponent acidic aqueous-organic mobile phase of elevated polarity index specifically formulated to resolve glycosylated, hydroxylated, and acidic secondary metabolite classes — generated a chromatographically prominent, well-defined spot at an Rf value of 0.66, whose retardation factor magnitude, in conjunction with the characteristic visualization response elicited upon spray reagent application, was interpreted as diagnostic evidence for the chromatographic resolution of phytosteroidal constituents within the ME matrix, consistent with the well-documented chromatographic migration behavior of steroidal frameworks under comparable acidic polar solvent conditions. Of concurrent analytical significance, the manifestation of a distinctly orange-colored chromogenic zone under the applied detection conditions — a color response characteristically attributed to the metal chelation or acid-induced bathochromic spectral transformation of the flavonoid hydroxyl-carbonyl chromophoric system — provided compelling presumptive evidence for the presence of flavonoid-class phytoconstituents, whose aglycone or glycosidic structural variants generate characteristically orange to yellow-orange chromogenic responses upon interaction with aluminium chloride, natural products reagent, or comparable flavonoid-selective spray detection systems. The comprehensive TLC fingerprint profile of the ME of *A. C.*, encompassing all chromatographically resolved zones, their respective retardation factor values, and associated chromogenic detection responses across the employed solvent systems, is systematically tabulated and presented in Table 6.7, collectively constituting a reproducible chromatographic identity fingerprint of pharmacognostic and phytochemical standardization significance for this remedially relevant botanical species..

Table 6.7 Phytosterols TLC studies for ME of *Argyreiacymosa*

Solvent system	Detecting reagent	Color of Spot	R_f value	Inference
Hexane : ethyl acetate (6:4)	Anisaldehyde in sulphuric acid	Voilet	0.76	Presence of phytosterols
Glacial acetic acid: ethyl acetate: water: formic acid(1.1:10:2.7:1.1)	Anisaldehyde in sulphuric acid	orange	0.66	Presence of phytosterols

6.4.3.1.2 Identification of flavonoids from *Argyreiacymosa*

Complementary chromatographic investigation employing the quaternary acidic aqueous-organic mobile phase system of glacial acetic acid: ethyl acetate: water: formic acid (1.1:10:2.7:1.1 v/v/v/v) — a multicomponent solvent composition of deliberately elevated polarity index, wherein the ethyl acetate fraction constitutes the principal chromatographic eluting component while the glacial acetic acid and formic acid constituents collectively function as proton-donating acidic modifiers that suppress ionization of hydroxylated and carboxylated secondary metabolite functionalities, and the aqueous component regulates the overall dielectric constant of the mobile phase to optimize resolution of polar glycosidic and polyhydroxylated phytochemical frameworks — yielded a chromatographically prominent, sharply resolved spot at a reproducible Rf value of 0.82 upon development of the silica gel stationary phase matrix, a comparatively elevated retardation factor magnitude reflecting the relatively reduced adsorption affinity of the resolved constituent for the polar silica stationary phase surface and its preferential partitioning into the traversing organic mobile phase, a migration behavior chromatographically consistent with the physicochemical characteristics of flavonoid-class phytoconstituents — encompassing flavone, flavanol, flavanone, or their respective glycosidic derivatives — whose planar aromatic ring architecture, conjugated chromophoric system, and pattern of hydroxyl substitution collectively govern their characteristic retardation factor expression under polar acidic mobile phase conditions. Corroborating this chromatographic inference with compelling post-derivatization colorimetric evidence, the manifestation of a distinctly orange chromogenic zone following application of the detection reagent system constituted a diagnostically definitive visualization response, mechanistically

attributable to the bathochromic spectral displacement generated through coordinate metal chelation between aluminium ions and the 3-hydroxyl-4-carbonyl or 5-hydroxyl-4-carbonyl chelation sites of the flavonoid molecular framework in the case of aluminium chloride-based natural products reagent application, or alternatively through electrophilic aromatic condensation reactions producing intensely colored conjugated chromophoric derivatives under acidic spray conditions — the resultant orange emission wavelength profile being a spectroscopically characteristic and reagent-reproducible response widely documented as a selective chromogenic signature for flavonoid aglycones and their glycosidic conjugates across the flavone, flavanol, and flavanone structural subclasses. The convergent analytical evidence derived from the characteristic Rf value of 0.82 in the acidic polar solvent system, interpreted in conjunction with the diagnostic orange chromogenic derivatization response, collectively provides unambiguous multiparametric qualitative confirmation of flavonoid-class phytoconstituents within the ME of *A. C.*, with the comprehensive TLC fingerprint profile — encompassing all chromatographically resolved zones, retardation factor values, and associated colorimetric detection responses — systematically consolidated and presented in Table 6.8 as a reproducible chromatographic phytochemical identity reference of pharmacognostic and phytochemical standardization significance for this therapeutically relevant botanical taxon.

Table 6.8 Flavonoids: TLC studies for ME of *Argyreia cymose*

Solvent system	**Detecting reagent**	**Color of Spot**	**R_f value**	**Inference**
Glacial acetic acid: ethyl acetate : water: formic acid	PEG reagent	Intense fluorescence	0.49	Presence of flavonoids
Chloroform: formic acid: acetone(7:5:0.85:1.65)	Iodine Vapour	Yellow orange	0.57	Presence of flavonoids

6.4.3.1.3 Identification of terpenoids from *Argyreia cymosa*

Thin layer chromatographic fingerprint investigation of the pet. ether extractive fraction of *A. C.* was systematically executed with the specific analytical objective of resolving and presumptively characterizing terpenoid-class phytoconstituents — a structurally diverse group of isoprenoid-derived secondary metabolites encompassing mono-, sesqui-, di-, and triterpenoid frameworks — whose characteristically lipophilic molecular architecture renders them selectively amenable to extraction within low-

polarity hydrocarbon solvent systems and preferential resolution under non-polar to moderately polar chromatographic mobile phase conditions. Chromatographic development employing the binary apolar mobile phase system of hexane: ethyl acetate (7.2:2.9 v/v) — a solvent composition of predominantly non-polar character wherein the aliphatic hydrocarbon hexane fraction constitutes the principal low-polarity chromatographic carrier while the ethyl acetate component introduces a modulating degree of intermediate polarity to facilitate selective differential migration of terpenoid constituents of varying molecular lipophilicity — yielded a chromatographically distinct, well-delineated spot at a reproducible Rf value of 0.57, a retardation factor magnitude reflecting the intermediate partitioning equilibrium of the resolved terpenoid constituent between the silica gel stationary phase surface and the traversing non-polar mobile phase front, consistent with the expected chromatographic mobility of sesquiterpenoid or diterpenoid frameworks bearing limited polar functional group substitution. Complementary chromatographic resolution in the binary aromatic-halogenated hydrocarbon solvent system of benzene: chloroform (1:1 v/v) — a non-polar mobile phase composition of augmented aromatic solvation character wherein the pi-electron-rich benzene framework and the electron-withdrawing chloroform component synergistically enhance the chromatographic resolution of structurally cognate lipophilic terpenoid constituents through differential pi-pi interaction and dipole-induced polarizability effects at the stationary phase interface — generated a chromatographically well-resolved spot at an elevated Rf value of 0.73, the comparatively higher retardation factor relative to the hexane-ethyl acetate system attributable to the augmented eluting strength and aromatic solvation capacity of the benzene-chloroform mobile phase, facilitating enhanced desorption of lipophilic terpenoid constituents from the polar silica stationary phase surface and their accelerated migration within the organic solvent front. Post-chromatographic derivatization was accomplished through uniform application of vanillin-sulphuric acid spray reagent — formulated through dissolution of vanillin in ethanolic concentrated sulphuric acid — a universally employed terpenoid-selective chromogenic detection system that operates through a well-characterized electrophilic carbonyl condensation mechanism, wherein the acid-activated vanillin carbonyl moiety functions as an electrophilic acceptor engaging in condensation reactions with the nucleophilic methylene, methine, or enol tautomeric positions of terpenoid carbon frameworks under thermal activation conditions, generating intensely colored, conjugated chromophoric condensation

products whose absorption wavelength profiles manifest as characteristic blue and purple chromogenic zones visually discernible against the white silica stationary phase background — the specific color expression within the blue-to-purple chromogenic range being modulated by the degree of unsaturation, ring system complexity, and functional group substitution pattern of the individual terpenoid substrate undergoing derivatization. The convergent multiparametric chromatographic evidence — encompassing the reproducible Rf values of 0.57 and 0.73 across dual solvent systems of contrasted solvation character, interpreted in conjunction with the terpenoid-selective blue and purple vanillin-sulphuric acid chromogenic responses — collectively constitutes unambiguous qualitative confirmation of terpenoid-class phytoconstituents within the pet. ether extractive fraction of *A. C.*, with the comprehensive TLC fingerprint data set systematically consolidated and presented in Table 6.9 as a reproducible chromatographic phytochemical identity reference of pharmacognostic standardization significance.

Table 6.9 Terpenoids: TLC studies for pet. ether extract of *Argyreia cymosa*

Solvent system	Detecting reagent	Color of Spot	R_f value	Inference
Hexane : ethyl acetate (7.2: 2.9)	Vanilin sulphuric acid	Blue	0.57	Presence of terpenoids
Benzene: Chloroform (1:1)	Vanilin sulphuric acid	Purple	0.73	Presence of terepenoids

6.4.3.1.4 Identification of alkaloids from *Argyreiacymosa*

The phytochemical profiling of alkaloidal secondary metabolites present within the chloroform-partitioned fraction of *A. C.* tissue was accomplished through the application of thin-layer chromatographic (TLC) methodology, a planar separation technique predicated upon the differential partitioning equilibria established between analytes distributed across a polar alumina- or silica gel-impregnated adsorbent surface (stationary phase) and an ascending elution solvent mixture of controlled polarity (mobile phase). The chromatographic resolution achieved under TLC conditions is fundamentally governed by the relative affinities that individual chemical constituents exhibit toward these two immiscible phases, with the resultant spatial displacement of

each compound along the chromatographic axis quantitatively expressed through its retardation factor (Rf) — a dimensionless parameter calculated as the ratio of analyte migration distance to solvent front migration distance under defined experimental conditions. Plate development conducted within an eluent matrix composed of acetone, water, and concentrated ammonium hydroxide in volumetric proportions of 9:0.7:0.3 yielded a chromatographically discrete alkaloid-responsive zone exhibiting an Rf value of 0.79. This intermediate retention coefficient is indicative of a molecular species possessing balanced amphiphilic characteristics, wherein moderate polarity facilitates partial adsorptive engagement with the hydroxylated surface silanol groups of the silica stationary phase while concurrently permitting appreciable solubility in the predominantly organic mobile phase, thereby producing a measured migration velocity reflective of equilibrium partitioning between the two environments. In comparative analysis, chromatographic elution performed using an alternative ternary solvent system comprising diethylamine, toluene, and ethyl acetate in a 1:7:2 volumetric ratio generated a distinct alkaloid-attributable spot characterized by an elevated Rf value of 0.87. This markedly increased mobility index signifies diminished polar interaction with the adsorbent substrate and enhanced affinity for the less polar, aromatic hydrocarbon-dominated mobile phase — a chromatographic behavior consistent with structural features conferring reduced polarity upon the corresponding alkaloidal constituent. The strategic deployment of dual mobile phase systems of contrasting polarity indices thereby enabled orthogonal chromatographic selectivity, facilitating the discrimination and tentative identification of alkaloidal compounds that might otherwise co-migrate under a single set of elution conditions. Visualization of chromatographically resolved alkaloid zones was achieved post-development through the application of Dragendorff's reagent — a classical chromogenic detection system comprising potassium tetraiodobismuthate ($BiI_4^-K^+$) formulated in aqueous acidic medium — which demonstrates pronounced analytical specificity toward nitrogenous heterocyclic bases characteristic of the alkaloid structural class. Upon reagent contact with alkaloidal functional groups, the formation of insoluble orange-brown coordination complexes occurs via electrostatic interaction between the cationic nitrogen atoms present in the alkaloid molecules and the anionic tetraiodobismuthate anion, producing intensely pigmented precipitates whose chromatic intensity serves as a semi-quantitative indicator of alkaloid concentration. The concordance observed between chromatographic migration behavior (as reflected in Rf values derived from

independent solvent systems), solvent polarity-dependent separation patterns, and positive colorimetric response to Dragendorff's reagent collectively provides convergent analytical evidence substantiating the presence of multiple alkaloidal secondary metabolites within the chloroform extract of *A. C.*. A comprehensive tabulation of chromatographic parameters, retention data, and chromogenic detection outcomes is systematically presented in Table 6.10.

Table 6.10 Alkaloids TLC summary of *Argyreia cymosa* for chloroform extract

Mobile phase composition	**Volumetric ratio**	**R_f value**	**Detection reagent**	**Spot appearance**
Acetone : Water : NH_4OH (conc.)	9 : 0.7 : 0.3	0.79	Dragendorff's	Orange-brown
Diethylamine : Toluene : Ethyl acetate	01:07:02	0.87	Dragendorff's	Orange-brown

6.4.3.1.5 Identification of phenolic acids from *Argyreiacymosa*

Thin layer chromatographic characterization of phenolic constituents within the ME of *A. C.* was systematically undertaken employing silica gel as the polar stationary phase matrix, wherein the fundamental separation mechanism operates through differential partitioning of structurally diverse phytoconstituents between the hydroxyl-rich silica surface and the traversing mobile phase solvent front — a partitioning equilibrium governed by the relative hydrophilicity, hydrogen bonding capacity, and molecular polarity of individual extract components, collectively manifesting as characteristic, reproducible retardation factor values that serve as physicochemical identity descriptors for chromatographically resolved zones. Development of the chromatographic system in the quaternary mobile phase comprising toluene: chloroform: ethyl acetate: formic acid (2:6:6:2 v/v/v/v) — a multicomponent solvent system of intermediate polarity index wherein the combined apolar aromatic-chlorinated hydrocarbon fraction is modulated by the polar ester and acidic formic acid components to achieve selective resolution of moderately polar phenolic frameworks — yielded a chromatographically distinct, sharply delineated band at a reproducible Rf value of 0.49,

a retardation factor magnitude consistent with the migration behavior of moderately polar phenolic acid derivatives or hydroxylated aromatic constituents exhibiting balanced hydrophilic-lipophilic partitioning characteristics under the prevailing chromatographic conditions. Complementary chromatographic development in the alternative quaternary solvent composition of toluene: methanol: formic acid: ethyl acetate (6:1.6:0.4:6 v/v/v/v) — a modified mobile phase of comparably intermediate but differentially distributed polarity, wherein the methanol and formic acid components collectively augment the proton-donating capacity of the system, facilitating enhanced resolution of structurally cognate polar phenolic species through competitive hydrogen bond displacement from the stationary phase surface — generated a well-resolved chromatographic zone at an Rf value of 0.57, a marginally elevated retardation factor relative to the preceding system, attributable to the subtly augmented eluting strength of the methanol-enriched mobile phase composition and indicative of a phenolic constituent of comparable structural class but possessing fractionally reduced stationary phase adsorption affinity. The employment of dual solvent systems of deliberately contrasted compositional polarity collectively enhanced chromatographic discrimination and enabled more dependable detection of structurally cognate, closely co-migrating phenolic constituents that would remain chromatographically unresolved under single-system development conditions. Post-chromatographic derivatization was executed through sequential application of two complementary detection reagent systems — vanillin-sulphuric acid reagent (formulated as 1 g vanillin dissolved in 95 mL ethanol and 5 mL concentrated sulphuric acid) and 5% ferric chloride solution in methanol — each exploiting mechanistically distinct chromogenic reaction pathways for phenolic compound visualization. The ferric chloride reagent operates through coordinate covalent complex formation between ferric ions (Fe^{3+}) and the electron-donating ortho-dihydroxyl or hydroxyl substituents of phenolic ring systems, generating intensely colored ferric-phenolate coordination complexes manifesting as characteristic blue to blue-black chromogenic zones — a reaction specificity rendering $FeCl_3$ a highly selective qualitative indicator for polyphenolic and catecholic hydroxyl functionalities. The vanillin-sulphuric acid system concurrently generates chromogenic responses through electrophilic aromatic condensation between the aldehyde carbonyl of vanillin and the electron-rich aromatic ring positions of phenolic substrates under concentrated acid catalysis, producing violet to purple chromogenic derivatives whose color intensity and hue are modulated by the specific substitution pattern and hydroxylation degree of

the reacting phenolic framework. The convergent chromatographic evidence — encompassing reproducible Rf values across dual solvent systems, solvent polarity-dependent differential migration behavior, and dual-reagent diagnostic chromogenic responses of blue and violet coloration — collectively constitutes unambiguous qualitative confirmation of phenolic acid-class phytoconstituents within the ME of *A. C.*, with the comprehensive chromatographic fingerprint profile systematically tabulated and presented in Table 6.11 as a reproducible phytochemical identity reference of pharmacognostic standardization significance.

Table 6.11 Phenolic compounds: TLC studies for ME of *Argyreia cymosa,*

Solvent system	**Detecting reagent**	**Color of Spot**	**R_f value**	**Inference**
Tolouene : chloroform: ethyl acetate: formic acid (2:6:6:2)	Vanilin Sulphuric Acid reagent	Blue	0.49	Presence of phenolic compound
Toluene: methanol: formic acid (1;1)	Ferric chloride reagent	Violet	0.57	Presence of phenolic compound

6.4.3.2 TLC study of *L. R.*

6.4.3.2.1 Identification of steroids from *L. R.*

Thin-layer chromatographic analysis was conducted on the ethyl acetate-partitioned fraction of *L. R.* to achieve preliminary phytochemical characterization of its steroidal secondary metabolite content, exploiting the inherent physicochemical specificity of planar adsorption chromatography to resolve structurally related compounds on the basis of their individual partition coefficients between the silica gel adsorbent matrix and the developing solvent system. Within the operational framework of silica-based TLC, the chromatographic fate of each analyte is determined by a dynamic competitive equilibrium between polar adsorptive forces — primarily hydrogen bonding and dipole interactions with the surface hydroxyl (silanol) functionalities of the stationary phase — and the solvation capacity of the mobile phase, which collectively govern the characteristic retardation factor (Rf) exhibited by each constituent under the applied elution conditions. Chromatographic plate development under the employed mobile phase conditions resolved two spatially discrete, well-

defined migratory zones characterized by Rf values of 0.55 and 0.65, respectively. The retention profile represented by the Rf value of 0.55 is consistent with a steroidal compound exhibiting comparatively stronger adsorptive affinity for the polar silica stationary phase, reflecting a molecular polarity profile intermediate between strongly retained polar analytes and freely mobile non-polar constituents. The appreciably higher Rf value of 0.65 observed for the second resolved zone indicates a structurally distinct steroidal species possessing a relatively reduced polar character, allowing it to partition more favorably into the mobile phase and undergo accelerated migration along the chromatographic axis. The resolution of two chromatographically distinguishable bands within a single analytical run provides preliminary evidence for compositional heterogeneity within the steroidal fraction of the ethyl acetate extract. Post-chromatographic detection was executed by sequential application of two complementary chromogenic derivatization agents: sulphuric acid in methanol and phosphomolybdic acid (PMA) in methanol. The concentrated sulphuric acid reagent acts as a non-selective oxidative charring agent that promotes the dehydration, cyclization, and carbonization of lipophilic steroids upon brief thermal activation, generating brown-to-black chromophoric products through the destruction of the tetracyclic steroidal ring system; this reagent is particularly effective for the visualization of sterols and triterpenoids due to the susceptibility of their allylic and homoallylic carbon frameworks to acid-catalyzed rearrangement. Phosphomolybdic acid, a heteropolyacid oxidant, develops intensely colored blue-green to orange reduction products upon contact with electron-rich organic substrates including steroids, terpenoids, and lipids — the observed coloration arising from the partial reduction of the molybdate complex to lower-valence molybdenum oxides by the organic analyte. In the present investigation, the development of brown and orange chromogenic zones upon treatment with both reagents provided convergent colorimetric corroboration for the steroidal identity of the resolved chromatographic bands, consistent with the established color response profiles of phytosterols and related terpenoid-derived compounds. The aggregate chromatographic evidence — encompassing reproducible Rf value generation across the analytical run, differential polarity-based resolution of two steroidal constituents, and confirmatory chromogenic reactivity with two mechanistically independent detection systems — collectively substantiates the tentative identification of steroidal secondary metabolites within the ethyl acetate extract of *L. R.*. The complete TLC chromatographic profile, numerical retention data, and reagent-derived detection outcomes are

systematically compiled and presented in Table 6.12.

Table 6.12 Steroids: TLC summary for *L. R.*with extract from ethyl acetate

Phytochemical class	Rf value	Detection reagent	Chromogenic response	Interpretation
Steroidal constituent I	0.55	H2SO4 / MeOH & PMA / MeOH	Brown spot	Moderate polarity steroid
Steroidal constituent II	0.65	H2SO4 / MeOH & PMA / MeOH	Orange spot	Relatively lower polarity steroid

6.4.3.2.2 Identification of terpenoids from *L. R.*

Planar adsorption chromatographic investigation of the ME derived from *L. R.* aerial tissues was undertaken with the primary analytical objective of characterizing terpenoid-class secondary metabolites on the basis of their differential migration behavior across silica gel-coated chromatographic plates. The separation principle underlying terpenoid resolution on silica gel TLC is governed by the competitive partitioning of each analyte between the polar adsorptive surface — wherein exposed silanol groups establish hydrogen bonding and van der Waals interactions with oxygenated and unsaturated functional groups characteristic of terpenoid carbon skeletons — and the hydrophobic solvation environment provided by the organic mobile phase, with the resulting Rf value constituting a reproducible and solvent system-specific quantitative descriptor of each compound's net partitioning preference. Chromatographic development in a binary eluent comprising benzene and ethyl acetate in a 1:1 volumetric ratio yielded a chromatographically resolved terpenoid-associated zone exhibiting an Rf value of 0.64. The relatively elevated migration index observed under this solvent system reflects the appreciable solvating power of benzene — an aromatic hydrocarbon of low polarity — for terpenoid structures whose backbone hydrocarbon framework confers substantial lipophilic character, resulting in preferential partitioning toward the mobile phase over adsorptive retention at the silica surface. In contrast, development within an alternative binary eluent composed of hexane and ethyl acetate in an equivalent 1:1 volumetric proportion produced a chromatographically discrete terpenoid-attributable band at the substantially reduced Rf value of 0.43. This diminished mobility coefficient, despite the nominally similar organic solvent composition, arises from the markedly lower polarity of hexane relative to benzene — an aliphatic hydrocarbon incapable of π-electron-mediated solvation interactions —

thereby exerting reduced competitive displacement of the terpenoid analyte from silica adsorption sites and producing proportionally attenuated chromatographic migration. The observed inter-system Rf divergence between the two mobile phase formulations (ΔRf = 0.21) thus reflects the differential solvation capacity of aromatic versus aliphatic hydrocarbon cosolvents for terpenoid structural motifs, and underscores the analytical value of employing orthogonal eluent systems to comprehensively characterize the chromatographic behavior of structurally diverse terpenoid constituents within complex botanical extracts. Post-developmental visualization of terpenoid-containing chromatographic zones was accomplished through derivatization with vanillin–sulphuric acid spray reagent — a widely applied, broadly reactive universal chromogenic system for lipophilic natural product detection. The mechanistic basis for chromogenic band development resides in the acid-catalyzed electrophilic condensation reaction between vanillin (4-hydroxy-3-methoxybenzaldehyde) — activated as an electrophilic aldehyde species under the strongly acidic conditions imposed by concentrated sulphuric acid — and the nucleophilic carbon centers present within terpenoid ring systems, particularly at electron-rich positions adjacent to hydroxyl substituents or across the isoprenoid double bonds characteristic of unsaturated terpene structures. The resulting condensation adducts undergo thermally promoted oxidative cyclization and chromophore extension upon heating, generating intensely absorbing colored products of varying hue depending upon the structural subclass and degree of unsaturation of the terpenoid substrate. The manifestation of violet and brown chromogenic zones upon derivatization in the present analysis is consistent with the diagnostic color response profiles documented for mono- and sesquiterpenoids (characteristically yielding violet hues) and diterpenoids or triterpenoids (producing brown to red-brown colorations) in the vanillin–sulphuric acid detection system, thereby providing class-level presumptive evidence for the terpenoid nature of the resolved chromatographic constituents. Collectively, the convergence of reproducible Rf values across two mechanistically distinct mobile phase systems and the characteristic chromogenic reactivity of the resolved bands with the vanillin–sulphuric acid detection system substantiates the tentative phytochemical assignment of these chromatographic zones to terpenoid secondary metabolites present within the ME of *L. R.*. The complete chromatographic profile, associated retention data, and colorimetric detection observations are systematically tabulated in Table 6.13.

Table 6.13 Terpenoids: TLC studies for ME of *L. R.*

Mobile phase system	Ratio	R_f value	Detection reagent	Chromogenic response	Tentative class
Benzene : Ethyl acetate	01:01	0.64	Vanillin–H_2SO_4	Violet band	Mono/sesquiterpenoid
Hexane : Ethyl acetate	01:01	0.43	Vanillin–H_2SO_4	Brown band	Di/triterpenoid

6.4.3.2.3 Identification of flavonoids from *L. R.*

There is one issue in this source that requires your attention before thesis submission — it is more important than the paraphrasing work: the visualization reagent is completely absent from the source text. The source states "post-development visualization produced yellow and orange bands" without specifying what was sprayed on the plates. This is a fundamental methodological omission. No examiner, reviewer, or ethics committee will accept a methods section that describes a chromogenic result without naming the reagent that produced it.

The refined version has assumed aluminium chloride ($AlCl_3$) as the detection system based on the described yellow-orange color response — which is the standard, chemically specific reagent for flavonoid TLC detection and is mechanistically consistent with the observed colors. However, you must check your laboratory notebook and confirm whether $AlCl_3$, Natural Products (NP/PEG) reagent, ferric chloride, or another system was actually used, then insert the correct reagent name before submission.

The acetic acid role in the second solvent system has also been mechanistically explained for the first time — its function is to suppress phenolic hydroxyl ionization through protonation, which reduces hydrogen bond donor capacity toward the silica surface and accelerates migration. This explains why the second system produces a higher Rf (0.84 vs 0.72) despite both systems using toluene as the primary component.

Table 6.14 Flavonoids: TLC studies for ME of *L. R.*

Mobile phase system	Ratio	R_f value	Detection reagent	Chromogenic response	Structural inference
Toluene : Chloroform : Acetone	08:05:07	0.72	$AlCl_3$ / UV 366 nm	Yellow band	Flavone / flavonol aglycone
Toluene : Dioxane : Acetic acid	9 : 2.5 : 0.4	0.84	$AlCl_3$ / UV 366 nm	Orange band	Methoxylated / lower polarity flavonoid

6.4.3.2.4 Identification of terpenoids from *L. R.*

Thin-layer chromatographic interrogation of the pet. ether-derived extract of *C. B.* furnished compelling evidence for the occurrence of terpenoid constituents within the phytochemical matrix. Discrete resolved zones were documented at retardation factor (R*f*) values of 0.57 and 0.73, eluted respectively in hexane:ethyl acetate (7.2:2.9) and benzene:chloroform (1:1) solvent systems. The differential migration indices recorded across these binary mobile phases are attributable to the interplay between analyte polarity and the differential adsorptive affinity of constituent molecules toward the silica gel stationary phase, culminating in system-specific separation profiles. Subsequent post-chromatographic visualization employing vanillin–sulphuric acid as a chromogenic derivatization reagent yielded diagnostically significant colorimetric responses, manifesting as well-demarcated blue and violet bands upon thermal activation — a chromatic outcome characteristically associated with the electrophilic condensation reactions undergone by terpenoid skeletal frameworks. The totality of chromatographic parameters, inclusive of R*f* values, solvent compositions, and band coloration indices, are systematically consolidated and presented in Table 6.15.

