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Botanical Insights: From Traditional Knowledge to Modern Science

Volume- II

Editors

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Dr. R. Rajalakshmi
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VOLUME II

Editors

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Preface

The intricate relationship between plants and human civilization stretches back millennia. From ancient healing systems to contemporary pharmaceutical research, botanical knowledge has played a pivotal role in shaping our health, culture, and understanding of nature. The edited volume "Botanical Insights: From Traditional Knowledge to Modern Science" is a scholarly endeavor to bridge traditional ethnobotanical wisdom with modern scientific advancements, bringing together interdisciplinary research from diverse fields of plant science. This compilation opens with an exploration into the therapeutic potential of essential oils, presenting their diverse applications in aromatherapy, antimicrobial formulations, and alternative medicine. With growing interest in natural remedies, essential oils are gaining traction for their bioactive compounds, reinforcing the value of traditional plant-based treatments in modern healthcare.

Furthering this theme, the volume includes phytochemical and pharmacological studies of medicinal plants, shedding light on the biochemical properties, therapeutic efficacy, and traditional usage of specific species. These studies form the core of natural product research, offering insight into plant-derived compounds with significant potential in drug development and disease management.

A groundbreaking contribution in this volume focuses on computational approaches to phytochemical drug discovery, highlighting the fusion of plant genomics, molecular modeling, and pharmaceutical chemistry. This approach signifies a shift from conventional screening to precision-based drug design, enabling researchers to virtually identify, test, and optimize bioactive molecules from plant sources.

Expanding the technological frontier, a chapter on CdS nanomaterials presents their synthesis, characterization, and biological applications. By integrating nanotechnology with phytoscience, this work underscores innovative

possibilities in targeted drug delivery, imaging, and biosensors, opening a new dimension in plant-based biomedical applications.

Another noteworthy study offers a comparative analysis of morphological, phytochemical, and pharmacological features in Curcuma species, emphasizing the genetic and biochemical diversity within this vital genus. This research not only enriches our understanding of turmeric and its relatives but also supports the development of species-specific therapeutics.

Plant biotechnology is addressed through a tissue culture-based investigation of organogenic responses in Brassica rapa sp. Pekinensis. The study showcases the potential of in vitro techniques for crop improvement, genetic conservation, and rapid propagation of elite lines with medicinal and nutritional value.

Ethnobotany and regional plant diversity are highlighted in multiple contributions. One such work evaluates the phytochemical and nutritional composition of Solanum diphyllum L., positioning it as a promising candidate for future medicinal use. Another explores the diversity of corticioid fungi in the family Peniophoraceae from Chamba, Himachal Pradesh, bringing fungal biodiversity into the botanical dialogue and hinting at untapped bioresource potential.

Adding a cultural and historical dimension, a detailed account of the ethnobotanical significance of Mirabilis jalapa provides insights into how traditional communities have used this plant for generations. Such documentation not only preserves indigenous knowledge but also guides scientific inquiry into underexplored species.

Finally, a comparative phytochemical screening of Averrhoa carambola and Averrhoa bilimbi adds to the existing body of nutritional and pharmacognostic knowledge, advocating for the broader use and conservation of these tropical fruit species with dual food-medicinal value.

Together, these chapters form a cohesive narrative that honors the legacy of traditional botanical knowledge while celebrating the advances made possible by modern science. This volume aims to serve as a valuable reference for

researchers, academicians, practitioners of ethnomedicine, and all those interested in the dynamic interface between plants and human health.

We extend our deepest gratitude to the contributing authors whose rigorous research and dedication have shaped this publication. We also acknowledge the guidance of experts in the fields of phytochemistry, pharmacognosy, biotechnology, and ethnobotany, without whom this endeavor would not have reached fruition.

As we navigate the challenges of modern healthcare and environmental sustainability, it is our hope that this book will inspire further research, foster interdisciplinary collaboration, and promote the sustainable use of botanical resources grounded in both heritage and innovation.

Editors

Botanical Insights: From Traditional Knowledge to Modern Science Volume II

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Essential Oils And Their Therapeutic Applications

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Abstract

Essential oils (EOs) are aromatic, volatile compounds derived from plants that have been utilized for centuries for their therapeutic benefits. These oils are typically extracted through methods such as steam distillation, cold pressing, and solvent extraction. With growing interest in natural medicine, EOs are being increasingly integrated into modern healthcare systems for their antimicrobial, anti-inflammatory, analgesic, and anxiolytic properties. This chapter explores the chemical composition, mechanisms of action, and therapeutic applications of essential oils, focusing on commonly used oils like lavender, peppermint, tea tree, eucalyptus, and rosemary. The mechanisms by which essential oils exert their effects are multifaceted, involving interactions with cellular receptors, ion channels, and enzymes. Clinical and preclinical evidence supports their efficacy in treating a variety of conditions, such as anxiety, depression, gastrointestinal disorders, and respiratory issues. However, the use of essential oils requires caution due to potential adverse effects and toxicity, particularly when misused. Future research is needed to better understand the full therapeutic potential of essential oils and establish safe, standardized protocols for their application in clinical practice.

Keywords: Essential oils, Therapeutic applications, Aromatherapy, Lavender oil antimicrobial properties, Anti-inflammatory, Safety and toxicity

Introduction

Essential oils (EOs) are concentrated, volatile compounds derived from various parts of plants such as flowers, leaves, stems, bark, roots, and seeds. The extraction process typically involves methods like steam distillation, cold pressing, and solvent extraction. Essential oils have been used for centuries in traditional medicine, notably in Ayurveda, Traditional Chinese Medicine, and Ancient Egyptian healing practices. In modern times, essential oils are experiencing a renaissance, finding applications not just in aromatherapy, but

also in mainstream healthcare, where they are used for their antimicrobial, anti-inflammatory, analgesic, and anxiolytic properties.

As more people seek holistic and complementary approaches to health, essential oils have gained prominence in both clinical and self-care practices. However, with increasing use, it is crucial to understand their chemical composition, therapeutic mechanisms, safety profiles, and evidence supporting their efficacy.

Objectives

This chapter aims to:

- Provide an overview of essential oils, including their chemical composition and methods of extraction.
- Discuss the pharmacological mechanisms through which essential oils exert their therapeutic effects.
- Examine specific examples of essential oils that have been validated for clinical use.
- Review preclinical and clinical data regarding the safety and effectiveness of essential oils.
- Address safety concerns, toxicity risks, and proper usage protocols.

Data and Methodology

A comprehensive literature review was performed to gather information from peer-reviewed journal articles and clinical trials. Databases such as PubMed, Scopus, and Google Scholar were used, with keywords including "essential oils," "mechanism of action," "pharmacology," and "clinical evidence." Studies from 2000 to 2024 were selected for inclusion, focusing on experimental, clinical, and review articles.

Analytical methods for identifying and quantifying essential oil constituents were also examined, including Gas Chromatography-Mass Spectrometry (GC-MS), High-Performance Liquid Chromatography (HPLC), and Nuclear Magnetic Resonance (NMR) spectroscopy. These tools are essential for ensuring the quality, purity, and bioactivity of essential oils.

Results and Discussion

Chemical Composition of Essential Oils

The chemical composition of essential oils is highly variable depending on the plant species and the extraction method used. However, the primary components are typically terpenes (monoterpenes and sesquiterpenes), terpenoids, phenols, aldehydes, ketones, and esters. For example, lavender oil contains significant amounts of linalool and linalyl acetate, both of which are responsible for its calming effects. In contrast, peppermint oil is rich in menthol and menthone, compounds that have analgesic and anti-inflammatory properties.

The bioactivity of essential oils is directly linked to the synergistic action of these compounds. The composition of an essential oil can also influence its therapeutic application, with some oils being more effective for certain conditions due to their specific constituents.

Mechanisms of Action

Essential oils exert their effects through multiple mechanisms that often involve cellular receptors, enzymes, and ion channels. Their pharmacodynamics depend on both the direct interaction of oil components with biological targets and their ability to trigger secondary signaling cascades that influence cellular processes.

Lavender Oil (*Lavandula angustifolia*)

- **Active components:** Linalool, Linalyl acetate
- **Mechanism:** These compounds interact with GABA receptors, enhancing inhibitory neurotransmission in the brain. This mechanism results in anxiolytic, sedative, and anticonvulsant effects.
- **Therapeutic uses:** Lavender oil has demonstrated efficacy in reducing anxiety, improving sleep quality, and relieving stress. A study by Koulivand et al. (2013) showed significant reductions in anxiety levels in patients with generalized anxiety disorder when treated with lavender oil.

Peppermint Oil (*Mentha piperita*)

- **Active components:** Menthol, Menthone
- Mechanism: Menthol acts on transient receptor potential melastatin 8 (TRPM8) channels, providing a cooling sensation. It also induces smooth muscle relaxation, alleviating gastrointestinal discomfort.
- **Therapeutic uses:** Peppermint oil is widely used for gastrointestinal disorders, such as irritable bowel syndrome (IBS), and for providing relief from tension headaches. Cash et al. (2016) demonstrated that enteric-coated peppermint oil capsules significantly reduced IBS symptoms.

Tea Tree Oil (*Melaleuca alternifolia*)

- **Active components:** Terpinen-4-ol, α-Terpinene
- **Mechanism:** These constituents disrupt microbial cell membranes, leading to cell lysis and inhibiting microbial growth.
- **Therapeutic uses:** Tea tree oil is a potent antimicrobial agent. It has been used in the treatment of acne, fungal infections, and even dandruff. Hammer et al. (2003) confirmed its antimicrobial activity against both bacteria and fungi, including pathogens like *Staphylococcus aureus* and *Candida albicans.*

Eucalyptus Oil (*Eucalyptus globulus*)

- **Active components:** 1,8-Cineole
- **Mechanism:** 1,8-Cineole reduces inflammation by inhibiting pro-inflammatory cytokines and suppressing the NF-κB pathway. It also promotes mucociliary clearance in the respiratory system.
- **Therapeutic uses:** Eucalyptus oil is commonly used to treat respiratory conditions such as asthma, bronchitis, and sinusitis. Juergens et al. (2004) found that inhalation of eucalyptus oil led to significant improvements in lung function in asthma patients.

Rosemary Oil (*Rosmarinus officinalis*)

- **Active components:** 1,8-Cineole, Camphor, Rosmarinic acid
- **Mechanism:** These compounds enhance acetylcholine activity, which plays a crucial role in memory and cognitive function. Rosemary oil also has antioxidant properties, which protect cells from oxidative stress.
- **Therapeutic uses:** Rosemary oil is commonly used for cognitive enhancement and memory improvement. Moss et al. (2003) showed that exposure to rosemary oil aroma improved memory performance in subjects.

Additional Therapeutic Benefits

Essential oils also exhibit other therapeutic actions:

- **Anti-inflammatory:** Many oils, such as chamomile and frankincense, inhibit cyclooxygenase (COX-2) enzymes and reduce the production of pro-inflammatory cytokines.
- **Antioxidant:** Oils like rosemary and lemon are rich in compounds that neutralize free radicals and prevent oxidative stress.
- **Analgesic:** Oils such as clove and ginger inhibit pain signaling pathways, providing relief for headaches and muscle pain.
- **Wound healing:** Certain oils like tea tree and lavender stimulate fibroblast activity, accelerating tissue repair and collagen formation.

Clinical and Preclinical Evidence

Clinical studies have demonstrated the efficacy of essential oils in various therapeutic areas. Notable examples include:

- **Lavender oil:** Used preoperatively to reduce anxiety and improve sleep (Koulivand et al., 2013).
- **Peppermint oil:** Efficacious in reducing symptoms of irritable bowel syndrome (IBS) (Cash et al., 2016).
- **Tea tree oil:** Effective in treating acne and fungal infections (Hammer et al.,

2003).

- **Eucalyptus oil:** Inhalation therapy improves lung function in asthma patients (Juergens et al., 2004).

These studies provide robust evidence for the clinical utility of essential oils. However, many studies suffer from small sample sizes and methodological limitations, emphasizing the need for further research to confirm their widespread applicability.

Safety and Toxicity

Despite their natural origin, essential oils can be toxic if misused. Adverse effects may include allergic reactions, skin irritation, and phototoxicity. Ingesting certain oils, such as eucalyptus or wintergreen, can lead to poisoning, especially in children. Furthermore, some oils are contraindicated during pregnancy or breastfeeding.

To ensure safe use:

- Essential oils should always be diluted in carrier oils before topical application (typically 1–5% dilution).
- Ingestion of essential oils should be avoided unless under the supervision of a trained healthcare professional.
- Patch testing should be done before applying oils to large areas of skin.

Conclusion

Essential oils represent a fascinating and effective natural therapeutic option, with a broad range of biological activities supported by both traditional knowledge and modern scientific research. From their antimicrobial properties to their effects on cognitive function, essential oils hold significant promise in integrated healthcare. However, due to potential safety concerns, their use must be approached with caution, ensuring proper dilution and adherence to recommended guidelines. As more clinical studies are conducted, the therapeutic potential of essential oils will continue to expand, offering valuable alternatives and adjuncts to conventional medicine.

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Phytochemical And Pharmacological Studies Of Medicinal Plants

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Abstract

Medicinal plants have historically served as indispensable resources for treating various diseases and maintaining human health. They continue to be a foundation for drug discovery, especially as modern science seeks alternative, natural therapeutic agents. This research investigates the phytochemical composition and pharmacological activities of selected medicinal plants widely used in traditional medicine systems. Through systematic phytochemical screening and extensive literature analysis, this study highlights the relationship between phytoconstituents and their bioactivities. Samples of medicinal plants were collected based on ethnobotanical relevance and subjected to qualitative and quantitative phytochemical analyses, identifying alkaloids, flavonoids, tannins, saponins, terpenoids, and glycosides. Simultaneously, existing pharmacological literature was reviewed to associate specific compounds with biological activities such as antimicrobial, antioxidant, anti-inflammatory, and anticancer effects. The findings revealed a significant correlation between phytochemical diversity and pharmacological potential, validating many traditional therapeutic claims.

This research underscores the importance of medicinal plants as a valuable reservoir of bioactive compounds. The study advocates for integrating traditional knowledge with modern scientific validation to promote evidence-based applications of herbal medicine. Additionally, it emphasizes the necessity of sustainable conservation practices to preserve biodiversity and cultural heritage. The results highlight promising avenues for further exploration, including bioassay-guided isolation, mechanistic studies, and clinical trials to substantiate therapeutic efficacy and safety.

Keywords: Medicinal plants, Phytochemicals, Pharmacological activity, Ethnobotany.

Introduction

Medicinal plants have occupied a central role in human civilization, functioning as the backbone of various traditional healthcare systems across the world. From Ayurveda and Traditional Chinese Medicine (TCM) to indigenous practices in Africa and Latin America, plant-derived therapies have been used for millennia to treat a broad spectrum of ailments. According to the World Health Organization (WHO), approximately 80% of the world's population, especially in developing regions, relies on traditional plant-based medicines for primary healthcare needs.

The success of medicinal plants in therapeutic applications is largely attributed to their rich array of secondary metabolites, commonly termed phytochemicals. Unlike primary metabolites essential for plant growth, secondary metabolites serve ecological functions, including defense against herbivores, pathogens, and environmental stress. These bioactive compounds include alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, and others, many of which demonstrate significant pharmacological activities such as antimicrobial, antioxidant, anti-inflammatory, anticancer, and analgesic effects.

Historically, numerous modern drugs owe their origins to plant sources. For example, quinine from Cinchona bark, morphine from Papaver somniferum (opium poppy), and aspirin derived from salicylic acid in willow bark exemplify how traditional plant knowledge has led to pharmacological breakthroughs. This enduring link between ethnobotany and pharmacology underpins the increasing scientific interest in validating and harnessing traditional remedies for modern therapeutic applications.

The current global health landscape, marked by emerging diseases, antibiotic resistance, and chronic health conditions, has intensified the demand for novel, safe, and effective drugs. Given their structural diversity and biocompatibility, plant-derived phytochemicals are promising leads for pharmaceutical innovation (Aktar & Foyzun,2017). Despite the wealth of traditional knowledge, many medicinal plants remain underexplored scientifically.

This study focuses on bridging the gap between ethnomedicine and pharmacology by exploring the phytochemical and pharmacological properties of select medicinal plants commonly used in traditional healthcare systems. By combining phytochemical screening with pharmacological data analysis, the research aims to validate traditional uses and identify promising bioactive compounds with therapeutic Potential (Shukla et al., 2021).

Objectives

The primary objectives of this research are as follows:

- To identify and categorize the phytochemical constituents of selected

medicinal plants using established laboratory methods and literature data.

- To evaluate the pharmacological activities associated with these phytochemicals, focusing on antimicrobial, antioxidant, anti-inflammatory, and anticancer properties.
- To assess the correlation between traditional ethnomedicinal uses and scientifically validated bioactivities.
- To promote the rational use and conservation of medicinal plants while advocating for advanced research, including bioassay-guided isolation, toxicity studies, and clinical trials to ensure efficacy and safety.
- These objectives aim to strengthen the scientific foundation for traditional medicine practices and facilitate the discovery of novel therapeutic agents from natural sources.

Data And Methodology

a. Selection of plant samples
b. Sample collection and preparation
c. Phytochemical screening
d. Pharmacological analysis

Selection of plant sample

- Plant species were selected based on ethnobotanical importance, availability, and documentation of traditional uses in treating ailments such as infections, inflammation, digestive disorders, and cancers. Primary data were gathered from interviews with local healers and herbalists, alongside secondary data from ethnobotanical literature and electronic databases (PubMed, Scopus, Google Scholar).
- The selected species included:
  - *Terminalia chebula* (Chebulic Myrobalan)
  - *Glycyrrhiza glabra* (Licorice)
  - *Azadirachta indica* (Neem)
  - *Ocimum sanctum* (Holy Basil)
  - *Catharanthus roseus* (Madagascar Periwinkle)

Sample collection and preparation

Plant materials (leaves, bark, roots, and seeds) were collected from verified locations and authenticated by a botanist. The collected samples were washed, air-dried under shade to preserve bioactive constituents, and pulverized using a mechanical grinder. The powdered samples were stored in airtight containers at room temperature until analysis.

Phytochemical screening

- Qualitative analysis

- Quantitative analysis

Qualitative Analysis
Standard phytochemical tests were conducted to detect the presence of major secondary metabolites:

Alkaloids

Tests Used: Dragendorff's test and Wagner's test

Principle
Alkaloids are nitrogen-containing compounds that form precipitates with specific reagents containing heavy metal ions (like bismuth or iodine). These reagents react with the alkaloid's basic nitrogen to form colored or insoluble complexes.

Procedure
- **Dragendorff's reagent** (contains bismuth subnitrate and potassium iodide):
  A small amount of the plant extract is acidified with dilute hydrochloric acid and a few drops of Dragendorff's reagent are added.
  Positive result: Orange or reddish-brown precipitate indicates the presence of alkaloids.
- **Wagner's reagent** (iodine in potassium iodide solution):
  A few drops of Wagner's reagent are added to the acidic plant extract.
  Positive result: Reddish-brown precipitate confirms alkaloids.