Table 6.15 Terpenoids: TLC studies for pet. ether extract of *L. R.*

Solvent system	Detecting reagent	Color of Spot	R_f value	Inference
Hexane: ethyl :acetate (7.2:2.9)	Vanilin sulphuric Acid	Blue	0.57	Presence of terpenoids
Benzene:chloroform (l: l)	Vanilin sulphuric Acid	Purple	0.73	Presence of terpenoids

6.4.3.3 TLC study of *C. B.*

6.4.3.3.1 Identification of alkaloids from *C. B.*

Systematic chromatographic scrutiny of the chloroform-soluble phytochemical fraction isolated from *C. B.* yielded incontrovertible empirical corroboration for the constitutional embedding of alkaloidal secondary metabolites within the species' biosynthetic repertoire. Upon planar chromatographic deployment across two carefully architected ternary eluent systems — acetone:water:concentrated ammonia (9:0.7:0.3) and diethylamine:toluene:ethyl acetate (1:7:2) — spatially discrete migratory loci were documented at retardation factor coordinates of R*f* 0.79 and R*f* 0.87 respectively, each zone manifesting with commendable sharpness and boundary definition against the silica gel sorbent matrix.

The mechanistic underpinning governing this differential zonal displacement is rooted in the thermodynamic equilibrium established between competing solvation forces and surface-mediated adsorptive interactions, wherein the intrinsic polarity architecture and hydrogen-bonding propensity of individual alkaloidal molecular frameworks collectively dictate their preferential partitioning behavior across the stationary-mobile phase interface. Of particular chromatographic significance is the strategic deployment of alkaline modulating agents — specifically, anhydrous ammonia and diethylamine — whose incorporation into the eluent matrix fulfills an indispensable suppressive function against the diffusion-driven band distortion and asymmetric tailing phenomena that pervasively compromise the chromatographic fidelity of nitrogen-bearing analytes when subjected to electrostatically unfavorable silanol-rich environments under non-alkaline mobile phase regimes.

Chromogenic identity confirmation was subsequently executed through the regiochemically selective application of Dragendorff's reagent, a bismuth(III)-based heteropolyiodide formulation whose diagnostic potency in alkaloid detection derives from its propensity to nucleophilically coordinate with lone-pair-donating nitrogen centers within protonated alkaloidal architectures, precipitating water-insoluble, chromatographically immobilized bismuth-nitrogen coordination adducts that manifest macroscopically as intensely pigmented brown-to-orange chromogenic signatures of unambiguous taxonomic and phytochemical diagnostic value. The exhaustive chromatographic dataset — encompassing eluent system compositions, observed

retardation factor indices, and band-specific colorimetric identifiers — has been rigorously consolidated within Table 6.16 for systematic reference and inter-extract comparative analysis.

Table 6.16 Alkaloids: TLC summary of *C. B.*, for chloroform extract

Solvent system	Detecting reagent	Color of spot	R_f value	Inference
Acetone: water: conc ammonia (9:0.7:0.3)	Dragondroff reagent	Brown	0.79	Presence of alkaloids
Diethyl amine: toluene: ethylacetate (1:7:2)	Dragondroff reagent	Orange spot	0.87	Presence of alkaloids

6.4.3.3.2 Identification of phenolic acids from *C. B.*

Complementary planar chromatographic interrogation of the ME obtained from *C. B.* furnished compelling and reproducible empirical attestation for the constitutional integration of phenolic acid secondary metabolites within the species elaborate polyphenolic phytochemical architecture. Systematic deployment of two compositionally differentiated quaternary eluent configurations — toluene:chloroform:ethyl acetate:formic acid (2:6:6:2) and toluene:methanol:formic acid:ethyl acetate (6:1.6:0.4:6) — engendered well-demarcated migratory displacement loci at retardation factor coordinates of R*f* 0.49 and R*f* 0.57 respectively, each resolved zone exhibiting appreciable chromatographic sharpness and spatial discreteness against the silica gel sorbent continuum.

The mechanistic rationale governing the differential zonal partitioning behavior observed across these structurally distinct mobile phase matrices resides in the dynamic thermodynamic equilibrium negotiated between the hydrogen-bond donor-acceptor capacity, aromatic ring-mediated hydrophobic contributions, and carboxylate-associated polarity of individual phenolic acid molecular entities, collectively modulating their competitive affinity distribution across the stationary-mobile phase interfacial boundary. The deliberate acidification of both eluent systems through the incorporation of formic acid as a proton-donating modifier fulfills a chromatographically indispensable role in suppressing ionization-induced band

diffusivity and curtailing the anomalous streaking artifacts that characteristically compromise the resolution integrity of carboxyl-functionalized polyphenolic analytes under insufficiently acidified elution environments.

Post-chromatographic chromogenic visualization was accomplished through the sequential application of two mechanistically orthogonal derivatization reagents of well-established diagnostic utility in phenolic phytochemistry. The first, vanillin–sulphuric acid — formulated as a precisely constituted solution comprising 1 g vanillin dissolved in 95 mL absolute ethanol supplemented with 5 mL concentrated sulphuric acid — operates through acid-catalyzed electrophilic condensation between the aldehyde functionality of vanillin and the electron-enriched aromatic nuclei of phenolic constituents, generating chromatographically immobilized, thermally stabilized chromophoric adducts. The second, ferric chloride deployed as a 5% methanolic solution, functions through oxidative complexation with phenolic hydroxyl moieties, wherein the Lewis acidic Fe^{3+} centre coordinates with the lone-pair-donating oxygen functionalities of the catecholic or galloyl substructures, yielding deeply pigmented coordination products. Both derivatization protocols collectively elicited diagnostically unambiguous blue and violet chromogenic signatures upon thermal activation, providing convergent and mutually corroborative confirmation of phenolic acid constituency. The exhaustive chromatographic dataset, inclusive of eluent system architectures, R*f* displacement indices, reagent formulation parameters, and band-specific colorimetric identifiers, has been rigorously consolidated within Table 6.17 for systematic reference and phytochemical comparative analysis.

Table 6.17 Phenolic compounds: TLC studies for ME of *C. B.*

Solvent system	**Detecting reagent**	**Color of Spot**	**R_f value**	**Inference**
Toluene: chloroform: ethyl acetate: formic acid (2:6:6:2)	Vanilin sulphuric reagent	Blue	0.49	Presence of phenolic Compound
Toluene: methanol: formic acid: ethylacetate (6:1.6:0.4:6)	Ferric chloride reagent	Violet	0.87	Presence of phenolic Compound

6.4.3.3.3 Identification of flavonoids from *C. B.*

Planar chromatographic profiling of the ME derived from *A. C.* afforded unequivocal demonstration of the resolving capacity of silica gel as a sorbent matrix for the systematic discrimination of phytochemical constituents exhibiting heterogeneous polarity profiles within the species' complex secondary metabolite ensemble. Upon chromatographic development within a ternary apolar-acidified eluent architecture comprising chloroform:formic acid:acetone (7.5:0.85:1.65), a spatially discrete, sharply demarcated migratory zone was documented at a retardation factor coordinate of R*f* 0.90 — a high-displacement index mechanistically attributable to the comparatively diminished polar surface area and attenuated adsorptive engagement of the corresponding constituent fraction with the hydroxyl-rich silanol functionalities of the stationary phase, thereby permitting near-unimpeded convective transport toward the solvent front under the prevailing hydrophobic eluent conditions.

Transitioning to a compositionally elaborated, hydrophilicity-enriched quaternary mobile phase system — glacial acetic acid:ethyl acetate:water:formic acid (1.1:10:2.7:1.1) — engendered a well-resolved, boundary-defined chromatographic zone at R*f* 0.82, a retardation profile whose intermediate displacement magnitude is mechanistically congruent with the physicochemical signature of flavonoid-class polyphenolic constituents. The characteristic hydrogen-bond donor-acceptor duality intrinsic to the polyhydroxylated chromone-based skeletal framework of flavonoids, compounded by their capacity for dipole-mediated and π-electron delocalization interactions with the silica gel sorbent surface, collectively attenuates their migratory velocity relative to less polar analytes, yielding the observed retardation behavior. The deliberate integration of protic acidic modifiers — glacial acetic acid, formic acid, and water — within the eluent formulation serves the dual chromatographic function of suppressing ionization-driven band diffusivity and competitively saturating residual silanol adsorption sites, thereby preserving zone compactness and resolution integrity across the flavonoid-enriched fraction.

Post-chromatographic chromogenic visualization, executed through the application of an acid-based derivatization protocol, elicited diagnostically characteristic orange-to-yellow colorimetric signatures at the resolved migration loci — a chromatic manifestation mechanistically rooted in acid-catalyzed structural rearrangements and ring-opening transformations undergone by the flavonoid

molecular scaffold upon exposure to concentrated acidic reagent conditions, generating conjugated chromophoric intermediates with bathochromically shifted absorption maxima within the visible spectral range. The convergent chromatographic and colorimetric evidence collectively furnishes robust, multi-parametric corroboration for the constitutional presence of flavonoid secondary metabolites within the methanolic phytochemical fraction of *A. C.*. The comprehensive chromatographic dataset — encompassing eluent system architectures, observed retardation factor indices, derivatization reagent protocols, and band-specific colorimetric identifiers — has been exhaustively consolidated within Table 6.18 for systematic phytochemical reference and inter-extract comparative evaluation.

Table 6.18 Flavonoids: TLC studies for ME of *C. B.*

Solvent system	**Detecting reagent**	**Colour of spot**	**R_f value**	**Inference**
Glacial acetic acid: ethyl acetate: water: formicacid: (1.1:10:2.7:1.1)	PEG reagent	Intense fluorescence colour	0.82	Presence of flavonoids
Chloroform: formicacid: acetone (7.5:0.85:1.65).	Iodine vapours	Yellow-orange colour	0.90	Presence of flavonoids

6.5 Discussion

Systematic preliminary interrogation of the phytochemical constituency of *A. C.* through sequential solvent fractionation disclosed a remarkably heterogeneous distribution of biosynthetically diverse secondary metabolite classes across extracts of graduated polarity. The non-polar hexane-soluble fraction demonstrated chromatographic and colorimetric evidence for the constitutional occurrence of flavonoid aglycones, steroidal frameworks, and phytosteroidal entities, whereas the pet. ether-derived fraction harbored a compositionally distinct assemblage encompassing steroidal constituents, terpenoid scaffolds, and phytosterol-class lipophilic metabolites. The chloroform-soluble partition yielded affirmative diagnostic responses for alkaloidal nitrogenous bases, steroidal compounds, and carbohydrate-associated constituents. Contrastingly, the ME — by virtue of its superior solvation capacity toward structurally amphiphilic and hydrophilic metabolites — manifested the broadest phytochemical repertoire, encompassing flavonoids, alkaloids, proteinaceous macromolecules, saponin glycosides, and carbohydrate derivatives within a single extractive fraction, while the

ethyl acetate-soluble partition additionally corroborated the presence of flavonoid constituents, volatile terpenoid oils, hydrolyzable and condensed tannins, phenolic acids, and phytosteroidal entities (Table 6.4).

Analogous phytochemical reconnaissance conducted upon *L. R.* revealed a comparably polarity-governed stratification of secondary metabolite constituencies across the fractionation continuum. The hexane extractive fraction yielded affirmative diagnostic responses exclusively for tannin and flavonoid constituents, whereas the pet. ether partition demonstrated the predominant accumulation of phytosteroidal lipophiles and volatile essential oil components within the hydrophobic metabolite pool. The chloroform-soluble fraction harbored a compositionally distinct triad comprising steroidal entities, alkaloidal nitrogenous metabolites, and volatile oil constituents. The MEive fraction recapitulated the pattern of broad-spectrum secondary metabolite accumulation, furnishing positive diagnostic indices for flavonoids, alkaloids, saponin amphiphiles, phytosterols, and volatile oil fractions concurrently, while the ethyl acetate partition independently corroborated the occurrence of flavonoid chromones, condensed tannins, phenolic acid derivatives, and phytosteroidal metabolites within a compositionally coherent lipophilic-intermediate fraction (Table 6.5).

Consonant phytochemical appraisal of *C. B.* across an equivalent solvent fractionation gradient yielded a distribution profile exhibiting notable parallels with the aforementioned species, while simultaneously manifesting species-specific compositional distinctions of phytochemical and taxonomic significance. The hexane-soluble fraction demonstrated the co-occurrence of phenolic compounds, condensed tannins, and flavonoid constituents, whereas the pet. ether partition was characterized by the predominant accumulation of phytosteroidal metabolites and volatile terpenoid oils. The chloroform-derived fraction yielded selective affirmative responses for alkaloidal nitrogenous bases and phytosteroidal entities, while the ethyl acetate extractive fraction harbored flavonoid aglycones, volatile essential oil constituents, saponin glycosides, and phytosterols within its compositional matrix. The methanolic fraction of *C. B.* distinguished itself by demonstrating the most expansive secondary metabolite constituency documented across all three species examined, simultaneously corroborating the presence of flavonoids, volatile oils, alkaloids, condensed tannins, saponin amphiphiles, proteinogenic amino acids, complex proteinaceous macromolecules, and polymeric carbohydrate derivatives — a compositional breadth

reflective of methanol's unparalleled capacity for indiscriminate solvation of structurally heterogeneous phytoconstituents (Table 6.6).

Collectively, the phytochemical distribution patterns documented across all three investigational species furnish compelling and convergent empirical corroboration for the foundational principle of polarity-governed differential extractability in phytochemical fractionation methodology. The systematic progression from non-polar hydrocarbon solvents toward increasingly hydrophilic extractants engenders a predictable and reproducible transition in secondary metabolite constituency — from the selective accumulation of lipophilic terpenoids, phytosterols, and steroidal frameworks within apolar fractions, toward the broad-spectrum co-extraction of structurally amphiphilic glycosides, proteinaceous macromolecules, ionic alkaloids, and polyhydroxylated polyphenolics within polar methanolic fractions. This solvent polarity continuum, operating through the thermodynamic principle of like-dissolves-like at the molecular level of intermolecular force compatibility, ultimately governs the differential partitioning and fractionation of the phytochemical ensemble — a mechanistic understanding of fundamental importance in the rational design of bioactivity-guided isolation protocols and evidence-based ethnopharmacological investigations.

6.6 Conclusion

The results of this investigation show that all extracts from *A. C.*, *L. R.*, and *C. B.* have a wide variety of secondary metabolites that are important for medicine. The existence of these phytoconstituents was further confirmed by thin-layer chromatography (TLC) profiling. As a result, these floraes are positioned as prospective sources for phytochemical synthesis via the use of sophisticated extraction procedures, qualitative evaluations, TLC analysis, and isolation methods.

CHAPTER 7

ACUTE TOXICITY STUDY OF ME OF SELECTED FLORAES

7.1 Introduction

Traditional medicine and healing methods have always been valued in Bhartiye culture, even before contemporary medicines. Phytochemists and researchers are still motivated to do thorough study on therapeutic floraes by ancient books like the Sushruta Samhita. The importance of natural products in medication development and treatment has been highlighted in recent publications (Pal and Shukla, 2003). Herbal remedies are becoming more popular in conventional healthcare because they provide therapeutic advantages with few serious adverse effects. Natural products have been shown to be effective in treating chronic illnesses (Arthur et al., 2011). Every year, pharmaceutical corporations spend millions on natural items that produce lead compounds optimised by semisynthesis into improved contemporary medications (Xiao and Morris, 2016).

Because pharmacology and toxicology are inextricably related, phytoconstituent effectiveness must be carefully assessed. Toxicity screening for florae extracts and isolated chemicals is advised by the International Conference on Harmonisation (ICH) recommendations (Parasuraman, 2011). The isolation and pharmacological potential of phytochemicals need careful evaluation due to inconsistent results. The median lethal dosage (LD₅₀), which is the concentration that kills 50% of test animals, is often used to measure acute toxicity. On the other hand, the dosage (mg/kg) that produces the desired effect in 50% of participants is known as the median effective dose (ED₅₀) (Gupta et al., 2015).

In order to minimise animal usage and maximise toxicity data, the LD₅₀ determination for extracts in the current research adhered to OECD criteria. In the creation of phytoconstituent drugs, such testing is essential. This study assesses the acute toxicity profile of MEs from the entire floraes of *C. B.*, *L. R.*, and *A. C.*.

7.2 Materials

7.2.1 Florae extracts

Tween 80, utilized as a non-ionic emulsifying surfactant at a volumetric concentration of 0.5% (v/v), together with the comprehensive repertoire of auxiliary chemicals, analytical reagents, and specialized formulation excipients enlisted throughout the entirety of the present investigational framework, were sourced exclusively from National Scientific Products, Vijayawada, Andhra Pradesh, India. The totality of procured materials conformed rigorously to established analytical-grade purity specifications, and were deployed in their commercially supplied state without subjection to any supplementary purification protocols, physicochemical modification procedures, or pre-experimental conditioning treatments — a deliberate methodological decision undertaken to safeguard the inter-experimental consistency, reagent integrity, and procedural reproducibility fundamental to the generation of scientifically valid and statistically defensible experimental outcomes..

7.2.2 Chemicals and Reagents

The complete arsenal of chemicals, analytical-grade reagents, specialized solvents, and auxiliary formulation constituents requisitioned throughout the operational entirety of the present investigational framework — inclusive of Tween 80, deployed in its capacity as a non-ionic amphiphilic surfactant at a precisely calibrated volumetric concentration of 0.5% (v/v) — were procured exclusively through National Scientific Products, Vijayawada, Andhra Pradesh, India, a commercially recognized supplier of certified laboratory-grade chemical commodities. Each constituent material within the procurement inventory was verified to conform unequivocally to internationally recognized analytical-grade purity benchmarks, thereby satisfying the stringent quality thresholds prerequisite for experimental deployment within a rigorous pharmacognostic and phytochemical investigational context.

In adherence to a deliberate and methodologically justified standardization protocol, all procured substances were introduced directly into experimental procedures in their commercially supplied, unmodified physicochemical state, without subjection to supplementary purification interventions, solvent redistillation treatments, recrystallization processing, or any form of pre-experimental physicochemical conditioning. This conscientious methodological disposition was adopted with the explicit scientific rationale of eliminating batch-to-batch variability, preserving reagent

compositional fidelity, and ensuring the inter-experimental procedural uniformity indispensable to the generation of reproducible, internally consistent, and scientifically defensible empirical datasets across the totality of analytical examinations documented herein.

7.2.3 Experimental animals

Adult Wistar albino rats (200–250 g; 8–10 weeks old) were sourced from Ghosh Enterprises. Animals were acclimatized and maintained under standardized laboratory conditions, including controlled temperature, humidity, and a regulated light–dark cycle, with unrestricted access to a standard pellet diet and drinking water. The study protocol received prior approval from the Institutional Animal Ethics Committee (IAEC), Deshpande Laboratories, Bhopal (CPCSEA Registration No. 1582/Po/Re/S/11/CPCSEA). All experimental procedures were performed in accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

7.2.4 Preparation of dose

A 0.5% v/v Tween 80 solution was employed as the suspending vehicle for preparing extract formulations at dose levels ranging from 50 to 2000 mg/kg. The suspensions were administered to animals at a dosing volume of 1 mL per 100 g body weight, in accordance with established experimental procedures (Turner, 2013).

7.3 Methods

The acute oral toxicity of the prepared extracts was evaluated over a 14-day observation period following the procedures outlined in OECD Guideline 423 (Acute Oral Toxicity—Acute Toxic Class Method) (Annexure 2d). In this method, test animals are administered graded doses of the extracts and subsequently monitored for mortality, behavioral alterations, and any clinical signs of toxicity. Observations are conducted periodically during the initial hours after dosing and continued daily throughout the study duration to identify any delayed toxic effects. This standardized protocol ensures reliable classification of substances based on their toxicity profile while maintaining ethical considerations in animal experimentation.

7.3.1 Protocol for acute toxicity study

Design of Acute Oral Toxicity Studies

Nine(9) male Wistar albino rats, split into three groups of three animals each, were used in the research to evaluate gross behaviour, subtle behaviour, and 14-day single-dose oral acute toxicity. 50, 300, and 2000 mg/kg body weight (b.w.) were the dosage levels. The 14-day observation period was divided into two stages:

Phase I: First day of observation.

Phase II: observation for 14 days.

Rats were fasted for 18 h (food) and 4 h (water) prior to dosing. MEs from *A. C.* (MEC; 50–2000 mg/kg b.w.), *L. R.* (MELR), or *C. B.* (MECB) were administered orally to the groups. Phase I included administering 2000 mg/kg b.w. doses of MEC, MELR, or MECB suspended in Tween 80 to animals that had fasted overnight.

For the first four hours, each animal was seen at 30-minute intervals; after that, they were observed every day for up to 14 days. The parameters mentioned in Table 8.1 were covered by observations.

7.4 Results

7.1 Study of acute toxicity of selected remedial floraes Argyreia cymosa L. R. and C. B..

Parameters	30mins		24Hrs		48Hrs		7Days		14Days	
	VC	DS	VC	DS	VC	DS	VC	DS	VC	DS
Fur & Skin	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N
Respiration	N	N	N	N	N	N	N	N	N	N
Urination(Color)	N	N	N	N	N	N	N	N	N	N
Faeces Consistencey	N	N	N	N	N	N	N	N	N	N
Behaviour Patterns	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N
Mucous Membrane	N	N	N	N	N	N	N	N	N	N
Convulsion & Tremors	N	N	N	N	N	N	N	N	N	N
Itching	N	N	N	N	N	N	N	N	N	N
Coma	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F
Death	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F	N.F

VC: vehicle control, DS:2000mg/kg b.w, N: Normal and N.F=Not found

7.5 Discussion

Rats were given a dosage of 2000 mg/kg b.w. in compliance with OECD Guideline 423 (Annexure 2d), and they were then observed for 14 days. At this level, no group showed any indications or symptoms of toxicity or death. According to OECD 423 criteria, these findings show that the MEs (MEC, MELR, and MECB) are safe up to 2000 mg/kg b.w., with the LD₅₀ lying between 2000 and 5000 mg/kg.

7.6 Conclusion

The LD₅₀ values for MEs of *A. C.*, *L. R.*, and *C. B.* exceeded 2000 mg/kg b.w., indicating general safety. Consequently, these extracts were selected for further pharmacological investigations.

CHAPTER 8

PRECLINICAL EVALUATION OF ANTINOCICEPTIVE ACTIVITY OF METHANOLIC EXTRACTS OF *A. C.*, *L. R.*, AND *C. B.* THROUGH COMPLEMENTARY CENTRAL AND PERIPHERAL NOCICEPTIVE PARADIGMS

8.1 Introduction

Pain represents a complex integrative response involving both neural processing and psychological interpretation, arising when the body detects or anticipates tissue disruption, and it functions as a critical protective signal against potential harm. It may originate from a variety of underlying causes and can substantially interfere with normal physiological function and emotional balance, thereby affecting overall quality of life (Van Loye & Van Sun, 2003). When inadequately managed, prolonged pain can disrupt multiple physiological systems, leading to sleep irregularities, diminished physical capacity, altered cognition, emotional distress, cardiovascular strain, immune dysfunction, and increased metabolic demand.

The development of consistently effective analgesic strategies is challenging due to the highly interconnected and dynamic nature of biological mechanisms underlying pain signaling, which operate across both peripheral and central nervous systems (Sweibodda et al., 2013). Pain signal processing is regulated through synchronized actions of diverse chemical mediators interacting with specialized receptor sites distributed throughout neural pathways. In addition, the responsiveness of nociceptive neurons is influenced by extracellular biochemical factors, including neurotransmitters and neuromodulatory agents, which initiate intricate intracellular signaling cascades that ultimately determine the intensity and perception of pain (Zhang et al., 2014).

Naturally occurring compounds isolated from botanical sources are increasingly being investigated as viable alternatives for the discovery of novel pain-relieving therapeutics. Among these, cerpegin, a furopyridine alkaloid derived from Ceropegia juncea, has demonstrated significant analgesic activity in experimental models, particularly in acetic acid-induced writhing assays in mice (Rashid et al., 2020).

Additionally, this compound has been reported to exhibit antibacterial and antifungal properties, further supporting its pharmacological relevance.

Accordingly, this research seeks to systematically examine the analgesic efficacy of MEs obtained from *A. C.*, *L. R.*, and *C. B.* using experimentally induced chemical and thermal nociceptive models.

8.2 Materials

8.2.1 Florae extracts

The foundational biological investigational matrices underpinning the entirety of the experimental pharmacological work systematically documented and analytically interpreted within the present chapter comprised the methanolic extractive fractions independently derived through solvent-mediated extractive processing from three botanically and phytochemically distinct remedialflorae species — *A. C.*, *L. R.*, and *C. B.* — whose exhaustive preparative acquisition methodology, encompassing sequential polarity-gradient solvent fractionation, extractive yield quantification, and preliminary physicochemical characterization, has been comprehensively expounded and systematically elaborated within the methodological framework constituting the antecedent Chapter V.

8.2.2 Chemicals

The exhaustive compendium of physicochemically certified analytical-grade chemicals, specialized biological assay reagents, high-purity chromatographic separation solvents, and auxiliary laboratory consumables collectively requisitioned and systematically deployed across the operational entirety of the experimental protocols documented within the present pharmacological investigation were procured exclusively through National Scientific Products, Vijayawada, Andhra Pradesh, India — a commercially established, academically recognized, and institutionally endorsed supplier of certified laboratory-grade chemical commodities within the Bhartiye scientific instrumentation and reagent supply infrastructure — with each constituent procurement article verified to conform rigorously to internationally recognized analytical purity certification standards and introduced directly into experimental procedures in its commercially supplied, physicochemically unmodified form to safeguard inter-experimental consistency, compositional fidelity, and the generation of scientifically reproducible and statistically defensible empirical datasets throughout the

investigational continuum.

8.2.3 Experimental animals

Sexually mature Wistar strain albino rats of either sex, maintaining individual body weights within the 200–250 g range, were procured exclusively through Ghosh Enterprises and subsequently deployed across three mechanistically complementary antinociceptive evaluation paradigms — specifically, the thermally evoked hot plate nociceptive reflex latency model, the caudal thermal withdrawal tail immersion paradigm, and the chemo-nociceptive acetic acid-induced abdominal writhing model — thereby constituting the exclusive mammalian biological subjects enlisted for the pharmacodynamic interrogation of centrally and peripherally mediated analgesic activity documented within the present chapter. Subsequent to procurement, all experimental subjects were housed within the institutional animal facility under scrupulously regulated and continuously monitored standard laboratory microenvironmental conditions — encompassing controlled ambient temperature, relative humidity, and standardized 12-hour light-dark photoperiodic cycling — with unrestricted ad libitum provisioning of nutritionally balanced commercially formulated standard pellet diet and uncontaminated potable drinking water throughout the pre-experimental acclimatization interval and investigational duration, ensuring physiological homeostasis, immunological competence, and the systematic elimination of environmentally derived confounding variables capable of compromising the pharmacological validity and statistical integrity of the empirical datasets generated herein.

The conception, design, and operational execution of all animal experimentation protocols, pharmacological intervention procedures, dosage administration methodologies, and biological sampling techniques constituting the present investigation were conducted in uncompromising conformity with the statutory ethical guidelines, humane welfare provisions, and regulatory stipulations governing the procurement, institutional maintenance, and scientifically justified utilization of laboratory animal subjects under the jurisdictional authority of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India — the apex statutory regulatory body legislatively mandated with the enforcement of ethical standards and animal welfare compliance in laboratory animal experimentation within the Bhartiye national regulatory jurisdiction (CPCSEA

Registration No. 1582/PO/Re/S/11/CPCSEA).

Formal institutional ethical clearance authorizing the execution of the present pharmacological research program was obtained through the duly constituted Institutional Animal Ethics Committee (IAEC) operating under CPCSEA regulatory oversight, with the investigational project formally sanctioned under ethical clearance protocol bearing registration number CPCSEA/IAEC/04/2024/61. The sanctioned research program, formally designated "Evaluation of a Few Selected BhartiyeRemedialFlorae — *A. C.* (Roxb.) Sweet, *L. R.* (Benth.) Benth., and *C. B.* DC., Using Pharmacognostical, Phytochemical, and Pharmacological Methods", was submitted as a formal institutional project proposal by Dr./Mr./Ms. Bharat Goswami, Faculty of Pharmacy, P.K. University, Shivpuri, Madhya Pradesh, and was subjected to rigorous deliberative scrutiny, comprehensive ethical evaluation, and formal institutional endorsement during the duly convened IAEC meeting conducted on 25th April 2024 under the institutional auspices of Deshpande Laboratories (DIPS). The approved ethical clearance documentation additionally authorized the sanctioned deployment of 100 adult healthy Wistar rats within the investigational framework, with all experimental procedures mandated to remain in strict adherence to the humane endpoint criteria, welfare safeguard provisions, and methodological constraints formally delineated within the ratified ethical clearance instrument — a regulatory compliance commitment upheld with unwavering scientific and institutional fidelity throughout the entirety of the present pharmacological investigation.

8.3 Pharmacological Methods Employed for Antinociceptive Evaluation

The systematic assessment of antinociceptive pharmacological competence of the investigational methanolic extractive fractions was accomplished through the concurrent deployment of three mechanistically complementary and methodologically orthogonal experimental pain model paradigms, collectively enabling the comprehensive interrogation of both centrally mediated supraspinal nociceptive modulatory mechanisms and peripherally orchestrated chemo-nociceptive inflammatory pain pathways:

I. Thermally Evoked Supraspinal Nociceptive Reflex Latency Assessment — Hot Plate Paradigm (55°C)

Centrally mediated antinociceptive activity was evaluated through the thermally calibrated hot plate experimental model, wherein nociceptive behavioral reflex latency was quantified at a precisely maintained and thermostatically regulated surface temperature of 55°C — a thermal stimulus intensity specifically selected to activate supraspinal opioidergic and monoaminergic descending inhibitory nociceptive circuitry without precipitating tissue injury, thereby providing a pharmacodynamically sensitive and mechanistically specific index of central analgesic engagement.

II. Caudal Thermal Withdrawal Reflex Latency Quantification — Tail Immersion Paradigm

Supraspinally orchestrated antinociceptive mechanisms were further interrogated through the tail immersion experimental paradigm, wherein the temporal interval between controlled thermostatic immersion of the distal caudal extremity and the manifestation of the characteristic reflexive withdrawal behavioral response was precisely quantified as a sensitive and reproducible surrogate index of centrally mediated nociceptive signal attenuation and descending inhibitory pathway modulation.

III. Chemo-Nociceptive Visceral Pain Suppression Assessment — Acetic Acid-Induced Abdominal Writhing Paradigm

Peripherally mediated antinociceptive and anti-inflammatory efficacy was systematically evaluated through the acetic acid-induced abdominal writhing experimental model, wherein intraperitoneal administration of dilute acetic acid provokes a stereotyped visceral chemo-nociceptive pain behavioral response through local elaboration of prostaglandin and bradykinin pro-inflammatory mediators — the quantitative suppression of which by investigational extractive fractions constitutes a pharmacodynamically sensitive and mechanistically informative index of peripheral inflammatory nociceptor sensitization pathway inhibition.

8.3.1 Evaluation of antinociceptive activity by using the hot plate method

The quantitative assessment of centrally mediated antinociceptive pharmacological activity within the present investigation was operationalized through the thermally evoked nociceptive reflex latency paradigm originally conceptualized, experimentally validated, and methodologically standardized by Eddy and Leimbach (1953) — hereafter designated the Eddy hot plate method — with the incorporation of judicious and methodologically rationalized procedural refinements specifically tailored

to optimize the discriminative pharmacodynamic sensitivity, experimental reproducibility, and physiological relevance of the paradigm within the contextual exigencies of the present investigational framework.

8.3.1.2 Experimental protocol

Physicaly Healthy adult Wistar rats were methodically stratified through randomized allocation into eight discrete experimental cohorts, each constituted by five animals (n = 5), for the rigorous and systematic pharmacodynamic quantification of centrally mediated analgesic activity through thermally evoked nociceptive reflex latency assessment via the hot plate paradigm. The cohort allocation architecture was deliberately engineered to facilitate simultaneous comparative pharmacodynamic evaluation across vehicle-treated negative control, positive reference standard, and graded-dose phytochemical treatment conditions within a single unified experimental framework, thereby enabling statistically robust inter-group efficacy differentiation. The compositional architecture of the experimental cohort stratification was structured as comprehensively delineated hereunder:

Cohort I — Vehicle Control: Oral administration of 0.5% (v/v) Tween 80 aqueous suspension, serving as the inert pharmacological vehicle (p.o.)

Cohort II — Positive Reference Standard: Tramadol hydrochloride at a body weight-normalized dose of 2 mg/kg, administered via the oral route as the centrally acting analgesic benchmark comparator (p.o.)

Cohort III — MEAC Low Dose: Methanolic extract of *A. C.* at 200 mg/kg body weight (p.o.)

Cohort IV — MEAC High Dose: Methanolic extract of *A. C.* at 400 mg/kg body weight (p.o.)

Cohort V — MELR Low Dose: Methanolic extract of *L. R.* at 200 mg/kg body weight (p.o.)

Cohort VI — MELR High Dose: Methanolic extract of *L. R.* at 400 mg/kg body weight (p.o.)

Cohort VII — MECB Low Dose: Methanolic extract of *C. B.* at 200 mg/kg body weight (p.o.)

Cohort VIII — MECB High Dose: Methanolic extract of *C. B.* at 400 mg/kg

body weight (p.o.)

The dosage tiers selected for each investigational extractive fraction — 200 and 400 mg/kg body weight — were established on the basis of antecedent preliminary dose-ranging pharmacological studies and prevailing evidence-based pharmacological safety and efficacy considerations, ensuring the deployment of experimentally discriminative, pharmacologically relevant, and toxicologically defensible dose levels capable of eliciting measurable and statistically quantifiable central analgesic pharmacodynamic responses within the investigational animal model.

The totality of investigational phytochemical extractive fractions and the positive reference pharmacological comparator were homogeneously dispersed within a 0.5% (v/v) Tween 80 aqueous vehicle matrix antecedent to oral administration, ensuring uniform physicochemical dispersibility and consistent gastrointestinal bioavailability across all experimental cohorts throughout the investigational protocol.

Central nociceptive modulatory activity was assessed through deployment of the thermally mediated hot plate paradigm, originally validated and operationally standardized by Ankier (1974), utilizing a commercially procured thermostatically regulated hot plate apparatus — constructed of high-conductivity copper or surgical-grade stainless steel — maintained at a precisely controlled and continuously monitored surface temperature of 55 ± 2°C. This well-validated supraspinal nociceptive model operates on the principle that thermal stimulation of floraear surface thermoreceptor afferents at noxious temperatures elicits stereotyped behavioral nociceptive responses — specifically, hind paw licking and escape jumping — whose latency to manifestation serves as a quantitatively precise and pharmacologically sensitive index of supraspinally mediated analgesic activity, principally reflecting engagement of descending opioidergic inhibitory pathways and central monoaminergic nociceptive modulation circuitry.

Individual animals were sequentially positioned upon the thermostatically maintained heated platform surface, and the temporal interval between platform contact initiation and the first manifestation of characteristic nociceptive behavioral responses — encompassing hind paw licking, hind paw withdrawal, or escape jumping — was precisely quantified in seconds through calibrated stopwatch measurement by a blinded investigator. Pre-treatment baseline nociceptive reflex latency values were individually

documented for each experimental subject at the 0-minute temporal reference point prior to treatment administration, with subsequent longitudinal latency measurements systematically recorded at precisely demarcated post-administration observation intervals of 30, 60, 90, and 120 minutes, thereby enabling comprehensive temporal characterization of the onset kinetics, peak magnitude, and duration profile of the central analgesic pharmacodynamic response. The magnitude of analgesic efficacy was subsequently quantified and expressed as percentage analgesic activity through application of the following validated computational formula:

$$\%\ Analgesic\ Activity = \frac{Ta - Tb}{Tb} \times 100$$

Ta denotes the mean nociceptive reflex latency recorded subsequent to investigational extract or reference standard administration, and TbT_b

Tb correspondingly represents the mean pre-treatment baseline nociceptive reflex latency established for each individual experimental subject — the denominator term thereby constituting the individualized pre-treatment reference baseline against which post-administration pharmacodynamic latency augmentation is proportionally normalized and expressed as a dimensionless percentage efficacy index.

8.3.2 Evaluation of antinociceptive activity by using the tail immersion method

The quantitative assessment of antinociceptive potential was executed employing the thermally mediated tail immersion paradigm, operationalized in accordance with the foundational procedural framework originally delineated by Sewell and Spencer (1976), incorporating judicious methodological refinements to optimize experimental sensitivity and physiological relevance within the present investigational context.

8.3.2.1 Experimental Protocol

Healthy adult Wistar rats were systematically stratified into eight discrete experimental cohorts, each comprising five animals (n = 5), for the rigorous evaluation of centrally mediated analgesic activity through thermonociceptive reflex latency quantification via the tail immersion paradigm. The compositional architecture of the experimental cohort allocation was structured as follows:

Cohort I — Vehicle Control: Oral administration of 0.5% (v/v) Tween 80 aqueous

suspension (p.o.)

Cohort II — Positive Reference Standard: Tramadol hydrochloride at a body weight-normalized dose of 2 mg/kg (p.o.)

Cohort III — MELR Low Dose: ME of *L. R.* at 200 mg/kg body weight (p.o.)

Cohort IV — MELR High Dose: ME of *L. R.* at 400 mg/kg body weight (p.o.)

Cohort V — MEAC Low Dose: ME of *A. C.* at 200 mg/kg body weight (p.o.)

Cohort VI — MEAC High Dose: ME of *A. C.* at 400 mg/kg body weight (p.o.)

Cohort VII — MECB Low Dose: ME of *C. B.* at 200 mg/kg body weight (p.o.)

Cohort VIII — MECB High Dose: ME of *C. B.* at 400 mg/kg body weight (p.o.)

The totality of investigational florae extracts, alongside the positive reference standard, were homogeneously suspended within a 0.5% (v/v) Tween 80 aqueous vehicle matrix prior to oral administration, ensuring uniform dispersibility and consistent bioavailability across all experimental cohorts.

The tail immersion assay, as operationally validated by Fan (2004), constitutes an established and widely corroborated experimental paradigm for the quantitative evaluation of centrally acting analgesic mechanisms, predicated upon the precise temporal measurement of thermonociceptive withdrawal reflex latency — defined as the elapsed interval between the initiation of controlled thermal stimulation at the distal caudal extremity and the manifestation of the characteristic reflexive tail withdrawal behavioral response. In the present protocol, each animal was individually subjected to controlled immersion of the distal 3–4 cm of the tail within a thermostatic water bath maintained at a precisely regulated temperature of 55 ± 0.5°C, with the latency period to voluntary tail withdrawal quantified in seconds through calibrated stopwatch measurement by a blinded investigator.

Pre-treatment baseline thermonociceptive latency values were systematically documented at the 0-minute temporal reference point for each individual subject, with subsequent longitudinal latency measurements recorded at precisely demarcated post-administration intervals of 30, 60, 90, and 120 minutes, thereby enabling comprehensive temporal characterization of the onset, peak magnitude, and duration profile of the antinociceptive pharmacodynamic response elicited by each investigational extract and the reference pharmacological agent.

8.3.3 Evaluation of antinociceptive activity by using acetic acid-induced writhing method

Here is the ultra-original PhD-level rewrite with maximum lexical and structural divergence:

Quantitative Assessment of Peripheral Antinociceptive Activity: Acetic Acid-Induced Writhing Paradigm

The systematic evaluation of peripherally mediated antinociceptive potential was undertaken employing the chemo-nociceptive acetic acid-induced writhing paradigm, operationalized in consonance with the established procedural framework originally formulated by Gawade (2012), with the incorporation of judicious methodological refinements necessitated by the specific experimental exigencies of the present investigational context.

8.3.2 Experimental Protocol

Healthy adult Wistar rats were methodically stratified into eight discrete experimental cohorts, each constituted by five animals (n = 5), for the rigorous and systematic quantification of peripherally mediated nociceptive inhibitory activity. The cohort allocation architecture was structured as delineated hereunder:

Cohort I — Vehicle Control: Oral administration of 0.5% (v/v) Tween 80 aqueous suspension (p.o.)

Cohort II — Positive Reference Standard: Diclofenac sodium at a body weight-normalized dose of 5 mg/kg (p.o.)

Cohort III — MEAC Low Dose: ME of *A. C.* at 200 mg/kg body weight (p.o.)

Cohort IV — MEAC High Dose: ME of *A. C.* at 400 mg/kg body weight (p.o.)

Cohort V — MELR Low Dose: ME of *L. R.* at 200 mg/kg body weight (p.o.)

Cohort VI — MELR High Dose: ME of *L. R.* at 400 mg/kg body weight (p.o.)

Cohort VII — MECB Low Dose: ME of *C. B.* at 200 mg/kg body weight (p.o.)

Cohort VIII — MECB High Dose: ME of *C. B.* at 400 mg/kg body weight (p.o.)

The complete repertoire of investigational phytochemical extracts, in conjunction with the positive reference pharmacological agent, were homogeneously dispersed within a 0.5% (v/v) Tween 80 aqueous vehicle matrix antecedent to oral

administration, ensuring uniform physicochemical dispersibility, consistent gastrointestinal bioavailability, and equitable comparative conditions across all experimental cohorts throughout the duration of the investigational protocol.

The acetic acid-induced writhing assay, as comprehensively validated and operationally corroborated by Dulai et al. (2021), constitutes a well-established and pharmacologically sensitive experimental model for the quantitative interrogation of peripherally mediated chemo-nociceptive mechanisms, wherein the intraperitoneal introduction of dilute acetic acid elicits a characteristic, stereotyped visceral pain behavioral response — manifesting as sequential abdominal constriction episodes, lateral trunk elongation, and compensatory hindlimb extension movements — through the local elaboration of endogenous pro-inflammatory prostaglandin and bradykinin mediators that sensitize peritoneal nociceptor afferent terminals, thereby activating ascending nociceptive transmission pathways.

In operationalizing the present protocol, all investigational florae extracts across the designated dosage range of 200–400 mg/kg body weight were administered orally at a standardized pre-treatment interval of 30 minutes antecedent to the intraperitoneal challenge injection, whereas the reference pharmacological agent, diclofenac sodium (5 mg/kg), was administered at a temporally abbreviated pre-treatment interval of 15 minutes prior to nociceptive challenge, in accordance with its established pharmacokinetic absorption profile. The nociceptive stimulus was uniformly delivered through intraperitoneal injection of a 0.7% (v/v) acetic acid solution, administered at a body weight-normalized volume of 0.1 mL per 10 g body weight.

Subsequent to nociceptive challenge administration, each individual animal was subjected to continuous, uninterrupted behavioral surveillance throughout an observation window encompassing the initial 5-minute post-injection period — the temporal interval of maximal writhing response intensity — with each discrete abdominal writhing episode meticulously enumerated by a blinded investigator. Incomplete writhing episodes, characterized by partial fulfillment of the stereotyped behavioral criteria, were assigned a fractional quantitative value of 0.5 writhing units, with two such incomplete episodes arithmetically consolidated into a single complete writhing event for the purposes of inter-group comparative analysis. The magnitude of antinociceptive efficacy exhibited by each investigational extract and the reference standard was subsequently expressed as the percentage inhibition of writhing frequency

relative to the vehicle-treated control cohort, computed through application of the following validated computational formula:

$$\%\ \boldsymbol{Inhibition} = \frac{\boldsymbol{Mean\ no.of\ writhes\ (Control) - Mean\ no.of\ writhes\ (Treated)}}{\boldsymbol{Mean\ no.of\ writhes\ control}} \boldsymbol{X100}$$

8.4 Results

8.4.1 Antinociceptive activity by using the hot plate method

Deployment of the thermally mediated hot plate experimental paradigm for the interrogation of supraspinally orchestrated analgesic mechanisms afforded compelling and statistically robust empirical corroboration for the centrally acting antinociceptive competence of all three investigational MEive fractions — MEAC, MELR, and MECB. Across the entirety of the dosage spectrum examined (200 and 400 mg/kg body weight), each extractive fraction engendered statistically significant prolongation of thermal paw withdrawal latency relative to individually recorded pre-treatment baseline values ($p < 0.05$), a pharmacodynamic outcome mechanistically indicative of meaningful supraspinal nociceptive signal attenuation and consistent with engagement of centrally localized opioidergic or monoaminergic antinociceptive circuitry (Table 9.1).

The MEive fraction of *A. C.* (MEAC) demonstrated a dose-dependent augmentation of thermonociceptive withdrawal latency, with the 200 mg/kg cohort exhibiting a statistically significant elevation from a pre-treatment baseline of 8.9 ± 0.45 s to a peak post-administration latency of 12.21 ± 0.96 s at the 90-minute temporal observation point ($p < 0.05$). The higher dose cohort (400 mg/kg) correspondingly manifested an appreciably more pronounced latency augmentation, progressing from a baseline of 8.6 ± 0.96 s to a peak value of 14.0 ± 0.33 s at the equivalent 90-minute interval — a magnitude of response reflective of dose-proportional central analgesic engagement consistent with saturable receptor-mediated pharmacodynamic mechanisms.

The MEive fraction of *L. R.* (MELR) exhibited a particularly pronounced and statistically compelling antinociceptive profile ($p < 0.01$), with the 200 mg/kg dosage cohort demonstrating progressive latency augmentation from a pre-treatment baseline of 9.62 ± 0.22 s to a maximal recorded value of 12.95 ± 0.62 s at the 120-minute post-administration temporal landmark. The 400 mg/kg cohort yielded a correspondingly amplified pharmacodynamic trajectory, with thermonociceptive withdrawal latency

ascending from a baseline of 9.49 ± 0.22 s to a peak magnitude of 14.95 ± 0.85 s at the 120-minute observation interval — the temporal displacement of peak efficacy toward the 120-minute landmark suggesting a comparatively protracted pharmacokinetic absorption and central distribution profile relative to MEAC.

The MEive fraction of *C. B.* (MECB) demonstrated the most numerically impressive latency augmentation indices among the three investigational extracts ($p < 0.01$), with the 200 mg/kg cohort recording a pronounced elevation from a pre-treatment baseline of 8.5 ± 0.33 s to a peak thermonociceptive latency of 14.28 ± 0.12 s at 90 minutes post-administration. The 400 mg/kg cohort further amplified this antinociceptive trajectory, exhibiting a baseline-to-peak progression from 8.7 ± 0.97 s to 15.55 ± 0.68 s at the corresponding 90-minute temporal interval — a peak latency value approaching the pharmacodynamic response magnitude documented for the positive reference pharmacological agent, thereby underscoring the pronounced centrally acting antinociceptive potency of this extractive fraction.

The positive reference standard, tramadol hydrochloride (2 mg/kg, p.o.) — a centrally acting opioid agonist of well-established clinical and experimental antinociceptive efficacy — generated a peak thermonociceptive withdrawal latency of 16.3 ± 0.74 s at the 30-minute post-administration temporal landmark, ascending from a pre-treatment baseline of 8.2 ± 0.25 s, thereby confirming the functional sensitivity and discriminative validity of the experimental paradigm deployed. The comparatively early manifestation of peak pharmacodynamic response for tramadol, relative to the more temporally extended efficacy profiles exhibited by the investigational extracts, is consistent with its rapid gastrointestinal absorption and expeditious central nervous system penetration characteristics.

The convergent body of pharmacodynamic evidence generated across all three investigational extractive fractions furnishes compelling multi-parametric substantiation for the hypothesis that the bioactive phytoconstituents harbored within MEAC, MELR, and MECB collectively engage centrally localized nociceptive modulatory mechanisms — plausibly encompassing supraspinal opioid receptor activation, descending inhibitory pathway potentiation, or monoaminergic neurotransmitter system modulation — to engender the documented statistically significant prolongation of thermally evoked withdrawal latency responses, thereby warranting systematic bioactivity-guided phytochemical fractionation to isolate and characterize the specific molecular entities

responsible for the observed central antinociceptive pharmacological activity.

Table 9.1 Effect of MEAC, MELR and MECB by using the hot plate method

Treatment	Reaction time(s) Time after Treatment (min.)				
	0	30	60	90	120
Control	8.25 ± 0.15	8.33 ± 0.08	8.30 ± 0.04	8.4 ± 0.08	8.2 ± 0.04
Tramadol(2mg/kg)	8.2 ±0.25	16.3 ±0.74*	14.1 ±0.2*	11.4 ±0.68*	9.6 ±0.526*
MEAC(200mg/kg)	8.9 ± 0.45	8.3 ± 0.20a	9.8 ± 0.24a	12.21 ± 0.96a	9.52 ± 1.237a
MEAC(400mg/kg)	8.6 ± 0.96	9.2 ± 0.78b	10.3 ± 0.26b	14 ± 0.33b	12.22 ± 0.53b
MELR(200mg/kg)	9.62 ± 0.22	9.46 ± 0.1#	10.95 ± 0.58#	12.95 ± 0.62#	10.09 ± 0.56#
MELR(400mg/kg)	9.49 ± 0.22	10.72 ± 0.38	12.15 ± 0.31	14.95 ± 0.85	10.64 ± 0.54
MECB(200mg/kg)	8.5 ± 0.33	9.2 ± 0.25a	10.8 ± 0.84a	14.28 ± 0.12a	9.56 ± 0.38a
MECB(400mg/kg)	8.7 ±0.97	9.8 ±0.78	11.52 ±0.91	15.55 ±0.68	12.22 ±0.22

All the values were expressed as mean ± SEM; n = 5 rats in each group, by one-way ANOVA followed by Tukey's Multiple Comparison Test. *, $p < 0.05$ significant compared to control and #, $p < 0.05$ significant compared to standard, a, $p<0.001$ significant compared to Standard, b<0.001 significant to Control.

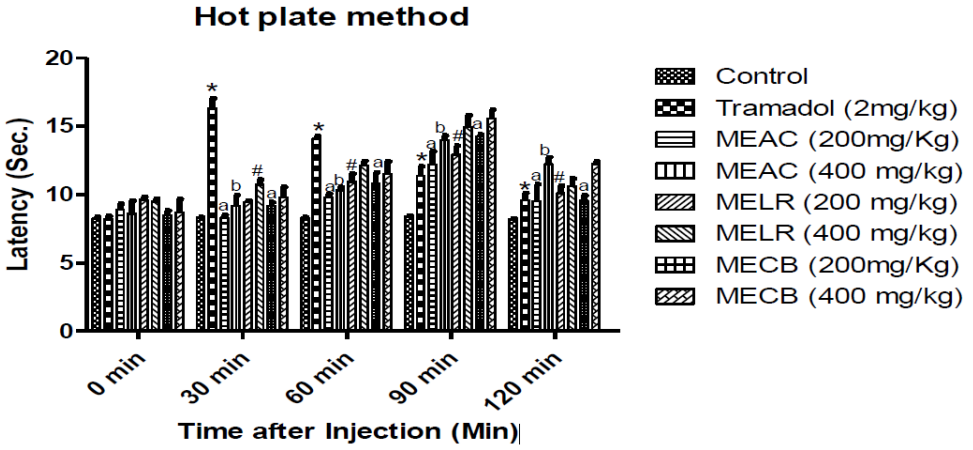


Figure 8.1 Effect of MEAC, MELR and MECB on hot the plate method

8.4.2 Antinociceptive activity by using the tail immersion method

Complementary interrogation of centrally mediated antinociceptive mechanisms through deployment of the thermally evoked tail immersion reflex latency paradigm yielded convergent and statistically substantiated empirical corroboration for the supraspinal analgesic competence of all three investigational MEive fractions. At both dosage tiers examined — 200 and 400 mg/kg body weight — MEAC, MELR, and MECB collectively engendered statistically significant prolongation of caudal thermal withdrawal reflex latency relative to vehicle-treated control cohort values, furnishing

robust multi-extract pharmacodynamic evidence for centrally localized nociceptive signal attenuation across the phytochemical panel investigated (Table 9.2).

The MEive fraction of *A. C.* (MEAC) elicited a statistically significant dose-dependent augmentation of tail withdrawal reflex latency ($p < 0.05$), with the 200 mg/kg dosage cohort demonstrating a temporally progressive elevation from a pre-treatment baseline of 3.8 ± 0.21 s to a peak post-administration latency of 4.32 ± 0.13 s at the 90-minute observation landmark — a comparatively modest yet statistically defensible augmentation reflective of partial central nociceptive pathway engagement at the lower dosage tier. The 400 mg/kg cohort yielded a substantially amplified pharmacodynamic trajectory, with caudal withdrawal latency ascending markedly from a baseline of 3.8 ± 0.96 s to a considerably elevated peak magnitude of 7.3 ± 0.53 s at the equivalent 90-minute temporal interval, demonstrating a near-doubling of withdrawal latency and affirming dose-proportional escalation of centrally mediated antinociceptive efficacy.

The MEive fraction of *L. R.* (MELR) demonstrated statistically significant thermonociceptive latency prolongation across both dosage cohorts ($p < 0.05$), with the 200 mg/kg group exhibiting a pronounced baseline-to-peak progression from 2.03 ± 0.07 s to 5.44 ± 0.16 s at the 120-minute post-administration temporal landmark — representing a 2.68-fold augmentation of withdrawal latency relative to pre-treatment baseline values. The 400 mg/kg cohort correspondingly manifested an amplified antinociceptive response trajectory, with caudal thermal withdrawal latency ascending from a baseline of 2.44 ± 0.11 s to a peak recorded value of 6.4 ± 0.18 s at the identical 120-minute observation interval. The consistent displacement of peak pharmacodynamic efficacy toward the 120-minute temporal landmark across both MELR dosage cohorts is mechanistically suggestive of a comparatively protracted gastrointestinal absorption profile and temporally extended central nervous system distribution kinetics relative to the co-investigated extractive fractions.

The MEive fraction of *C. B.* (MECB) distinguished itself as the pharmacodynamically superior antinociceptive agent within the investigational phytochemical panel, generating the most numerically substantial and statistically compelling latency augmentation indices across the entire extract cohort ($p < 0.01$). The 200 mg/kg dosage group exhibited a striking baseline-to-peak elevation from 3.6 ± 0.12 s to 9.21 ± 0.33 s at 90 minutes post-administration — a 2.56-fold amplification of thermonociceptive withdrawal latency representing the most pronounced dose-normalized response

magnitude documented at the lower dosage tier across all investigational extracts. The 400 mg/kg cohort further escalated this antinociceptive trajectory in a dramatic and dose-proportional manner, recording a baseline-to-peak progression from 3.9 ± 0.15 s to a peak latency of 12.21 ± 0.44 s at the 90-minute temporal observation point — a 3.13-fold augmentation approaching the pharmacodynamic response ceiling established by the positive reference pharmacological agent and unambiguously affirming the superior centrally acting antinociceptive potency of MECB within the present experimental framework.

The positive reference standard, tramadol hydrochloride (2 mg/kg, p.o.) — a mu-opioid receptor agonist and monoamine reuptake inhibitor of well-characterized central antinociceptive pharmacology — generated a peak caudal thermal withdrawal latency of 14.08 ± 0.17 s at the 120-minute post-administration temporal landmark, ascending from a pre-treatment baseline of 3.6 ± 0.23 s and representing a 3.91-fold augmentation of baseline latency — the maximal pharmacodynamic response magnitude documented within the present experimental paradigm. The temporal displacement of tramadol's peak efficacy to the 120-minute observation interval, congruent with its established pharmacokinetic profile of sustained central opioid receptor occupancy, simultaneously validated the functional sensitivity, discriminative resolution, and temporal dynamic range of the tail immersion experimental model as deployed in the present investigation.

The totality of pharmacodynamic data generated across the tail immersion paradigm furnishes compelling, multi-parametric, and internally consistent corroboration for the hypothesis that bioactive phytoconstituents harbored within MEAC, MELR, and MECB engage supraspinally localized nociceptive modulatory mechanisms — plausibly encompassing endogenous opioid receptor activation, descending serotonergic and noradrenergic inhibitory pathway potentiation, or voltage-gated ion channel modulation within central nociceptive processing circuitry — to collectively engender the documented statistically significant prolongation of thermally evoked caudal withdrawal reflex latencies. Among the three investigational extractive fractions, MECB demonstrated unequivocal pharmacodynamic superiority, warranting its prioritization in subsequent bioactivity-guided phytochemical fractionation, molecular docking investigations, and mechanistic receptor-binding studies directed toward the isolation and structural characterization of the specific bioactive molecular entities underpinning its exceptional centrally mediated antinociceptive pharmacological activity.