Flavonoids

Tests Used: Alkaline reagent test and Lead acetate test

Principle
Flavonoids (polyphenolic compounds) can complex with metal ions (e.g., lead) or exhibit color changes under alkaline conditions due to the presence of hydroxyl groups.

Procedure
- **Alkaline reagent test:** The plant extract is treated with a few drops of sodium hydroxide solution.
  Positive result: Intense yellow color (due to flavonoid ionization), which disappears on adding dilute acid.
- **Lead acetate test:** A few drops of 10% lead acetate solution are added to the extract.
  Positive result: Formation of a yellow precipitate indicates flavonoids.

Tannins

Test Used: Ferric chloride test

Principle
Tannins are polyphenolic compounds that form complexes with iron (III) ions, resulting in characteristic color changes.

Procedure

- A small quantity of plant extract is mixed with a few drops of 5% ferric chloride solution.

  Positive result:
  - **Blue-black color** indicates hydrolyzable tannins
  - **Greenish-black color** suggests condensed tannins

Saponins

Test Used: Froth test

Principle
Saponins are glycosides that reduce surface tension and form stable foams when agitated in water.

Procedure

- About 2 mL of the extract is shaken vigorously in 5 mL of distilled water in a test tube for 5 minutes.

  Positive result: Formation of stable, persistent froth (foam) indicates the presence of saponins.

Glycosides

Test Used: Keller-Killiani test (for cardiac glycosides)

Principle
Cardiac glycosides contain a deoxy sugar linked to a steroid nucleus. The Keller-Killiani test detects deoxy sugars through oxidation and complex formation with iron salts, producing a characteristic color.

Procedure

- 2 mL of extract is treated with glacial acetic acid containing a trace of ferric chloride. Carefully, 1 mL of concentrated sulfuric acid is added along the sides of the test tube (without mixing).

  Positive result:
  - A brown ring forms at the interface (due to deoxy sugar)

- A violet-blue ring appears below the brown ring (indicating cardiac glycosides)

Terpenoids

Test Used: Salkowski's test

Principle
Terpenoids react with concentrated sulfuric acid to form reddish-brown or other coloured complexes, indicating the presence of unsaturated compounds like terpenes.

Procedure
- 2 mL of extract is mixed with 2 mL of chloroform.
- Carefully, 3 mL of concentrated sulfuric acid is added along the wall of the test tube to form a layer.
  Positive result: A reddish-brown coloration at the interface confirms the presence of terpenoids.

Quantitative Analysis
Selected metabolites were quantified using:

Total Phenolic Content (TPC)

Method Used: Folin–Ciocalteu method

Principle
The Folin–Ciocalteu reagent (a mixture of phosphomolybdic and phosphotungstic acids) reacts with phenolic compounds in the plant extract. In alkaline conditions, phenols reduce the reagent, producing a blue-colored complex (molybdenum-tungsten blue) (Alonso-Castro et al., 2010). The intensity of the blue color is proportional to the total phenolic content and is measured spectrophotometrically.

Reagents Required
- Folin–Ciocalteu reagent (commercially available)
- Sodium carbonate solution (usually 7.5% w/v)
- Standard: Gallic acid (commonly used for calibration curve)

Procedure
1. **Preparation of standard curve**
   - Prepare a series of gallic acid solutions of known concentrations (e.g., 20–100 µg/mL).
2. **Sample preparation**
   - Dilute the plant extract suitably.

3. **Reaction**
   - Add 0.5 mL of extract (or standard) to 2.5 mL of 10% diluted Folin–Ciocalteu reagent.
   - After 5 minutes, add 2 mL of 7.5% sodium carbonate solution.
   - Mix thoroughly and incubate for **30 minutes at room temperature** (in the dark).
4. **Measurement**
   - Measure the absorbance at **760 nm** using a UV–Vi's spectrophotometer.
5. **Calculation**
   - The total phenolic content is expressed as **mg gallic acid equivalents** (GAE) per gram of extract (mg GAE/g), using the calibration curve.

Advantages
- Simple, sensitive, and reproducible method.
- Suitable for crude extracts with a mixture of phenolic compounds.

Total Flavonoid Content (TFC)

Method Used: Aluminum chloride colorimetric method

Principle
Flavonoids form stable complexes with aluminum chloride ($AlCl_3$) in the presence of alkaline conditions. The complex exhibits a yellow coloration, whose intensity correlates directly with the concentration of flavonoids (Verma, 2013). The absorbance of the yellow complex is measured spectrophotometrically.

Reagents Required
- 5% Sodium nitrite ($NaNO_2$)
- 10% Aluminum chloride ($AlCl_3$)
- 1 M Sodium hydroxide (NaOH)
- Distilled water
- Standard: Quercetin or Rutin (commonly used for calibration curve)

Procedure

1. Preparation of standard curve
Prepare a series of quercetin (or rutin) solutions of known concentrations (e.g., 20–100 µg/mL).

2. Sample preparation
Dilute the plant extract appropriately.

3. Reaction
- Mix 0.5 mL of extract (or standard) with 2 mL distilled water.

- Add 0.15 mL of 5% sodium nitrite and let it stand for 5 minutes.
- Add 0.15 mL of 10% aluminum chloride and incubate for another 6 minutes.
- Add 1 mL of 1 M sodium hydroxide and 1.2 mL distilled water to make the final volume.
- Mix well.

4. Measurement

Measure the absorbance at 510 nm using a UV–Vi's spectrophotometer.

5. Calculation

The total flavonoid content is expressed as mg quercetin equivalents (QE) per gram of extract (mg QE/g), using the calibration curve.

Advantages

- Rapid and specific for flavonoids.
- Allows comparative quantification across samples.

Pharmacological analysis

Secondary data from peer-reviewed articles and pharmacological databases were systematically reviewed to correlate identified phytochemicals with reported bioactivities.

Inclusion criteria for literature:

- Experimental studies (in vitro, in vivo)
- Peer-reviewed journals
- Clear association between phytochemical and bioactivity

Pharmacological activities of interest included:

- **Antimicrobial:** Minimum inhibitory concentration (MIC) studies
- **Antioxidant:** DPPH, ABTS radical scavenging assays
- **Anti-inflammatory:** Carrageenan-induced paw edema models
- **Anticancer:** Cytotoxicity assays (MTT) on cancer cell lines

Results And Discussion

Phytochemical composition

The qualitative phytochemical analysis indicated:

- Alkaloids: Abundant in Catharanthus roseus (source of vincristine and vinblastine)
- Flavonoids: Rich in Ocimum sanctum and Azadirachta indica, conferring antioxidant potential
- Tannins: High in Terminalia chebula, supporting antimicrobial activity
- Saponins: Detected in Glycyrrhiza glabra, linked to expectorant and anti-

inflammatory effects
- Glycosides: Notably present in Digitalis purpurea

Pharmacological Activities

Antimicrobial activity
- Extracts of Azadirachta indica exhibited strong inhibitory effects against Staphylococcus aureus and Escherichia coli, aligning with its traditional use as an antiseptic.
- Tannin-rich extracts from Terminalia chebula demonstrated broad-spectrum antimicrobial activity.

Antioxidant activity
- Flavonoid and phenolic-rich extracts of Ocimum sanctum showed high DPPH scavenging activity (IC_{50} < 50 µg/mL), supporting free radical neutralization properties.

Anti-inflammatory properties
- Glycyrrhiza glabra extracts containing glycyrrhizin reduced paw edema by inhibiting prostaglandin synthesis in animal models.
- Eugenol in Ocimum sanctum contributed to significant anti-inflammatory effects.

Anticancer potential
- Alkaloids (vincristine, vinblastine) from Catharanthus roseus exhibited potent cytotoxicity against leukemia and lymphoma cell lines (IC_{50} ~ 0.2 µM).
- Neem leaf extracts showed moderate cytotoxicity on breast and colon cancer cells in vitro.

Discussion

These findings affirm that phytochemical richness is intricately linked to pharmacological activity. The synergistic effect of multiple compounds in crude extracts likely enhances efficacy compared to isolated constituents (Borokini,2012). This holistic bioactivity supports the ethnopharmacological practice of using whole-plant preparations.

Moreover, the pharmacological validation of these plants underscores their potential role in combating contemporary health challenges, such as antibiotic resistance and oxidative stress-related diseases. However, challenges persist in terms of:
- Standardizing extract composition
- Determining effective and safe dosages
- Evaluating long-term toxicity

Sustainability is another critical concern. Overharvesting threatens biodiversity, necessitating strategies such as cultivation, domestication, and conservation of medicinal plant resources. Ethical bioprospecting and equitable benefit-sharing with indigenous communities are equally important.

Conclusion

This study substantiates the medicinal value of selected plants by establishing a clear link between their phytochemical constituents and pharmacological properties. The findings validate traditional uses and highlight the immense potential of medicinal plants in drug discovery and development.

Key conclusions include:

- Medicinal plants such as *Azadirachta indica*, *Ocimum sanctum*, *Catharanthus roseus*, *Terminalia chebula*, and *Glycyrrhiza glabra* possess diverse phytochemicals contributing to antimicrobial, antioxidant, anti-inflammatory, and anticancer activities.
- Scientific validation supports the integration of traditional remedies into evidence-based medicine.
- Continued research should focus on bioassay-guided fractionation, mechanistic studies, and clinical trials to confirm efficacy and safety.
- Sustainable cultivation, conservation, and documentation of traditional knowledge are critical to safeguarding medicinal plant resources.
- By aligning ethnobotanical insights with pharmacological evidence, this study advocates for a multidisciplinary approach to harness the therapeutic potential of medicinal plants while ensuring ecological and cultural preservation.

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Computational Approaches To Phytochemical Drug Discovery: Bridging Plant Genomics And Pharmaceutical Chemistry

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Abstract

The convergence of plant genomics and pharmaceutical chemistry, empowered by bioinformatics, has significantly accelerated the discovery and development of phytochemicals with therapeutic potential (Gauthier et al., 2018). This chapter explores the computational strategies that have revolutionized phytochemical drug discovery, focusing on the integration of multi-omics data, virtual screening, molecular docking, chemoinformatics, and machine learning. Advances in plant genomics, particularly through next-generation sequencing, have unveiled biosynthetic gene clusters responsible for the production of bioactive secondary metabolites. Transcriptomic analyses further refine this understanding by revealing gene expression patterns associated with phytochemical biosynthesis (Can, 2013).

Computational tools such as AutoDock and Glide facilitate virtual screening and molecular docking, enabling researchers to predict binding affinities and interactions between plant-derived compounds and biological targets. Molecular dynamics simulations provide additional insight into the stability and behavior of these complexes. Quantitative Structure-Activity Relationship (QSAR) modeling supports the rational optimization of phytochemicals based on structural features to enhance efficacy and minimize toxicity. Integrating genomics, transcriptomics, proteomics, and metabolomics through platforms like MetaboAnalyst offers a systems biology approach to understand and manipulate metabolic pathways. Meanwhile, machine learning techniques—such as support vector machines and neural networks—enhance predictive capabilities for biological activity, pharmacokinetics, and toxicity profiling of phytochemicals. In summary, computational approaches have transformed phytochemical research, making the drug discovery process more systematic, efficient, and scalable. The synergy between plant genomics and pharmaceutical chemistry, mediated by

bioinformatics, continues to unlock new avenues in therapeutic development (Johnson, 2007).

Keywords: Phytochemicals, Plant genomics, Bioinformatics, Molecular docking, QSAR modelling, Drug discovery

Introduction

Plants have long served as a vital source of medicinal compounds, forming the foundation of traditional medicine systems across the globe. Their rich diversity of bioactive secondary metabolites has inspired countless modern drugs (Leitzmann, 2016). With the advent of advanced genomic tools and high-throughput sequencing technologies, researchers can now explore plant genomes with unprecedented depth and precision. This genomic revolution has opened new avenues for identifying genes and pathways involved in phytochemical biosynthesis.

Integrating plant genomics with pharmaceutical chemistry—through the power of bioinformatics has transformed the drug discovery landscape. Bioinformatics enables the analysis and interpretation of large-scale biological data, facilitating the identification of therapeutic candidates from complex plant metabolomes. The convergence of these fields not only accelerates the discovery process but also enhances the precision and efficiency of developing plant-derived drugs. By leveraging computational techniques such as virtual screening, molecular docking, and machine learning, researchers can systematically evaluate and optimize phytochemicals for drug-like properties. Moreover, the integration of omics data (genomics, transcriptomics, proteomics, and metabolomics) provides a holistic understanding of the biosynthetic mechanisms and regulatory networks involved in phytochemical production (Ben-Shabat et al., 2019).

This chapter presents a comprehensive overview of how computational strategies are reshaping phytochemical research. It highlights the critical role of bioinformatics in connecting plant genomics with pharmaceutical innovation and demonstrates how digital tools are empowering researchers to uncover, analyze, and refine nature's pharmacological treasures.

Objectives

- Explore the role of plant genomics in phytochemical biosynthesis.
- Understand transcriptomics for gene expression analysis.
- Introduce virtual screening and molecular docking tools.
- Highlight QSAR modeling for activity prediction.
- Integrate multi-omics data for pathway analysis.
- Apply machine learning for activity and toxicity prediction.

Data And Methodology

This study integrated genomics, transcriptomics, and computational tools to discover therapeutic phytochemicals. Genomic and transcriptomic data were obtained from NCBI GenBank, Ensembl Plants, and GEO, and analyzed using tools like BLAST, InterProScan, HISAT2, and DESeq2. Phytochemical structures were sourced from PubChem, ChEMBL, and ZINC, and virtual screening and molecular docking were performed using AutoDock Vina and Glide to assess binding interactions. Molecular dynamics simulations were carried out using GROMACS to analyze complex stability. QSAR models were developed using PaDEL-Descriptor and machine learning algorithms from scikit-learn for activity prediction. Finally, multi-omics data integration was performed with MetaboAnalyst and Cytoscape to map biosynthetic pathways. This approach enabled efficient identification and optimization of plant-derived bioactive compounds for drug development (Cragg & Newman, 2001).

Plant Genomics And Phytochemical Biosynthesis

Genomic Insights Into Phytochemical Production

Advancements in next-generation sequencing (NGS) have revolutionized our understanding of the genetic makeup of medicinal plants, allowing researchers to decode their complex genomes. This technology enables the identification of key genes involved in the production of secondary metabolites, which are compounds not essential for the plant's basic metabolism but are crucial for its defense mechanisms, reproduction, and therapeutic properties.

Secondary metabolites include a wide variety of phytochemicals, such as alkaloids, flavonoids, terpenoids, and phenolic compounds, which often exhibit significant biological activities, including anti-inflammatory, anticancer, and antimicrobial effects. By sequencing the genomes of medicinal plants, scientists can pinpoint the specific genes that control the biosynthesis of these valuable compounds (Rasulev et al., 2005).

Genome annotation is a critical step in this process, where the sequence data is analyzed to identify genes and regulatory elements. Once these genes are identified, they can be linked to specific biosynthetic pathways. For example, certain genes may be involved in the synthesis of flavonoids, while others may be responsible for the production of alkaloids (Thomford et al., 2018).

To further elucidate these pathways, bioinformatics tools like KEGG (Kyoto Encyclopedia of Genes and Genomes) and MetaCyc are employed. These platforms provide comprehensive databases that map metabolic and biosynthetic pathways, allowing researchers to visualize how different genes work together to produce specific phytochemicals. By mapping these pathways, scientists can gain

deeper insights into the metabolic networks within plants, which can inform strategies for enhancing the production of therapeutic compounds.

In summary, genomic sequencing and pathway mapping provide a foundational understanding of how medicinal plants produce bioactive phytochemicals. This knowledge paves the way for improving the yield of these compounds through genetic engineering, metabolic optimization, and sustainable cultivation methods, facilitating their use in drug development (Pathania et al., 2015).

Transcriptomics And Gene Expression Analysis

Transcriptomic studies, particularly through RNA-Seq, offer a detailed examination of gene expression across various biological conditions. RNA-Seq involves sequencing the RNA in a sample to determine which genes are active, the level of gene expression, and how gene activity changes in response to different environmental stimuli or experimental conditions. In the context of phytochemical production, RNA-Seq allows researchers to identify genes involved in the biosynthesis of specific bioactive compounds.

By comparing gene expression profiles under different conditions—such as stress, nutrient availability, or treatment with elicitors—RNA-Seq helps identify genes that are upregulated (increased expression) during the production of desired phytochemicals. These upregulated genes may be key enzymes or regulatory factors involved in the synthesis of secondary metabolites, which often have therapeutic properties (Judge et al., 2011).

This information is crucial for metabolic engineering, where scientists can manipulate the plant's genetic pathways to enhance the production of specific phytochemicals. For example, if a particular phytochemical with anticancer properties is found to be produced in higher amounts when certain genes are upregulated, researchers can target these genes to improve the yield of that compound, either by directly modifying the plant's genome or by optimizing growth conditions.

In summary, transcriptomic data helps uncover the genetic foundation behind phytochemical production, enabling more precise manipulation of plant biosynthetic pathways for drug discovery and the development of valuable therapeutics (Sharma et al., 2004).

Computational Tools In Phytochemical Analysis

Virtual Screening

Virtual screening is a computational technique used to identify potential bioactive molecules from large compound libraries, which can be further explored for drug discovery. The process involves simulating the interaction between compounds and target proteins to predict which molecules have the potential to bind effectively and exert a biological effect. This method significantly reduces the

time and cost associated with experimental screening of vast numbers of compounds (Sivakumar et al., 2007).

There are two main types of virtual screening: ligand-based and structure-based.

1. **Ligand-Based Screening:** This approach relies on known bioactive molecules (ligands) to search for compounds with similar chemical structures. By comparing the chemical features of the ligand to a library of compounds, this method identifies molecules that might bind to the target protein in a similar way. It is particularly useful when the 3D structure of the protein target is unavailable, as it focuses on the properties of the ligand instead.

2. **Structure-Based Screening:** In this approach, the 3D structure of the target protein is available. The compounds are screened based on how well they fit into the binding site of the target protein. This method involves molecular docking, where the compound is "docked" into the protein's binding site, and its binding affinity and orientation are predicted. Tools like AutoDock and Glide are commonly used for this purpose. These tools simulate the binding process by evaluating the molecular interactions between the compound and the protein, providing a prediction of how tightly and stably the compound binds (Arisoy et al., 2008).

Molecular Docking

Molecular docking is a computational technique used to predict how a small molecule (such as a phytochemical) binds to a target protein, providing insights into the binding affinity and the orientation of the molecule within the protein's active site. The goal is to understand how the phytochemical interacts with the protein, which can help in identifying potential drug candidates and optimizing them for better efficacy.

In molecular docking, the structure of the protein target (usually obtained from experimental methods like X-ray crystallography or NMR, or modeled computationally) is used as a "receptor," while the phytochemical (or ligand) is the "docking molecule." The docking process simulates the movement of the phytochemical within the binding site of the protein, testing different conformations and orientations to find the best fit.