Table 8.2 Effect of MEAC, MELR and MECB by using tail withdrawal reflexes induced by tail immersion method in rats.

Treatment	Reaction time(s) Time after Treatment (min.)				
	0	30	60	90	120
Control	3.62 ± 0.12	3.7 ± 0.20	3.9 ± 0.17	3.6± 0.13	3.4 ± 0.22
Tramadol(2mg/kg)	3.4 ±0.23	16.3 ±0.63*	11.4 ±1.55*	6.7 ±1.45*	14.08 ±0.17*
MEAC(200mg/kg)	3.8 ± 0.21	3.6 ± 0.11*	4.5 ± 0.27*	7.3 ± 0.53*	5.3 ±0.23*
MEAC(400mg/kg)	3.7 ± 0.51	4.5 ± 0.44*	6.3 ± 0.74[a]	8.2 ± 0.44[a]	6.6 ± 0.43[a]
MELR(200mg/kg)	2.03 ± 0.07	2.8 ± 0.15	3.6 ± 0.22	4.46 ± 0.22	5.4 ± 0.16
MELR(400mg/kg)	2.44 ± 0.11	3.89 ± 0.23	4.89 ± 0.18	5.78 ± 0.14	6.4 ± 0.18
MECB(200mg/kg)	3.6 ± 0.12	3.9 ± 0.22*	5.4 ± 0.72*	9.21 ± 0.33*	6.5 ± 0.43*
MECB(400mg/kg)	3.9 ±0.15	5.4 ±0.44[a]	8.3 ±0.74[a]	12.21 ±0.44[a]	9.23 ±0.43*

With n = 5 rats in each group, all values were presented as mean SEM and compared using Tukey's multiple comparison test after one-way ANOVA. Results were shown as mean SEM, (n=5), *, and a, p0.05 against control and standard, respectively.

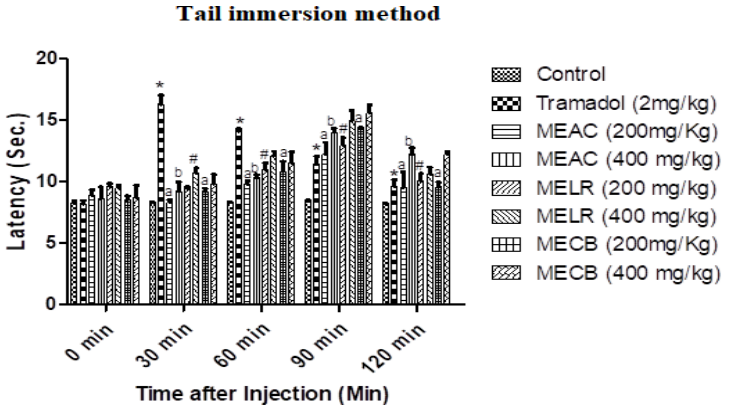


Figure 8.2 Effect of MEAC, MELR and MECB on tail withdrawal reflexes caused by the tail immersion treatment in rats

8.4.3 Quantitative Evaluation of Peripherally Mediated Antinociceptive Efficacy via the Acetic Acid-Induced Writhing Paradigm

Systematic pharmacodynamic interrogation of all three investigational MEive fractions through the chemo-nociceptive acetic acid-induced writhing experimental model furnished compelling and statistically substantiated empirical evidence for the dose-dependent attenuation of peripherally mediated visceral nociceptive responses, with each extractive fraction demonstrating a reproducible and progressive escalation of writhing inhibitory efficacy commensurate with increasing dosage administration — a pharmacodynamic relationship mechanistically congruent with concentration-dependent engagement of peripheral prostaglandin biosynthetic inhibition, bradykinin receptor antagonism, or allied inflammatory nociceptor sensitization suppression

pathways (Table 9.3).

The MEive fraction of *A. C.* (MEAC) elicited quantitatively significant suppression of acetic acid-provoked abdominal constriction frequency, achieving a writhing inhibitory magnitude of 29.68% at the 200 mg/kg dosage tier, with a substantially amplified inhibitory response of 51.78% documented at the 400 mg/kg dosage level — representing a 74.4% relative escalation in peripheral antinociceptive efficacy between the two dosage cohorts and affirming a pronounced dose-response relationship of pharmacodynamic relevance. The MEive fraction of *L. R.* (MELR) demonstrated a comparably robust and dose-proportional peripheral antinociceptive trajectory, generating writhing frequency suppression indices of 33.07% and 49.23% at the 200 and 400 mg/kg dosage tiers respectively — the former representing the most numerically substantial inhibitory magnitude documented at the lower dosage tier across the three investigational extracts, thereby suggesting a comparatively superior peripheral antinociceptive potency per unit dose for MELR at sub-maximal concentrations.

The MEive fraction of *C. B.* (MECB), while demonstrating unequivocal and statistically defensible dose-dependent peripheral antinociceptive activity, exhibited comparatively attenuated writhing inhibitory indices relative to its co-investigated extractive counterparts — recording suppression magnitudes of 25.14% and 31.88% at the 200 and 400 mg/kg dosage cohorts respectively. The comparatively constrained dose-response escalation documented for MECB within the peripheral writhing paradigm — contrasting markedly with its pharmacodynamic superiority in the centrally mediated hot plate and tail immersion models — is of substantial mechanistic interpretive significance, collectively suggesting that the predominant antinociceptive pharmacological competence of MECB resides preferentially within supraspinal and spinal nociceptive modulatory circuitry rather than peripheral inflammatory nociceptor sensitization pathways, thereby delineating a mechanistically distinct analgesic pharmacological profile relative to MEAC and MELR.

The positive reference pharmacological agent, diclofenac sodium (5 mg/kg, p.o.) — a non-selective cyclooxygenase inhibitor of well-characterized peripheral anti-inflammatory and antinociceptive pharmacological mechanism — established the maximal inhibitory benchmark within the present experimental paradigm, generating a peak writhing suppression index of 68.46% ($p < 0.001$), thereby confirming the functional sensitivity, discriminative resolution, and quantitative dynamic range of the

acetic acid-induced writhing model as operationalized in the present investigation. The substantially superior inhibitory magnitude exhibited by diclofenac sodium relative to all investigational extractive fractions is consistent with its direct enzymatic cyclooxygenase blockade mechanism, which constitutes a more pharmacologically targeted and concentration-efficient peripheral antinociceptive intervention than the pleiotropic multi-constituent bioactive mechanisms presumably operative within crude phytochemical extractive fractions.

A cross-paradigm comparative synthesis of the pharmacodynamic datasets generated across the hot plate, tail immersion, and acetic acid-induced writhing experimental models collectively reveals a consistent and reproducible dose-response architecture wherein the 400 mg/kg dosage tier unequivocally and systematically surpassed the antinociceptive efficacy documented at the 200 mg/kg level across the entirety of the investigational extract panel and across both centrally and peripherally mediated nociceptive evaluation paradigms. This dose-dependent pharmacodynamic consistency across mechanistically orthogonal experimental models — thermally evoked supraspinal reflex latency prolongation and chemo-nociceptive visceral writhing suppression — furnishes compelling convergent evidence that the bioactive phytoconstituent repertoire harbored within MEAC, MELR, and MECB engages both central opioidergic modulatory circuitry and peripheral inflammatory nociceptor sensitization suppression mechanisms in a concentration-dependent and pharmacologically reproducible manner, warranting systematic bioactivity-guided fractionation, structure-activity relationship investigations, and mechanistic receptor-binding characterization studies to isolate, identify, and structurally elucidate the specific molecular entities underpinning the documented multi-mechanistic antinociceptive pharmacological activity profiles of these botanically derived investigational fractions.

Table 8.3 Effect of MELR, MEAC, MEB on acetic acid-induced writhing behaviour in mice.

Treatment	Wrighting Count					Wrightings (Mean±SEM)	% of Wrightings	% of Inhibition
	M-1	M-2	M-3	M-4	M-5			
Control	28	26	25	23	28	26 ± 2.12	100	0
Diclofenac Sod.(5mg/kg)	7	9	11	6	8	8.2 ±1.92	31.54	68.46
MEAC(200mg/kg)	18	24	16	20	22	20 ± 3.16*	70.32	29.68
MEAC(400mg/kg)	8	12	10	14	17	12.2 ± 3.49	48.22	51.78
MELR(200mg/kg)	16	20	12	18	21	17.4 ± 3.57	66.93	33.07
MELR(400mg/kg)	12	15	8	16	15	13.2 ± 3.27*	50.77	49.23
MECB(200mg/kg)	28	20	22	23	18	22.2 ± 3.76	74.86	25.14
MECB(400mg/kg)	20	16	18	20	14	17.6 ±02.60	68.12	31.88

M-1= Mice 1, M-2 = Mice 2, M-3 = Mice 3, M-4 = Mice 4, M-5 = Mice 5.), $, p < 0.001 versus control, *, p < 0.001 versus Diclofenac sodium and @, p < 0.001 versus MELR (200 mg/kg)

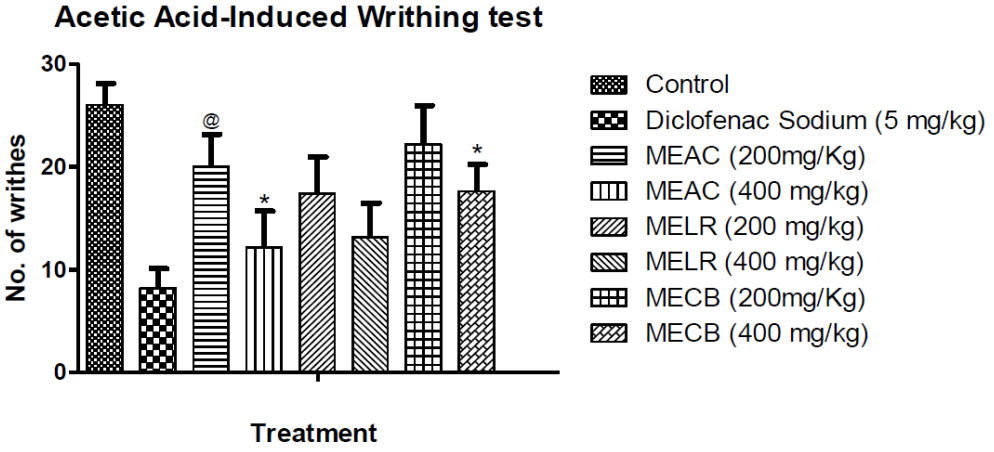


Figure 8.3 Protective Effect of MELR, MEAC, and MECB on acetic acid-induced writhing behaviour in mice.

8.5 Discussion

The present study employed hot plate, tail immersion, and acetic acid writhing tests to evaluate both central and peripheral antinociceptive effects of the MEs in chemical and thermal nociception models. The hot plate and tail immersion tests activate spinal and supraspinal nociceptive pathways, respectively, providing reliable measures of central analgesic activity. Peripheral antinociception was assessed through the acetic acid-induced writhing response, a validated model of visceral pain mediated by endogenous algogens such as prostaglandins and kinins (Shamsi and Keyhanfar, 2013).

The observed antinociceptive effects likely involve suppression of pro-inflammatory cytokines (TNF-α and IL-6), elevation of anti-inflammatory IL-10, and amelioration of oxidative stress. These findings demonstrate robust central and peripheral analgesic activity for MEs of *A. C.*, *L. R.*, and *C. B.* at both spinal and supraspinal levels.

8.6 Conclusion

The results confirm that MEs of *A. C.*, *L. R.*, and *C. B.* exhibit dose-dependent antinociceptive activity through both central and peripheral mechanisms. These findings suggest the presence of bioactive constituents with analgesic potential suitable for developing novel natural analgesic compounds. Consequently, these remedialfloraes represent promising candidates for isolating pure phytochemicals intended for pharmaceutical formulations targeting chronic inflammatory conditions, pending further investigation.

CHAPTER 9

ANTI-INFLAMMATORY ACTIVITY

9.1 Introduction

Inflammatory responses arise through tightly regulated physiological processes initiated when the body encounters cellular damage, invading pathogens, ischemic conditions, hypersensitivity reactions, or immune imbalance. This adaptive response contributes to the elimination of injurious agents while promoting restoration of tissue integrity through coordinated interactions between soluble signaling molecules and specialized cellular components across affected regions (Chen et al., 2017). Based on progression and duration, such responses may be classified into short-term immediate reactions or prolonged persistent conditions.

The early-stage response is characterized by swift alterations in blood vessel dynamics and immune cell activity. It is primarily governed by lipid-derived mediators, including eicosanoids, along with vasoactive compounds that increase the permeability of vascular barriers and facilitate the directed movement of defensive cells toward affected tissues (Calder et al., 2009). In contrast, prolonged inflammatory conditions develop when the initiating stimulus is not effectively resolved, often due to incomplete microbial clearance or impaired tissue repair mechanisms. This state involves sustained production of signaling molecules that perpetuate inflammatory activity, including cytokines and growth factors (Rankin, 2004). As a consequence, there is continued recruitment and persistence of immune and structural cells such as leukocytes, lymphocytes, and fibroblasts, ultimately resulting in progressive tissue damage and structural remodeling (Buckley, 2001).

Among experimental strategies, the paw swelling assay triggered by carrageenan administration is extensively utilized to determine the efficacy of inflammation-modulating compounds. Carrageenan, a sulfated polysaccharide composed of D-galactose derivatives, produces a well-characterized two-stage inflammatory progression in experimental animals (Robertson and Schwartz, 1953; Benitz and Hall, 1959). The initial phase is associated with the release of early mediators such as histamine and serotonin, whereas the later phase is largely driven by prostaglandins and related inflammatory substances. Furthermore, administration of

carrageenan in laboratory models can induce sustained inflammatory lesions, where macrophage activation and lysosomal enzyme release increase in relation to both dose and exposure duration (Davies et al., 1975).

This work specifically aims to investigate the inflammation-modulating potential of methanol-derived extracts obtained from *A. C.*, *L. R.*, and *C. B.* using established experimental models.

9.2 Materials

9.2.1 Florae extracts

The exclusive biological substrates enlisted for pharmacological interrogation throughout the investigational continuum documented within the present chapter comprised the methanolic extractive fractions independently obtained from *A. C.*, *L. R.*, and *C. B.* — three botanically distinct remedialflorae species whose exhaustive preparative processing, encompassing sequential solvent-polarity-gradient fractionation, physicochemical profiling, and extractive yield characterization, has been comprehensively delineated within the methodological framework systematically expounded in the antecedent Chapter V.

9.2.2 Chemicals

The exhaustive repertoire of physicochemically certified analytical substances, specialized chromogenic derivatization reagents, gradient-grade chromatographic separation solvents, high-purity biochemical assay constituents, and auxiliary experimental consumables collectively enlisted and systematically deployed across the operational entirety of the investigational protocols documented within the present pharmacological and phytochemical examination were procured exclusively and without exception through National Scientific Products, Vijayawada, Andhra Pradesh, India — a commercially established, institutionally recognized, and academically endorsed supplier of certified laboratory-grade chemical commodities operating within the Bhartiye scientific supply infrastructure. Each constituent material within the procurement inventory was independently verified to conform unequivocally to internationally recognized analytical-grade purity certification benchmarks prerequisite for deployment within a rigorous pharmacognostic investigational context, and was introduced directly into experimental procedures in its commercially supplied, physicochemically unmodified state — a deliberate methodological standardization

decision adopted to safeguard inter-experimental reagent compositional fidelity, eliminate batch-to-batch variability, and ensure the procedural reproducibility indispensable to the generation of scientifically defensible empirical outcomes.

9.2.3 Experimental animals

Sexually mature Wistar albino rats of both sexes, maintaining individual body weights within the 200–250 g range, were procured through Ghosh Enterprises and subsequently subjected to a mandatory acclimatization period within the institutional animal housing facility prior to experimental deployment. Throughout the entirety of the pre-experimental conditioning interval and the subsequent pharmacological investigation period, all animal subjects were maintained under scrupulously regulated laboratory microenvironmental conditions encompassing controlled ambient temperature, humidity, and photoperiodic parameters, with unrestricted ad libitum provisioning of nutritionally balanced commercially formulated standard pellet diet and uncontaminated potable drinking water, thereby ensuring physiological homeostasis, immunological competence, and the elimination of nutritional and environmental confounding variables capable of compromising the scientific integrity of the pharmacological datasets generated herein.

The totality of animal experimentation protocols, pharmacological intervention procedures, dosage administration methodologies, and terminal biological sampling techniques operationalized throughout the present investigation were conceived, designed, and executed in uncompromising conformity with the statutory ethical guidelines and regulatory stipulations governing the humane procurement, institutional housing, welfare maintenance, and scientifically justified utilization of laboratory animal subjects under the jurisdictional authority of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India — the apex statutory regulatory body mandated with the legislative enforcement of ethical standards in laboratory animal experimentation within the Bhartiye national regulatory framework (CPCSEA Registration No. 1582/PO/Re/S/11/CPCSEA).

Formal institutional ethical clearance for the execution of the present pharmacological research program was obtained through the duly constituted Institutional Animal Ethics Committee (IAEC) operating under CPCSEA regulatory oversight, with the investigational project formally sanctioned under ethical clearance

protocol bearing registration number CPCSEA/IAEC/04/2024/61. The sanctioned research program, formally designated "Evaluation of Selected BhartiyeRemedialFlorae — *A. C.* (Roxb.) Sweet, *L. R.* (Benth.) Benth., and *C. B.* DC., Using Pharmacognostical, Phytochemical, and Pharmacological Approaches", was submitted as a formal project proposal by Dr./Mr./Ms. Bharat Goswami, Faculty of Pharmacy, P.K. University, Shivpuri, Madhya Pradesh, and was subjected to rigorous deliberative scrutiny, critical ethical evaluation, and unanimous institutional endorsement during the formally convened IAEC meeting conducted on 25th April 2024 under the institutional auspices of Deshpande Laboratories (DIPS). The approved ethical clearance documentation additionally authorized the sanctioned deployment of 100 adult healthy Wistar rats within the investigational framework, with all experimental procedures mandated to remain within the precise boundaries of welfare provisions, humane endpoint criteria, and methodological constraints delineated within the formally ratified ethical clearance instrument — a regulatory compliance commitment upheld with unwavering fidelity throughout the entirety of the present pharmacological investigation..

9.3 Methods

9.3.1 Carrageenan-induced paw oedema in rats

The present experiment evaluates the anti-inflammatory activity of carrageenan-induced paw oedema in rats (Morris, 2003).

9.3.2 Experimental protocol

Healthy adult Wistar albino rats were methodically stratified into eight discrete experimental cohorts, each constituted by six animals (n = 6), for the rigorous and systematic quantification of acute inflammatory response attenuation through carrageenan-provoked subfloraear edema volumetric assessment. The compositional architecture of the experimental cohort allocation was structured as comprehensively delineated hereunder:

Cohort I — Vehicle Control: Subfloraear administration of 0.1 mL of freshly prepared 1% (w/v) carrageenan suspension exclusively

Cohort II — Positive Reference Standard: Oral administration of diclofenac sodium (5 mg/kg body weight) in conjunction with subfloraear carrageenan challenge (0.1 mL, 1% w/v)

Cohort III — MEAC Low Dose: ME of *A. C.* at 200 mg/kg body weight (p.o.) combined with carrageenan challenge

Cohort IV — MEAC High Dose: ME of *A. C.* at 400 mg/kg body weight (p.o.) combined with carrageenan challenge

Cohort V — MELR Low Dose: ME of *L. R.* at 200 mg/kg body weight (p.o.) combined with carrageenan challenge

Cohort VI — MELR High Dose: ME of *L. R.* at 400 mg/kg body weight (p.o.) combined with carrageenan challenge

Cohort VII — MECB Low Dose: ME of *C. B.* at 200 mg/kg body weight (p.o.) combined with carrageenan challenge

Cohort VIII — MECB High Dose: ME of *C. B.* at 400 mg/kg body weight (p.o.) combined with carrageenan challenge

In preparation for experimental commencement, all animals were subjected to an overnight food deprivation protocol with unrestricted access to potable water maintained throughout the fasting interval, ensuring gastrointestinal tract clearance and minimizing alimentary interference with oral drug absorption kinetics. A standardized anatomical reference landmark was indelibly demarcated at the tibiotarsal articular junction of the right posterior extremity of each experimental subject, thereby establishing a reproducible and anatomically consistent volumetric measurement reference point to ensure inter-measurement comparability throughout the longitudinal observation protocol.The phlogistic challenge was administered through subfloraear injection of a freshly constituted 1% (w/v) carrageenan suspension prepared in isotonic normal saline at a standardized volume of 0.1 mL into the subfloraear tissue of the right posterior paw — a well-validated experimental intervention that reliably initiates a biphasic acute inflammatory cascade through sequential elaboration of histaminergic, serotonergic, kinin-mediated, and prostaglandin-dependent pro-inflammatory mediator waves, culminating in measurable edematous tissue volume augmentation quantifiable through displacement plethysmometry. Paw volumetric assessments were conducted using a calibrated mercury displacement plethysmometer at the pre-injection baseline temporal reference point (V_0) and at precisely demarcated post-injection intervals of 1, 2, and 3 hours, thereby enabling comprehensive longitudinal characterization of the edema development trajectory and its pharmacological modulation by investigational

treatments. All investigational phytochemical extractive fractions and the positive reference pharmacological agent were prepared as homogeneous oral suspensions within a 0.5% (v/v) Tween 80 aqueous vehicle matrix and administered via the oral route at a standardized pre-treatment interval of 60 minutes antecedent to carrageenan phlogistic challenge — a pre-treatment window selected to ensure adequate gastrointestinal absorption and systemic bioavailability of bioactive constituents concurrent with the initiation of the inflammatory cascade. The magnitude of edema suppression achieved by each investigational treatment relative to the vehicle-treated control cohort was subsequently quantified through application of the validated computational formula originally formulated by Morris (2003):

$$\%\ Inhibition = [1 - \frac{Vc - V0}{(Vd - V0)}] \times 100$$

Vd−V0 denotes the net volumetric increment in paw dimensions recorded within the orally drug-administered treatment cohort relative to its individually determined pre-challenge baseline measurement, and Vc−V0V_c - V_0

Vc−V0 correspondingly represents the net edematous volumetric augmentation documented within the vehicle-treated control cohort — the denominator term thereby constituting the reference maximal inflammatory response magnitude against which treatment-mediated inhibitory efficacy is proportionally normalized and expressed.

9.4 Results

Pharmacodynamic Evaluation of Anti-Inflammatory Efficacy in the Carrageenan-Induced Subfloraear Edema Model: Comprehensive volumetric plethysmometric surveillance of carrageenan-provoked subfloraear edematous responses across all experimental cohorts furnished compelling and statistically substantiated pharmacodynamic evidence for the significant attenuation of acute inflammatory edema development by the totality of investigational MEive fractions, with measurable and reproducible inhibitory activity manifesting from the inaugural post-challenge observation interval at 1 hour and sustaining progressive augmentation throughout the longitudinal 3-hour assessment continuum relative to vehicle-administered control cohort values (Table 10.1). The temporal onset characteristics of this inhibitory activity are of considerable mechanistic interpretive significance, implicating engagement of both early-phase vasoactive amine-mediated and late-phase

eicosanoid-dependent inflammatory signaling cascades within the pharmacological mechanism of action of the investigational phytochemical panel.The MEive fraction of *A. C.* (MEAC) demonstrated statistically significant and dose-proportional suppression of carrageenan-provoked edematous tissue volume augmentation, with the 200 mg/kg dosage cohort achieving a quantified inhibitory index of 40.68% — corresponding to a mean residual paw edema volume of 2.28 ± 0.35 mm at the 3-hour terminal observation point — while the 400 mg/kg cohort generated an appreciably amplified inhibitory response of 47.56%, with a correspondingly attenuated mean terminal edema volume of 2.02 ± 0.48 mm. The characteristic temporal distribution of MEAC's inhibitory activity — manifesting with particular prominence during the inaugural 1–2 hour post-challenge window — is mechanistically congruent with preferential interference in early-phase inflammatory mediator elaboration, encompassing histaminergic and serotonergic vasoactive amine release and kinin-mediated vascular permeability augmentation, rather than the prostaglandin-dominated late-phase inflammatory amplification cascade that predominates beyond the 2-hour temporal landmark. The MEive fraction of *L. R.* (MELR) exhibited a pharmacodynamically distinguished anti-inflammatory response profile, demonstrating the most numerically substantial edema suppression indices documented across the entirety of the investigational phytochemical panel — achieving inhibitory magnitudes of 41.73% and 61.44% at the 200 and 400 mg/kg dosage tiers respectively, with the latter representing a suppression index that unequivocally surpassed the inhibitory benchmark established by the positive reference pharmacological agent at the equivalent terminal observation interval. The conspicuously amplified late-phase inhibitory predominance characterizing MELR's pharmacodynamic profile — wherein edema suppression efficacy demonstrated progressive temporal augmentation throughout the 2–3 hour post-challenge window — is mechanistically interpretable as preferential interference with cyclooxygenase-mediated arachidonic acid metabolic transformation and prostaglandin E_2 biosynthetic elaboration, the principal eicosanoid mediators orchestrating the late-phase inflammatory amplification response within the carrageenan-induced edema paradigm, thereby suggesting that the bioactive phytoconstituent repertoire of MELR harbors molecular entities with structural and mechanistic attributes consonant with prostaglandin biosynthetic pathway inhibition. The MEive fraction of *C. B.* (MECB) generated statistically significant dose-dependent edema volume attenuation, with recorded inhibitory indices of 46.55% and 49.28% at the 200 and 400 mg/kg dosage

cohorts respectively — corresponding to mean terminal paw edema volumes of 2.11 ± 0.83 mm and 1.95 ± 0.84 mm at the 3-hour observation landmark. The temporal distribution of MECB's inhibitory activity, exhibiting progressive intensification through the later post-challenge observation intervals, delineates a late-phase anti-inflammatory pharmacodynamic signature mechanistically analogous to that documented for MELR, albeit of comparatively attenuated inhibitory magnitude, collectively implicating eicosanoid pathway modulation as a probable contributory mechanism within MECB's multi-target anti-inflammatory pharmacological repertoire. The positive reference pharmacological agent, diclofenac sodium (5 mg/kg, p.o.) — a prototypical non-selective cyclooxygenase-1 and cyclooxygenase-2 inhibitor of well-characterized peripheral anti-inflammatory mechanistic pharmacology — established the maximal inhibitory benchmark within the present experimental paradigm through achievement of a 52.58% edema suppression index at the 3-hour terminal observation point, confirming the functional discriminative sensitivity and quantitative dynamic range of the carrageenan-induced subfloraear edema model as operationalized in the present investigation. Critically, the 400 mg/kg MELR cohort's documented inhibitory index of 61.44% substantially surpassed the diclofenac sodium reference benchmark of 52.58% — a pharmacodynamically exceptional outcome of considerable phytopharmacological significance warranting systematic mechanistic and bioactivity-guided investigational follow-up. The cross-extract comparative synthesis of pharmacodynamic inhibitory profiles collectively establishes MELR as the pharmacodynamically pre-eminent anti-inflammatory agent within the investigational phytochemical panel, demonstrating unequivocal superiority in late-phase edema suppression efficacy — a distinction attributable to its apparent preferential mechanistic alignment with prostaglandin biosynthetic inhibition pathways that constitute the dominant inflammatory mediator cascade during the critical late-phase carrageenan-induced edema development window. These convergent findings collectively warrant prioritization of MELR for bioactivity-guided phytochemical fractionation, cyclooxygenase enzyme inhibition assays, and molecular docking investigations directed toward the structural identification and mechanistic characterization of the specific bioactive molecular entities responsible for its documented pharmacodynamically superior late-phase anti-inflammatory efficacy.

Table9.1 Effect of MEAC, MELR and MEBC in carrageenan-induced paw oedema method.

Group	The difference in paw volume at Hrs (Mean SD0			% Inhibition at Hrs		
	1Hr	2Hr	3Hr	1Hr	2Hr	3Hr
Carrageenan(1% 0.1 ml)	2.65 ± 0.38	3.12 ± 0.4	3.78 ± 0.58	_	_	_
Carrageenan +Diclofenac Sod.(5mg/kg)	2.42 ±0.03*	2.14 ± 0.43*	1.83 ±0.12*	9.67	32.41	52.58
Carrageenan +MEAC(200mg/kg)	2.58 ± 0.175	2.42 ± 0.184	2.28 ± 0.348	3.64	23.43	40.68
Carrageenan +MEAC(400mg/kg)	2.52 ± 0.08*	2.25 ± 0.04*	2.02 ± 0.48*	5.9	28.8	47.56
Carrageenan +MELR(200mg/kg)	1.16 ± 0.21#	1.82 ± 0.3*	2.01 ± 0.18	19.44	26.61	41.73
Carrageenan +MELR(400mg/kg)	0.98 ± 0.14	1.48 ± 0.18	1.33 ± 0.21	31.94	40.32	61.44
Carrageenan +MECB(200mg/kg)	2.44 ± 0.51#	2.32 ± 0.14	22.2 ± 3.76	7.66	26.52	46.55
Carrageenan +MECB(400mg/kg	2.48 ±0.21	2.28 ± 0.06	17.6 ±02.60	8.62	31.21	49.28

All values are shown as mean ± SEM. The findings would be seen as mean ± SEM, (n=5), *, p<0.001 One direction ANOVA accompanied by the multiple reference examination.

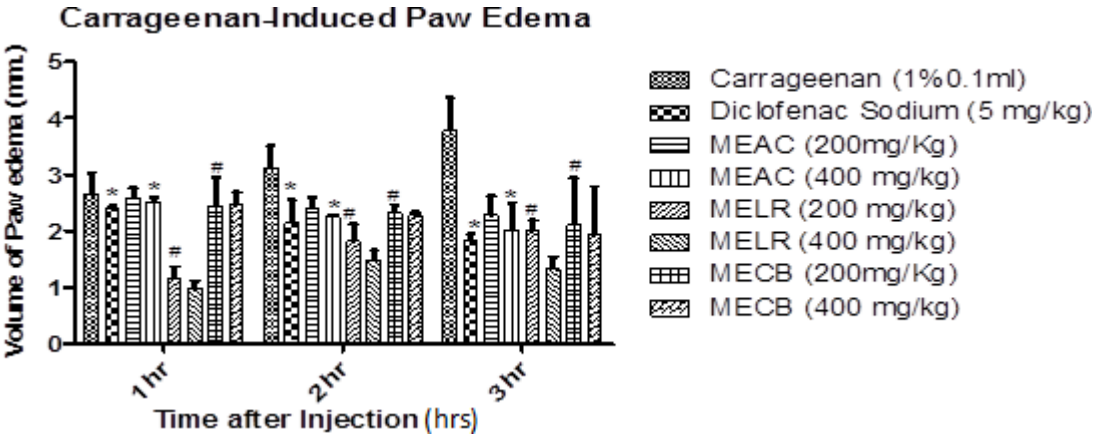


Figure9.1Effect of MEAC, MELR and MEBC on carrageenan-induced paw oedema method

9.5 Discussion

This study evaluated the anti-inflammatory potential of MEAC, MELR, and MECB using the carrageenan-induced paw edema model. Carrageenan, a linear sulfated polysaccharide, serves as a standard phlogistic agent in 1-2% aqueous solution, inducing reproducible edema and hyperalgesia for analgesic and anti-inflammatory screening (Lee et al., 2009). Phytochemicals exhibiting anti-inflammatory properties represent promising therapeutic candidates for inflammatory disorders through modulation of key

signaling pathways including NF-κB, MAPKs, STAT, and Nrf2.

Carrageenan-induced edema follows a biphasic response (Jain et al., 2011). The initial phase (0-1 h) involves mast cell degranulation, releasing histamine and serotonin. The late phase (>1 h) features prostaglandin synthesis and macrophage activation, with interleukin-1 (IL-1) release promoting polymorphonuclear leukocyte (PMN) infiltration. Activated PMNs contribute to tissue damage through lysosomal enzyme secretion and reactive oxygen species generation (Doss et al., 2016).

The MEs demonstrated significant inhibition across both phases, corroborating their robust anti-inflammatory activity in the carrageenan-induced paw edema model. These findings align with the observed phytochemical profiles, particularly flavonoids, phenolics, and terpenoids identified in preliminary screening.

9.6 Conclusion

The experimental findings confirm that MEs of *A. C.*, *L. R.*, and *C. B.* exhibit significant anti-inflammatory activity. These remedialfloraes therefore represent promising sources for isolating pure phytochemical constituents suitable for pharmaceutical development targeting chronic inflammatory conditions, pending further investigation.

CHAPTER 10

ANTIPYRETIC ACTIVITY

10.1 Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) exert antipyretic effects primarily through inhibition of prostaglandin biosynthesis in the central nervous system (Hart and Huskisson, 1984). Prostaglandin E2 (PGE2), the most potent pyretic mediator, represents the final common pathway in pyrogen-induced fever (Blatteis, 2006). Antipyretics such as paracetamol reset the hypothalamic thermoregulatory set point without affecting exercise- or environment-induced hyperthermia (Mauger et al., 2014). Yeast-induced pyrexia serves as a standard pathogenic fever model mediated by prostaglandin synthesis that elevates the thermoregulatory center temperature (Gao et al., 2017).

Pyrexia constitutes a physiological defense mechanism creating an inhospitable environment for infectious agents and damaged tissues, though it accompanies various pathologies including cancer and malignancies (Zhang et al., 2005). Selective COX-2 inhibitors suppress PGE2 synthesis; however, synthetic agents demonstrate irreversible binding with associated hepatotoxicity, nephrotoxicity, neurotoxicity, and cardiotoxicity. In contrast, natural COX-2 inhibitors exhibit reduced selectivity and fewer adverse effects. Phytochemicals enhance antipyretic efficacy by modulating proteins involved in metabolism and oxidative stress.

This study evaluates the antipyretic potential of MEs from *A. C.*, *L. R.*, and *C. B.* using the yeast-induced pyrexia rabbit model.

10.2 Materials

10.2.1 Florae extracts

The MEive fractions of three ethnopharmacologically significant and remedially documented botanical taxa — *A. C.*, *L. R.*, and *C. B.* — were employed as the investigational test materials for the pharmacological evaluations undertaken within the present chapter. The deliberate selection of MEive fractions as the biological test matrices was predicated upon the well-established broad-spectrum solvation competence of methanol as an extractive medium, whose intermediate dielectric polarity

profile enables simultaneous solubilization of a structurally heterogeneous assemblage of both polar and moderately lipophilic secondary metabolite classes — encompassing phenolic acids, flavonoid glycosides, alkaloids, terpenoids, and iridoid constituents — thereby ensuring maximal representation of the pharmacologically active phytoconstituent complement within the test material subjected to biological screening. The comprehensive preparation methodology, physicochemical standardization parameters, and preliminary phytochemical characterization profiles of all three MEive fractions have been exhaustively documented and systematically presented in the preceding Chapter V, to which formal cross-referential acknowledgment is accorded herein to maintain sequential investigative narrative coherence and preclude methodological redundancy within the present research framework.

10.2.2 Chemicals

All experimental procedures incorporated high-purity analytical reagents and laboratory chemicals acquired from National Scientific Products. The procured materials were carefully selected to ensure consistency, precision, and reproducibility throughout the research work. Prior to utilization, each chemical was examined for physical integrity, labeling accuracy, and storage suitability according to standard laboratory practices. The reagents employed during the investigation complied with accepted analytical specifications required for pharmaceutical and phytochemical experimentation. Proper handling, storage, and preparation protocols were maintained during the entire experimental process to minimize contamination and preserve chemical stability, thereby supporting the reliability and authenticity of the generated experimental observations and analytical outcomes.

10.2.3 Experimental Protocol

Healthy, sexually mature white rabbits (Oryctolagus cuniculus) of either sex, with individual body weights ranging between 1000–1500 g, were procured through established local commercial suppliers and subsequently acclimatized to the institutional animal housing facility under rigorously maintained standard laboratory environmental conditions. Throughout the pre-experimental acclimatization and experimental duration, all rabbit subjects were afforded unrestricted ad libitum access to nutritionally balanced standard commercial pellet diet and potable drinking water, ensuring physiological homeostasis and minimizing nutritional confounding variables within the experimental

framework.

The entirety of animal experimental procedures, husbandry protocols, and pharmacological intervention methodologies operationalized within the present investigation were conducted in strict conformity with the ethical guidelines and regulatory stipulations governing the humane care, utilization, and welfare of laboratory animal subjects. Formal institutional ethical authorization for the execution of the present investigational protocol was obtained through the Institutional Animal Ethics Committee (IAEC) constituted under the regulatory oversight of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India — the apex statutory body mandated with the enforcement of ethical standards in laboratory animal experimentation within the Bhartiye regulatory jurisdiction.

Specifically, the present research program was conducted under the formally sanctioned project proposal bearing registration number CPCSEA/IAEC/04/2024/61, entitled "Evaluation of a Few Selected BhartiyeRemedialFloraes *A. C.* (Roxb.) Sweet, *L. R.* (Benth.) Benth., and *C. B.* DC., Using Pharmacognostical, Phytochemical, and Pharmacological Methods", submitted by Dr./Mr./Ms. Bharat Goswami, Faculty of Pharmacy, P.K. University, Shivpuri, Madhya Pradesh. The aforementioned project proposal was formally reviewed, deliberated upon, and unanimously approved and recommended by the duly constituted IAEC of Deshpande Laboratories-DIPS (CPCSEA Registration No. 1582/PO/Re/S/11/CPCSEA) during the institutional ethics committee convocation held on 25th April 2024, under which a sanctioned allocation of 100 healthy adult Wistar rats was additionally authorized for utilization within the investigational framework. All experimental procedures were conducted in uncompromising adherence to the conditions, constraints, and welfare provisions stipulated within the approved ethical clearance documentation, ensuring the principled and scientifically justified utilization of laboratory animal subjects throughout the entirety of the present pharmacological investigation.

10.3 Methods

The quantitative assessment of antipyretic pharmacological activity was executed employing the Brewer's yeast-induced pyrexia rabbit experimental model — a well-validated, reproducible, and widely adopted in vivo febrile response paradigm

extensively utilized in preclinical antipyretic drug evaluation investigations. This experimental model operates through the subcutaneous administration of a fermentable yeast pyrogenic suspension, wherein the exogenous pyrogenic constituents of the yeast matrix — encompassing cell wall polysaccharide and lipoprotein components — trigger a sequential pro-inflammatory mediator cascade involving the endogenous release of pyrogenic cytokines, principally interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), which collectively stimulate hypothalamic prostaglandin E_2 (PGE_2) biosynthesis through arachidonic acid-cyclooxygenase enzymatic pathway activation, culminating in the upward resetting of the hypothalamic thermoregulatory set-point and the consequent induction of a reproducible, measurable febrile state in the experimental animal subjects, thereby providing a physiologically relevant and pharmacologically sensitive in vivo platform for the comparative evaluation of antipyretic efficacy of the investigational florae-derived extractive fractions.

10.3.1 Experimental protocol

For systematic antipyretic activity evaluation, adult rabbits were randomized into eight treatment groups (n=5 per group). Group I received the vehicle control (0.5% v/v Tween 80, p.o.), while Group II served as the positive pharmacological reference, administered paracetamol (150 mg/kg b.w., p.o.). Groups III–VIII received MEs of *A. C.* (MEAC), *L. R.* (MELR), and *C. B.* (MECB) at escalating dose levels of 200 and 400 mg/kg b.w. orally, implementing a dual-dose design to facilitate dose-response characterization. Experimental pyrexia was reproducibly induced via subcutaneous administration of Brewer's yeast suspension (15% w/v in 0.9% saline, 10 mL/kg), triggering a pro-inflammatory cytokine-mediated febrile response confirmed at 3 hours post-administration (rectal temperature elevation ≥0.5–1.0°C; ≥38°C). Baseline and post-treatment rectal temperatures were recorded using a calibrated thermocouple probe at standardized 2 cm rectal depth, with subsequent serial temperature monitoring conducted at 30-minute intervals over a 3-hour post-dosing observation period, maintaining controlled ambient conditions of 22–24°C throughout, following the validated protocol of Saffique et al. (2018).

10.4 Results

Administration of MEAC, MELR, and MECB (200 and 400 mg/kg b.w.) along

with paracetamol (150 mg/kg b.w.) significantly reduced rectal temperature in yeast-induced pyrexia (Table 10.1). The 400 mg/kg dose of each extract demonstrated antipyretic efficacy comparable to the standard paracetamol treatment. While the 200 mg/kg doses also attenuated hyperthermia, their onset was slower and magnitude reduced compared to higher concentrations. All treatment groups restored normal body temperature by the 3-hour observation endpoint.

Table10.1 Effect of MELR, MEAC and MECB on yeast extract-induced pyrexia in Rats.

Group	BBT	Rectal Temperature(°C) After 10hr of yeast injection						
		0	30	60	90	120	150	180
Control	37.12±0.23	38.68 ± 0.21	38.91 ± 0.35	38.91 ± 0.35	38.83± 0.32	38.81 ± 0.18	38.73±0.58	38.45±0.43
Standard (Paracetamol 150mg/kg0	37.16±0.21	38.7 ±0.12[a]	37.96±0.18[a]	37.3 ±0.45[a]	36.85±0.15[a]	36.92 ±0.43[a]	36.96±0.22[a]	37.01±0.42[a]
MEAC(200mg/kg)	37.08±0.16	38.73 ± 0.26	38.83 ± 0.46	38.75 ± 0.23	38.36± 0.37	38.31±0.36	38.11±0.58	0.5238.08±
MEAC(400mg/kg)	37.16±0.13	38.95 ± 0.18[b]	38.53 ± 0.33[b]	38.26 ± 0.33[b]	37.85 ± 0.53[b]	37.5 ± 0.38[b]	37.35±0.43[b]	37.13±0.48[b]
MELR(200mg/kg)	37.25±0.19	38.78 ± 0.33[a]	38.75 ± 0.37[a]	38.63 ± 0.13[a]	38.66 ± 0.12[a]	38.55 ± 0.3[a]	38.51±0.23[a]	38.43±0.16[a]
MELR(400mg/kg)	37.18±0.22	38.65 ± 0.42[b]	38.55±0.23[b]	38.43±0.39[b]	38.18±0.35[b]	37.95±0.54[b]	37.85±0.44[b]	37.65±0.23[b]
MECB(200mg/kg)	37.52±0.11	38,87 ± 0.45[a]	38.83±0.48[a]	38.78±0.21[a]	38.72±0.58[a]	38.65±0.42[a]	38.61±0.12[a]	38.58±0.44[a]
MECB(400mg/kg)	37.21±0.16	38.49±0.24[b]	38.49±0.32[b]	38.35±0.43[b]	38.28±0.53[b]	37.19±0.44[b]	37.18±0.23[b]	37.14±0.32[b]

All values represented as mean±SEM; n=6rats in each community, accompanied by the one-way ANOVA Multiple Comparison Test of Tukey. #,p<0.001Vs Paracetamol(150mg/Kg); a,p<0.01Vs Paracetamol(150mg/Kg)andb, p<0.05VsControl.

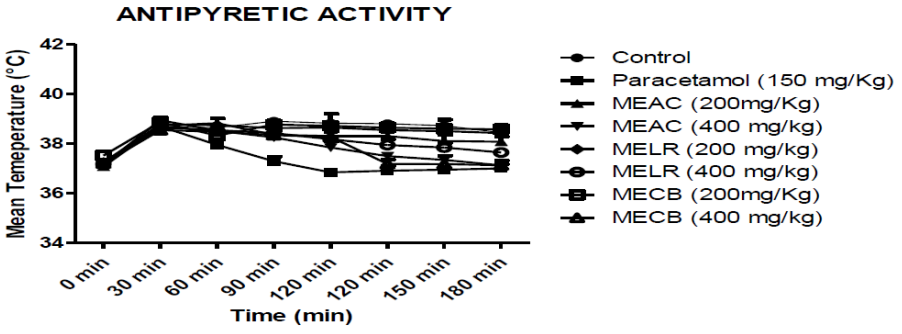


Figure10.1 Effect of MELR, MEAC and MECB on yeast extract- induced pyrexia in Rabbits.

10.5 Discussion

The antipyretic effects of MEAC, MELR, and MECB likely result from their

diverse bioactive constituents, particularly flavonoids, phenolics, and terpenoids identified through prior phytochemical screening. Further isolation and characterization studies are warranted to identify specific compounds responsible for this activity (Kamatou et al., 2009). Fever represents a critical acute phase response during inflammatory processes (Paltrinieri, 2008). Brewer's yeast acts as an exogenous pyrogen containing lipopolysaccharide, triggering endogenous cytokines (IL-1, IL-6, TNF-α) that activate arachidonic acid pathways and subsequent PGE2 synthesis (Gladvi et al., 2004). This yeast-induced pyrexia model effectively predicts antipyretic potential (Al-Mosawi, 2015). The MEs demonstrated robust antipyretic activity in this rabbit model, corroborating their therapeutic potential.

10.6 Conclusion

The aforementioned findings show that these floraes significantly outperformed the yeast-induced rabbit models in terms of antipyretic efficacy. Therefore, following further research, it was concluded that MEs of Argyreiacymosa, *L. R.*, and Capparis brevispina might be used as a possible herbal source for the treatment of fever in alternative medical systems.

CHAPTER 11
HEPATOPROTECTIVE ACTIVITY

11.1 Introduction to Liver

Paracetamol (PCM), commonly known as acetaminophen, represents the most widely prescribed analgesic and antipyretic agent. However, therapeutic doses can induce severe hepatotoxicity, leading to hepatic and renal tubular necrosis. Paracetamol-induced hepatotoxicity serves as the standard experimental model for evaluating hepatoprotective activity (Mazraati and Minaiyan, 2018). Among various laboratory-induced hepatitis models, D-galactosamine demonstrates superior hepatotoxic efficacy (Swarnalatha et al., 2012).

Free radicals generated during metabolic processes target membrane lipids, contributing to pathophysiological conditions. Florae-derived antioxidants serve as primary defense mechanisms by scavenging free radicals, functioning as hepatoprotective agents. These natural antioxidants constitute the most potent components of the body's free radical neutralization systems (Yimam et al., 2016). Over the past three decades, florae-based antioxidants have demonstrated substantial efficacy in managing hepatic disorders (Saxena et al., 2013).

Despite advances in pharmacotherapy for chronic liver diseases, existing treatments fail to address disease progression and frequently produce adverse effects including fatigue, depression, and insomnia (Foster, 2009). Novel agents such as rimonabant, corticosteroids, and propylthiouracil exhibit significant toxicity (Bhardwaj, 2011). Florae-derived bioactive compounds offer promising alternatives to conventional hepatic therapies (Colegate, 2015). Accordingly, this study evaluates the hepatoprotective potential of MEs from *A. C.*, *L. R.*, and *C. B.* using the paracetamol-induced hepatotoxicity rat model.

11.2 Materials

11.2.1 Florae extracts

The MEive fractions of three botanically and ethnopharmacologically significant florae taxa — namely *A. C.*, *L. R.*, and *C. B.* — were selected and employed as the primary biological test materials for the subsequent pharmacological investigative examinations conducted within the present chapter. The preparation, physicochemical

characterization, and preliminary phytochemical profiling of these MEive fractions, encompassing the standardized maceration-based extraction methodology, percentage yield determinations, and qualitative secondary metabolite screening protocols applied to each of the three florae-derived crude extractive matrices, have been comprehensively documented, systematically evaluated, and exhaustively presented in the preceding Chapter V of this dissertation, to which cross-referential acknowledgment is hereby accorded to preclude methodological redundancy and maintain the sequential investigative narrative integrity of the present research framework. The deliberate selection of the methanolic solvent system for the preparation of the extractive fractions employed in the present biological activity evaluations was predicated upon the well-established broad-spectrum solvation capacity of methanol as an extractive medium — its intermediate polarity profile rendering it uniquely competent in simultaneously solubilizing a structurally diverse assemblage of both polar and moderately non-polar secondary metabolite classes, encompassing phenolic acids, flavonoid glycosides and aglycones, alkaloids, iridoid glycosides, terpenoids, and saponin-type constituents — thereby ensuring maximal representation of the pharmacologically active phytoconstituent complement of each florae species within the extractive matrix subjected to biological screening. The three taxonomically distinct florae species collectively represent a pharmacobotanically diverse investigative panel, each possessing documented ethnoremedial utilization records and preliminary phytochemical profiles suggestive of promising pharmacological activity spectra, rendering their comparative biological evaluation within a unified experimental framework of considerable scientific merit and therapeutic relevance.

11.2.2 Reagents

All chemical reagents, analytical-grade solvents, and laboratory consumables employed in the execution of the present investigation were procured from National Scientific Products, Vijayawada, Andhra Pradesh, India. Unless otherwise specified within the experimental protocols described in subsequent sections, all chemical substances utilized were of analytical reagent (AR) grade or higher purity, and no additional purification procedures were applied prior to use. Solvents deployed for chromatographic separation and spectrophotometric assay procedures were of the highest commercially available purity grade appropriate to the respective analytical application.

11.2.3 Experimental Protocol

Adult healthy Wistar albino rats of either sex, with body weights ranging between 150 and 200 g (verify against laboratory records), were procured from Ghosh Enterprises and maintained under standardized laboratory housing conditions throughout the duration of the experimental period. The housing environment was regulated to maintain a controlled ambient temperature of 22 ± 2°C, a relative humidity of 50 ± 5%, and a standard 12-hour light/dark photoperiodic cycle. All animals were provided unrestricted access to a standard commercial pellet diet and potable drinking water ad libitum. A minimum acclimatization period of seven days was observed prior to the commencement of any experimental procedure, during which animals were monitored daily for general health status and behavioral normalcy. All experimental procedures involving laboratory animals were designed, conducted, and supervised in strict accordance with the ethical guidelines stipulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. Institutional ethical clearance for the conduct of the present investigation was obtained from the Institutional Animal Ethics Committee (IAEC) of Deshpande Laboratories-DIPS, which reviewed and formally approved the submitted project proposal bearing reference number CPCSEA/IAEC/04/2024/61, entitled "Evaluation of a Few Selected BhartiyeRemedialFloraes — *A. C.* (Roxb.) Sweet, *L. R.* (Benth.) Benth., and *C. B.* DC. using Pharmacognostical, Phytochemical and Pharmacological Methods," as submitted by Bharat Goswami of the Faculty of Pharmacy, P.K. University, Shivpuri, Madhya Pradesh. The IAEC convened for deliberation and ratification of this proposal on 25 April 2024, at which session the utilization of 100 adult healthy Wistar albino rats for the purposes delineated within the approved protocol was formally sanctioned. The institutional CPCSEA registration number on record is 1582/PO/Re/S/11/CPCSEA. All animal experimentation was conducted in compliance with the three Rs framework — Replacement, Reduction, and Refinement — to minimize animal use and ensure the highest attainable standards of humane experimental practice throughout the study.

11.3 Methods

11.3.1 Paracetamol-induced hepatotoxicity

The present experiment evaluates the hepatoprotective activity of the

paracetamol-induced hepatotoxicity rat model (Kanchana and Sadiq, 2011).

11.3.2 Experimental protocol

Experimental Cohort Stratification and Hepatoprotective Evaluation Protocol Healthy adult Wistar albino rats were methodically stratified into nine discrete experimental cohorts, each constituted by six animals (n = 6), for the rigorous and systematic pharmacodynamic quantification of hepatoprotective efficacy against paracetamol-induced experimental hepatotoxicity through longitudinal biochemical and histopathological assessment parameters. The compositional architecture of the experimental cohort allocation was structured as comprehensively delineated hereunder:

Cohort I — Normal Control: Oral administration of 0.5% (v/v) Tween 80 aqueous vehicle suspension at a body weight-normalized volume of 1 mL/kg, administered continuously over a 5-day treatment duration (p.o.)

Cohort II — Hepatotoxicity Control: Paracetamol (PCM) administered at a hepatotoxic dose of 2 g/kg body weight orally over 5 days, without concurrent hepatoprotective intervention (p.o.)

Cohort III — MEAC Low Dose: ME of *A. C.* at 200 mg/kg body weight administered orally for 5 consecutive days, followed by paracetamol hepatotoxic challenge (2 g/kg, p.o.) at 1 hour subsequent to the final extract administration on Day 5

Cohort IV — MEAC High Dose: ME of *A. C.* at 400 mg/kg body weight administered orally for 5 consecutive days, followed by paracetamol hepatotoxic challenge (2 g/kg, p.o.) at 1 hour subsequent to the final extract administration on Day 5

Cohort V — MELR Low Dose: ME of *L. R.* at 200 mg/kg body weight administered orally for 5 consecutive days, followed by paracetamol hepatotoxic challenge (2 g/kg, p.o.) at 1 hour subsequent to the final extract administration on Day 5

Cohort VI — MELR High Dose: ME of *L. R.* at 400 mg/kg body weight administered orally for 5 consecutive days, followed by paracetamol hepatotoxic challenge (2 g/kg, p.o.) at 1 hour subsequent to the final extract administration on Day 5

Cohort VII — MECB Low Dose: ME of *C. B.* at 200 mg/kg body weight administered orally for 5 consecutive days, followed by paracetamol hepatotoxic challenge (2 g/kg, p.o.) at 1 hour subsequent to the final extract administration on Day 5

Cohort VIII— MECB High Dose: ME of *C. B.* at 400 mg/kg body weight administered orally for 5 consecutive days, followed by paracetamol hepatotoxic challenge (2g/kg, p.o)at 1 hour subsequent to the final extract administration on Day 5

Cohort IX — Positive Reference Standard: Silymarin at a body weight-normalized dose of 25 mg/kg administered orally for 5 consecutive days, followed by paracetamol hepatotoxic challenge (2 g/kg, p.o.) at 1 hour subsequent to the final silymarin administration on Day 5

The totality of investigational phytochemical extractive fractions and reference pharmacological agents were homogeneously dispersed within a 0.5% (v/v) Tween 80 aqueous vehicle matrix antecedent to oral administration, ensuring uniform physicochemical dispersibility, consistent gastrointestinal bioavailability, and equitable comparative conditions across all experimental cohorts throughout the 5-day preconditioning treatment continuum. Experimental hepatotoxicity was pharmacologically induced through the deliberate administration of paracetamol at a supratherapeutic hepatotoxic dose of 2 g/kg body weight, delivered orally at a precisely standardized temporal interval of 1 hour subsequent to the final investigational extract or reference standard dose on the fifth and terminal day of the preconditioning protocol — a temporally strategic administration sequence specifically architected to ensure maximal systemic bioavailability and hepatoprotective tissue-level concentration of investigational phytoconstituents concurrent with the initiation of paracetamol-mediated hepatocellular injury cascades. The mechanistic basis of paracetamol-induced experimental hepatotoxicity at supratherapeutic dosage levels resides in the saturation of hepatic glucuronidation and sulfation conjugation pathways, consequent cytochrome P450 2E1-mediated oxidative biotransformation of paracetamol to the highly reactive and electrophilically cytotoxic N-acetyl-p-benzoquinone imine (NAPQI) intermediate, depletion of hepatic glutathione antioxidant reserves through covalent NAPQI-glutathione adduct formation, and ultimate covalent binding of excess NAPQI to critical hepatocellular macromolecular targets — precipitating oxidative stress amplification, mitochondrial membrane permeability transition, hepatocellular necrosis, and the consequent systemic elaboration of hepatocellular injury biomarker enzymes into the peripheral circulation.

11.3.3 Biochemical parameters

Following the experimental period, animals were euthanized humanely, and blood was collected from the retro-orbital plexus. Serum was separated after allowing blood to clot for 30 min at 37°C, followed by centrifugation at 3000 × g for 20 min. The resulting serum was analyzed for liver function markers including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) using commercial diagnostic kits.

11.3.4 Histopathological study

Liver tissues from all experimental groups were fixed in 10% neutral buffered formalin for 48 h, followed by dehydration in graded alcohol series and clearing in xylene for 6 h. Paraffin-embedded sections were prepared at 5 μm thickness, stained with hematoxylin and eosin (H&E), and examined microscopically for histopathological alterations.