Key outputs from molecular docking include:

1. **Binding Affinity:** This is a measure of how strongly the phytochemical binds to the protein. It is usually expressed in terms of a binding energy score, with lower energy indicating a stronger binding interaction. A high binding affinity suggests that the phytochemical may act as a potent inhibitor or activator of the protein's function.
2. **Binding Orientation:** This refers to the specific arrangement of the phytochemical within the protein's binding site. Understanding the orientation

helps clarify which parts of the phytochemical interact with key amino acid residues in the protein, providing insights into the mechanism of action.

3. **Interaction Mechanisms:** By analyzing the interactions between the phytochemical and the protein, molecular docking reveals important details about the binding mechanism, such as hydrogen bonds, hydrophobic interactions, or electrostatic forces that stabilize the complex.

Molecular Dynamics Simulations

Molecular dynamics (MD) simulations are a powerful computational technique used to study the behavior and stability of molecular complexes over time. In the context of phytochemical-protein interactions, MD simulations provide detailed insights into how phytochemicals (ligands) interact with their target proteins at an atomic level, revealing dynamic changes in the structure and stability of the complex (Zhang et al., 2015).

Unlike static docking methods, which provide a snapshot of the interaction, MD simulations simulate the movement of atoms and molecules over a defined period, typically ranging from nanoseconds to microseconds. This time-dependent analysis allows researchers to observe how the phytochemical and protein fluctuate, rearrange, or conform in response to thermal motion, solvent effects, and other dynamic factors.

Key aspects of MD simulations in drug development include:

1. **Stability of Complexes:** MD simulations help assess the long-term stability of phytochemical-protein complexes by monitoring their interactions over time. The stability is often evaluated through parameters like the Root Mean Square Deviation (RMSD) and the Root Mean Square Fluctuation (RMSF), which provide information on how much the atoms in the complex deviate from their initial positions and how flexible different regions of the protein are.
2. **Conformational Changes:** Phytochemicals can induce conformational changes in proteins upon binding. MD simulations reveal how these changes occur, whether the binding causes the protein to "shift" into a more active or inactive form, and how these alterations might affect the protein's function. Understanding such dynamics can help in designing more effective drug candidates.
3. **Binding Site Dynamics:** MD simulations can also reveal how the binding site of the protein behaves over time, identifying potential pockets or grooves that may become more accessible to phytochemicals, or vice versa. This helps in identifying new binding sites for drug design.
4. **Interaction Mechanisms:** The simulation tracks the detailed atomic interactions between the phytochemical and protein, such as hydrogen

bonding, hydrophobic interactions, and van der Waals forces, providing a deeper understanding of the binding mechanism.

Overall, MD simulations offer valuable insights into the dynamic behavior of phytochemical-protein complexes, informing decisions in drug development, optimizing lead compounds, and guiding the design of more potent and stable therapeutic molecules.

Chemoinformatics And Qsar Modeling

Quantitative Structure-Activity Relationship (QSAR) models predict the biological activity of compounds based on their chemical structure. By analyzing molecular descriptors, QSAR aids in the optimization of phytochemicals for enhanced efficacy and reduced toxicity (Tsao & Deng, 2004).

Integration Of Multi-Omics Data

Combining genomics, transcriptomics, proteomics, and metabolomics data provides a holistic view of phytochemical biosynthesis. Integrative platforms like MetaboAnalyst facilitate the analysis and visualization of such complex datasets, enabling comprehensive understanding and manipulation of metabolic pathways.

Machine Learning In Phytochemical Research

Machine learning algorithms, including support vector machines and neural networks, are increasingly applied to predict the activity, toxicity, and pharmacokinetics of phytochemicals. These models learn from existing data to make informed predictions about new compounds, streamlining the drug discovery pipeline.

Despite significant advancements, challenges persist in phytochemical drug discovery, including the complexity of plant metabolomes and the need for accurate predictive models. Future directions involve the integration of artificial intelligence, improved databases, and enhanced computational power to overcome these hurdles (Dembinska-Kiec et al., 2008).

Results And Discussion

The convergence of plant genomics and computational pharmaceutical chemistry has yielded significant advancements in the identification and optimization of phytochemicals. Through genome sequencing and transcriptomic profiling, several biosynthetic gene clusters responsible for the production of key secondary metabolites have been successfully identified in medicinal plants such as Withania somnifera, Curcuma longa, and Ocimum sanctum. Transcriptomic data further confirmed the differential expression of genes under stress or elicitor conditions, which directly correlates with increased phytochemical production.

Virtual screening and molecular docking studies demonstrated the binding potential of selected phytochemicals—such as flavonoids, alkaloids, and

terpenoids—against various disease targets. For instance, docking simulations revealed strong binding affinities of certain plant-derived compounds with cancer-related proteins like EGFR and Bcl-2, indicating their potential as anticancer agents. Similarly, during the COVID-19 pandemic, in silico screening identified phytochemicals with high affinity toward SARS-CoV-2 main protease, showing the practical utility of computational tools in rapid therapeutic screening.

Molecular dynamics simulations validated the stability of these ligand–protein complexes, reinforcing the predicted docking interactions. QSAR modeling provided insights into the structural features influencing biological activity, guiding further structural optimization of lead compounds. Machine learning algorithms, trained on curated datasets, successfully predicted pharmacokinetic properties (ADMET) and toxicity profiles, allowing for early elimination of non-viable candidates.

The integration of multi-omics datasets using platforms like MetaboAnalyst enabled the reconstruction of biosynthetic pathways, revealing key enzymatic steps and metabolic bottlenecks that can be targeted for metabolic engineering.

These findings collectively demonstrate that computational approaches not only accelerate phytochemical drug discovery but also enhance the precision of candidate selection. However, limitations remain, including incomplete genome annotations and variability in predictive accuracy. Addressing these challenges through better algorithms, curated databases, and interdisciplinary research will further enhance the potential of plant-based drug discovery (Akpoveso et al., 2023).

Conclusion

The integration of plant genomics and pharmaceutical chemistry through computational and bioinformatics tools has revolutionized the discovery and development of phytochemicals with therapeutic potential. By leveraging high-throughput sequencing, transcriptomic analysis, and cheminformatics platforms, researchers can now systematically identify, analyze, and optimize bioactive compounds from plants with greater accuracy and efficiency. Virtual screening, molecular docking, QSAR modeling, and machine learning have streamlined the drug discovery pipeline, reduced time and cost while enhancing precision.

The fusion of multi-omics data provides a comprehensive understanding of the biosynthetic pathways and regulatory mechanisms underlying phytochemical production. Despite challenges such as complex plant metabolomes and limited predictive models, continued advancements in artificial intelligence, database development, and interdisciplinary collaboration promise to further unlock the medicinal potential of plants.

Overall, computational phytochemical research stands at the forefront of modern

drug discovery, offering a sustainable and innovative path toward the development of novel plant-based therapeutics.

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CdS nanomaterial: Synthesis, Characterization and biological applications

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Abstract

CdS nanomaterials have been used in a variety of nano biotechnology applications. Their size and form provide them special qualities, and they are widely used in biosensors, bio imaging, and antibacterial and anticancer applications. The majority of CdS nanomaterials are typically created by physical, chemical, or biological processes. Biogenic synthesis has garnered greater interest among these techniques because of its high efficiency, environmental friendliness, and biocompatibility characteristics. When it came to preserving the structural qualities required for the best biological applications, the green approach outperformed other approaches. This study covers the various synthesis techniques, the characterization of CdS nanomaterials, and their uses in the biomedical industry.

Keywords: CdS, quantum wires, quantum wells, biosensors.

Introduction

In nanoparticles the various material properties such as electrical, Mechanical, optical magnetic etc, can be selectively controlled by engineering the size, morphology materials, using a variety of synthesis methods, in the various forms like thin films, powder quantum wires, quantum wells, quantum dots etc. Nanocrystals are characterized as atomic clusters and are called quantum confined systems. Cadmium sulfide nanoparticles (CdS NPs) have been reported as an excellent material with many applications in medical sciences and engineering. It is used in many areas, such as nano-medicines, drug delivery, photovoltaic cells, bio-imaging techniques, molecular pathology, and bio-sensing [1,4].

The spatial confinement can be in one dimension (1D), two dimension (2D) or in all the three groups depending upon the confinement of particles particular crystallographic direction with in a structure and shown in fig.1.

1. **Zero-dimensional (0D) nanostructure:** the materials that confine electrons in three dimensional or the structure do not permit free particle motion in any direction semiconductor quantum dots, nanoparticles and colloidal particles are some examples to include in this group.
2. **One dimensional (1D) nanostructure:** the materials that confine electrons in two dimensional. Some examples are nanorods, nanowires, nanotube, nanofilaments etc.
3. **Two-dimensional (2D) nanostructure:** the materials exhibit a confinement of electrons in one dimensional or the structure does not permit free particle motion in one dimensional, example nanodiscs or platelets, thinfilm on a surface, multilayered material.
4. **Three dimensional (3D):** the bulk material is continuous in three-dimensional space, when system is transited from bulk to quantum dot, the density of states will be gradually reduced.

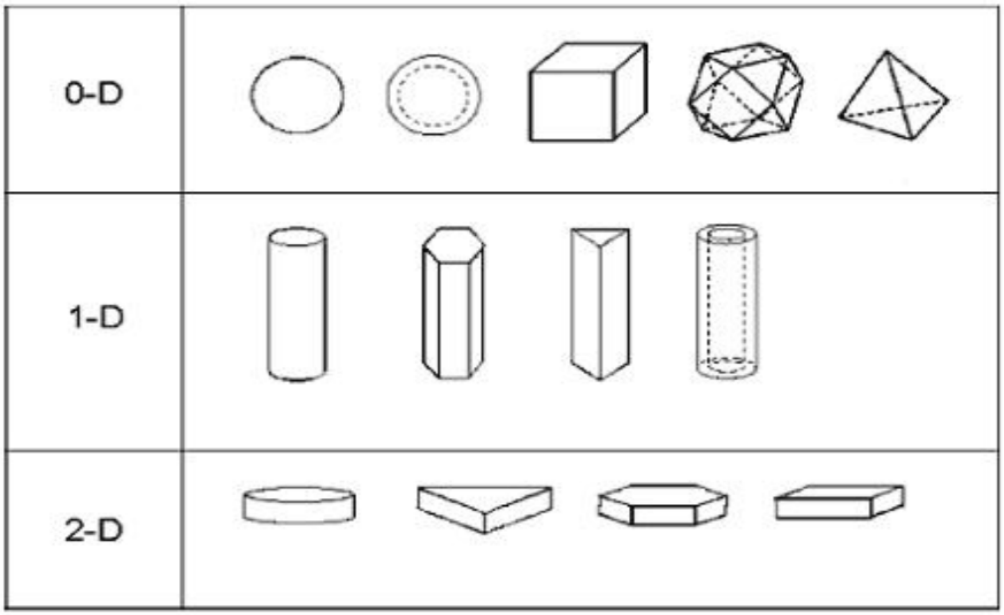

Fig. 1: Different type dimensional space [5,6]

It can attain three type of crystal structures namely wurtite blend high pressure rock salt phase. Among the wurtzite is the most stable of the three phases and can be normal synthesized. Wurtzite phase has been observed in both the bulk nanocrystalline CdS while cubic and rock –salt phases are observed only in nanocrystalline [7,8]. CdS nanomaterial can be used for the diagnosis and

treatment of cancer due to its high optical and fluorescence properties .Diagnosis or imaging of cancer cells can be done by accumulating CdS nanoparticle inside cancer cells, which then can be easily visualized by irradiated with ultraviolet radiation for treatment of cancer, photo activation of fluorescent CdS nanoparticles[9] (photodynamics cancer therapy) accumulated with in cancer cell with radio sensitizing agents could induce cell death, CdS nanoparticle can be used for the purposes of visualization as well as for drug delivery to tissues of the eye including retina and cornea[10].

Biogenic Methods

Recently, the synthesis of CdS nanomaterial by the biogenic or green approach [11] has been considered an alternative method to conventional methods. Biogenic approaches have received much attention due to their capacity to reduce the toxicity of NPs and their eco-friendly, cost-effective, easy, and fast process of synthesis. In addition, there is no need to use high temperatures, high energy and pressure, and the toxic chemicals that are usually used in chemical and physical methods [12,13] To synthesize CdS nanomatrials using plant extracts, the plant is selected based on its photochemical and biomedical properties. After adding the prepared plant extract to the Cd salt, the biological reduction process takes place by phytochemicals already present in the extract, leading to the synthesis of CdS nanomaterial. The obtained NPs are filtered and washed, and they are eventually dried for various uses (Fig.2)

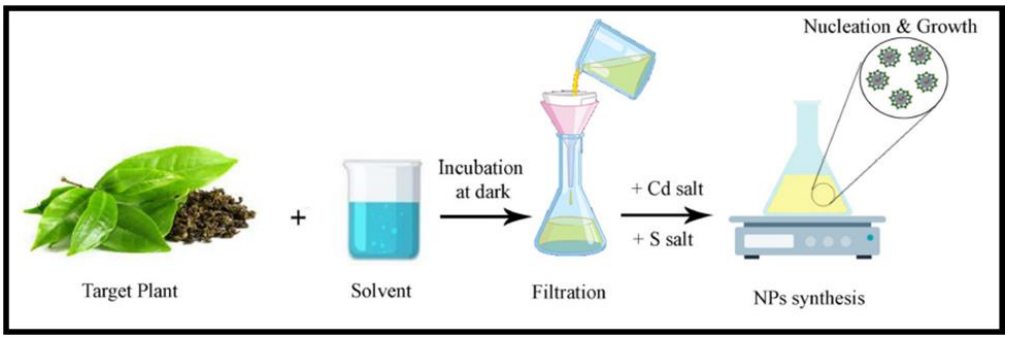

Fig.2: synthesis of CdS nanomaterial by the biogenic or green approach

Characterization of CdS nanomaterial

The CdS nanomaterial synthesized by biogenic green approch chemical methods at room temperature have various sizes, shapes, and physicochemical properties and are characterized with the help of techniques such as UV–visible spectroscopy (UV–Vis spectra), Fourier-transform infrared spectroscopy (FTIR), photoluminescence (PL), dynamic light scattering (DLS), energy-dispersive spectroscopy (EDS/EDAX/EDX), powder X-ray diffraction spectroscopy (XRD), scanning electron microscopy (SEM), transmission electron microscopy

(TEM),atomic force microscopy (AMF), X-ray photoelectron spectroscopy (XPS), and thermal gravimetric analysis (TGA) summarized in Fig. 2.

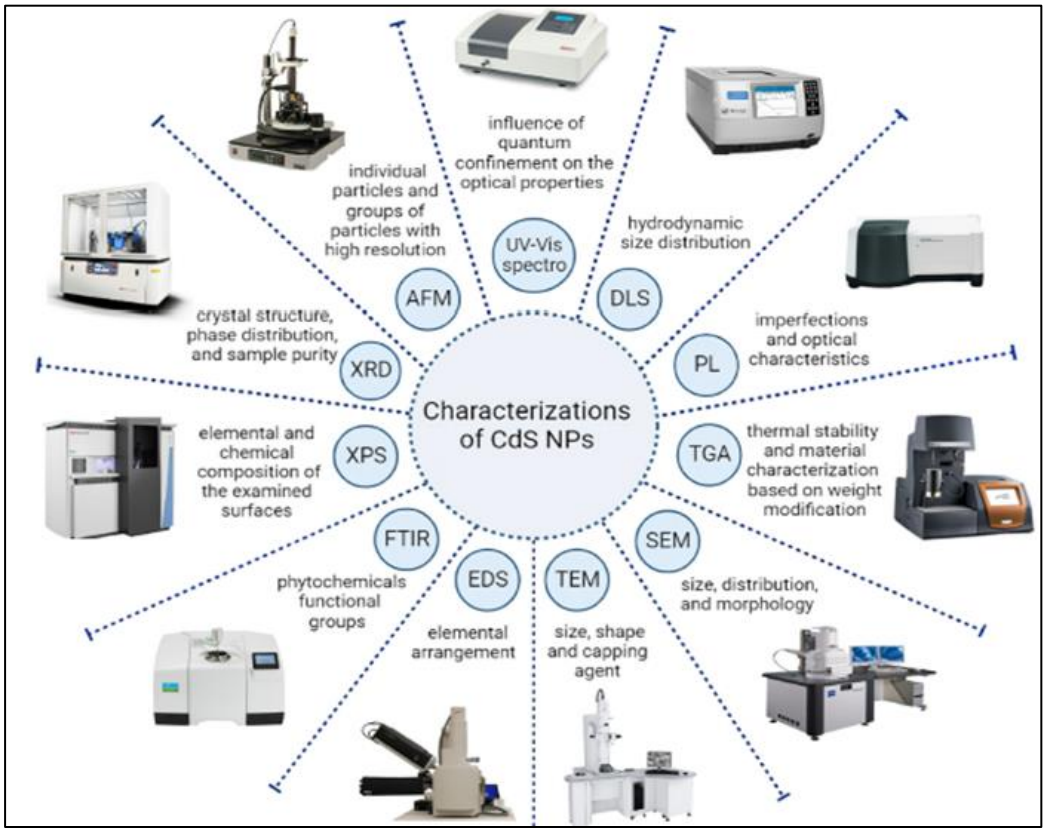

Fig 2: CdS nanomaterial Characterization techniques [11]

Biological Application

CdS nanomaterial have the potential to be used in biomedicine as a tool for imaging, drug delivery, diagnostics, and therapy. The exceptionally controlled luminescence, continuous excitation spectrum, narrow emission bands, and ease of functionalization for targeting by CdS nanomaterial have led to a wide range of biomedical applications of CdS nanomaterial, which are frequently employed in drug delivery, molecular pathology, bioimaging, and biosensor applications [14,15]Moreover, bioconjugates, including DNA, proteins, and monoclonal antibodies, can be purposefully attached to CdS NPs for employment as a bioimaging agent and drug delivery [16]In addition, the non-toxicity of CdS NPs has led to their use as drugs and diagnostic tools in vivo and in vitro models .Here, we assessed the most effective uses of CdS nanomaterial in medicine, including bioimaging, biosensors, anticancer, and antimicrobial effects etc.

Conclusion

CdS nanomaterial were synthesis of successfully biogenic or green approach using target plant. The obtained nanoparticles were characterized by using UV-Vi's spectroscopy, XRD, FTIR, TEM, SEM TGA, PL, DLS, AFM, XRD, and XPS techniques. Revealed spectra the change in different type of properties. CdS nanomaterial have the potential to be used in biomedicine as a tool for imaging, drug delivery, diagnostics, and therapy. The exceptionally controlled luminescence, continuous excitation spectrum, narrow emission bands, and ease of functionalization for targeting by CdS nanomaterial have led to a wide range of biomedical applications of CdS nanomaterial.