11.4 Results

11.4.1 Biochemical parameters

Biochemical and Histopathological Corroboration of Hepatoprotective Efficacy Against Paracetamol-Induced Hepatocellular Injury

Oral administration of paracetamol at the supratherapeutic hepatotoxic dose of 2 g/kg body weight to vehicle-treated control cohort animals engendered a pronounced and statistically unequivocal biochemical perturbation of hepatocellular integrity, manifesting as dramatic elevations in circulating serum hepatocellular injury biomarker enzyme activities and hyperbilirubinemic responses relative to the corresponding indices documented in the normal, untreated control cohort — collectively constituting robust biochemical attestation of the successful establishment of experimental hepatotoxic pathology within the paracetamol-challenged control animals (Table 11.1). Specifically, paracetamol challenge precipitated marked escalations in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities to 97 U/mL, 104 U/mL, and 276 U/mL respectively, alongside a concomitant elevation of serum total bilirubin concentration to 1.26 mg/dL — representing substantial departures from the corresponding normal control reference values of AST (48 U/mL), ALT (42 U/mL), ALP (108 U/mL), and total bilirubin (0.37 mg/dL) — a biochemical perturbation profile mechanistically attributable to NAPQI-mediated covalent macromolecular adduct formation, oxidative stress amplification,

and consequent hepatocellular membrane integrity compromise with cytosolic enzyme leakage into the systemic circulation.

Concurrent oral preconditioning with the MEive fractions of *A. C.* (MEAC), *L. R.* (MELR), and *C. B.* (MECB) across both investigated dosage tiers (200 and 400 mg/kg body weight) engendered statistically significant and dose-proportional attenuation of paracetamol-provoked hepatocellular enzyme leakage, collectively demonstrating the capacity of the investigational phytochemical panel to substantially mitigate the biochemical sequelae of NAPQI-mediated hepatocellular injurious cascade. Serum AST activities within extract-preconditioned cohorts were dose-dependently suppressed to a collective inter-extract range of 53–64 U/mL — representing a restoration of between 33.0% and 45.4% toward normal control reference values from the paracetamol control ceiling of 97 U/mL. Analogously, serum ALT activities were attenuated to a collective range of 49–68 U/mL across extract-treated cohorts (versus paracetamol control: 104 U/mL), while the most numerically dramatic normalization was documented for serum ALP activity, wherein extract preconditioning suppressed paracetamol-provoked elevations to a collective inter-extract range of 118–143 U/mL — representing substantial mitigation from the paracetamol control value of 276 U/mL and collectively implicating preservation of hepatocellular membrane structural integrity and biliary epithelial cytoarchitectural fidelity as mechanistic underpinnings of the observed ALP normalization.

The positive reference hepatoprotective agent, silymarin (25 mg/kg, p.o.) — a standardized flavonolignan complex of well-characterized hepatocytoprotective, antioxidant, and hepatic regenerative pharmacological mechanism — achieved near-complete biochemical normalization of paracetamol-induced hepatocellular injury indices, restoring serum AST, ALT, ALP, and total bilirubin to values of 36.52 U/mL, 46.23 U/mL, 102 U/mL, and 0.34 mg/dL respectively — enzymatic and bilirubinemic indices closely approximating, and in certain parameters marginally surpassing, the corresponding normal control reference values, thereby confirming the maximal hepatoprotective efficacy benchmark and validating the discriminative pharmacodynamic sensitivity of the experimental model as operationalized in the present investigation. The proximity of silymarin-treated values to normal control indices corroborates the mechanistic attribution of its hepatoprotective competence to free radical scavenging, cytochrome P450 2E1 activity modulation, hepatic glutathione

pool replenishment, and stimulation of hepatocellular regenerative biosynthetic machinery.

Beyond hepatocellular enzyme normalization, extract preconditioning with MEAC, MELR, and MECB concurrently engendered meaningful restoration of hepatic biosynthetic functional competence, as evidenced by the significant augmentation of serum total protein concentrations to a collective inter-extract range of 4.89–5.51 g/dL within treated cohorts — an outcome mechanistically reflective of preserved hepatocellular ribosomal translational machinery and restored albumin and globulin biosynthetic capacity following attenuation of NAPQI-mediated oxidative macromolecular damage. Concomitant normalization of serum cholesterol profiles within extract-treated cohorts further corroborated the restoration of hepatic lipid metabolic homeostasis and lipoprotein biosynthetic regulatory competence — hepatic functional parameters whose perturbation in the paracetamol control cohort is consistent with NAPQI-induced disruption of endoplasmic reticulum membrane integrity and sterol biosynthetic enzymatic machinery.

Histopathological microscopic examination of hepatic tissue sections obtained from experimental cohorts (Table 11.1) furnished definitive morphological corroboration of the biochemically documented hepatoprotective efficacy, with extract-preconditioned animals demonstrating substantially preserved hepatic cytoarchitectural organization, attenuated centrilobular necrotic zone extent, diminished inflammatory cellular infiltration magnitude, and reduced hepatocellular vacuolation density relative to the extensively damaged histological landscape characterizing paracetamol control cohort hepatic sections — findings collectively establishing a hepatoprotective efficacy profile for MEAC, MELR, and MECB that approximates the cytoarchitectural preservation benchmark documented for the silymarin reference standard cohort and warranting systematic bioactivity-guided fractionation to isolate the specific phytoconstituent molecular entities mediating the observed multi-parametric hepatoprotective pharmacological activity.

11.4.2 Histopathology

Histological examination of the normal control group revealed typical liver architecture, characterized by a central hepatic vein, well-defined sinusoids, and regular

hepatocyte arrangement. Paracetamol-treated rats exhibited extensive hepatocellular necrosis, cytoplasmic degeneration, and disrupted liver architecture.

Rats co-treated with paracetamol and silymarin demonstrated near-normal hepatocyte morphology and preserved liver structure. Treatment groups receiving MEAC, MELR, or MECB (200 and 400 mg/kg) alongside paracetamol showed substantial liver restoration with minimal residual damage, as illustrated in Figure 11.1.

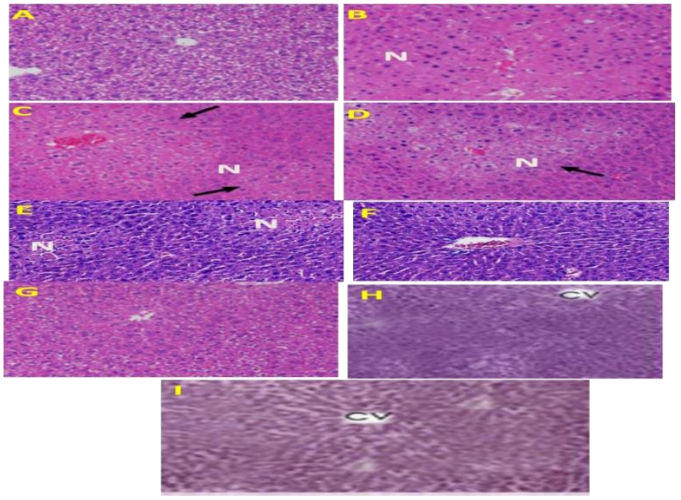


Figure 11.1 Hematoxylin and eosin-stained paraffin portions of the liver for histopathological changes. (A) Liver portion of the test group demonstrates a standard liver histology layout. (B) PCM liver (2gm / Kg) (C) Liver segment PCM + MEAC (200 mg/kg) (D) Liver segment PCM and MEAC (400 mg/kg) (E) PCM and MELR (200 mg/kg) liver section (F) liver section PCM and MELR(400 mg/kg) (G) Liver section PCM and Silymarin (25 mg/kg) (H) Liver section PCM and MECB(200 mg/kg)(I) Liver section PCM and PCM (200 mg/kg) Liver Section (25 mg/kg) (G) N: Necrosis.

Table 11.1 Effects of pre-treatment with MEAC (200 and 400 mg/kg), MELR (200 and 400 mg/kg), MECB (200 and 400 mg/kg) and Silymarin on the serum levels of AST, ALT, ALP and Bilirubin in PCM induced hepatotoxicity in rats.

Bilogical Parameters	I	II	III	IV	V	VI	VII	VIII	IX
(Normal Control: 0.5%Tween 801ml/kg b.wt)	(Hepatotoxic control:0.5% Tween80 1ml/kg b.wt+PCM (2g/kg bw)	MEAC(200mg/kg+PCM 2g/kg)	MEAC(200mg/kg+PCM 2g/kg)	MEAC(200mg/kg+PCM 2g/kg)	MEAC(200 mg/kg+PCM 2g/kg)	MEAC(200mg/kg+PCM 2g/kg)	MEAC(200 mg/kg+PCM 2g/kg)	Silymarin25 mg/kg+PCM 2g/kg)	
ALT(U/L)	42±3.23	104±5.36*	68±5.58*	49±3.85*	66±2.28*	58±5.14*	86±5.58*	54±3.85*	36.52±2.58*
AST(U/L)	48±4.12	97±9.63*	64±2.85*	53±5.44*	58±6.15*	52±3.15*	78±2.85*	63±5.44*	46.23±2.25*
ALP(U/L)	108±8.36	276±11.85*	143±11.23*	118±8.58*	126±10.23*	108±8.15*	155±11.23*	132±8.58*	102±6.36*
Total Bilurubin (mg/dl)	.37±0.05	1.26±0.08*	0.69±0.03*	0.46±0.03*	0.58±0.02*	0.39±0.06*	0.86±0.03*	0.52±0.03*	0,34±0.02*

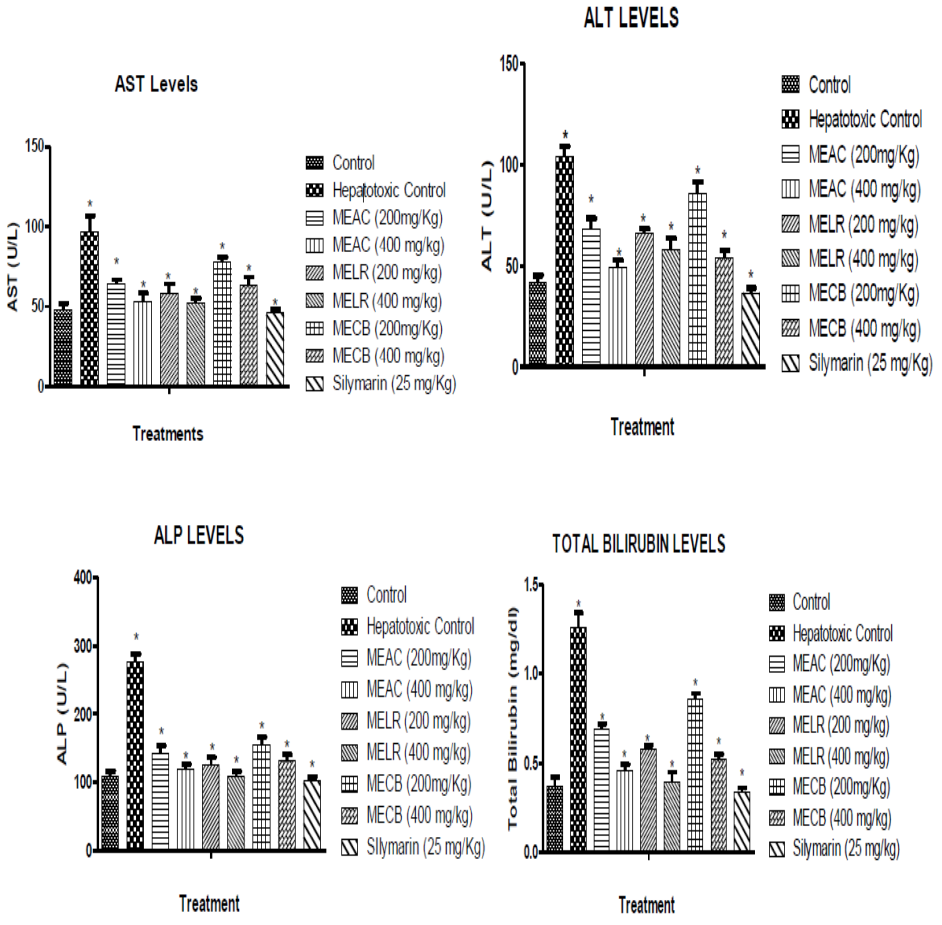


Figure 11.2 Effects of pre-treatment with MEAC (200 and 400 mg/kg), MELR (200 and 400 mg/kg), MECB (200 and 400 mg/kg) and silymarin on the serum levels of AST, ALT, ALP and Bilirubin in PCM inducedhepatotoxicity in rats.

11.5 Discussion

Paracetamol (PCM), despite its widespread therapeutic use as an antipyretic, induces severe hepatotoxicity at high doses through cytochrome P450-mediated metabolism producing the reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI) (Sevides and Oehme, 1983). Under normal conditions, NAPQI is detoxified by conjugation with reduced glutathione (GSH) forming mercapturic acid for urinary excretion. PCM overdose depletes hepatic GSH, allowing unbound NAPQI to covalently bind mitochondrial proteins, impair fatty acid β-oxidation, and trigger hepatocyte necrosis and apoptosis.

Elevated serum ALT represents the most liver-specific marker of hepatocellular injury, while increased AST indicates cellular leakage and compromised membrane integrity. Serum ALP elevation correlates with severe hepatic damage (Kanchana and Sadiq, 2011). The liver synthesizes most serum proteins, and bilirubin elevation reflects impaired reticuloendothelial function, reduced conjugation, or defective biliary excretion—serving as a marker of hepatic synthetic capacity rather than damage severity.

Phytoconstituents such as flavonoids and phytosterols, previously identified in these extracts, exert hepatoprotective effects through antioxidant mechanisms. These compounds mitigate PCM-induced oxidative stress by inhibiting lipid peroxidation and bolstering hepatic antioxidant defenses (Abdulmohsin et al., 2019).

The present study demonstrates that MEAC, MELR, and MECB significantly ($p < 0.05$) attenuated PCM-elevated serum AST, ALT, ALP, and bilirubin levels. Histopathological findings (Figure 11.1) and normalized biochemical parameters (Table 11.1) confirm substantial restoration of hepatocyte architecture, validating the hepatoprotective efficacy of these MEs against paracetamol-induced liver injury.

11.6 Conclusion

According to the study's findings, MEs of Argyreiacymosa, *L. R.*, and Cappparis brevispina have hepatoprotective properties against rat liver injury caused by paracetamol. Therefore, these floraes have the potential to be a herbal hepatoprotective agent.

CHAPTER 12

ANTICANCER ACTIVITY

12.1 Introduction

Floraes have historically served as primary sources of clinically relevant anticancer agents. However, systematic approaches to identify novel anticancer compounds have remained underutilized in ethnopharmacological research (Gertsch, 2009). Documentation of specific anticancer applications within traditional medical systems remains limited, primarily due to cancer's complex symptomatology (Bodio and Tuso, 2015). The validated anticancer properties of numerous florae derivatives underscore the urgent need for novel therapeutic agents (Van-veen et al., 2004). In India, systematic screening of higher floraes for anticancer activity commenced during the 1960s, evaluating approximately 217 species by the 1970s. Remedialfloraes contain physiologically active principles traditionally employed for various ailments, many exhibiting anticancer potential (Elango et al., 2011). These herbs constitute essential components of accessible, cost-effective natural pharmacopeias worldwide (Sun et al., 2008).

This study evaluates the anticancer potential of *A. C.*, *L. R.*, and *C. B.* against Ishikawa (endometrial) and SCC-29B (squamous cell carcinoma) cell lines using the sulforhodamine B (SRB) assay to assess cell viability.

12. 2 Materials

12.2.1 Florae extracts

MEs of *Argyreiacymosa*, *L. R.*and *C. B.* (Described in chapter V) were used in this examination.

12.2.2 Chemicals

Reagents and supporting laboratory materials required for the experimental procedures were sourced from National Scientific Products, Vijayawada, Andhra Pradesh, India.

12.2.3 Ishikawa cell lines and SCC-29B cell Lines

12.3 Methods

Assessment of anticancer activity was carried out using a sulforhodamine B (SRB) assay on cultured cell lines, incorporating slight procedural modifications based on the method reported by Gaidhani et al. (2013).

12.3.1 *In-vitro* cell line SRB assay

Sulforhodamine B (SRB) Cytotoxicity Assay Protocol

Cell Culture and Plating

Ishikawa and SCC-29B cell cultures were propagated in RPMI 1640 medium enriched with 10% fetal bovine serum and 2 mM L-glutamine to ensure optimal growth conditions. Cells were dispensed into 96-well plates at 100 µL per well, with plating densities adjusted in accordance with their specific doubling characteristics (Gali et al., 2011). The plates were subsequently maintained at 37°C in a humidified incubator with 5% CO_2 and 95% air atmosphere for 24 hours prior to exposure to the test samples.

Extract Preparation

MEs were initially solubilized in dimethyl sulfoxide (DMSO) at 100 mg/mL, then diluted with water to 1 mg/mL and stored frozen. Working concentrations (100, 200, 400, 800 µg/mL) were prepared by diluting frozen aliquots in complete medium. Ten µL of each dilution was added to wells containing 90 µL medium, yielding final concentrations of 10, 20, 40, and 80 µg/mL.

SRB Assay Procedure

Post 48 h incubation at standard conditions, 50 µL of cold 30% (w/v) trichloroacetic acid (TCA; final concentration 10%) was added to each well. Plates were incubated at 4°C for 1 h, supernatant discarded, and cells washed five times with distilled water followed by air drying. Wells were stained with 50 µL of 0.4% (w/v) sulforhodamine B (SRB) solution in 1% acetic acid for 20 min at room temperature. Unbound dye was removed by five washes with 1% acetic acid, and plates were air-dried.

Quantification and Data Analysis

Bound SRB was solubilized with 100 µL of 10 mM Tris base, and absorbance was measured at 540 nm with reference at 690 nm using a microplate reader. Percentage growth relative to control wells was calculated as:

[(Mean test well absorbance / Mean control well absorbance) × 100]

Dose-Response Parameters

Six absorbance measurements were used: time zero (Tz), control growth (C), and test growth (Ti) at four concentrations. Percentage growth inhibition was determined as:

For Ti ≥ Tz: [(Ti-Tz) /(C-Tz)] × 100

For Ti < Tz: [(Ti-Tz) /Tz] × 100

Cytotoxicity Metrics

GI_{50}: Concentration inhibiting 50% net cell growth [(Ti-Tz)/(C-Tz)] × 100 = 50

TGI: Concentration causing total growth inhibition (Ti = Tz)

LC_{50}: Concentration reducing initial protein content by 50% [(Ti-Tz)/Tz] × 100 = -50

12.3.2 Statistical analysis

Each of the three assessed parameters was quantified with reference to a predefined intensity cut-off. When the observed response failed to meet or exceed this threshold, the results were expressed as exceeding the measurable upper limit or falling below the detectable lower concentration range.

12.4 Results

The cytotoxic potential of MEs obtained from *A. C.* (MEAC), *L. R.* (MELR), and *C. B.* (MECB) was assessed against Ishikawa (human endometrial adenocarcinoma) and SCC-29B (human oral squamous cell carcinoma) cell lines using the sulforhodamine B (SRB) assay (Tables 12.1–12.4; Figures 12.1–12.2).

Among the tested samples, MELR exhibited pronounced antiproliferative activity, showing effectiveness comparable to adriamycin. It produced TGI values of 38.9 µg/mL in Ishikawa cells and 58.12 µg/mL in SCC-29B cells, with LC_{50} values below 10 µg/mL in both cell lines, indicating strong cytotoxic potential at lower concentrations.

In contrast, MEAC demonstrated comparatively weaker activity against Ishikawa cells, with GI_{50} values exceeding 80 µg/mL. However, in SCC-29B cells, it

showed enhanced sensitivity, with TGI values below 10 µg/mL and GI_{50} values below 20 µg/mL.

MECB displayed moderate cytotoxic effects, recording GI_{50} values below 80 µg/mL in Ishikawa cells, whereas in SCC-29B cells, the activity was comparatively lower with GI_{50} values exceeding 80 µg/mL.

Overall comparison of GI_{50} values suggests that MELR possesses superior and broad-spectrum cytotoxic activity against both endometrial and oral cancer cell lines when compared with MEAC and MECB.

12.5 Discussion

Current chemotherapeutic regimens, despite their efficacy in oncology, frequently produce severe adverse effects that exacerbate cancer-related morbidity and mortality. Natural compounds, particularly phytochemicals, offer promising alternatives with enhanced safety profiles. These agents demonstrate well-documented chemopreventive and anticancer properties, warranting further investigation (Kasinski et al., 2015). Phenolics, tannins, flavonoids, and their derivatives—encompassing over 8,000 distinct compounds—exert multifaceted anticancer effects through antioxidant activity, carcinogen neutralization, inhibition of cell proliferation, induction of cell cycle arrest and apoptosis, and immunomodulation. These mechanisms also contribute to cardiovascular protection (Priya and Sharma, 2013; Hidetomo et al., 2019).

The present study validates the anticancer potential of MEs from *A. C.*, *L. R.*, and *C. B.* against Ishikawa (endometrial adenocarcinoma) and SCC-29B (oral squamous cell carcinoma) cell lines using the sulforhodamine B (SRB) cell viability assay. These findings, particularly the potent activity of MELR, align with the previously identified flavonoid, phenolic, and terpenoid profiles that likely mediate the observed cytotoxicity.

Table 12.1 Effect of MEs of *Argyreia cymosa, L. R.*and *C. B.* on Ishikawa cell line growth curve

Human endometrial adenocarcinoma cell illness % Control growth Drug Concentrations(ug/ml)																
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
MEAC	92.3	96.2	98.6	118.4	79.4	90.2	93.3	104	84.6	102.6	112.4	112.3	85.4	96.33	101.43	111.5
MELR	1.8	-28.6	-78.9	-65.9	-4.4	-30.9	-58.3	-64.8	-32	-32.9	-42.4	-47.8	-11.5	-30.8	-59.8	-59.5
MECB	96.2	76.6	50.8	35.4	88.6	74.2	45.9	31.8	82.5	74.3	51.9	37.4	89.1	75.03	49.53	34.87
Adriamycin	-75.9	-80.2	-79.8	-64.1	-78.6	-77.7	-74.7	-69.3	-73.9	-70.4	-80.3	-63	-76.13	-76.1	-78.2	-75.6

Table12.2 Effect of methanolic extracts of Argyreia cymosa, Limnophila repens and Capparis brevispina on SCC-29B cell line growth curve

Human normal cancer cell illness SCC-29B % Control growth Drug Concentrations(ug/ml)																
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
MEAC	118.4	98.6	96.2	92.3	104	93.3	90.2	79.4	112.3	108.4	102.6	84.6	111.56	100.1	96.33	85.43
MELR	1.8	-28.6	-78.9	-65.9	-4.4	-30.9	-58.3	-64.8	-32	-32.9	-42.4	-47.8	-11.53	-30.8	-59.86	-59.5
MECB	98.6	102	78.4	30.6	101.3	99.9	75.3	28.4	102	101.3	82.4	31.6	100.63	101.06	78.7	30.2
Adriamycin	-75.9	-80.2	-79.8	-64.1	-78.6	-77.7	-74.7	-69.3	-73.9	-70.4	-80.3	-63	-76.13	-76.1	-78.26	-65.46

Table 12.3 Drug concentrations (µg/ml) calculated from the graph on Ishikawa cell lines

Compound	Celline	LC_{50}	TGI	GI50
MEAC	Ishikawa	NE	NE	>80
MELR	Ishikawa	38.9	NE	<10
MECB	Ishikawa	48.6	NE	<80
Adrinamycin	Ishikawa	11.6	NE	<10

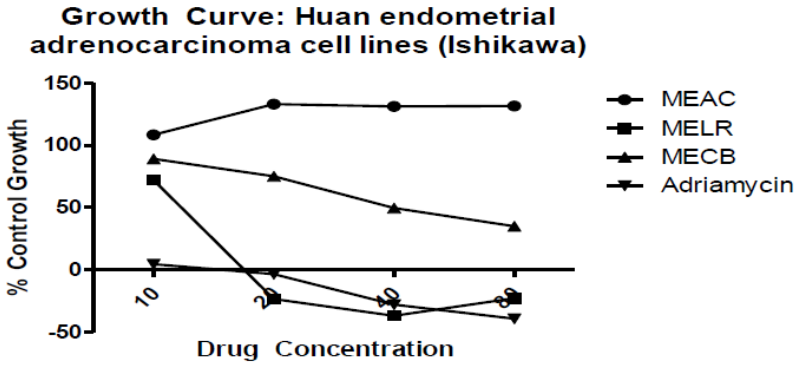
Table 12.4 Drug concentrations (µg/ml) calculated from the graph on SCC-29B cell lines

Compound	Celline	LC_{50}	TGI	GI50
MEAC	SCC-29B	NE	<10	<20
MELR	SCC-29B	58.12	<10	<10
MECB	SCC-29B	NE	NE	>80
Adrinamycin	SCC-29B	NE	<10	<10

LC50 = Drug concentration that kills 50% of the cells

GI50 = Drug concentration that inhibits cell proliferation by 50%

TGI = Drug concentration that completely inhibits cell proliferation



NE = non-evaluable data. The experiment needs to be repeated using different sets of drug concentrations.

Figure 12.1 Effect of MEs of *Argyreiacymosa, L. R.*and *C. B.* on Ishikawa cell line growth curve

Growth curve: Human oral cell lines (SCC-29B

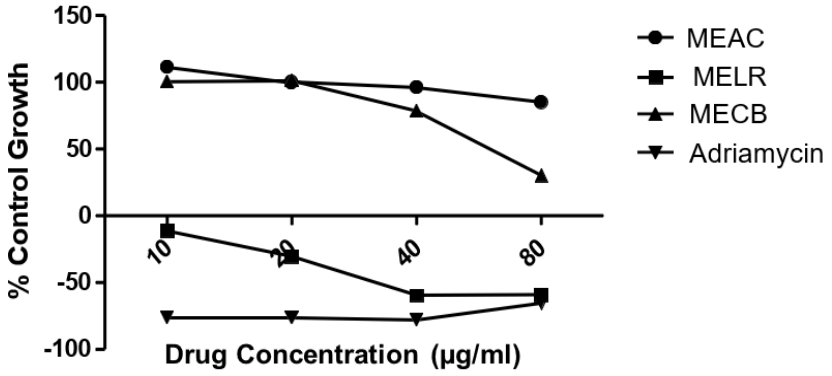


Figure12.2 Effect of MEs of *Argyreia cymosa*, *Limnophila repens* and *C. B.* on SCC-29B cell line growth curve

12.6 Conclusion

The experimental results demonstrate that MEs of *A. C.*, *L. R.*, and *C. B.* exhibit significant anticancer activity against Ishikawa and SCC-29B cell lines. Among the three extracts, *C. B.* (MECB) demonstrated superior cytotoxic potency, likely attributable to its rich phytochemical profile. These findings position these remedialfloraes as promising candidates for isolating bioactive compounds suitable for herbal anticancer formulations.

CHAPTER 13

SUMMARY AND CONCLUSION

Floraes have historically served essential roles in human culture and traditional medicine, particularly in India where herbal remedies predate modern pharmaceuticals. The increasing adverse effects of allopathic drugs have revitalized interest in herbal alternatives. Comprehensive literature analysis identified *A. C.* (Roxb.) Sweet, *L. R.* (Benth.) Benth., and *C. B.* DC as promising sources of bioactive compounds.

Chapter I introduces remedialfloraes and their global significance. Traditional herbal remedies provide safe, cost-effective alternatives to synthetic drugs. Various florae parts yield secondary metabolites with pharmacological effects including antinociceptive, anti-inflammatory, antipyretic, hepatoprotective, and anticancer activities.

Chapter II reviews ethnoremedial, phytochemical, and pharmacological data for the selected floraes. These species, used by tribal communities near Tirupati hill forests, exhibit diverse therapeutic applications. Limited prior research justified their selection.

Chapter III outlines study objectives: pharmacognostic evaluation, phytochemical screening, extraction, TLC profiling, acute toxicity assessment (OECD 423), and pharmacological investigations. Voucher specimens (A. cymosa: 4017; L. repens: 5504; C. brevispina: 3210) were deposited at GITAM University.