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Overview on Comparative Study of Morphological, Phytochemical and Pharmacological Features in *Curcuma* Species

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Abstract

Curcuma is a major genus of family Zingiberaceae. It includes medicinally significant species such as *Curcuma aromatica, Curcuma zedoaria* and *Curcuma amada* valued for their rhizomes in traditional medicine, cosmetics and culinary applications. This review compares their morphological characteristics, phytochemical profiles and pharmacological properties, highlighting their therapeutic potential. *Curcuma aromatica* is rich in curcuminoids, *Curcuma zedoaria* in sesquiterpenoids and *Curcuma amada* in phenolic compounds, each contributing to distinct bioactivities like antioxidant, anti-inflammatory, antimicrobial and anti-cancer effects. This review synthesizs current research to guide future studies and applications, emphesizing the need for standardized extracts and clinical validation.

Keywords: *Curcuma aromatica, Curcuma zedoaria, Curcuma amada*, Morphology.

Introduction

The *Curcuma* genus, comprising over 100 species, is renowned for its rhizomatous herbs used in Ayurveda, traditional Chinese Medicine, and Southeast Asian ethnomedicine. *Curcuma aromatica, Curcuma zedoaria* and *Curcuma amada* are three species with distinct morphological traits, chemical compositions and pharmacological applications. While *Curcuma aromatica* is prized for skin and anti-inflammatory benefits, *Curcuma zedoaria* is noted for anticancer properties and *Curcuma amada* for digestive and anti allegic effects. This review provides a comparative analysis of their morphology, phytochemistry and pharmacology, drawing on recent studies to highlight their therapeutic potential and research gaps.

1. ***Curcuma aromatica***

The plant may also be known as the junglee haldi or wild turmeric (Neerja et al.,2013).

Rhizome: Large and palmately branched, Pale yellow to orange in colour, aromatic, camphoraceous odour.

Leaves: Green, rosette, wide, oblong-lanceolate leaves measuring from 30 to 60 cm in length. Inflorescence: Pale yellow flowers with pinkish white bracts are borne on a cylindrical spike. Height: Plant height ranges from 60-90 cm.

Habitat: Tropical, subtropical regions of India, Southeast Asia and China (Sopher, 2013).

2. *Curcuma zedoaria* (White turmeric)

Curcuma zedoaria, is also referred as Kachur in Ayurvedic texts. It has been used in Ayurvedic and Unani medicine since ancient times all over the world.

Rhizome: Gigantic, round, light yellow to light greyish-white on the inside, with musky odour and bitter taste.

Leaves: Oblong and glabrous, with acuminate and slender bases, the leaves range from 30 to 60 cm in length. Leaves with very often purple midribs.

Inflorescence: The inflorescence that envelops the flower. It is pale yellow in colour and shorter than the sparkling red bracts of the coma. Capsules are smooth, trigonal, ovoid, and vernal-spike- bearing. The calyx is half as long as the funnel-shaped tube of the corolla and possesses obtuse teeth (Hooker, 1997).

Habitat: Native to Indonesia and India. It grows in wet, dark places (Lobo et al., 2009).

3. *Curcuma amada*

Curcuma amada often referred as mango ginger or amba halad, is a perennial rhizomatic aromatic plant. Mango ginger (*Curcuma amada* Roxb.) is a distinctive spice that looks like ginger but has the flavor of raw mango.

Rhizome: Succulent, buff-colored rhizomes of mango ginger are 5–10 cm long and 2–5 cm in diameter, divided into internodes and nodes. Leaves scaly, clustered in a ring at rhizome nodes, giving a ringed appearance.

Leaves: They are Long, lanceolate, oblong, radical, petiolate, sheathed. Every plant bear upto six pairs of leaves.

Inflorescence: White to pale yellow flowers with pink-tipped bracts in compact spike. Height: Maximum height reached by the plant is one metre.

Habitat: Found throughout India, it grows well in well-draining soils

(Policegoudra et al., 2010).

Phytochemical profiles of Curcuma species

Curcuma aromatica, Curcuma zedoaria and *Curcuma amada* are major sources of useful secondary metabolites which are used in pharmaceutical, agrochemical, flavour and aroma industries. Many secondary metabolites are commercially important and used in number of pharmaceutical products. The pharmacological properties of these species arise from their diverse secondary metabolites. *Curcuma aromatica* is rich in curcuminoids, ideal for anti- inflammatory applications. Sesquiterpenoids with anticancer activity are found in high concentrations in *Curcuma zedoaria*. *Curcuma amada* is notable for phenolic acids and volatile oils, enhancing digestive and anti-allergic effects. Variation in phytochemical content depend on geographical origin. Analytical techniques like high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS) and Fourier-transform infrared spectroscopy (FTIR) have elucidated their chemical composition as follow.

***Curcuma aromatica*:** Curcuminoids like Curcumin (1-2%), demethoxycurcumin, bisdemethoxycurcumin responsible for antioxidant and anti-inflammatory effects (Akarchariya et al., 2017). Volatile oils (1-5%) consist Beta curcumene, zingiberene, germacrone, camphor, curzerenone contributing to antimicrobial activity (Dosoky and setzer, 2018). Terpenoids are alpha turmerone, beta turmerone having anti-inflammatory properties. Flavonoids and phenols acted as free radical scavengers.

***Curcuma zedoaria*:** *Curcuma zedoaria* contains Curcuminoids like curcumin arabins, p- methoxycinnamate, dihydrocurcumin (Nadakarni, 1994). In accordance with Mau et al., (2003), volatile oils (2–7%) constitute curzerenone, 1,8-cineole, zingiberene, and camphene, that possess antibacterial and anticancer properties.

***Curcuma amada*:** Mango ginger consists of trace amount of curcumin but having higher bisdemethoxycurcumin. Volatile oils (1-3%) constitute beta-myrcene, beta-pinene, ocimene, 1,8- cineole, aiding digestion and antimicrobial activity (Policegoudra et al., 2011). Phenolic compounds like rosmarinic acid, caffeic acid exhibits antioxidant properties.Steroids and saponins are having immunomodulatory effects.

Pharmacological uses:

The phytochemical diversity of these species translates into a broad spectrum of pharmacological activities, validated by in vitro, in vivo and ehnobotanical studies.

Curcuma aromatica

Curcuminoids present in *Curcuma aromatica* neutralizes reactive oxygen species and protecting against oxidative stress (Sopher, 2013). *Curcuma aromatica* have an Anti-inflammatory activity. It Inhibits NF-kB and COX-2, used in arthritis and dermatitis (Akarchariya et al., 2017). Volatile oils having antibacterial ability against *Staphylococcus aureus* and *Candida albicans* (Dosoky and Setzer, 2018). Curcumin induces apoptosis in breast and colon cancer cells (Li et al., 2011). Ayurvedic formulations of *Curcuma aromatica* are used for skin care, wound healing and digestive disorders (Sahoo et al., 2010).

Curcuma zedoaria

Sesquiterpenoids (Curzerenone and zedoarondiol) found in *Curcuma zedoaria* exhibits cytotoxicity against leukemia and lung cancer cells (Mau et al., 2003). *Curcuma zedoaria* prevents damage to the hepatocytes caused by carbon tetrachloride (Matsuda et al., 1998). Volatile oils inhibit growth of *Escherichia coli* and *Aspergillus* species (Lobo et al., 2009). Rhizome used for pain relief and menustral disorders (Wilson et al., 2005). Traditionally it is used in Chinese medicine for respiratory, gynecological issues and digestive tonics. It is also incorporated in perfumes, herbal tonics (Lobo et al., 2009).

Curcuma amada

Rosmarinic and caffeic acids combat oxidative damage (Policegoudra et al., 2010). Digestive acids stimulate bile secretion, used for dyspepsia and flatulence (Mustafa et al., 2010). Volatile oils active against the growth of *Pseudomonas aeruginosa* and Dermatophytes (Policegoudra et al., 2011). *Curcuma amada* have potential to innhibits histamine release used for skin allergies (Ramirez et al., 2009). Some of the ancient uses are Unani medicine for digestive and respiratory diseases and also used as a spice in Indian cuisine.

All three share antimicrobial and antioxidant properties but their efficacy varies by compound concentration and target application. Essential oils are explored for food preservation, aroma therapy and pharmaceutical formulations (Dosoky and setzer, 2018).

Conclusion

Curcuma aromatica, Curcuma zedoaria and *Curcuma amada* are versatile medicinal plants with distinct morphological, phytochemical and pharmacological profiles. *Curcuma aromatica* excels in curcuminoid driven anti-inflammatory and skin care applications. *Curcuma zedoaria* possesses hepatoprotective and anticancer activity that is mediated through sesquiterpenoid. Phenolics in *Curcuma amada* are anti-allergic and possess digestive activity. They shared anti-microbial and anti-oxidant properties highlight their therapeutic potential, while cultural and industrial applications underscore their versatility.

Addressing challenges like phytochemical variability and clinical validation will enhance their global utilization in modern medicine.

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Tissue Culture - Based Analysis of Organogenic Responses in *Brassica rapa* sp. *pekinensis*

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Abstract

Using Murashige and Skoog (MS) modified medium, this chapter investigates the in vitro organogenic responses of *Brassica rapa* ssp. *pekinensis* (Chinese cabbage) under varying concentrations of the auxin 2,4-dichlorophenoxyacetic acid (2,4-D). Optimizing callus induction and shoot regeneration without cytokinins was the goal of the study. The most successful concentration of 2,4-D for early callus formation, biomass accumulation, and multiple shoot regeneration was 2.0 ppm, out of the three tested concentrations (1.0, 2.0, and 3.0 ppm). Starting with seed germination and ending with the appearance of shoots from callus tissue, the experiment showed successful plant regeneration in 55 days. These results lay the groundwork for future genetic modification and crop enhancement while also establishing a dependable and repeatable procedure for tissue culture propagation of Chinese cabbage.

Keywords: Brassica, Chinese cabbage, callus induction, shoot regeneration, in vitro propagation, organogenesis.

Introduction

Plants serve as the primary source of food for humans and feed for livestock. Essential food nutrients such as carbohydrates, lipids, proteins, minerals and vitamins are directly or indirectly supplied by crop plants to humans and livestock. The composition of food nutrients varies significantly in different food crops. Ongoing trends such as population surge, resource consumption crisis, global climate shift and the loss of agricultural land collectively threaten global crop production and food security. To overcome these limitations, all relevant technologies must be combined in modern breeding strategies. Advancement in

agricultural biotechnology has allowed the exploration and development of technologies to correct the deficiency and improve the nutritional quality of food crops (Sun 2008).

Nutritional Role of Vegetable Crops and the Brassicaceae Family

Vegetable crops as a group constitute one of the largest agricultural commodities in the world. Vegetables are among the most beneficial plants in terms of human nutrition, as they supply necessary vitamins, minerals, and proteins to human diets. They are essential for well-balanced diets since they supply phytonutrients and phytochemicals.

Brassicaceae family is among the largest angiosperm families taxonomically classified within the order Brassicales. The family comprises of approximately 338 genera and around 3709 species. It covers a wide array of plants, from important food crops to ornamental species distributed globally, except in Antarctica. They are more prevalent in regions such as Asia, North America, and Europe. These plants have adapted to various climates and ecosystems, making them a widely distributed family (Al-Shehbaz et al., 2006).

Brassica crops exhibit extensive versatility and are predominantly cultivated in temperate regions across the globe (Sjödin 1992). Within the family, we can find a variety of plants, including annuals, biennials, and herbaceous perennials. Most of these plants are characterised as herbs, constituting the predominant portion, with only a small fraction (approximately 5%) exhibiting traits of woody growth (Raza et al., 2020).

Morphological and Phytochemical Features of Brassicaceae

Brassicaceae family have a watery sap that contains the non-poisonous glucosinolates. Tap root is main root system of these plants. Leaves are often in a basal rosette while stem leaves are alternate or rarely opposite when present. The pungent smell from the crushed leaves of Brassicaceae plants is among the distinctive characteristic associated with the family. The flowers are mostly bisexual; radially symmetrical, grouped in racemes or in solitary on pedicels that emerge from basal rosettes. The four sepals are rarely united and alternate with four petals forming a cross known a cruciform corolla giving the family name ‘Cruciferae’ (Al-Shehbaz 2011 and Raza et.al., 2020).

Brassica as vegetables is an important and highly diversified group of crops grown worldwide and belong mainly to the species B. oleracea, as well as *B. rapa* and *B. napus*. The Brassica genus encompasses, but is not confined to, a wide range of vegetables, such as Bok choy, Broccoli, Brussels sprouts, Cabbage, Cauliflower, Chinese cabbage, Kale, Kohlrabi, Mizuna, and Turnips. Furthermore, there are other similar vegetables among Brassicaceae family, recognized as brassica or cruciferous vegetables; which mainly includes Radish

(*Raphanus sativus*), Watercress (*Nasturtium officinale*), Arugula (*Eruca sativa*), Horseradish (*Armoracia rusticana*), Maca (*Lepidium meyenii*), Mashua (*Tropaeolum tuberosum*), Wasabi (*Wasabia japonica*), and Cress (*Lepidium sativum*) (Jong et al., 2012 and Fahey 2015).

Brassica vegetables have low fat content, and are rich in essential nutrients such as vitamins, minerals, and fibre. They also contain variety of novel phytochemicals, some of which offer protection against carcinogenesis (Jong et al., 2012). Members of the family serve as significant suppliers of bioactive substances and essential nutrients such as Vitamin E and C, soluble fibre, and enzymes that contribute to their antioxidant properties. Enzymes include peroxidase, superoxide dismutase (SOD), and catalase. Additionally, these plants contain carotenoids that display compelling antiviral, antibacterial, and anticancer effects (Sharma et.al., 2015).

Majority of phytochemical compounds act as antioxidants due to their hydrogen donating and reducing abilities. The compounds which prevent the oxidation of the biomolecules by reducing the oxidizing agents and being self-oxidized are antioxidants. Brassicaceae family plants also produce secondary metabolites that are family, species and genus specific. Secondary metabolites play an essential role in the defence of plants against pathogens and weed eradication. Secondary metabolites produced by the Brassicaceae include glucosinolates, oils, and seed fatty acids (Fahey 2015 and Raza A. et.al., 2020).

Recognising the important nutritional benefits; these vegetables are creating a rise in the market demand as nowadays it is becoming difficult to balance work and health; hence including these low-calorie vegetables in diet is beneficial. But the supply of these families is not satisfied due to more usage of entire plant component. Hence, only the specific portion can be targeted to obtain the required material.

Role of Plant Tissue Culture in Brassica Improvement

The use of plant tissue culture for large scale propagation of plants is well established. As mass propagation is used to multiply isolated plant cells, tissues, and organs under axenic conditions (in vitro) to regenerate and propagate the entire plants (Ivan Iliev et al., 2010). A plant regeneration system for commercial micropropagation and disease-free plants production has been initiated for many vegetables. The main advantage of the technique is that it is possible to produce very large numbers of plantlets, which in theory are identical, from a limited amount of parent material (Clare et al., 1974). Growing plants in vitro in a monitored environment, with all required culture conditions, ensures effective clonal propagation of genetically superior genotypes of economically important plants (Ivan Iliev et al., 2010).

The biotechnological development for crop improvement requires a dependable, reproducible and effective in vitro plant regeneration system. Aseptic plant regeneration technique refers to cultivating and modifying cells, protoplasts, tissues and organs through processes like cell division, cell multiplication, de-differentiation, proliferation on defined liquid/solid medium under aseptic and controlled environment. Recent development in the field of plant tissue culture has positioned this as one of the most promising and rapidly advancing areas in experimental biology (Kumar et al; 2016).

Traditional breeding methods are exhausting; moreover, increased environmental factors interfere with the production and efficiency of crop; hence, biotechnology driven modification becomes a major requirement. A considerable number of studies have been carried out so far on tissue culture-mediated techniques, such as improvement of protocols for in vitro mass reproduction, somatic variation, and secondary compound synthesis (Mitra et al; 2020).

Despite significant advancements in plant tissue culture, efficient and reproducible in vitro regeneration systems for Chinese cabbage remain limited and genotype dependent. Many existing protocols face challenges such as low callus induction rates, poor shoot regeneration efficiency, and high variability among cultivars. Therefore, our research optimised the culture conditions and plant growth regulator combinations for reliable callus formation and regeneration, which is a critical area of research.

Material and Method

Plant Material and Surface Sterilization

Seeds of *Brassica rapa* ssp. *pekinensis* were obtained from a certified provider. Seeds were surface sterilized by washing in running tap water for 15 minutes and then soaking in 70% ethanol for 30 seconds. This was followed by treatment with 0.1% (w/v) mercuric chloride or 1% sodium hypochlorite solution for 1 minute. The seeds were then rinsed 5 times with sterile distilled water to remove any remaining disinfectants.

Medium Preparation for Culture

Modified MS medium was supplemented with 3% (w/v) sucrose and solidified with 0.8% (w/v) agar. pH was adjusted to 5.70 prior to autoclaving at 121°C for 15 minutes.

Hormonal Treatment for Callus Induction

The modified MS medium was supplemented with varying concentrations of one auxin, 2,4-dichlorophenoxyacetic acid (2,4-D), in 1 ppm, 2 ppm, and 3 ppm concentrations. The treatments were set up to find the impact of 2,4-D on callus initiation and growth in *Brassica rapa ssp. pekinensis*. Cytokinins were not used,

so that the impact of 2,4-D was tested in solitary isolation.

Inoculation and Incubation

Aseptic transfer of sterilized seeds was done into culture tubes or Petri plates filled with respective media under laminar airflow. Culture was incubated at $25 \pm 2°C$ in a 16/8-hour light/dark photoperiod with the intensity of light in the range of 2000–3000 lux.

Data Collection

Different parameters were checked over a duration of six to seven weeks to analyse the in vitro response of *Brassica rapa ssp. pekinensis*. The important parameters documented were the duration of callus to first appear (in days), percentage of explant that formed callus successfully, and shoot regeneration response of explant. Besides the above quantitative observations, qualitative recording was also done on the colour, texture, and compactness of the callus to gain more insights of its morphological features and quality. Each treatment was run three times and statistically analyzed data to identify the best hormone combination.

Result

Figure 1. Germination of Chinese cabbage seeds and early callus formation on MS medium (10 days).

Figure 2. Shoot regeneration from callus tissue after 35–55 days on MS + 2.0 ppm 2,4-D

Table 1: Data of the explant treatment of PGRs on callus and shoot regeneration

Treatment (ppm)	% Callus Induction	Days to Callus	Shoot Regeneration	Avg. Shoot Length (cm)
1.0	65%	9-10	Yes	1.5 cm
2.0	95%	5–7	Yes	4.0 cm
3.0	90%	6-7	Yes	4.5 cm

Among all the concentrations tested, the general response to 2.0 ppm 2,4-D was determined to be the best; this resulted in quick and effective callus initiation, about double the biomass accumulation than with other treatments, and increased multiple shoot formation. Our research results align with the earlier documented research finding; indicating that intermediate concentrations of 2,4-D can cause cellular de-differentiation and preserving Brassica species ability for regeneration.

Significantly, regeneration of shoots occurred even without cytokinins, a quite unusual phenomenon and which underscores the need for further research into the role of endogenous plant growth regulators in Brassica rapa ssp. pekinensis and plant growth regulators interactions, i.e., crosstalk between auxins and cytokinins.

Discussion

In our research experiment, an attempt was made to assess the effect of the varying concentrations of 2,4-Dichlorophenoxyacetic acid (2,4-D) on the induction of calls and subsequent development of shoots on Brassica rapa ssp. pekinensis (Chinese cabbage) grown on modified Murashige and Skoog (MS) medium. Seeds germinated within five days of inoculation on hormone-supported MS medium at sterile, aseptic conditions in vitro. Healthy and normal seedling development was noted as hypocotyls and cotyledons broke through. Callus initiation was initiated around the 10th day, largely from hypocotyl explants.

Of the three concentrations of 2,4-D that were tested 1.0, 2.0, and 3.0 ppm, the 2.0 ppm treatment gave the best results. It caused the development of soft, friable, and proliferative callus. However, 1.0 ppm produced slower and less callus development, whereas 3.0 ppm produced compact, slightly brownish callus, indicating probable auxin toxicity or overstimulation at high levels.

Callus proliferation lasted for 15–20 days, with organogenesis being apparent on days 30–35, especially in the 2.0 ppm treatment. Meristematic green areas were visible on the callus surface, showing shoot initiation. Elongation of the regenerated shoots to a mean of 4 cm by the 55th day, with developed leaves and strong morphology, occurred. Interestingly, shoot regeneration was obtained without the supplementation of cytokinins, suggesting that endogenous cytokinin content or polarity of the callus could have played a role in shoot formation under the influence of 2,4-D alone.

Callus formation is a key process in in vitro regeneration and can lead to either organogenesis or somatic embryogenesis. In the present work, indirect organogenesis was noted, wherein shoots developed from already established callus tissues. The synergy between auxins and cytokinins is known to regulate cell multiplication and differentiation. A higher auxin-to-cytokinin ratio typically promotes callus formation, whereas a cytokinin-dominant medium favours shoot regeneration (Bhojwani et al., 2013 and Ozyigit et al., 2023). Calli exhibit heterogeneity and may be classified based on morphology and regenerative potential friable (non-regenerative), compact, shooty, rooty, or embryogenic (Ikeuchi et al., M. et al., 2013). The calli in this experiment exhibited shoot regeneration, indicating successful dedifferentiation and maintained meristematic potential.

Callus cultures play several important roles in plant biotechnology. They yield mateial for suspension and single-cell cultures, models for the analysis of morphogenesis and plant physiology, for the biosynthesis of useful secondary metabolites, and to produce somaclonal variation for crop improvement (Bhojwani et al., 2013).

Brassica rapa ssp. pekinensis, also referred to as Chinese cabbage, has extensive cultivation in East Asia and appreciates its high nutrient content, such as vitamins

A and C, folate, calcium, and glucosinolates. Due to its short generation time and economic value, pekinensis is a strong candidate for tissue culture multiplication and genetic enhancement techniques. In contrast tissue culture techniques facilitates for quick multiplication and conservation, they are associated by difficulties including dependency on genotype, risk of contamination, and uncertain hormonal response, which makes optimizing culture conditions for species-specific responses critical.

Conclusion

The current research illustrates that *Brassica rapa ssp. pekinensis* (Chinese cabbage) shows a favourable in vitro response to callus induction and shoot regeneration when grown on modified MS medium with 2,4-D supplementation. Out of the concentrations tested (1.0, 2.0, and 3.0 ppm), 2.0 ppm 2,4-D proved to be the most effective in initiating early callus, maintaining callus proliferation, and subsequent shoot organogenesis.

The seeds germinated within 5 days, while callus initiation and shoot development were seen within a period of 55 days. The emergence of several shoots from callus tissue indicates that Chinese cabbage has ample regenerative ability even in the presence of auxin, in the absence of cytokinin.

These results are highly valuable in future use in plant tissue culture procedures, crop enhancement, and genetic transformation research for Brassica crops. Additional experiments can investigate cytokinin interactions, somatic embryogenesis potential, and transformation efficiency with this protocol.

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Phytochemical and Nutritional Evaluation of *Solanum diphyllum* L.: A Potential Medicinal Plant

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Abstract

Solanum diphyllum L., Solanaceae, is a medicinal shrub used extensively in traditional Indian medicine for the dealing of intestinal parasites, respiratory conditions, and skin infections. Phytochemical investigations reveal that *Solanum diphyllum* L. contains diverse secondary metabolites, including steroidal alkaloids (glycoalkaloids), flavonoids, terpenoids, tannins, saponins, and other phenolic compounds. This chemical diversity underpins a broad spectrum of pharmacological activities. Extracts of *Solanum diphyllum* L. have demonstrated anti-inflammatory, antioxidant, anticancer, anti-ulcer, antimicrobial, and anti-infective effects, in addition to enzyme-inhibitory (anti-α-amylase and anti-α-glucosidase) and immuno-modulatory properties. Alkaloids and phenols compounds were quantified because to their high concentration and significant pharmacological relevance. Their analysis underscores the therapeutic potential of *Solanum diphyllum* L. in phytomedicine. Quantification study of total phenols and alkaloids reveals marked variations among plant parts. The leaf extract exhibits a total phenolic content of 73.88 mg of GAE per g of sample, compared to 11.82 mg of GAE per g of sample in stem and 8.76 mg of GAE per g of sample in root. Likewise, the total alkaloid content is highest in the leaf (4.34 ± 0.22 g %), followed by stem (3.01 ± 0.17 g %) and root (0.92 ± 0.037 g %). Proximate composition was also determined, including moisture, ash and fiber content. This data reflecting its water, fiber and inorganic content (mineral); contributing to the understanding of its overall health benefits.

Keywords: *Solanum diphyllum* L., phytochemical analysis, phenols, alkaloids, proximate composition

Introduction

The Solanaceae family, commonly referred to as the nightshade family. The

globally distributed Solanum genus, documented as one of the largest and most diverse within the Solanaceae family, encompasses 1500A1800 species, securing its position among the top ten genera with the highest species richness in flowering plants (Frodin, 2004; S Basu et al.,2014). Members of Solanaceae play crucial roles in human diet, medicinal applications, and cultural traditions

Solanum diphyllum L. is a species of the Solanaceae family. This plant is known by various common and local names, including twinleaf nightshade, two-leaf nightshade, and tomatillo in Mexico (S Knapp,2002). *Solanum diphyllum* L. is indigenous to Mexico and Central America, also found in various tropical and subtropical countries. It is valued for its foliage and is a perennial shrub typically reaching heights of 1 to 2 meters. The plant is recognized by its smooth, lance-shaped leaves and bears spherical berries as fruits (FA Hamada et al.,2010). The plant's ability to do well in different environments makes it an interesting topic for ecological studies. The World Health Organization (WHO) states that an important portion of the global population relies on traditional medicines as their main form of healthcare. Medicinal plants are considered crucial for the expansion of life-saving medications. Secondary metabolites found in plants show various biological properties, comprising antioxidant, anti-apoptotic, anti-aging, anti-carcinogenic, anti-inflammatory, anti-atherosclerotic, cardiovascular protective, angiogenesis inhibition, and cell proliferation activity (DS Fabricant & NR Farnsworth, 2001). *Solanum diphyllum* L. shows anti-inflammatory, anticancer, antiulcer, antioxidant, antimicrobial, antiinfection, anti-alpha amylase, anti-alpha glucosidase, and immuno-modulatory activities and also exhibited encouraging cytotoxic effects on colon and breast cancer cell lines, which demonstrates its potential as an important reservoir of anticancer compounds (MA El-Sayed et al.,2009).

Objectives

- To conduct a comprehensive phytochemical screening of *Solanum diphyllum* L. using solvent extracts derived from methanol, n-hexane, ethyl acetate, and water.
- To quantitatively determine the concentrations of key secondary metabolites, specifically total phenols and total alkaloids
- To evaluate the fundamental composition of *Solanum diphyllum* L. by assessing percentage of moisture, overall ash composition, fiber content, acid-insoluble ash, and water-soluble ash, thereby elucidating its nutritional and physicochemical properties.

Data and Methodology

Collection plant material

Plant material of *Solanum diphyllum* L. was obtained from Hanging Garden located in Simla Nagar, Malabar Hill, Mumbai, Maharashtra (N 18° 57' 25.596", E 72° 48' 17.2872"). The plant material taxonomically Classified and confirmed in the Blatter herbarium at the Department of Botany by Dr. Pravin V. Kale, St. Xavier's college, Mumbai. The entire plant sample was dehydrated in exposure to air in the shade at ambient temperature and subsequently subjected to an oven at 40ºC to eliminate moisture. The dried plant was then finely grounded, sieved and stored in airtight containers.

Phytochemical profiling

Phytochemical profiling of *Solanum diphyllum* L. involved using established methods to detect various bioactive compounds. Different tests were conducted to examine the phytochemical content of extracts obtained from methanol, n-hexane, ethyl acetate, and water. Solvents were chosen according to their ability to extract efficiently and their polarity to maximize yield. The tests aimed to identify Sugars, peptides, lipids, and amino acids, Steroids, Glycosides, Terpenoids, Saponin, Flavonoids, Alkaloids, Phenols, and Tannins within the plant material (RNS Yadav et.al.,2011; Ayoola et al.,2008; Harborne,1998).

Evaluation of carbohydrates

Benedict's examination

A reddish-brown precipitate formed when the extract was heated with two milliliters of Benedict's reagent, indicating the presence of carbohydrates.

Test of Fehling solutions

One milliliter of Fehling's solutions A and B were added to two milliliters of the test solution. After that, the mixture was boiled for five to ten minutes. Carbohydrates were present because a brick-red precipitate formed.

Evaluation of amino acids

Test for ninhydrin

Two milliliters of a 0.2% ninhydrin solution were used to boil the extract. The presence of amino acids was indicated by the appearance of a violet color.

Evaluation of lipids

Sudan 4 test

A few drops of Sudan IV were added to the test solution, shaken thoroughly, and then water was added. Lipids were present because a reddish-stained oil layer

formed.

Evaluation of protein

Xanthoproteic examination
A few drops of concentrated nitric acid (HNO_3) were added to the extract. Proteins were present when a yellow tint appeared.

Evaluation of tannins

Braymer's examination
A few drops of a 1% ferric chloride ($FeCl_3$) solution were added to the extract. Tannins were present when a blue or greenish tint appeared.

Evaluation of alkaloids

Wagner's examination
A few drops of Wagner's reagent (iodine solution in potassium iodide) were added to the extract. Alkaloids were present because a reddish-brown precipitate formed.

The Dragendroff test
A few drops of Dragendroff's reagent were added to the extract. Alkaloids were present because a reddish-brown precipitate formed.

Evaluation of glycosides

The Liebermann test
After combining the extract with acetic acid and chloroform, concentrated H_2SO_4 was added along the side of the test tube. Glycosides were indicated by a blue-green ring at the junction.

The Keller-Kiliani test
The extract was subjected to concentrated H_2SO_4 after being treated with glacial acetic acid and ferric chloride in the Keller-Kiliani test. The presence of glycosides was indicated by a reddish-brown ring at the interface.

Evaluation of saponin

Test for foam
Water was used to vigorously shake the extract. Saponins were present because of the persistent foam formation.

Evaluation of flavonoids

Test with an alkaline reagent

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Indicating flavonoids, the extract turned yellow when NaOH was added and turned colorless when diluted acid was added.

Test for lead acetate
Lead acetate solution was used to treat the extract. Flavonoids were present because a yellow precipitate formed.

Evaluation of terpenoids

Salkowski's examination
Salkowski's test involved combining the extract with concentrated H_2SO_4 and chloroform. Steroids were visible at the interface as a reddish-brown tint.

Evaluation of phenols

Test for ferric chloride
A few drops of a 10% $FeCl_3$ solution were added to the extract. Phenolic compounds were present when a blue, green, or purple hue appeared

Evaluation of steroids

The Burchard test by Liebermann
Acetic anhydride and concentrated H_2SO_4 were used to treat the extract. Steroids or sterols were present when a blue-green tint developed.

Estimation of Phenols
The Folin-Ciocalteu reagent, as outlined by Slinkard and Singleton (1977), was used to measure the concentration of total phenolics in water extracts. Gallic acid was used as the external calibration standard. The solution was made by vigorously mixing 0.1 ml of the extract with 0.8 ml of DW and 0.1 ml of Folin-Ciocalteu reagent. Three milliliters of 2% Na2CO3 were added after three minutes of shaking. After that, the mixture was left to stand for two hours at room temperature. A UV-VIS spectrophotometer was used to measure the absorbance at 760 nm. Using a calibration curve derived from Gallic acid, the total phenolic content was expressed as Gallic acid equivalents.

Estimation of Alkaloids
The gravimetric method was used to identify the alkaloids present in the leaf, stem, and root. One liter of 10% acetic acid in ethanol was added to a 500-milliliter conical flask containing a 200-gram sample of plant material. Before being filtered, the mixture was covered and allowed to stand for four hours. To precipitate the alkaloids, concentrated aqueous ammonium hydroxide was added drop wise to the filtrate, which had been concentrated to a quarter of its initial volume on a water bath set at 60°C. After centrifuging the mixture, the precipitate was gathered and cleaned with 15% ammonium hydroxide. After 30

minutes of oven drying at 60°C, the alkaloid precipitate was weighed. According to Nimenibo-Uadia et al. (2017), the alkaloid content was computed as a percentage of the initial sample weight.

Proximate analysis

Ayurvedic pharmacopeia methods were used to determine the proximate composition in order to determine moisture, crude fiber, total ash, acid insoluble ash, water soluble ash, and total solid content.

Results and Discussion

One essential technique for locating bioactive substances is phytochemical analysis. It is a quick, affordable, and simple method for identifying different classes of phytochemicals found in plants. Phytochemicals indicate that a plant may hold promise as a potential reservoir of precursor compounds for drug development (GA Ayoola et al., 2008). Plant synthesizes a large range of metabolites, both primary and secondary with different functional groups. Phytochemicals suggests both physiological and medicinal activities. The qualitative phytochemical profiling of *Solanum diphyllum* L. was studied as mentioned in Table.1. (Here, PP = moderately to high, P = Low, A = absent or negligible, L=Leaf, S= Stem, R=Root)

Sr.No.	**Primary & Secondary metabolites**	**Phytochemical tests**	**Water**			**Methanol**			**n Hexane**			**Ethyl acetate**		
			L	**S**	**R**	**L**	**S**	**R**	**L**	**S**	**R**	**L**	**S**	**R**
1	Carbohydrates	Fehling's sol.test	P	P	P	P	P	A	P	A	A	A	A	A
		Benedict's test	P	A	P	P	A	A	A	A	A	A	A	A
2	Amino acids	Ninhydrin test	P	P	P	P	A	A	A	A	A	A	A	A
3	Lipids	Sudan 4 test	A	A	A	A	A	A	A	A	A	A	A	A
4	Proteins	Xenthoproteic test	A	A	A	A	A	A	A	A	A	A	A	A
5	Tannins	Braymer's test	P	A	A	P	A	A	A	A	A	A	A	A
6	Alkaloids	Dragendroff's test	PP	P	P	PP	P	A	P	A	A	P	A	A
		Wagner's reagent	PP	A	P	P	P	A	P	A	P	A	A	P

		Liebemann's test	A	A	A	P	P	A	A	A	A	A	A	A
7	Glycosides	KellerKiliani test	P	A	A	A	A	A	P	A	A	A	A	A
8	Saponin	Foam test	P	P	A	A	A	A	A	A	A	A	A	A
		Lead acetate test	P	P	A	A	P	P	A	A	A	A	A	A
9	Flavonoids	Alkline reagent test	P	P	P	A	P	P	A	A	A	A	A	A
10	Terpenoids	Salkowski's test	P	A	A	A	A	A	A	A	A	A	A	A
11	Steroids	Liebermann's Burchard	A	A	A	A	A	A	A	A	A	A	A	A
12	Phenols	Ferric chloride test	A	A	A	P P	P	A	A	A	A	P	A	A

Table.1: Phytochemical profiling of Solanum diphyllum L.

Phytochemical profiling of the plant samples showed that water and methanol extracts had more primary and secondary metabolites than hexane and ethyl acetate.

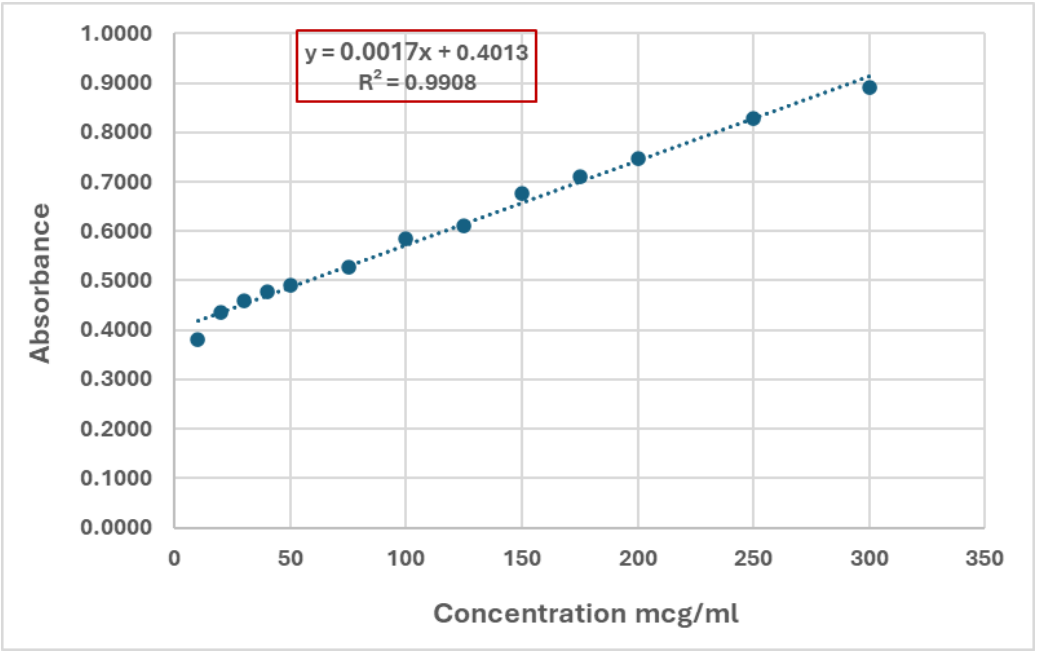
Sample	**y value**	**Regression formula**	**Conc. in 50ml/gm extract (mg)**
Leaf	0.6525	y = 0.0017x P 0.4013	73.88
Stem	0.4415		11.82
Root	0.3715		8.764

Table.2: Estimation of phenols of *Solanum diphyllum* L.