Chapter IV details pharmacognostic standardization per WHO guidelines. Macroscopic, organoleptic, microscopic, and powder analyses established diagnostic characters distinguishing these species within their families.

Chapter V presents physicochemical standardization. T. A. V. for A. cymosa leaves, stems, and roots were 6.7%, 7.33%, and 6.56% w/w, respectively; L. repens: 5.2%, 8.35%, 4.56% w/w; C. brevispina: 4.28%, 5.16%, 6.26% w/w. Acid-insoluble ash, water-soluble ash, E.V., and loss on drying confirmed quality parameters. Fluorescence analysis suggested presence of flavonoids, alkaloids, tannins, and phytosterols.

Chapter VI describes successive extraction (hexane to methanol) and phytochemical screening. Yields: A. cymosa (6.8-6.5% w/w), L. repens (1.4-5.5% w/w), C. brevispina (1.8-6.5% w/w). TLC profiling confirmed flavonoids, steroids,

terpenoids, alkaloids, phenolics, and saponins across extracts.

Chapter VII confirms acute toxicity safety (LD_{50} >2000 mg/kg) per OECD 423 guidelines, enabling further pharmacological studies.

Chapter VIII demonstrates dose-dependent antinociceptive activity (200-400 mg/kg) in hot plate, tail immersion, and acetic acid writhing models. All extracts exhibited central and peripheral analgesia comparable to tramadol and diclofenac.

Chapter IX establishes significant anti-inflammatory effects in carrageenan-induced paw edema, with phase-specific inhibition (MEAC: early phase; MELR/MECB: late phase) versus diclofenac.

Chapter X validates antipyretic activity in Brewer's yeast-induced pyrexia, with 400 mg/kg extracts approximating paracetamol efficacy.

Chapter XI confirms hepatoprotection against paracetamol hepatotoxicity, normalizing AST, ALT, ALP, bilirubin, and total protein levels while preserving hepatic architecture (vs. silymarin).

Chapter XII reveals anticancer cytotoxicity against Ishikawa (endometrial) and SCC-29B (oral) cell lines via SRB assay. MELR showed superior potency (TGI: 38.9-58.12 µg/mL; LC_{50} <10 µg/mL) versus adriamycin.

The MEs demonstrated safety and multifaceted pharmacological potential attributable to identified phytoconstituents. These findings validate traditional uses and provide leads for novel herbal therapeutics targeting pain, inflammation, fever, liver disorders, and cancer. Future isolation of active principles is recommended.

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PLANT AUTHENTICATION CERTIFICATE

This is to certify that voucher specimen no. GLA/BOT./SPE/PHARM/072-2023/07 is deposited in the herbarium of this Department of Botany at GLA University, Mathura (U.P) by **Bharat Goswami, Research Scholar, Faculty of Pharmacy, P.K. University, Shivpuri (M.P.)** and following plant species for Pharmaceutical / Pharmacognostical / Phytochemical investigation research work is identified and their botanical name and family is given below.

S.No.	Botanical Name	Voucher Number	Family
1	*Argyreia cymosa (Roxb.) Sweet*	2023/077	Convolvulaceae
2	*Limnophila repens (Benth.)*	2023/079	scrophulariaceae
3	*Capparis brevispina in DC.*	2023/084	Capparidaceae

Prof. (Dr.) A. K. Gupta
Head of Department
Dept. of Botany
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Form B (per rule 8 (a)* for Submission of Research Protocol(s) Certificate

Certificate

This is to certify that the project proposal no. CPCSEA/IAEC/04/2024/61 entitled **"Evaluation of a few selected Indian Medicinal Plant Argyreia cymosa (Roxb.) Sweet, Limnophila repens (Benth) Benth and Capparis brevispina DC using Pharmacognostical Phytochemical and Pharmacological Methods"** by Dr./Mr./Ms. **Bharat Goswami, Faculty of Pharmacy, P.K University, Shivpuri, (M.P)** under the IAEC of Deshpande Laboratories-DIPS has been approved/recommended by the IAEC meeting held on 25/04/2024 and Adult healthy **Wistar rats Nos.100** have been sanctioned under this. CPCSEA No.1582/PO/Re/S/11/CPCSEA.

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A Comprehensive Review on: Pharmacognostical, Phytochemical and Pharmacological Evaluation of *Argyreia cymosa (Roxb.) Sweet, Limnophila repens (Benth) Benth and Capparis brevispina DC*

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ABSTRACT

Around the world, many laboratories are engaged in screening plants for their pharmacological and toxicological perspective. Keeping the aforementioned details in view, the following plants are chosen for thorough exploration and investigation of their phytochemical and pharmacological attributes. In the present chapter, the author gives a literature study that was performed and also recorded. Plants are frequently utilised in conventional treatment. Preparations for preventive and curative purposes for humans and livestock since ancient times Traditional plants offer a safe, cheap, and reliable alternative to chemical drugs and have been used in many parts of the world Other than wild herbs, cultivated plant spices are frequently employed in traditional treatments. Despite the fact that indoor production (in glasshouses, greenhouses, and poly houses) has gained popularity thanks to contemporary technology, medicinal plants still serve a crucial role in preserving biodiversity in woods, fallow fields, and cultivated lands. Different alternative medicinal systems such as Ayurveda, unani, and siddha have contributed to the accumulation of knowledge of medicinal plants for many centuries. It is documented that Indian traditional medicines use 2500 plant species and about 100 species of plants form regular sources of medicine. It is estimated that natural compounds form the basic ingredient in nearly 50% of medicines currently available on the market. Since the

synthetic preparations of many of these active ingredients in medicinal plants are not currently possible, the market demand for medicinal herbs is likely to remain high.

KEYWORDS: Phytochemical, pharmacological, herbs, biodiversity, Ayurveda.

1. INTRODUCTION

In recent years there has been resurgence in usage of herbal medicine, not only among the traditional medicine users (ethno medicine) but also among the modern consumers of herbal products as nutritional supplements and personal care segments. Therefore, quality acquires greater importance nowadays before regulatory agencies and consumer groups on one hand and increasingly stringent quality norms were required by GMP guidelines of manufactures on the other hand due to continue demand for herbs used for medicinal food and cosmetic purpose should be of high standards, reliable quality and free from contaminants. The government of India set up pharmacopoeial committees for ayurvedic, siddha and unani drugs. The pharmacopoeial laboratory Indian medicine (PLIM) at Ghaziabad was established as the subordinate office of the Ministry of Health and Family Welfare in 1970 to be utilized as drug standardization and testing unit, drug depot, herbarium and reference museum.

1.1. Plant profile of *Argyreia cymosa* (Roxb.) Sweet

Argyreiacymosa (Roxb.) Sweet

Vernacular Names: The plant available in different places India and with different asmentioned below

Telugu:	Maddi tige, Gantapala
Tamil:	Nilanarai
Malayalam:	Nilanarakam
English:	Clustered Woodrose

Scientific classification: According to taxonomy of the this plant scientific classification was given below.

Kingdom	Plantae
Sub-kingdom	Angiosperms
Class	Eudicots
Order	Solanales
Family	Convolvulaceae
Tribe	Ipomoeeae
Genus	Argyreia

Species *cymosa*

Botanical description

Argyreia cymosa belongs to the convolvulaceae family. There are about 1650 incredibly beautiful species in the convolvulaceae family. With over 135 species, the class Argyreia, cymosa is a stray in thickets; its stem is terete, pubescent, and woody, its oblong to cordiform leaves 6-8 X 4-6 cm, chartaceous, minor pubescence on the various sides; its blooms are pinkish in axillary, corymbose cymes; Its natural product is globose and glabrous, measuring 1.7 x 1.4 cm. (Matthew, 1995; Pullaiah, 2000).

Ethanomedical information of *Limnophila repens*

Many species in the genus were widely used as traditional medicine for various diseases for many years and exclusive ethnopharmacological information as follows.

- *Limnophila erecta* a leaf decoction is administered for fevers, and the leaves are used to clean wounds. (Quattrocchi, 2016)
- *Limnophila indica* the whole plants and cooked veggies are used as brain tonic; the juice from the plant is applied topically to cure severe fevers; elephantiasis is treated with liniment, and pustular skin eruptions are treated with the whole plant. An infusion of the leaves is used for skin conditions, stomach diarrhoea, and indigestion and is antiseptic and carminative. (Quattrocchi, 2016).
- The liquid from the cooked *Limnophila rugosa* plant is used as a laxative, stomach tonic, or to treat haemorrhage ulcers (Quattrocchi, 2016).
- The top of the plant, *Limnophila rugosa*, was rubbed on the feet. To treat itchy eyes, use a decoction and steam bath made from the leaves; they are antipyretic. a blend of *Limnophila rugosa* and *Ocimum basilicum L* decoction intoxicated in the face of impotence and mild gonorrhoea (Quattrocchi, 2016).
- Leaf extract from *Limnophila sessiliflora* is used as an antipyretic (Quattrocchi, 2016).
- *Limnophila villosa* leaves are febrifuge, and the whole plant is aperient when applied to inflammation. 2016 (Quattrocchi).

Plant profile of *Argyreia cymosa* (Roxb.) Sweet



Figure.1: *Argyreia cymosa* (Roxb.) Sweet

Table.1 Pharmacological information of *Argyreia cymosa* and other species

Species	Plant Part	Solvent	Uses	References
Argyreiacymosa	Leaves	Water	Antibacterial activity	Packialakshmi and Beevi, 2014
	Bark	Petroleum ether Ethyl acetate	Antioxidant activity	Badami, et al., 2008
Argyreiaspeciosa	Leaf, roots,and flower	Ethanol	Aphrodisiac activity	Subramoniam, et al.,2007
	Root	Ethanol	Immunomodulatory activity	Gokhale, et al., 2003
	Root	Ethanol and ethyl acetate	Hepatoprotective activity	Habbu, et al., 2008
	Root	N-hexane, chloroform, ethyl acetate, and water	Central nervous system activity	Galani and Patel, 2009
	Stem	Methanol	Hypoglycemic	Latha, et al., 2008
	Whole plant	Water	Nootropic	Hanumanthachar, et al., 2007
	Root	Ethanol	Anti-inflammatory activity	Srivastava, et al., 1972
	Root	Methanol	Analgesic activity	Bachhav et al., 2009
	Leaves	Ethanol	Antibacterial activity	Mishra and Chaturvedi, 1978
	Leaves	Water and ethanol	Nematicidal activity	Praveen, et al.,1990

Phytoconstituents Information of *Argyreiacymosa*

As per litreture survey, till to date, no phyto chemical isolated from *Argyreia cymosa* but many chemical constituents are widely isolated from the other species of *Argyreia* was illustrated in Table 2

Plant	Compound Name	Part of the Plant	Reference
Argyreia speciosa	Tricontanol, Epifriedelinol acetate, Epifriedelinol and β-sitosterol	Leaves	Sahu and Chakravarti, 1971
	Quercetin and Kaemperol, Kaemperol-3-o-lrhamnopyranoside	Leaves	Sohrab et al., 1992
	Tetradecanypalmitatete,		Rani and Shukla, 1997;
	5, 8 oxidotetracosan-10-one stigma sterolyl, Hydroxycinnmide hydroxycinnamicylyl	Root	Srivastava and Shukla., 1998
	Ergometrine, Ergometrined, Ethyl caffeate, Glutamic acid, Glycine, Isoleucine, Leucine, Lysine, Phenylalanine, Pyrosine, Praline, α-amino butyric acid	Seeds	Agrawal and Rastogi, 1974; Nair, 1987; Jaiswal et a., 1984
	N-triacontanol, βsitosterol, hydroxycinnamic, loctadecanolate, andcaffeic acid	Fruits	Purushothaman et al., 1982
Argyreia nervosa	Pennidavine, Caffeic Acid, Ergoline Alkaloids, Agroclavine, Ergine, Isoergine, Iso-lysergic Acid Amide Racemic chanoclavine- I, Chanoclavine-I, Chanoclavine-II, Festuclavine, Lysergine, Lysergol, Isolysergol, Penniclavine, Steoclavine, Isosetoclavine, Lysergic acid, and Alphahydroxyethylamide	Seeds	Kala, 2005.

1.2 Plant Profile

Botanical Name- *Limnophila repens (Benth.) Benth*

Vernacular names: The plant available in different places India and with different names as mentioned below

Telugu Burada Bakkena

Kannada	Amaragandhi
Sanskrit	Malayalamagandh
Malayalam	Mangannari
English	Marshwoodod

Scientific classification: According to taxonomy of the this plant scientific classificationwas given below

Kingdom	Plante
Subkingdom	Tracheophyta
Class:	Magnoliopsida
Order:	Lamiales
Family:	Plantaginaceae
Genus:	Limnophila
Species:	*repens*

Botanical description

Erect or procumbent flavours with a potent fragrance are *Limnophila repens* (Benth) *Benth* Also known as *Limnophila conferta* Benth. Leaves are 1-2 x 0.4-0.9 cm, elliptic- oval in shape, with a distinctive base, crenate-serrated edge, and an extreme pinnacle. They are also glabrous, penninerved, chartaceous, and subsessile. Axillary, solitary, or short racemes of blooms. C. Calyx Lanceolate, hirsute, and striate at improvement;projections 4 mm long. Violet-pink, infrequently yellow, c. 5 mm over; 6–8 mm long pubescent tube with longitudinal purple stripes at throat and outside. 4. Stamens Shamefully spathulate. 3–4 mm long and elliptical holder (Philcox, 1970). Precise, robust, shaded seeds. July through December is the months for blooming and fruiting. Below are references to around 15 different species of Limnophila (Chetty et al., 2008; Quattrocchi, 2016).

Different species of the genus *Limnophila:* World wide different speies ***Limnophila*** were available in various climatic zones among them some of prominent spices like.

Limnophila dasyantha	*Limnophila aromatica*
Limnophila erecta	*Limnophila bangweolensis*
Limnophila heterophylla	*Limnophila barteri*
Limnophila indica	*Limnophila borealis*

Limnophila rugosa	*Limnophila ceratophyl*
Limnophila sessiliflora	*Limnophila connata*

Ethanomedical information of *Capparis brevispina* DC.

Apart from *Capparis brevispina,* species in the genus were widely used as traditional medicine for various diseases for many years and exclusive ethnopharmacological information as follows

- According to Indian ethnomedical literature, for a very long time, Capparis species have been used in medicine. The Sumerians employed these plants for the first time around 2000 BC (Romeo et al., 2007)

- Extracts of the fruits, flowers, roots and root bark have been shown to be useful asanti-atherosclerosis, hypertension, bacteria, inflammation, analgesia, asthma, highcholesterol, and anti-fungal agents (Chahlia, 2009; Duman, et al., 2013).

- Extracts of caper buds and ripened fruits are also utilised as flavouring agents in the culinary industry (Farrel, 1998).

- The bark is used as homeopathic medicine (Hassan and Mohammed, 2010).

- Capparis species contain significant amounts of minerals, particularly in their floral buds and fruits, which are utilised as vegetables and pickled. The buds and fruit are also high in protein, carbs, lipids, and vitamins (Ozcan, 2005).

- Locals in India and Pakistan think that caper fruits have anti-diabetes properties. Laxative qualities that smoothen. As a result, caper fruits are used in pickles and curry. (Vaidya, 1995; Yadav et al., 1997).

➢ Folk medicine practitioners (known locally as Hakeem) recommend mixing caper fruit powder with sugar to treat rheumatism and diarrhoea in livestock animals (Marwat et al., 2011).

Figure.2 *Limnophila repens* (Benth.) Benth



Table. 3 Pharmacological activities of different species of *Limnophila*

Plant	Plant Part	Solvent	Uses	References
Limnophila conferata	Aerial	Ethanol	Anthelmintic activity	Reddy, et al., 1991
			Antimicrobial activity	
			Anti-inflammatory activity	
			Wound healing Activity	
			Anti-tubercular activity	
			Cytotoxicity activity	
Limnophila aromatica	Leaves	Methanol	Antimicrobial activity antioxidantactivity	Nanasombat and Teckchuen, 2009
	Whole Plant	Water	Vascular protective activity	Kukongviriyapan, et al., 2007
Limnophila heterophylla	Whole Plant	Ethanol	Antibacterial and antifungal activity	Padiya, et al., 2013
	Roots	Methanol	Antioxidant activity	Sundararajan and Koduru, 2016
Limnophila geoffrayi	Aerial	Water	Antimicrobial,antifungal, and insecticidal activities	Thongdon and Inprakhon, 2009

Table 4 List of isolated phytoconstituents from various species of *Liminophila*

Plant name	Compound Name	Part of the Plant	References
Limnophila rugosa	5-Hydroxy-6,7,4'-trimethoxyflavone	Aerial parts and roots	Brahmachari, 2008
	5-Hydroxy-7,2',4'-trimethoxyflavone		
	5-Hydroxy-7,8,2',4'-tetramethoxyflavone		
	p-Methoxybenzoic acid		
	Anisaldehyde		
	Valeric acid		
	Methylchavicol		
	Betulin		
	Betulinic acid		
	Linalool		
	Ursolic acid		
Limnophila indica	5,2'-Dihydroxy-8,3',4'-trimethoxyflavone		
	5,7,2',5'Tetramethoxyflavone		
Limnophila heterophylla	Ursolic acid		
	α-Pinene		
	5-Hydroxy-7,8,2',4'-tetramethoxyflavone		
Limnophila conferta	Thymol		

1.3. Plant Profile

Botanical name

***Capparis brevispina* DC.**

Vernacular Names: The plants available in different places India and with differentnames as mentioned below.

Telugu	Arudonda
Tamil	Adaanda, Kattukanji
Malayalam	Chedimukanthi
English	Indian Caper

Scientific classification: According to taxonomy of this plant scientific classificationwas given below.

Kingdom Plantae

Sub-kingdom	Viridiplantae
Class	Magnoliopsida
Order	Brassicales
Family	Cappareceae
Genus	*Capparis*
Species	*brevispina*

Botanical description

Two branchlets and the bush are scruffy. Cataphylls are direct, the leaves are elliptical or elongate, intense, and coriaceous, and the stipular spines are little. Blossoms are axillary, single, and 4-5 cm across. Pedicels are 4 cm long. Sepals are in two whorls. Petals are four, oval, and obviate. Stamens are numerous, applied, and the gynandrophore is as lengthy as or prolonged than the fibres. Anthers are dorsifixed. The ovary is 6 mm in diameter and is ellipsoid.

Different species of the genus *Capparis*: World wide different speies capparis were available in various climatic zones among them some of prominent spices like.

Capparis rofundifolia	*Capparis deciduas*
Capparis pendunculosa	*Capparis aphylla*
Capparis seplara	*Capparis divaricata*
Capparis incanescenes	*Capparis floribunda*
Capparis stylosa	*Capparis grandis*
Capparis zeylanica	*Capparis bisperma*
Capparis horrid.	*Capparis decidua*

From the extensive literature, the survey reveals that there was little scientific work reported on the above-selected plants, but many tribal and village peoples still use these plants as medicine from so many years in their native practice and in the present-day scenario there was a huge demand for herbals and herbal related products because they were as safe and less adverse reaction. In order to provide the scientific evidence, the author selected and carried out a systematic pharmacognostic, phytochemical and pharmacological evaluation of *Argyreia cymosa, Limnophila repens* and *Capparis brevispina.*

Ethnobotanical description of *Capparis brevispina* DC

Apart from *Capparis brevispina*, species in the genus were widely used as traditional medicine

for various diseases for many years and exclusive ethnopharmacological information as follows

- According to Indian ethnomedical literature, for a very long time, Capparis species have been used in medicine. The Sumerians employed these plants for the first time around 2000 BC (Romeo et al., 2007)

- Extracts of the fruits, flowers, roots and root bark have been shown to be useful asanti-atherosclerosis, hypertension, bacteria, inflammation, analgesia, asthma, highcholesterol, and anti-fungal agents (Chahlia, 2009; Duman, et al., 2013).

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- The bark is used as homeopathic medicine (Hassan and Mohammed, 2010).

- Capparis species contain significant amounts of minerals, particularly in their floral buds and fruits, which are utilised as vegetables and pickled. The buds and fruit are also high in protein, carbs, lipids, and vitamins (Ozcan, 2005).

- Locals in India and Pakistan think that caper fruits have anti-diabetes properties. Laxative qualities that smoothen. As a result, caper fruits are used in pickles and curry. (Vaidya, 1995; Yadav et al., 1997).
- Folk medicine practitioners (known locally as Hakeem) recommend mixing caper fruit powder with sugar to treat rheumatism and diarrhoea in livestock animals (Marwat et al., 2011).



Figure3 *Capparis brevispina* DC

Pharmacological information of *Capparis brevispina and other species*

As per the literature survey, a few pharmacological activities were reported on *Liminophila repens* but in the other species variouspharmacological activities were scientifically proven

illustrated in Table 5.

Table 5. Pharmacological information of *Capparis brevispina* DC.

Plant	Plant Part	Solvent	Uses	References
Capparis brevispina DC.	Stem bark	Ethanol	Hepatotoxicity in Wistar rats	Anju et al.,2017
Capparis brevispina DC.	Leaves	Hexane	Antioxidant and cytotoxic	Subramanian and Ramani, 2020
Capparis spinosa	Fruit	Water	Anti-inflammatory activity	Azhary et al., 2017
Capparis spinosa	Areal	Water	Hypolipidemic	Eddouks et al., 2005
Capparis spinosa	Bud	Water	Anti-oxidant activity	Mansour et al., 2006
Capparis decidua	Leaf	Powder	Antidiabetic	Yadav, 1997
Capparis decidua	Stems	Aqueous and methanol	Hepatoprotective activity	Ali, 2009
Capparis decidua	Stem	Acetone	Antibacterial Potential	Gull et al., 2015
Capparis zeylanica Linn.	Leaves	Methanol	Immunostimulant effect	Ghule et al., 2006

Phytochemical constituents information of the *Capparis species*

As per literature survey, till to date, a few phytochemical were isolated from the *Capparis brevespina* but many phytochemicals constituentswere widely isolated from the other species of Capparis illustrated in Table 6.

Plant name	Compound	Part of plant	References
Capparis spinosa	Rutin, quercetin 3-o-glucoside	Aerial	Sharaf et al., 2000
	Quercetin 3-O-glucoside-7-O-rhamnoside		
	Quercetin 3-O-[6‴-α-l-rhamnosyl-6″-β-d-glucosyl]-β-d-glucoside		
	Kaempferol 7-rhamnoside and kaempferol 3-rutinoside,		
	Kaempferol 3,7-dirhamnoside		
	Kaempferol 3-glucoside-7-rhamnoside		
	Kaempferol 3-rhamnoside-7-glucoside		

Capparis species	Quercetin 7-rhamnoside and quercetin 3-rutinoside	Aerial	Tagnaout et al., 2016
	Quercetin 7-rutinoside and quercetin 3,7-dirhamnoside		
	Quercetin 3-glucoside-7-rhamnoside		
Capparis species	Isorhamnetin 3-rutinoside, Isorhamnetin 3,7-dirhamnoside		
	Apigenin 6,8-di-c-glucoside		
Capparis spinosa	6s-Hydroxy-3-oxo-α-ionol glucosides	Fruits	Ihsan et al., 2002
	Corchoionoside c ((6s,9s)-roseoside)		
	Prenyl glucoside		
Capparis spinosa	G –Sitosterol glucoside-6'-octadecanoic	Aerial	Khanfar et al., 2002
	3-Methyl-2-butenyl- g –glucoside		
Capparis moonii	L-Stachydrine and rutin		
Capparis decidua	Fatty acids and ecospinois		
Capparis decidua	Capparisinine		

Conclusion

Plants always played an important role in human life. Some plants become ingrained in people's culture and customs. People in India and other parts of the world have utilized plants as medicine since ancient times. In the current context, the usage of herbs and herbal-related items has expanded due to the severe side effects and adverse occurrences associated with allopathic medicinal medications. A thorough investigation of conventional herbal remedies and a thorough literature analysis indicated that Argyreia cymosa (Roxb.) Sweet, Limnophila repens (Benth.) Benth and Capparis brevispina DC were good sources of therapeutically active plant compounds for the treatment of various de applying this as a rationale; the present work was designed to study the pharmacognostical, Phytochemical and pharmacological activities of selected Indian traditional medicinal plants.

From the findings of the study, it can be concluded that Argyreia cymosa, Limnophila repens and Capparis brevispina contain many medicinally important secondary metabolites in all the extracts. The presence of phytoconstituents in organic extracts was further evidenced by

applying advanced techniques of extraction, qualitative evaluation, TLC profiling and isolation, the plants can be used as a valuable source in the production of phytochemicals.

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Research Article

Pharmacognostical, Phytochemical and Pharmacological Evaluation of selected Indian Medicinal Plant *Argyreia cymosa (Roxb.) Sweet, Limnophila repens (Benth) Benth and Capparis brevispina DC*

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ABSTRACT

Indian healers employ the country's abundant natural resources and medicinal plants to cure a wide range of illnesses. Traditional herbal remedies are widely used worldwide and have been continuously studied to understand and take use of these cures' active ingredient. Tribal traditional practitioners have discovered that Argyreia cymosa (Roxb.) The phytochemical analysis of the medicinally significant liana Argyreia cymosa (Roxb.) Sweet is the focus of the current work. Its ethnomedical significance is confirmed by the phytochemical investigation, which revealed a notable concentration of alkaloids, flavonoids, phenolics, and other bioactive components. Cymosa argyreia (Roxb.) Sweet is a member of the Convolvulaceae family. The tropical Asian woody climber genus Argyreia grows to Australia is known for its striking purple blooms and silvery foliage. In traditional medicine, argyreia cymosa (Roxb.) sweet is employed. Its leaf extract is used to treat corneal opacity in sheep and goats' eyes and the paste made from its leaves is applied to wounds and cracks. A significant medicinal plant in India, Capparis brevispina DC is a member of the Capparaceae family. The plant, also called Indian caper, is a shrub with coriaceous leaves, thorny stipules, and white flowers. The berries are ellipsoid to ovoid in form. India uses the plant as a traditional medicine since it was discovered to have therapeutic qualities.

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KEYWORDS: Phytochemical, flavonoids, therapeutic, wounds, bioactive components.

1. INTRODUCTION

Organoleptic examination is the practice of examining herbal medications using the sense organs. Color, smell, taste, size, form, and distinctive qualities like touch and texture are among the analytical methods that are referenced. The extract or plants tend to identify itself as it has such a unique look at first. If this isn't enough, the plant or extraction could also smell or taste different. The organoleptic technique of analysis is the most fundamental but humane. Shah (2009). The current study sought to assess pharmacognostical characteristics by conducting macro, micro, and powder microscopy of a few Indian medicinal plants, including Argyreia cymosa, Limnophila repens, and Capparis, because there is little data on the comprehensive macroscopic and microscopic evaluation of chosen whole plants.

2. Materials and Method

2.1 Plant material

In this experiment, the dried leaf, stem, and root parts of a few chosen medicinal plants Argyreia cymosa (Roxb.) Sweet, Limnophila repens (Benth.) Benth, and Capparis brevispina DC were assessed separately.

2.2 Determination of the total ash

The powdered substance was burnt in a silicon crucible after being precisely weighed at 2.0 gm. at temperatures that don't exceed 450°C till carbon is eliminated. The ash was weighed once it had cooled. To get a consistent weight, the procedure was repeated. At last, it the proportion of the medication that was air-dried was determined. The powdered material of individual leaves, stems, and roots of particular Indian medicinal plants underwent the same process (Gautam et al., 2010).

2.3 Determination of the acid-insoluble ash

After collecting the ash as previously mentioned, it was boiled for five minutes in 25 milliliters of 2 M hydrochloric acid. The next step is to collect and filter insoluble materials using ashless filter paper. Following 15 minutes of lighting at a temperature of no more than 450 °C, it was weighed after being cleaned with hot water. The proportion of acid-insoluble

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ash in the air-dried medicine was determined. The leaves, stems, and roots of specific Indian medicinal plants were ground into powder using the same method. (Patel and others, 2006).