Phenolic compounds are critical secondary metabolites in plants, known for their anti-hyperglycemic, anti-inflammatory properties and inhibit the development of diabetes. Since phenols have been shown to donate hydrogen atoms or electrons, neutralize free radicals, and prevent oxidative stress, the higher phenolic content is consistent with improved antioxidant activity (MA El-Sayed et al.,2009). A significant range in phenolic concentrations was found in the most recent quantitative assessment of *Solanum diphyllum* L.'s total phenol content using Folin-Ciocalteu reagent assays. The leaves had the highest phenolic content of

any part examined, followed by the stem and the root.

Figure 1: Calibration curve of Gallic acid



The standard curve for measurement, shown in Figure 1, follows the regression equation y = 0.0017x P 0.4013 with an R^2 value of 0.9908, indicating a strong linear relationship. This standard curve equation is represented as y = mx P c, where m = 0.0017 and c = 0.4013.

Sample	**Calculation formula**	**Alkaloids (g %)**
Leaf	(Weight of Alkaloid / Weight of Sample) × 100 %	4.34%
Stem		3.10%
Root		0.92%

Table.3: Estimation of alkaloids of Solanum diphyllum L.

Solanum diphyllum L. contains alkaloids with a variety of pharmacological properties, such as analgesic, antibacterial, and anticancer effects. These bioactive substances help to reduce oxidative stress, induce cytotoxic reactions in cancerous cells, inhibit the growth of harmful microorganisms, and lessen inflammatory and nociceptive disorders (MA El-Sayed et al.,2009). Results obtained from the quantitative evaluation of alkaloids (Table 3) showed the leaf has the highest percentage of alkaloids, the stem and the root having lower levels.

Sr. no	Experimental Studies	Observations for powdered of *Solanum diphyllum* L.		
		Leaf	Stem	Root
1.	Total ash value	14.05±0.18	8.66±0.23	7.41±0.23
2.	Acid insoluble ash value	0.32±0.06	0.40±0.02	0.88±0.29
3.	Water soluble ash value	3.97±0.29	3.28±0.2	1.24±0.16
4.	Moisture content	78.63±0.94	63.32±2.24	56.82±0.35
5.	Total solid content	21.37±0.06	36.68±1.23	43.18±0.65
6.	Crude fiber content	10.67±0.09	19.89±0.34	31.2±0.25

Table.3: proximate composition of Solanum diphyllum L.

The proximate composition of *Solanum diphyllum* L. (leaf, stem, and root) reveals significant variations in moisture, ash, and solid content. The leaf has the highest moisture content, followed by the stem, with the root showing the lowest moisture. This suggests that the root is less prone to microbial spoilage if properly dried. The leaf has the highest total ash content, indicating a higher concentration of minerals, while the root contains a moderate level. The acid-insoluble ash is highest in the root, suggesting stronger mineral resistance to digestion. Water-soluble ash is highest in the leaf, which may enhance nutrient bioavailability. The root has the highest total solid content, indicating greater solid material for therapeutic use. The root's high fiber is likely beneficial for digestive health, weight management, and prevention of diseases such as cancer, diabetes, and cardiovascular disorders. The composition of *Solanum diphyllum* L. highlights its potential nutritional and therapeutic value.

Conclusion

The phytochemical investigation revealed the presence of carbohydrates, alkaloids, tannins, phenols, terpenoids, saponin and flavonoids bioactive compounds recognized for their diverse physiochemical and pharmacological effects. Their presence supports the traditional medicinal use of *Solanum diphyllum* L. in managing various health conditions. In addition to confirming conventional applications, this thorough biochemical profiling offers a strong basis for upcoming studies that seek to identify active ingredients and comprehend their modes of action in order to possibly develop pharmaceuticals. Additionally, proximate analysis highlighted significant nutritional properties, including high moisture, ash, and fiber content, which may enhance the plant's

therapeutic efficacy.

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Diversity Of Corticioid Fungi Belonging to The Family Peniophoraceae in District Chamba of Himachal Pradesh

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Abstract

The aim of the present chapter is to give an account of family Peniophoraceae from district Chamba (H.P.). Total 12 taxa belonging to 3 different genera have been reported from district Chamba (H.P.). These 12 taxa include *Gloiothele citrina, Peniophora cinerea, P. incarnata, P. lycii, P. limitata, P. ovalispora, P. pithya, P. pseudoversicolor, P. rufomarginata, P. suecica, P. violaceolivida* and *Metulodontia nivea*. Of the taxa named highlighted in bold were described by the author (Poonam et al. 2020) as new addition to corticioid mycoflora of India, while (*P. incarnata, P. lycii, P. limitata, P. ovalispora, P. rufomarginata and P. violaceolivida* were described for the first time from Himachal Pradesh (Poonam et al. 2020). *Gloiothele citrina* is described for the first time from district Chamba of Himachal Pradesh.

Keywords: Basidiomycota, Peniophoraceae Western Himalayas, Chamba, Corticioid Fungi.

Introduction

The Corticioid fungal family Peniophoraceae is distinguished by its members resupinate, adnate, effused, reflexed to orbicular to confluent; hymenial surface smooth to granulose to tuberculate, sometimes cracked. Hyphal system monomitic. Generative hyphae with or without clamps, subhyaline to light brown. Ancillary elements present. Basidia clavate to subclavate, with or without clamp at the base, 4–sterigmate. Basidiospores allantoid to suballantoid to subcylindrical to ellipsoid to broadly ellipsoid to subglobose to globose, smooth, positive/negative to Melzer Reagent and negative to Cotton Blue.

During the fungal forays conducted in various sub divisions of Chamba district in the years 2013-2018 many specimens of family Peniophoraceae were collected based on macro and microscopic morphological characteristics and comparison to the literature, these were identified and described as 12 taxa belonging to 3 genera of the family Peniophoraceae (Rehill and Bakshi,1965; Thind and Rttan 1968; Rattan,1977; Eriksson,1981; Dhingra,1993; Natarajan and

Kolandavelu,1998; Bhosle,2005; Dhingra and Kaur, 2005; Singh, 2007 ; Andreasen and Hallenberg,2009; Bernicchia and Gorjón, 2010; Dhingra, 2011; Ranadive, 2011; Ranadive,2013; Sharma, 2012; Kaur, 2012; Prashar and Ashok,2013; Prashar and Lalita, 2013; Samita and Dhingra;2013; Samita, 2014; Dhingra.,2014; Poonam et al. , 2017; Kaur, 2012; Devi, 2019; MycoBank, 2025.The present chapter provides an account of Family Peniophoraceae from Chamba district of Himachal Pradesh, based on 12 taxa spread over three genera. These 12 taxa include *Gloiothele citrina, Peniophora cinerea, P. incarnata, P. lycii, P. limitata, P. ovalispora, P. pithya, P. pseudoversicolor, P. rufomarginata, P. suecica and P. violaceolivida* and *Metulodontia nivea*.Of the twelve taxa described highlighted in bold were described by the author (Poonam et al. 2020) as new addition to corticioid mycoflora of India, while (*P. incarnata, P. lycii, P. limitata, P. ovalispora, P. rufomarginata and P. violaceolivida* were described for the first time (Poonam et al. 2020) from Himachal Pradesh. *Gloiothele citrina* is described for the first time from district Chamba of Himachal Pradesh.

Materials And Methods

Present studies are based on the collections made from different localities of district Chamba (Himachal Pradesh) during fungal forays conducted in various sub divisions of Chamba district in the years 2012-2018. The sporophores were collected along with a portion of the substrate with the help of a hammer and a chisel. The details pertaining to type of hymenial surface, colour, margins etc., were noted carefully with the help of a hand lens. A moist piece of the sporophore was used to get the spore print on a glass slide. These speciemens were dried either in sun or using an electric drier. The dried sporophores were packed in bond paper envelops carrying a standard herbarium label with required information. All the specimens have been deposited at the Herbarium, Department of Botany, Punjabi University, Patiala (PUN). The micromorphological details of the collected specimens were observed by making crush mounts/vertical sections of the sporophores in water, 3% KOH solution, 1% pholxine, 1% congo red, 1% cotton blue and Melzer's reagent (0.5gm Iodine + 1.5gm KI + 20gm Chloral hydrate + 20ml Distilled water). The outline of the microscopic structures was drawn with the help of a camera lucida mounted on compound microscope at 100X, 400X and 1000X magnifications. All specimens have been deposited at the Herbarium, Department of Botany, Punjabi University, Patiala (PUN). The colour citations are as per Kornerup and Wanscher (1978).

Family – Peniophoraceae Lotsy, Vorträge über botanische Stammesgeschichte: 687, 689 (1907).

Sporophores resupinate, adnate, effused, reflexed to orbicular to confluent; hymenial surface smooth to granulose to tuberculate, sometimed cracked. Hyphal system monomitic. Generative hyphae with or without clamps, subhyaline to light brown. Ancillary elements present. Basidia clavate to subclavate, with or without clamp at the base, 4–sterigmate. Basidiospores allantoid to suballantoid to subcylindrical to ellipsoid to broadly ellipsoid to subglobose to globose, smooth, positive/negative to Melzer Reagent and negative to Cotton Blue.

Key to the genera:

1. Generative hyphae without clamps.....……..…….....……... Gloiothele
1.Generative hyphae with clamps…….....2
2. Cystidia usually long, basidiospores ellipsoid.....……..... Metulodontia
2. Cystidia not as above, basidiospores allantoid to suballantoid to subcylindrical to ellipsoid to broadly ellipsoid to subglobose to globose ……....Peniophora

Gloiothele Bres., Annales Mycologici 18: 44, (1920).

Sporophores resupinate, adnate, effused; hymenial surface, smooth. Hyphal system monomitic. Generative hyphae without clamps. Ancillary elements present. Basidia clavate to subclavate, 4–sterigmate, without clamp at the base. Basidiospores globose to subglobose, smooth, thin-walled, positive to MR and negative to CB, with or without oily contents.

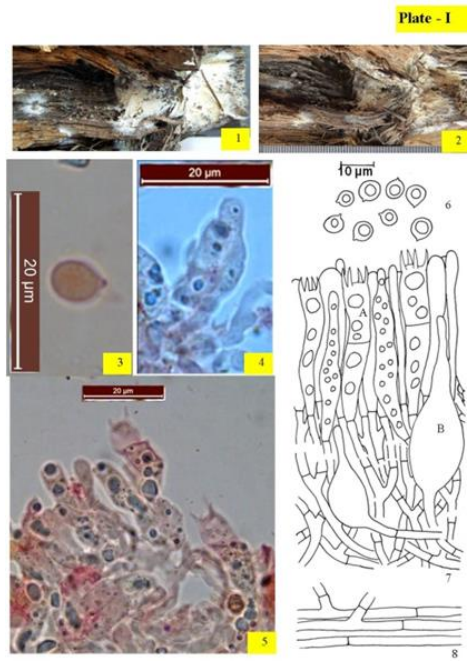
1. ***Gloiothele citrina*** (Pers.) Ginns & Freeman, Biblioth. Mycol., 157: 55, (1994). -*Thelephora citrina* Pers., Mycol.Eur.,1:136(1822**).** **Plate I**

Sporophores resupinate, effused, adnate, up to 280 µm thick in section; hymenial surface smooth both in fresh and dry states; orange white when fresh and orange white to pale orange on drying; margins fibrillose, paler concolorous when determinate. Generative hyphae ≤ 2.8 µm wide, subhyaline, simple septate, thin-walled, smooth; horizontal, less branched in the subicular zone; vertical, richly branched in the subhymenial zone. Ancillary elements of 2 kinds: (i) cystidia vesicular to subfusiform, tapering towards apex, without basal clamp, 52–65×6.6– 16 µm, thin-walled, without oily contents. (ii) *Gloeocystidia hyphoid* to subfusiform, without basal clamp, 40–45 × 5–7 µm, thin-walled. Basidia clavate to subclavate, with oily contents, 37–50 × 6.1– 7.2 µm; sterigma ≤ 5 µm long. Basidiospores 5.4–7.2 × 4.5–6.3 µm, globose to subglobose, thin-walled, smooth, acyanophilous, amyloid.

Collection examined: India, Himachal Pradesh: Chamba, Pangi, Sural, on stump of Betula utilis, Poonam 10182 (PUN), September 13, 2016.

Remarks: This species is being reported for the first time from district Chamba. It was earlier reported from India by Thind & Rattan (1968) district Kullu (Himachal Pradesh); Bhosle (2005) & Ranadive et al. (2011) Maharashtra; Kaur (2012) and Kaur (2018) District Shimla (Himachal Pradesh); Dhingra et al.

(2014) Districts Kullu and Shimla (Himachal Pradesh); Sharma (2012) and Samita (2014) Uttarakhand, Sharma (2017) Jammu & Kashmir.



Figs. 1– 8. *Gloiothele citrina*: 1-2. Sporophore showing hymenial surface (1. Fresh, 2. Dry); 3-5. Photomicrographs (3. Basidiospores, 4. Cystidium, 5. Basidia); 6. Basidiospores; 7. Reconstruction showing a portion of hymenium and subhymenium (*A. Basidium, B. Cystidium*); 8. Generative hyphae.

Metulodontia Parmasto, Conspectus Systematis Corticiacearum: 117 (1968).
Sporophores resupinate, adnate, effused, ceraceous; hymenial surface smooth to tuberculate to grandiniod. Hyphal system monomitic. Generative hyphae with clamped septa, thin- to thick-walled. Ancillary elements (long, encrusted cystidia) present. Basidia clavate to narrowly clavate, with clamped septa at the base, 4–sterigmate. Basidiospores ellipsoid, smooth, thin-walled, negative to both Melzer Reagen and Cotton Blue, with or without oily contents.

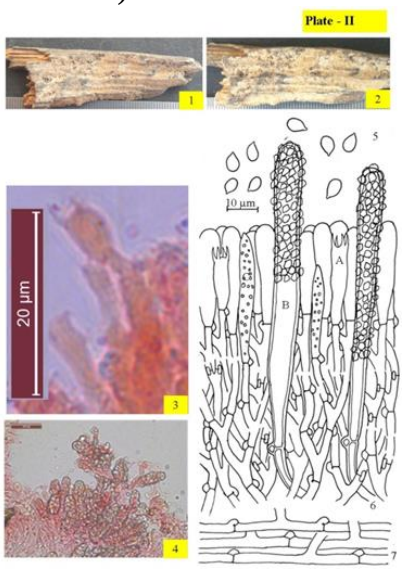
2. *Metulodontia nivea* (P. Karst.) Parmasto, Conspectus Systematis Corticiacearum: 118 (1968). - *Kneiffia nivea* Karst., Hedwigia 35: 173 (1896). **Plate II**

Sporophores resupinate, effused, adnate, up to 320 µm thick in section; hymenial surface smooth, mealy both in fresh and dry states; light orange to grayish orange to brownish orange both in fresh and dry states; fibrillose, whitish to paler concolorous. Generative hyphae ≤ 3.3 µm wide, subhyaline, septate, clamped, thin-walled, smooth; horizontal, less branched in the subicular zone; vertical, richly branched in the subhymenial zone. Ancillary elements of 2 kinds: (i) Cystidia cylindrical, with basal clamp, 92–104 × 8–10 µm, thick-walled, heavily

encrusted; projecting up to 40 µm out of the hymenium. (ii) Gloeocystidia fusiform, with basal clamp, 43–54 × 3.3–5.5 µm, with oily contents negative to sulphovanillin; embedded in the hymenium.Basidia clavate to subclavate, usually sinuous, 17–27 × 3.8– 6.1 µm; sterigma ≤ 4 µm lon. Basidiospores 4.6–7.2 × 4–4.8 µm, ellipsoid to broadly ellipsoid, thin-walled, smooth, acyanophilous, inamyloid.

Collections examined: India, Himachal Pradesh: Chamba, Dalhousie, Ahla, on log of *Cedrus deodara*, Poonam 10372 (PUN), October 13, 2012; Churah, Bhandal on sticks of Pinus wallichiana, Poonam 10373 (PUN), August 15, 2014 Kohlari on stump of *Cedrus deodara*, Poonam 10374 (PUN), August 29, 2014; Kohlari on stump of *Cedrus deodara*, Poonam 10375 (PUN), August 29, 2013; Churah, Bhandal on sticks of Rosa moschata, Poonam 10376 (PUN), August 15, 2014; Pangi, Sural, on sticks of Betula utilis, Poonam 10377 (PUN), September 13, 2016.

Remarks: Metulodontia nivea is being described for the first time from tehsil Churah. Earlier it was reported from India by Rattan (1977), districts Chamba, Shimla and Kullu (Himachal Pradesh); Singh (2007) district Kullu (Himachal Pradesh) Kaur (2012) district Sirmaur (Himachal Pradesh); Sharma (2012) Uttarakhand; Kaur (2018) district Shimla (Himachal Pradesh); Devi (2019), district Kangra (Himachal Pradesh).



Figs. 1–7. *Metulodontia nivea* :1-2. Sporophore showing hymenial surface (1. Fresh, 2. Dry); 3-4. Photomicrographs (3. Basidium 4. Metuloids); 5-7. Line diagrams [5. Basidiospores; 7. Reconstruction showing a portion of hymenium and subhymenium (*A. Basidium, B. Leptocystidium, C. Metuloid* 7. Generative hyphae

Peniophora Cooke, Grevillea 8 (45): 20 (1879).

Sporophores resupinate, adnate, orbicular to confluent to effused, sometimes reflexed at the margins; hymenial surface smooth to tuberculate, often cracked. Hyphal system monomitic. Generative hyphae with or without clamps, thin- to thick-walled, subhyaline to light brown. Ancillary elements (gloeocystidia, metuloids and dendrohyphidia) present or absent. Basidia clavate to subclavate, somewhat sinuous, 4–sterigmate, with or without clamp at the base. Basidiospores allantoid to suballantoid to subcylindrical to cylindrical to ellipsoid to broadly ellipsoid to subglobose, smooth, thin-walled, negative to both Melzer Reagen and Cotton Blue, with or without oily contents.

Key to the species:

1. Dendrohyphidia present …… P. lycii
1.Dendrohyphidia absent……..……..…. 2
2. Basidiospores broadly ellipsoid to subglobose……. P. ovalispora
2. Basidiospores allantoid to suballantoid to subcylindrical to cylindrical to ellipsoid.3
3. Sporophores very thin, subiculum poorly developed……....….. P. cinerea
3. Sporophores not as above, subiculum well developed.....…..... 4
4. Sporophores reddish orange to orange……..... P. pseudoversicolor
4. Sporophores differently coloured……. ….5
5. Reflexed abhymenial side of the margins blackish brown ……P. rufomarginata
5. Margins not of above type……. 6
6. Gloeocystidia subcylindrical …….....7
6. Gloeocystidia subfusiform to fusiform …….……8
7. Basidiospores cylindrical to ellipsoid to suballantoid, 7–11 × 3.4– 4. 1.... P. incarnata
7. Basidiospores shorter, allantoid to suballantoid, 7.7–8.8 × 2.8–3.3 µm..... P. violaceolivida
8. Gloeocystidia positive to sulphovanillin…….…….…....…… 9
8. Gloeocystidia negative to sulphovanillin……..…….....…… P. suecica
9. Subiculum well developed..... P. limitata
9. Subiculum not well developed …….....…..……..… P. pithya

3. *Peniophora cinerea* (Pers.) Cooke, Grevillea 8(45): 20 (1879). - *Corticium cinereum* Pers., Neues Mag. Bot. 1: 111 (1794).

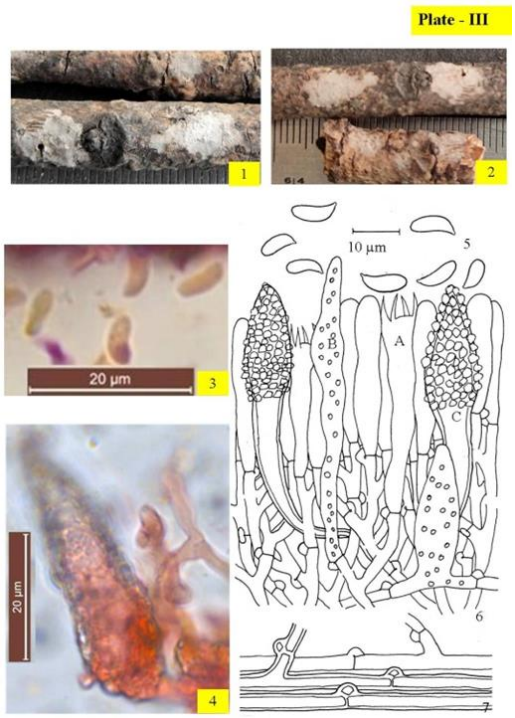
Plate III

Sporophores resupinate, effused, adnate, up to 160 µm thick in section; hymenial surface smooth, granulose both in fresh and dry states; yellowish white when fresh, grayish orange on drying; margins thinning, paler concolorous. Hyphal system monomitic. Generative hyphae septate, clamped, smooth; light brown,

horizontal, ≤ 3.2 µm wide, less branched, thin- to thick-walled in the subicular zone; vertical, subhyaline, ≤ 2.5 µm wide, richly branched, thin-walled in the subhymenial zone. Ancillary elements of 2 kinds: (i) Gloeocystidia usually sub fusiform, with basal clamp, 28–70 × 9–7.2 µm, thin-walled, with oily contents negative to sulphovanillin; projecting 20 µm out of the hymenium (ii) Metuloids conical to subfusiform, with basal clamp, 33–44 × 9.4–12 µm, thick-walled, heavily encrusted; projecting up to 25 µm out of the hymenium. Basidia clavate to subclavate, sinuous, 26–40 × 5–6.1 µm; sterigma ≤ 5 µm long. Basidiospores 6.5–9 × 2.8–3.3 µm, allantoid to suballantoid, thin-walled, smooth, acyanophilous, inamyloid.

Collections examined: India, Himachal Pradesh: Chamba, Hardaspura, on sticks of *Adhatoda vasica*, Poonam 10475 (PUN), September 3, 2014; Udaipur, on sticks of *Adhatoda vasica*, Poonam 10476 (PUN), September 4, 2014; Dalhousie, Kalatop, on sticks of *Pinus roxburghii*, Poonam 10477 (PUN), September 3, 2014; Kihar, Churah, on sticks of *Rosa macrophylla*, Poonam 10478 (PUN), September 04, 2016.

Remarks: This species is being described for the first time from tehsils Bharmour and Churah. Earlier it was reported from India by [Rehil and Bakshi (1965), Dhingra and Navneet (2005)] from Uttarakhand; Rattan (1977) District Chamba and Kullu (Himachal Pradesh) and Jammu & Kashmir; Bhosle et al.



(2005), Ranadive et al. (2011) and Ranadive (2013) Maharashtra; Kaur (2012) District Sirmaur (Himachal Pradesh); Dhingra et al. (2014) Districts Chamba, Kullu and Sirmaur (Himachal Pradesh); Samita (2014) Uttarakhand; Kaur (2017) Punjab and Sharma (2017) Jammu & Kashmir.

Figs. 1– 7. *Peniophira cinerea*:1-2. Sporophore showing hymenial surface (1. Fresh, 2. Dry); 3-4. Photomicrographs (3. Basidiospores, 4. Metuloid) 5-7. Line diagrams [5. Basidiospores; 6. Reconstruction showing a portion of hymenium and subhymenium (A. Basidium, B. Gloeocystidium, C. Metuloid); 7. Generative hyphae]

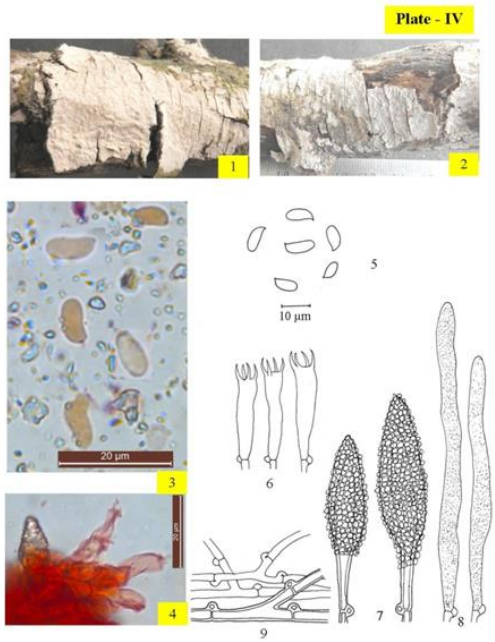
4. *Peniophora incarnata* (Pers.) P. Karst., Hedwigia 28: 27 (1889).- Thelephora incarnata Pers., Synopsis methodica fungorum: 573 (1801). **Plate IV**

Sporophores resupinate, effused, adnate, membranous, up to 280 µm thick in section; hymenial surface tuberculate, cracked both in fresh and dry states; orange white to pale orange when fresh, orange white on drying; margins thinning, paler concolorous, Hyphal system monomitic. Generative hyphae subhyaline, septate, clamped, smooth; horizontal, ≤ 5 µm wide, less branched, thick-walled in the subicular zone; vertical, ≤ 3 µm wide, richly branched, thin-walled in the subhymenial zone. Ancillary elements of 2 kinds: (i) Gloeocystidia subcylindrical, with basal clamp, 88–106 × 6.6–7.2 µm, thin-walled, with oily contents negative to sulphovanillin; projecting up to 30 µm out of the hymenium thin-walled, with oily contents negative to sulphovanillin; projecting 20 µm out of the hymenium (ii) Metuloids conical to subfusiform, with basal clamp, 60–74 × 8.9–12 µm, thick-walled, heavily encrusted; projecting 20 µm out of the hymenium. Basidia clavate to subclavate, somewhat sinuous, 29–33 × 6.1–6.7 µm, sterigma ≤ 5.5 µm long. Basidiospores 7–11 × 3.4– 4.1 µm ellipsoid to subcylindrical to suballantoid, thin-walled, smooth, acyanophilous, inamyloid.

Collections examined: India, Himachal Pradesh: Chamba, Hardaspura, Aadarsh Nagar, on branch of Robenia pseudoacacia, Poonam 9181 (PUN), November 04, 2015; Hardaspura, on sticks of Robenia pseudoacacia, Poonam 10485 (PUN), November 04, 2015.

Remarks: *P. incarnata* was described for the first time from the state of Himachal Pradesh by author (Poonam et al. 2020). Earlier it was reported from India by Rehill and Bakshi (1965) from different Indian localities; Natarajan and Kolendavelu (1998) from Tamil Nadu and Sharma (2012) from Uttarakhand.

Figs. 1– 9. *Peniophora incarnata* :1-2. Sporophore showing hymenial surface (1. Fresh, 2. Dry); 3-4. Photomicrographs (3. Basidiospores, 4. Metuloid); 5-9. Line diagrams [5. Basidiospores; 6. Basidia, 7. Metuloids, 8. Gloeocystidia; 9. Generative hyphae]



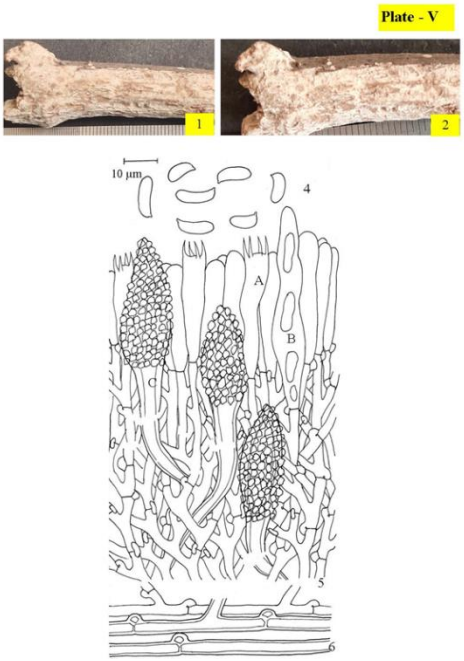
5. ***Peniophora limitata*** (Chaillet ex Fr.) Cooke, Grevillea, 8(45): 21 (1879). - *Thelephora limitata* Chaillet ex Fr., Elench. fung (Greifswald) 1: 222 (1828). Plate V

Sporophores resupinate, effused, adnate, up to 280 µm thick in section; hymenial surface smooth both in fresh and dry states; reddish white to pale red both in fresh and dry states; margins thinning, paler concolorous, Hyphal system monomitic. Generative hyphae septate, clamped, smooth; ≤ 4.1 µm wide, light brown, thin- to thick-walled, less branched in the subicular zone; ≤ 3.2 µm wide subhyaline, vertical, richly branched, thin-walled in the subhymenial zone. Ancillary elements of 2 kinds: (i) Gloeocystidia usually subfusiform to fusiform, with basal clamp, 55–62 × 8.3–9.9 µm, thin-walled, with oily contents negative to sulphovanillin; projecting 20 µm out of the hymenium (ii) Metuloids conical to subfusiform, with basal clamp, 41–54 × 12–14.4 µm, thick-walled, heavily encrusted; projecting up to 10 µm out of the hymenium. Basidia subclavate, sinuous, 44–50 × 11–13 µm; sterigma ≤ 6.6 µm long. Basidiospores 8.3–12 × 3.2–4 µm, allantoid to suballantoid, thin-walled, smooth, acyanophilous, inamyloid.

Collection examined: India, Himachal Pradesh, Chamba, Udaipur, on stick of Adhatoda vasica, Poonam 10471 (PUN), September 6, 2015.

Remarks: *Peniophora limitata* was described as new addition to corticioid mycoflora of district Chamba by author (Poonam et. al 2020). Previous reports from India are by Dhingra (1993), Dhingra et al. (2011) from Eastern Himalaya

and Devi (2019) from district Kangra (Himachal Pradesh).



Figs. 1– 5. *Peniophora limitata* :1-2. Sporophore showing hymenial surface (1. Fresh, 2. Dry); 3-6. Line diagrams [3. Basidiospores; 4. Reconstruction showing a portion of hymenium and subhymenium (A. Basidium, B. Gloeocystidium, C. Metuloid); 5. Generative hyphae]

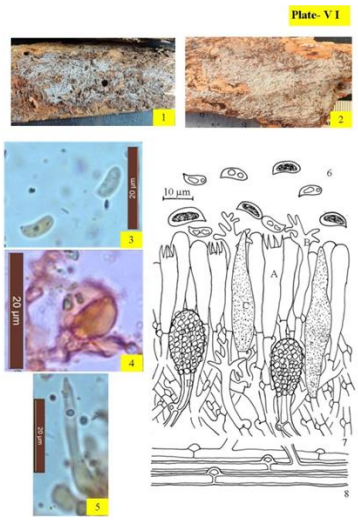
6. ***Peniophora lycii*** (Pers.) Höhn. & Litsch., Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften Math. -naturw. Klasse Abt. I 116: 747 (1907). -*Thelephora lycii* Pers., *Mycologia Europaea* 1: 148 (1822). Plate VI

Sporophores resupinate, effused, adnate, up to 200 µm thick in section; hymenial surface smooth both in fresh and dry states; yellowish gray to bluish gray when fresh, yellowish gray on drying; margins pruinose, paler concolorous, Hyphal system monomitic. Generative hyphae septate, clamped, smooth; light brown, thin- to thick-walled, horizontal, ≤ 3.1 µm wide, less branched in the subicular zone; subhyaline, vertical, ≤ 2.5 µm wide, richly branched, thin-walled in the subhymenial zone. Ancillary elements of 3 kinds: (i) Gloeocystidia fusiform, with basal clamp, 39–53 × 7–8.8 µm, thin-walled, with oily contents positive to sulphovanillin; embedded in the hymenium (ii) Metuloids obovate to oblong, with basal clamp, 24–29 × 9.2–11.6 µm, thick-walled, with crystalline deposits; embedded in the hymenium (iii) Dendrohyphidia richly branched in the apical region, with basal clamp, 36–50 × 3.3–4.4 µm, thin-walled. Basidia subclavate, sinuous, 21–32 × 5–6.6 µm; sterigma ≤ 5.5 µm long. Basidiospores 7.7–12.5 ×

3.8–5.5 µm, allantoid to suballantoid, thin-walled, smooth, acyanophilous, inamyloid.

Collections examined: India, Himachal Pradesh, Chamba, Churah, Bhandal, on stump of Picea smithiana, Poonam 10102 (PUN), August 15, 2014; Churah, Bhandal, on stump of Picea smithiana, Poonam 10741 (PUN), August 15, 2014.

Remarks: Peniophora lycii, was described as new record for India by the author (Poonam et al. 2020).



Figs. 1– 8. Peniophora lycii :1-2. Sporophore showing hymenial surface (1. Fresh, 2. Dry); 3-5. Photomicrographs (3. Basidiospores, 4. Metuloid, 5. Gloeocystidium); 6-8. Line diagrams [6. Basidiospores; 7. Reconstruction showing a portion of hymenium and subhymenium (A. Basidium, B. Dendrohyphidia, C. Gloeocystidium, D.Metuloid); 8. Generative hyphae]

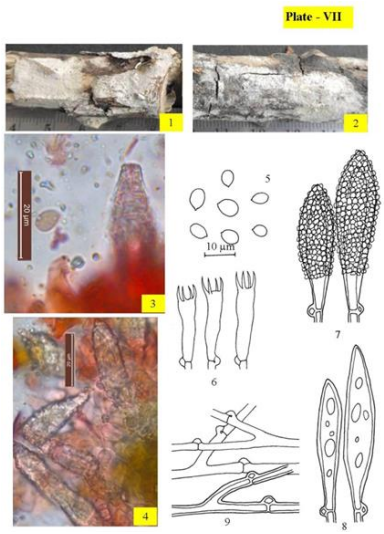
7. ***Peniophora ovalispora*** Boidin, Lanq. & Gilles, Bulletin de la Société Mycologique de France 107 (3): 108 (1991). Plate VII

Sporophores resupinate, effused, adnate, up to 240 µm thick in section; hymenial surface smooth both in fresh and dry states; orange white to reddish white both in fresh and dry states; margins fibrilliose, paler concolorous, Hyphal system monomitic. Generative hyphae septate, clamped, smooth; horizontal, light brown, ≤ 4 µm wide, less branched, thick-walled in the subicular zone; subhyaline, vertical, ≤ 3 µm wide, richly branched, thin-walled in the subhymenial zone. Ancillary elements of 2 kinds: (i) Gloeocystidia subfusiform, with basal clamp, 46–60 × 5.5–6.1 µm, thin-walled, with oily contents negative to sulphovanillin; projecting 20 µm out of the hymenium (ii) Metuloids conical to subfusiform, with basal lamp, 44–56 × 7.8–13 µm, thick-walled, heavily encrusted; projecting up to 15 µm out of the hymenium Basidia clavate to subclavate, somewhat sinuous, 21–26 × 5–6.1 µm; sterigma ≤ 5 µm long. Basidiospores 5.5–7.2 × 3.2–5.5 µm,

broadly ellipsoid to subglobose, thin-walled, smooth, acyanophilous, inamyloid.

Collections examined: India, Himachal Pradesh: Chamba, Udaipur, Chikryani on sticks of R. moschata, Poonam 9182 (PUN), September 06, 2015; Udaipur, Chikryani on sticks of Rosa moschata, Poonam 10472 (PUN), September 06, 2015.

Remarks: *P. ovalispora* was described for the first time from district Chamba (Poonam et al. 2020). Previous reports from India are from Uttarakhand (Samita, 2014) and district Kangra of Himachal Pradesh (Devi, 2019).



Figs. 1– 9. *Peniophora ovalispora* :1-2. Sporophore showing hymenial surface (1. Fresh, 2. Dry); 3-4. Photomicrographs (3. Basidiospores, 4. Metuloids); 5-9. Line diagrams [5. Basidiospores; 6. Basidia, 7. Metuloids, 8. Gloeocystidia); 9. Generative hyphae]

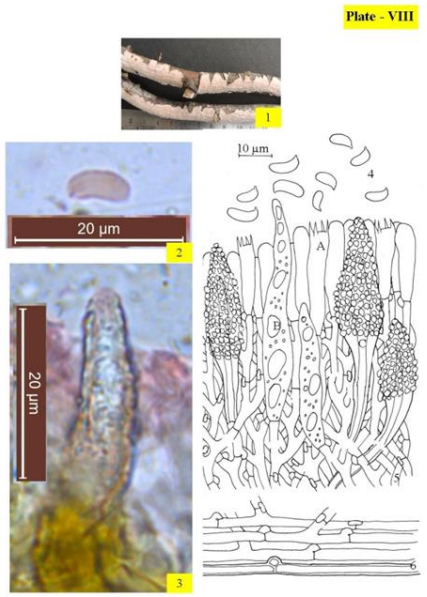
8. *Peniophora pithya* (Pers.) J. Erikss., Fungi Exsiccati Suecici Fasc. 37-38: p. 37 (1950). - Thelephora pithya Pers., Mycologia Europaea 1: 146 (1822). Plate VIII

Sporophores resupinate, effused, adnate, up to 240 µm thick in section; hymenial surface smooth both in fresh and dry states; grayish red both in fresh and dry states; margins thinning, paler concolorous, Hyphal system monomitic. Generative hyphae septate, clamped, smooth; light brown, horizontal, ≤ 3.5 µm wide, less branched, thin- to thick-walled in the subicular zone; subhyaline, vertical, ≤ 3 µm wide, richly branched in the subhymenial zone. Ancillary elements of 2 kinds: (i) Gloeocystidia subfusiform, with basal clamp, 41–62 × 7.2–9.4 µm, thin-walled, with oily contents negative to sulphovanillin; projecting up to 10 µm out of the hymenium (ii) Metuloids conical to subfusiform, with basal clamp, 35–49 × 11–14 µm, thick-walled, heavily encrusted; embedded in

the hymenium. Basidia 19–30 × 4.4–5.5 µm; sterigma ≤ 4 µm long. Basidiospores 5–9.2 × 2.1–3.5 µm, allantoid to suballantoid, thin-walled, smooth, acyanophilous, inamyloid.