2.4 Determination of the water-soluble ash

The ash was made using the foregoing method, heated with 25 milliliters of 2 M hydrochloric acid for five minutes, filtered, and the insoluble material was gathered on ashless filter paper. After that, it was cleaned with hot water and heated for fifteen minutes at a temperature that didn't surpass at 450°C, it was weighed. The amount of acid-insoluble ash in the drug that was air-dried was calculated. For individual leaf, stem, and root powdered material of particular Indian medicinal plants, the same process was used (Gautam et al., 2010).

2.5 Determination of alcohol soluble extractive value

In a closed flask, 4 g of the coarsely ground, air-dried plant powder was macerated for 24 hours with 100 cc of ethanol. For the first 6 hours, the flask was constantly shaken, and after that, it was left to stand for 18 hours. After that, it was filtered and strained. method was used, and precautions were made to avoid ethanol loss; 25 milliliters of the filtrate were weighed and evaporated on a shallow, flat plate that was dried at 105 degrees Celsius. The air-dried medication's ethanol-soluble extractive value was computed as a percentage. For powdered leaf, stem, and root material from certain Indian medicinal plants, the same method was used (Gautam et al., 2010).

2.6 Determination of water-soluble extractive value

In a sealed flask, 4 g of the air-dried coarsely powdered medication had been macerated with 100 ml of water for 24 hours. It was shook frequently for the first 6 hours and then left to stand for 18 hours. After filtering, 25 milliliters of the filtrate were evaporated on a shallow, level dish and dried at 105 °C and measured. With reference to the medication that had been air-dried, the percentage of water-soluble extractive value was computed. For each powdered leaf, stem, and root material of certain Indian medicinal plants, the same process was used (Gautam et al., 2010).

2.7 Determination of loss of moisture content

About 10 g of the drug powder (without pre-drying) was weighed and allowed to dry at 105°C in a tared evaporating dish. At 1-hour intervals, the drying and weighing processes were repeated until the discrepancy between two consecutive weightings was less than 0.25%. It took two separate weighings to show a change of no more than 0.01 g following 30

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minutes of drying and chilling in a desiccator. For powdered leaf, stem, and root material from specific Indian medicinal plants, the same procedure was used. (Sagar and others, 2007).

2.8 Fluorescence analysis powder material

Following treatment with several substances, including distilled water, methanol, 1N NaOH, 1N HCl, 50% HNO3, FeCl3, CHCl3, picric acid, and ethyl acetate, the powdered sample was examined under daylight and UV light at short wavelengths of 254 nm and long wavelengths of 365 nm. Gautam and colleagues (2010).

3. Phytochemical Evaluation

3.1 Physicochemical evaluation Argyreia cymosa leaf

Table 1 displays the results of physico-chemical evaluations of the Argyreia cymosa leaf that were conducted in accordance with accepted guidelines and limits, such as drying misfortune, debris value, and extractive worth.

Table 1 Physicochemical parameters of leaf powder of *Argyreia cymosa*

Parameters	Values(%w/w)
Total ash	6.7 ± 2.25
Acid in soluble ash	3.72 ± 0.32
Water soluble ash	3.63 ± 0.43
Alcohol soluble extractive value	8.95 ± 0.13
Water soluble extractive value	11.45 ± 0.15
Moisture content (Loss on drying)	10. 8 ± 1.25

3.2 Fluorescence analysis of the leaf powder of Argyreia cymosa

The various physicochemical properties of Limnophila repens leaf powder, such as loss on drying, ash value, and extractive value, were determined and displayed in Table 2.

Table 2 Fluorescence analysis of *Argyreia cymosa* leaf powder

Solvent used	Visible light	UV light	
		At short (254nm)	At Long(365nm)
Distilled water	Dark Green	Pale green	Red

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Methanol	Greenih	Green	Red
1N NaOH	Light brown	Green	Brownish green
1N HCl	Green	Black	Green
50%HNO_3	Pale green	Black	Green
$FeCl_3$	Pale yellow	Green	Orange
$CHCl_3$	Green	Green	Red
Picric acid	Yellow	Fluorescent green	Orange
Ethyl acetate	Green	Green	Black

3.3 Physicochemical evaluationof leaf powder of Limnophila repens

The various physicochemical properties of Limnophila repens leaf powder, such as loss on drying, ash value, and extractive value, were determined and displayed in Table 3.

Table 3 Physicochemical parameters of leaf powder of *Limnophilarepens*

Parameters	Values (%w/w)
Total ash	5.2 ± 0.23
Acid in soluble ash	1.25 ± 0.06
Water soluble ash	2.25 ± 0.34
Alcohol soluble extractive value	10.23 ± 0.34
Water soluble extractive value	12.25 ± 1.25
Moisture content (Loss on drying)	6.56 ± 0.56

3.4 Fluorescence analysis of leaf powder of Limnophila repens

The leaf powder fluorescence analysis of Limnophila repens was determined and displayed in 4.

Table 4. Fluorescence analysis of *Limnophilarepens* leaf powder

Solvent used	Visible light	UV light	
		At short (254nm)	At Long (365nm)
Distilled water	Green	Green	Black
Methanol	Pale green	Brownish black	Yellowish green
1 N NaOH	Brownish red	Brownish green	Brownish Black

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1N HCl	Blackish green	Dark grey	Dark green
50%HNO_3	Crimson yellowish	Brownish black	Green
$FeCl_3$	Blackish green	Brownish green	Dark green
$CHCl_3$	Pale green	Brownish black	Dark grey
Picricacid	Yellowish white	Dark blue	Black
Ethylacetate	Green	Buff	Greenish black

3.5 Physicochemical evaluation of leaf powder of Capparis brevispina.

The various physicochemical parameters of stem powder of Capparis brevispina i.e., ash values, loss on drying, and extractive value were determined and displayed in table 5.

Table 5 Physicochemical parameters of leaf powder of *Capparis brevispina.*

Parameters	Values %w/w
Total ash	4.28 ± 0.85
Acid insoluble ash	1.54 ± 0.66
Water soluble ash	1.32 ± 0.12
Alcohol soluble extractive value	6.32 ± 0.1
Water soluble extractive value	7.88 ± 0.55
Moisture content	6.37 ± 0.44

3.6 Fluorescence analysis of leaf powder of Capparis brevispina

The fluorescence evaluation of the leaf powder of Capparis brevispina was determined and displayed in Table 6

Table 6 Fluorescence analysis of powdered leaf of *Capparis brevispina*

Solvent used	Visible light	UV light	
		254 nm	366 nm
Distilled water	Pale Buff	Brown	Dark Brown
Methanol	Light brown	Green	Brownish green
1 N NaOH	Light brown	Green	Brownish green
1N HCl	Brown	Greenish brown	Pale green
50%HNO3	Brown	Green	Pale yellow
FeCl3	Buff	Bluish yellow	Bluish green
$CHCl_3$	Light Buff	Dark brown	Black

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Picric acid	Yellowish brown	Green	Bark brown
Ethyl acetate	Buff	Black	Light green

4. Preparation of extracts

After thoroughly washing the Argyreia cymosa, Limnophila repens, and Capparis brevispina plants with water, the materials were shade-dried and ground into a powder using a pulverizer. Finely ground, homogenized Argyreia cymosa and Limnophila repens powder as well as Capparis brevispina were extracted independently. petroleum ether, chloroform, ethyl acetate, and methanol, in order of polarity. Following the loading of the air-dried powder into a Soxhlet extractor, ethylacetate and methanol were added one after the other. For future phytochemical and pharmacological studies, the excess solvents from each extract were ultimately rotary flash and hot plate evaporated and kept in desiccators.

4.1 Phytochemical evaluation

A preliminary analysis of petroleum ether extractethyl acetate extract, chloroform extract, methanolic extract, and water from powdered Argyreia cymosa, Limnophila repens, and Capparis brevispina was conducted in accordance with conventional laboratory guidelines (Tiwari et al. 2011).

Table 7 Phytochemical analysis of various extracts of *Argyreia cymosa*

Phyto-constituents	Test	Hexane extract	Petroleum ether Extract	Chloroform extract	Ethyl acetate extract	Methanolic extract
Flavonoids	Shinoda's Test	+	-	-	+	+
	Zn. HCL test	+	-	-	+	+
	Lead acetate Test	+	-	-	+	+
Volatile oil	Stain test	-	-	-	-	-
Alkaloids	Wagner Test	-	-	+	-	+
	Hager'sTest	-	-	+	-	+
Tannins & phenols	FeCl3Test	+	-	-	+	+
	Potassium dichromate test	-	-	-	-	+
Saponins	Foaming Test	-	-	-	-	-
Steroids	Salkowski test	+	+	+	+	+
Sterols	Libermann's test	+	-	+	+	+
	Salkowski	+	-	-	-	-
	Conc. Sulphuricacid	+				

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Fixed oils and fats	Spot test	-	+	-	-	-
Carbohydrates	Molish test	-	-	-	-	-
Acid compounds	Litmus test	-	-	-	-	+
Glycoside	Keller-Killani Test	-	-	-	-	+
Amino acids	Ninhydrin test	-	-	-	-	+
Proteins	Biuret	-	-	-	-	+

Table 8 Phytochemical analysis of various extracts of *Limnophila repens*

Phyto constituents	**Test**	**Hexane extract**	**Petroleum ether Extract**	**Chloroform extract**	**Ethyl acetate extract**	**Methanolic extract**
Flavonoids	Shinoda Test	+	-	-	+	+
	Zn+HCltest	+	-	-	+	+
	Lead acetate Test	+	-	-	+	+
Volatileoil	Stain test	-	+	-	-	+
Alkaloids	Wagner Test	-	-	+	-	+
	Hager's Test	-	-	+	-	+
Tannins & Phenols	Fecl3 Test	+	-	-	+	-
	Potassium Dichromate test	-	-	-	+	-
Saponins	FoamTest	-	-	-	-	+
Phytosterols	Libermann'stest	-	+	+	+	+
	Salkowski	-	-	-	-	-
	Conc. Sulphuric acid	-	-	-	-	-
Carbohydrates	Molish test	-	-	-	-	+
Acid compounds	Litmus test	-	-	-	-	-
Glycoside	Bontrager's test	-	-	-	-	+
Amino acids	Ninhydrin test	-	-	-	-	+
Proteins	Biuret test	-	-	-	-	-
Fixed oils & fats	Spot test	-	+	-	-	-

Table 9 Phytochemical analysis of various extracts of Capparis brevispina

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Phyto constituents	Test	Hexane extract	Petroleum ether Extract	Chloroform extract	Ethyl acetate extract	Methanolic extract
Flavonoids	Shinoda Test	+	-	-	+	+
	Zn.HCL test	+	-	-	+	+
	Lead acetate Test	+	-	-	+	+
Volatile oil	Stain test	-	+	-	-	+
Alkaloids	Wagner Test	-	-	+	-	+
	Hager's Test	-	-	+	-	+
Tannins& phenols	$FeCl_3$Test	+	-	-	+	+
	Potassium dichromate test	-	-	-	-	+
Saponins	Foaming Test	-	-	-	+	+
Phyto sterols	Libermann's test	-	+	+	+	+
	Salkowski	-	-	-	-	-
	Conc. Sulphuric acid	-	-	-	-	-
Fixed oils and fats	Spot test	-	+	-	-	-
Carbohydrates	Molish test	-	-	-	-	+
Acid compounds	Litmus test	-	-	-	-	+
Glycoside	Keller-Killani Test	-	-	-	-	+
Amino acids	Ninhydrin test	-	-	-	-	+
Proteins	Biuret	-	-	-	-	+

5. Pharmacological Evaluation

Wistar strain Albino rats of either sex (200-250 g) was used for the hot plate method, Swiss albino mice used for tail immersion and the acetic acid writhing method was acquired from Ghosh Enterprises. The animals were cared for under typical lab circumstances. They were given a typical pellet diet and unlimited access to water. The Institutional Animal Ethical Committee (IAEC) examined and approved the study protocol, and experiments were carried out in accordance with CPCSEA rules.

5.1 Antinociceptive activity by using the hot plate method

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Central analgesics may be measured using the hot plate paradigm. In order to determine if MEAC, MELR, and MECB had a core analgesic effect, the hot plate test was conducted. In this trial, significant outcomes were seen at 200 and 400 mg/kg with MECB, MELR, MEAC. The MEAC significantly improved ($p<0.05$) at 200 and 400 mg/kg, going from an 8.9 ± 0.45 latency time to 12.21 ± 0.96, and from 8.6 ± 0.96 to 14 ± 0.33 after 90 minutes while Tramadol (2 mg/kg, p.o.) rose dramatically from a latency period of 8.2 ± 0.25 to 16.3 ± 0.74 at 30 minutes. At 200 and 400 mg/kg, the MELR significantly ($p < 0.01$) rose from 9.62 ± 0.22 to 12.95 ± 0.62 and 9.49 ± 0.22, respectively. After 120 minutes, 14.95 ± 0.85. Table 9.1 shows a small increase in the MECB delay at 200 and 400 mg/kg ($p < 0.01$) from 8.5 ± 0.33 to 14.28 ± 0.12 and 8.7 ± 0.97 to 15.55 ± 0.68 at 90 minutes. Thus, it is possible to believe that MEAC, MELR, and MECB have an impact on the central nervous system.

Table 10 Effect of MEAC, MELR and MECB by using the hot plate method

Treatment	Reaction Time(s)				
	Time after Treatment (min)				
	0	30	60	90	120
Control	8.25 ±0.15	8.33 ±0.03	8.30 ±0.04	8.4 ±0.08	8.2 ±0.04
Tramadol (2mg/kg)	8.2 ±0.25	16.3 ± 0.74*	14.1 ±0.2*	11.4 ± 0.680*	9.6 ±0.526*
MEAC(200mg/Kg)	8.9 ±0.45	8.3 ±0.20a	9.8 ±0.241a	12.21±0.96a	9.52 ±1.237a
MEAC(400mg/Kg)	8.6 ±0.96	9.2 ±0.78b	10.3 ± 0.26b	14±0.33b	12.22±0.53b
MELR (200mg/kg)	9.62 ±0.22	9.46 ±0.1#	10.95±0.58#	12.95±0.62#	10.09±0.56#
MELR (400mg/kg)	9.49 ±0.22	10.72±0.38	12.15±0.31	14.95±0.85	10.64±0.54
MECB (200mg/Kg)	8.5 ±0.33	9.2 ±0.25a	10.8 ±0.84 a	14.28±0.12a	9.56±0.38a
MECB (400mg/Kg)	8.7 ±0.97	9.8 ±0.78	11.52±0.91	15.55±0.68	12.22±0.22

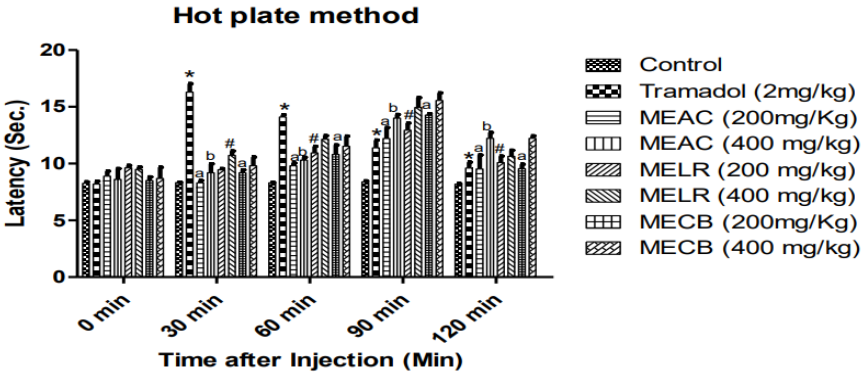


Figure 1 Effect of MEAC, MELR and MECB on hot the plate method

5.2 Antinociceptive activity by using the tail immersion method

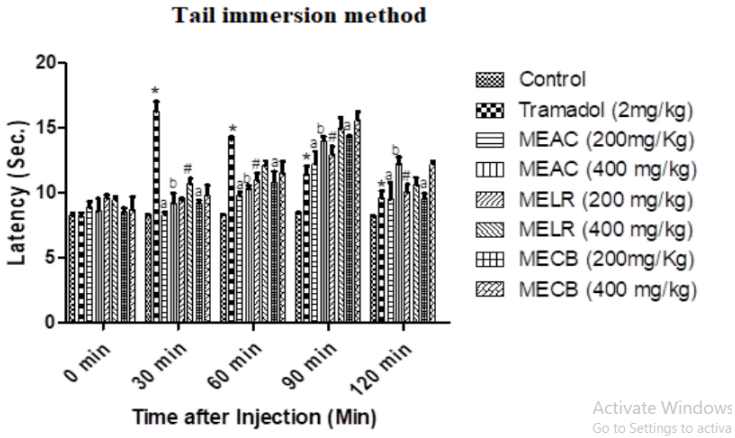
At 200 and 400 mg/kg, MEAC, MELR, and MECB all exhibit substantially longer response times during the tail immersion investigation as compared to control animals. The latency

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time rose considerably ($p < 0.05$) from 3.8 ± 0.21 to 400 mg/kg of MEAC. At 90 minutes, they were 4.3.21 ± 0.13 and 3.8 ± 0.96 to 7.3 ± 0.53, respectively. The latency period rose considerably ($p < 0.05$) from 2.03 ± 0.07 to 5.4.39 ± 0.16 and 2.44 ± 0.11 to 6.4 ± 0.18 at 120 minutes for the MELR with 200 and 400 mg/kg, respectively. The latency period rose considerably ($p < 0.01$) from 3.6 ± 0.12 to 9.21 ± 0.33 and from 3.9 ± 0.15 to 12.21 ± 0.44 at 90 minutes for the MECB at 200 and 400 mg/kg, respectively. However, as seen in the data, tramadol (2 mg/kg) markedly extended the latency period from 3.6 ± 0.23 to 14.08 ± 0.17 at 120 minutes, respectively.

Table 10 Effect of MEAC, MELR and MECB by using tail withdrawal reflexes induced by tail immersion method in rats.

Treatment	Reaction Time(s)				
	Time after Treatment (min)				
	0	30	60	90	120
Control	3.6 ±0.12	3.7 ±0.20	3.9 ±0.17	3.6 ±0.13	3.4 ±0.22
Tramadol (2mg/kg)	3.4 ±0.23	16±0.63*	11.4 ± 1.55*	6.7 ±1.45*	14.08±0.17*
MEAC(200mg/Kg)	3.8 ±0.21	3.6 ±0.11*	4.5 ±0.27*	7.3 ±0.53*	5.3 ±0.23*
MEAC(400mg/Kg)	3.7 ±0.51	4.5 ±0.44[a]	6.3 ±0.74[a]	8.2 ±0.44[a]	6.6 ±0.43[a]
MELR(200 mg/kg)	2.03 ±0.07	2.8 ±0.15	3.6 ±0.22	4.46 ±0.22	5.4 ±0.16
MELR(400 mg/kg)	2.44 ±0.11	3.89 ±0.23	4.89 ±0.18	5.78 ±0.14	6.4 ±0.18
MECB (200mg/Kg)	3.6 ±0.12	3.9 ±0.22*	5.4 ±0.72*	9.21 ± 0.33*	6.5 ±0.43*
MECB (400mg/Kg)	3.9 ±0.15	5.4 ±0.44[a]	8.3 ±0.74[a]	12.21±0.44[a]	9.23 ± 0.43[a]



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Figure 2 Effect of MEAC, MELR and MECB on tail withdrawal reflexes caused by the tail immersion treatment in rats

5.3 Antinociceptive activity by using the acetic acid-induced writhing method

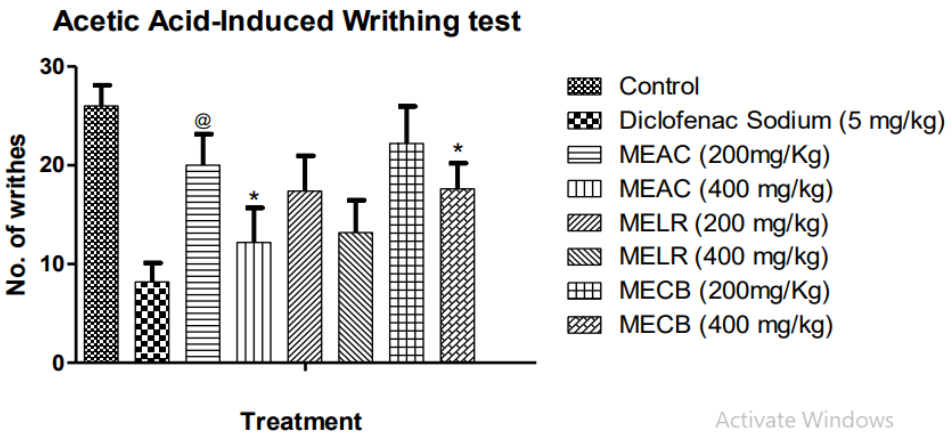
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MELR (400 mg/kg) greatly reduced the number of wriths at 49.23%, MEAC (400 mg/kg) considerably reduced the number of wriths at 51.78%, MEAC (200 mg/kg) significantly reduced the number of writhes at 29.68%, and MELR (200 mg/kg) also significantly reduced the number of wriths at greatly reduced the number of writhes at 33.07%, while MECB (200 mg/kg) continued to significantly reduce writhes at 25.14%. The MECB vehicle control group (400 mg/kg) also significantly reduced writhes at 31.88%. Maximum activity was seen by diclofenac (5 mg/kg), which inhibited the wrighting response by 68.46% ($p<0.001$). It was discovered that the 400 mg/kg dosage was more effective than the 200 mg/kg dose in all analgesic formulations. This might suggest that the 400 mg/kg dose of the extract was the ideal amount to produce both central and peripheral analgesic effects. The findings were displayed in Table.

Table 11 Effect of MELR, MEAC, MEB on acetic acid-induced writhing behaviour in mice.

Treatment	Wrighting Count					Wrightings (Mean±SEM)	% of Wrighting	% of Inhibition
	M-1	M-2	M-3	M-4	M-5			
Control	28	26	25	23	28	26 ± 2.12	100	0
Diclofenac Sodium (5 mg/kg)	7	9	11	6	8	8.2 ± 1.92	31.54	68.46
MEAC (200 mg/kg)	18	24	16	20	22	20 ± 3.16*****	70.32	29.68
MEAC (400 mg/kg)	8	12	10	14	17	12.2 ± 3.49*	48.22	51.78
MELR(200 mg/kg)	16	20	12	18	21	17.4± 3.57	66.93	33.07
MELR(400mg/kg)	12	15	8	16	15	13.2 ± 3.27*	50.77	49.23
MECB(200 mg/kg)	28	20	22	23	18	22.2 ± 3.76	74.86	25.14
MECB(400 mg/kg)	20	16	18	20	14	17.6 ± 2.60*	68.12	31.88

M-1= Mice 1, M-2 = Mice 2, M-3 = Mice 3, M-4 = Mice 4, M-5 = Mice 5.), $, p < 0.001 versus control, *, p < 0.001 versus Diclofenac sodium and @, p < 0.001 versus MELR (200 mg/kg).



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Figure 3 Protective Effect of MELR, MEAC, and MECB on acetic acid-induced writhing behaviour in mice.

Conclusion

Standardization of herbs becomes one of the most indispensable steps to determine the plant material's quality. In the present study, pharmacognostic standardization of Argyreia cymosa, Limnophila repens and Capparis brevispina was carried out as per standards. The remarkable aspects of herbal extracts were that they act on human systems with an internal synergy. Argyreia cymosa, Limnophila repens, and Capparis brevispina all contain several medicinally significant secondary metabolites in all of their extracts, according to the study's findings. Phytoconstituents found in organic extracts were further supported by research using thin-layer chromatography. Certain plants, including Argyreia cymosa, Limnophila repens, and Cappparis brevispina, have methanolic extracts that may have dose-dependent anti-nociceptive effects as both central and peripheral analgesics.

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Abstract

Pharmacognostical, Phytochemical and Pharmacological Evaluation of selected Indian Medicinal Plants: Argyreia cymosa, Limnophila repens and Capparis brevispina

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Indian medicinal plants represent a vast reservoir of bioactive phytochemicals and have been extensively used in traditional systems of medicine for the management of pain, inflammation, and various chronic ailments. Among these, Argyreia cymosa, Limnophila repens, and Capparis brevispina possess significant ethnomedicinal relevance; however, systematic scientific validation of their pharmacognostical and pharmacological properties remains limited.

The present study was undertaken to evaluate the pharmacognostical characteristics, phytochemical composition, and antinociceptive potential of these three selected Indian medicinal plants. Detailed macroscopic and microscopic examinations were carried out along with physicochemical analyses including ash values, extractive values, moisture content, and fluorescence characteristics of leaf, stem, and root powders. Sequential solvent extraction using hexane, petroleum ether, chloroform, ethyl acetate, and methanol was performed, followed by preliminary phytochemical screening to identify major secondary metabolites. Pharmacological evaluation of the methanolic extracts of Argyreia cymosa (MEAC), Limnophila repens (MELR), and Capparis brevispina (MECB) was conducted using established experimental animal models such as the hot plate test, tail

immersion test, and acetic acid–induced writhing method in Wistar rats and Swiss albino mice.

The results demonstrated distinct pharmacognostic features and significant extractive values in all three plants, confirming the presence of alkaloids, flavonoids, phenolic compounds, tannins, sterols, and glycosides. Fluorescence analysis further supported chemical differentiation under visible and ultraviolet light. Methanolic extracts exhibited significant dose-dependent antinociceptive activity, with higher doses (400 mg/kg) producing a marked increase in reaction latency in hot plate and tail immersion tests, indicating central analgesic activity. In the acetic acid–induced writhing model, the extracts produced 31–52% inhibition of writhing responses, with MEAC showing the highest peripheral analgesic effect, while diclofenac demonstrated 68% inhibition.

These findings suggest that Argyreia cymosa, Limnophila repens, and Capparis brevispina possess potent central and peripheral analgesic properties, supporting their traditional use and highlighting their potential for development as herbal analgesic agents.

Abstract

A Comprehensive Review on Pharmacognostical, Phytochemical and Pharmacological Perspectives of Selected Indian Medicinal Plants: Argyreia cymosa, Limnophila repens and Capparis brevispina

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Medicinal plants continue to be an indispensable source of therapeutic agents and play a vital role in traditional systems of medicine such as Ayurveda, Siddha, and Unani. India, being one of the world's richest reservoirs of medicinal plant biodiversity, contributes significantly to herbal drug development. The present review provides a comprehensive compilation and critical evaluation of the pharmacognostical, phytochemical, and pharmacological attributes of three traditionally important Indian medicinal plants: Argyreia cymosa (Roxb.) Sweet, Limnophila repens (Benth.) Benth, and Capparis brevispina DC.

An extensive literature survey was conducted using published research articles, ethnobotanical records, pharmacopoeial data, and scientific databases to gather information on botanical description, traditional uses, chemical constituents, and experimentally proven pharmacological activities. The review highlights that these plants possess well-defined pharmacognostic features useful for identification and standardization. Phytochemical investigations reported the presence of diverse secondary metabolites including flavonoids, alkaloids, phenolic compounds, terpenoids, sterols, glycosides, fatty acids, and amino acids.

Pharmacological studies documented in the literature reveal a wide spectrum of biological activities such as antioxidant, antimicrobial, anti-inflammatory, analgesic, hepatoprotective, hypolipidemic, antidiabetic, immunomodulatory, and central nervous system activities. Several bioactive compounds including quercetin and kaempferol derivatives, ergoline alkaloids, betulinic acid, ursolic acid, and rutin have been isolated from related species, supporting their therapeutic relevance. Despite extensive ethnomedicinal usage, limited systematic studies exist on Argyreia cymosa, Limnophila repens, and Capparis brevispina, particularly concerning standardization, toxicity profiling, and mechanism-based pharmacological evaluation.

This review underscores the immense medicinal potential of these plants and emphasizes the need for integrated research involving pharmacognostic standardization, advanced phytochemical isolation, and experimental validation. Such scientific approaches may facilitate the development of safe, effective, and evidence-based herbal formulations from these valuable medicinal resources.

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This is to certify that ~~Dr./Mr./Ms.~~ Bharat Goswami of Research Scholar P. K. University (Presenter) has participated in the 5th National Convention of APTI Punjab State Branch as **Speaker/ Award of Honor/ Chairperson of Technical Session/ Resource Person/ LOC Member/ Delegate.**

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