Collections examined: India, Himachal Pradesh: Chamba, Dalhousie, Jandrighat, on Cedrus deodara stump, 10473 Poonam (PUN), November 05, 2013; Pangi, Sural, on stick of Betula utilus, Poonam 10474 (PUN), September 13, 2016.

Remarks: *P. pithya* is being described for the first time from tehsil Pangi in the study area. Earlier it was reported from India by Dhingra (1993) and Dhingra et al. (2011) from Eastern Himalaya; Singh (2007) and Kaur (2012) as well as Kaur (2018) and Dhingra et al. (2014) from districts Chamba and Shimla (Himachal Pradesh) respectively; Ranadive et al. (2011) and Ranadive (2013) from Maharashtra; Samita (2014) from Uttarakhand and Kaur (2017) from Punjab.



Figs. 1– 6. *Peniophora pithya*:1. Sporophore showing hymenial surface; 2-3. Photomicrographs (3. Basidiospore, 4. Metuloid); 4-6. Line diagrams [4. Basidiospores; 5. Reconstruction showing a portion of hymenium and subhymenium (A. Basidium, B. Gloeocystidium, C. Metuloid); 6. Generative hyphae]

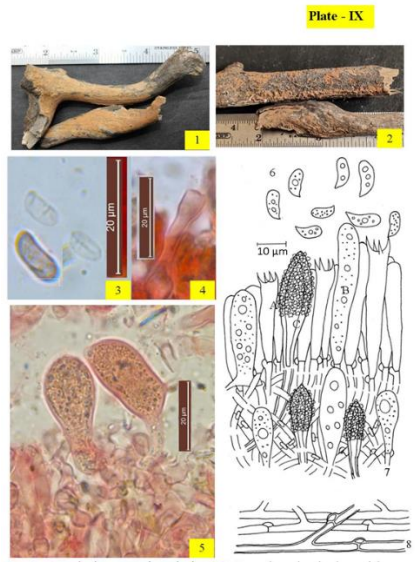
9. *Peniophora pseudoversicolor* Boidin, Bulletin Mensuel de la Société Linnéenne de Lyon 34: 162, (1965).
Plate IX

Sporophores resupinate, effused, adnate, up to 280 µm thick in section; hymenial surface smooth to somewhat tuberculate both in fresh and dry states; reddish

orange to brownish orange both in fresh and dry states; margins thinning, paler concolorous. Hyphal system monomitic. Generative hyphae septate, clamped, smooth; light brown, thin- to somewhat thick-walled, horizontal, ≤ 3.2 µm wide, less branched in the subicular zone; subhyaline, vertical, ≤ 2.5 µm wide, richly branched, thin-walled in the subhymenial zone. Ancillary elements of 2 kinds: (i) Gloeocystidia vesicular to subcylindrical, with basal clamp, 34–52 × 8.9–9.5 µm, thin-walled, with oily contents positive to sulphovanillin; projecting up to 20 µm out of the hymenium. (ii) Metuloids conical, with basal clamp, 31–45 × 6–9.5 µm, thick-walled, heavily encrusted; projecting up to 10 µm out of the hymenium. Basidia subclavate, somewhat sinuous, 27–45 × 5.5–6.1 µm; sterigma ≤ 5.6 µm long. Basidiospores 8–12 × 3.2–4.8 µm, allantoid to suballantoid, thin-walled, smooth, acyanophilous, inamyloid.

Collections examined: India, Himachal Pradesh, Chamba, Udaipur, on sticks of *Adhatoda vasica*, Poonam 7648 (PUN), September 06, 2015; Udaipur, on sticks of *Adhatoda vasica*, Poonam 10479 (PUN), September 06, 2015; Udaipur, on sticks of *A. vasica*, Poonam 10480 (PUN), September 06, 2015; Udaipur, on sticks of *Adhatoda vasica*, Poonam 10481 (PUN), September 06, 2015; Udaipur, on burnt branch of Prunu persica, Poonam 10482 (PUN), January 01, 2018.

Remarks: *P. pseudo* versicolor is a rereport for district Chamba of Himachal Pradesh. Earlier it was reported for the first time from India by Poonam et al. (2017) from district Chamba and Kaur (2018) from district Shimla of Himachal Pradesh.



Figs. 1– 8. *Peniophora pseudo* versicolor :1-2. Sporophore showing hymenial surface (1. Fresh, 2. Dry); 3-5. Photomicrographs (3. Basidiospores, 4. Basidium, 5. Gloeocystidia); 6-8. Line diagrams [6. Basidiospores; 7. Reconstruction showing a

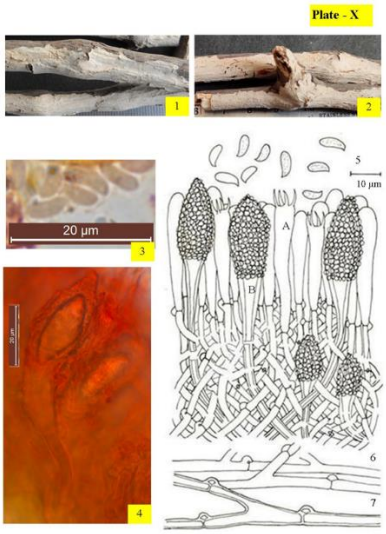
portion of hymenium and subhymenium (A. Basidium, B. Gloeocystidium, C. Metuloid); 8. Generative hyphae]

10. ***Peniophora rufomarginata*** (Pers.) Bourdot & Galzin, Bulletin de la Société Mycologique de France 28 (4): 408 (1913).- *Thelephora rufomarginata* Pers., Mycologia Europaea 1: 124 (1822).
Plate X

Sporophores resupinate, effused, adnate, occasionally reflexed at the margins, up to 400 µm thick in section; hymenial surface smooth both in fresh and dry states; orange white to reddish white when fresh, orange white to grayish orange on drying; margins thinning, paler concolorous, somewhat abrupt or reflexed, underside brownish black. Hyphal system monomitic. Generative hyphae septate, clamped, smooth; light brown, horizontal, ≤ 4 µm wide, less branched, thin- to thick-walled in the subicular zone; subhyaline, vertical, ≤ 3.3 µm wide, richly branched, thin-walled in the subhymenial zone. Metuloids conical to subfusiform, with basal clamp, 54–61 × 9.4–13 µm, thick-walled, heavily encrusted; projecting up to 20 µm out of the hymenium. Basidia clavate, sinuous, 32–44 × 4.9–7.2 µm; sterigma ≤ 5 µm long. Basidiospores 6.4–8.8 × 2.5–3.3 µm, allantoid to suballantoid, thin-walled, smooth, acyanophilous, inamyloid.

Collections examined: India, Himachal Pradesh: Chamba, Udaipur, Chikryani, on sticks of Adhatoda vasica, Poonam 9183 (PUN), July23, 2017; Udaipur, Chikryani, on sticks of Adhatoda vasica, Poonam 10739 (PUN), July 23, 2017; Udaipur, Chikryani, on sticks of Adhatoda vasica, Poonam 10740 (PUN), July 23, 2017.

Remarks: P. rufomarginata was described by author (Poonam et al. 2020) as new record from India.



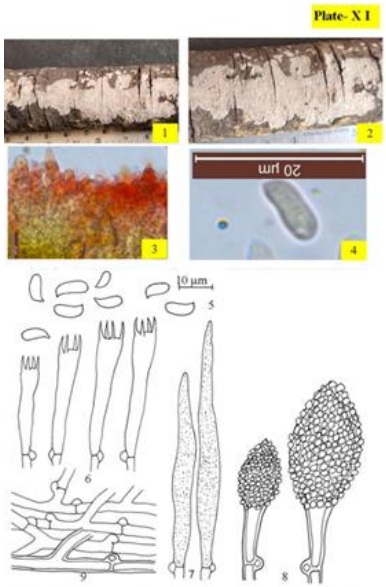
Figs. 1– 7. *Peniophora rufomarginata* :1-2. Sporophore showing hymenial surface (1. Fresh, 2. Dry); 3-4. Photomicrographs (3. Basidiospores, 4. Metuloids); 5-7. Line diagrams [5. Basidiospores; 6. Reconstruction showing a portion of hymenium and subhymenium (A. Basidium, B.Metuloid); 7. Generative hyphae]

11. Peniophora suecica Litsch., Annl. mycol. 39(2-3): 131, (1941).
Plate XI

Sporophores resupinate, effused, adnate, up to 140 µm thick in section; hymenial surface smooth both in fresh and dry states; reddish gray both in fresh and dry states; margins thinning, paler concolorous.. Hyphal system monomitic. Generative hyphae septate, clamped, smooth; light brown, horizontal, ≤ 3.5 µm wide, less branched, thick-walled in the subicular zone; vertical, subhyaline, ≤ 3 µm wide, richly branched, thin-walled in the subhymenial zone. Ancillary elements of 2 kinds: (i) Gloeocystidia subfusiform, with basal clamp, 56–63 × 6.1–7.2 µm, thin-walled, with oily contents negative to sulphovanillin; projecting 10 µm out of the hymenium (ii) Metuloids conical to subfusiform, with basal clamp, 35–53 × 12–24 µm, thick-walled, heavily encrusted; projecting up to 15 µm out of the hymenium. Basidia clavate to subclavate, somewhat sinuous, 24–35 × 5–6.1 µm; sterigma ≤ 5 µm long. Basidiospores 6.5–10.4 × 2.8–3.5 µm, allantoid to suballantoid, thin-walled, smooth, acyanophilous, inamyloid.

Collection examined: India, Himachal Pradesh: Chamba, Bharmour, Manimahesh, Tosh ka got, on stick of *Pinus roxburghii*, Poonam 10486 (PUN).

Remarks: *P. suecica* is being redescribed from the study area. Earlier it was reported from India by Singh (2007) and Dhingra et al. (2014) from district Chamba of Himachal Pradesh.



Figs. 1– 9. *Peniophora suecica* :1-2. Sporophore showing hymenial surface (1. Fresh, 2. Dry); 3-4. Photomicrographs (3. V.S. of sporophore showing gloeocystidia and metuloids, 4. Basidiospore); 5-9. Line diagrams [5. Basidiospores; 6. Basidia, 7. Gloeocystidia, 8. Metuloids); 9. Generative hyphae]

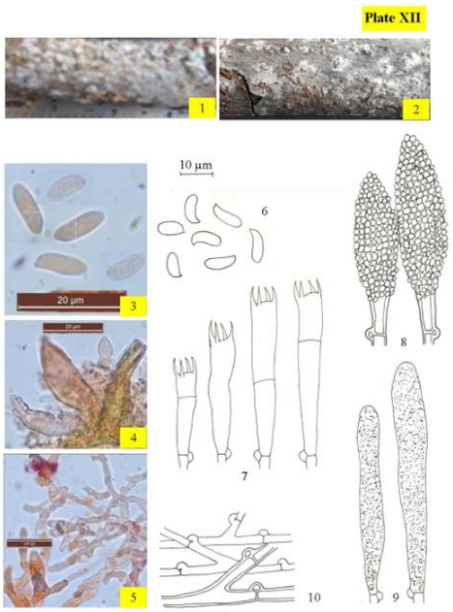
12. *Peniophora violaceolivida* (Sommerf.) Massee, Botanical Journal of the Linnean Society 25: 152 (1889). Thelephora violaceolivida Sommerf., Supplementum florae lapponicae: 283 (1826).
Plate XII

Sporophores resupinate, effused, adnate, up to 200 µm thick in section; hymenial surface smooth both in fresh and dry states; grayish orange both in fresh and dry states; margins thinning, paler concolorous. Hyphal system monomitic. Generative hyphae septate, clamped, smooth; light brown, horizontal, ≤ 4 µm wide, less branched, thick-walled in the subicular zone; subhyaline, vertical, ≤ 2.8 µm wide, richly branched, thin-walled in the subhymenial zone. Ancillary elements of 2 kinds: (i) Gloeocystidia subcylindrical, with basal clamp, 46–57 × 5.5–6.7 µm, thin-walled, oily contents positive to sulphovanillin; projecting up to 15 µm out of the hymenium (ii) Metuloids conical to subfusiform, with basal clamp, 48–60 × 6.7–11 µm, thick-walled, heavily encrusted; projecting up to 10 µm out of the hymenium. Basidia clavate, somewhat sinuous, sometime with adventitious septa, 24–49 × 6.1–7.2 µm; sterigma ≤ 5 µm long. Basidiospores 8–9.6 × 2.8–3.3 µm, allantoid to suballantoid to subcylindrical, thin-walled, smooth, acyanophilous, inamyloid.

Collections examined: India, Himachal Pradesh: Chamba, Manimahesh, Tosh ka got, on sticks of Juglans regia, Poonam 9180 (PUN), September 04, 2016; Manimahesh, Tosh ka got, on sticks of Juglans regia, Poonam 10483 (PUN), September 04, 2016; Manimahesh, Tosh ka got, on sticks of Juglans regia, Poonam 10484 (PUN), September 04, 2016.

Remarks: *P. violaceolivida* was described for the first time from the state of Himachal Pradesh (Poonam et al, 2020) It was earlier reported from India by Rehill and Bakshi (1965) from different Indian localities.

Figs. 1– 10. *Peniophora violaceolivida*: 1-2. Sporophore showing hymenial surface (1. Fresh, 2. Dry); 3-5. Photomicrographs (3. Basidiospores, 4. Metuloids, 5. Generative hyphae); 6-10. Line diagrams [6. Basidiospores, 7. Basidia, 8. Metuloids, 9. Gloeocystidia, 10. Generative hyphae]



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Ethnobotany of a medicinal plant *Mirabilis jalapa* L.

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Abstract

The *Mirabilis jalapa* L. has a large history of ethnobotanical studies, with a main focus on its connection with folk medicine practice in countries around the globe. Its leaves, roots, and flowers have been utilized for their purported medicinal properties, including anti-inflammatory, analgesic and laxative effects. Additionally, it is often used in herbal remedies for conditions such as fevers, skin ailments and digestive issues. Ethnobotanical research highlights the significance of *M. jalapa* in local herbal practices, its cultural relevance and the need for further scientific investigation to validate its traditional uses and pharmacological potentials.

Keywords: *M. jalapa,* anti-inflammatory, digestive issues.

Introduction

Mirabilis jalapa L. is a medicinal plant used for centuries for the treatment of various ailments, which belongs to family Nyctaginaceae (Saha et al., 2020). This plant is also known as Gul' A'bbas and is sometimes referred to as the Four O'clock plant because its blossoms usually arrive in the late afternoon or early evening. It is distributed throughout world, this plant is widely cultivated around the world for its ornamental value due to its vibrant, fragrant flowers (Rozina, 2016). It is a perennial, herbaceous, leafy, multibranched, shrub like bushy plant. It having showy flowers, coriaceous obovoid fruit and prominent tuberous roots which are planted as ornamental plant all over the world, which grows up to 1-meter height and just as wide. (Peiris et al., 2022). *M. jalapa* flowers can be yellow, pink, red, orange, white or any of these colors according to Akaji et al., (2016).

Mirabilis jalapa L.: Ethnobotanical Review:

Various parts of *Mirabilis jalapa* L. has reported to have medicinal uses in several regions of the world, such as Latin America, South Africa, Zaire, Madagascar, India, and Pakistan, where they are used as a laxative and to treat

infections, inflammation, allergies, and painful conditions. In Mexico and Madagascar, *M. jalapa* is employed as an analgesic to cure a range of unwanted diseases, including intestinal pain and bee and scorpion sting pain. Mexicans also use a number of plant preparations and decoctions for the alleviation of dysentery. (Kusamba et al., 1991; Encarnacion et al., 1998; Arroyo et al., 2004). In Malagasy, the plant was used to treat intestinal pains (Zhou et al., 2012). Individuals from numerous countries use the entire plant to treat diarrhoea, gastrointestinal colic and muscle pain, as per Holdsworth et al., (1992) the decoction of the entire plant is taken orally to treat kidney diseases for diuresis (Sharma et al., 2001). In Pakistan, juice of the whole plant used to relief from pain and also to provide a cure from typhoid (Ahmad et al., 2011).

The leaves used medicinally in Ayurveda, Siddha and other traditional systems of medicine for curing various ailments (Devi et al., 2011). An infusion prepared from the leaves is applied topically to alleviate swelling associated with bone fractures or sprains (Sharma et al., 2001; Khurian 2003; Al-Snafi 2021).

According to Goleniowski et al., (2006) the leaves of *M. jalapa* have been utilized in traditional medicine for the treatment of inflammation and boils and are recognized for exhibiting purgative and emetic activities. Leaves are taken as a laxative in southern Brazil and further used to treat inflammatory and painful diseases. (Bogle, 1974). According to Peiris et al., (2022) *M. jalapa* exhibits significant diuretic properties, enhancing urine production. Leaf juice is traditionally administered to individuals suffering from bladder inflammation and urinary retention. Furthermore, topical application of the juice is employed to manage skin infections including rashes and boils facilitating accelerated healing. Leaves of *M. jalapa* also utilized for treating wounds and skin lacerations. Leaves are crushed and mixed with salt in sprain and bruises (Boulogne et al., 2011). Bhatia et al., 2014 observed the leaves are fried in clarified butter and applied to abscesses, while boiled leaves are consumed to alleviate body pains. Warmly heated leaf juice is applied as a poultice to wounds and abscesses to assist in their healing. Additionally, the juice is also utilized as an eye drop to soothe eye inflammation. In women the paste of leaves is used in amenorrhea and dysmenorrhea (Srithi et al., 2012). Khurian, (2003) reports that leaves were employed as a poultice against boils and abscesses.

Acoording to Mahmood et al., (2012) and Vankar and Dhara, (2010) the leaves of *M. jalapa* has skin eruptions and emollient properties. Leaf juice is taken orally to treat Hepatitis (Mahmood et al., 2013 and Sharma et al., 2012). The decoction of the leaves is utilized to heal injuries and genitourinary system illnesses, as indicated by Weckerle et al., (2009). The juice extracted from the leaves is used to treat skin allergies, indigestion and earaches particularly in children. Decoction of *M. Jalapa* leaves was used orally as diuretic and in the treatment of kidney infections. Juices of the leaves were used in treatment of skin allergy (Al-Snafi,

2021). For depigmentation, the leaves and stems are utilized (Kamagaju et al., 2013). According to Chen et al., (2016) a decoction prepared from the roots and leaves of *Mirabilis jalapa* is utilized in the treatment of pain and inflammation in arthritis. *M. jalapa* leaves have been traditionally applied in folk medicine in southern Brazil to cure inflammatory and painful diseases.

Flowers are used in food colouring, edible crimson dye is used to colour cakes and jellies (Devi et al., 2011). The flower of *M. jalapa* exhales a strong odour at night, which can drive away mosquitos (Kirtikar and Basu, 1935).

The rhizome of *M. jalapa* is endowed with diuretic, purgative, healing of wounds, aphrodisiac, anti-inflammatory, anti-tumor, laxative, and anti-poison properties as cited by Peiris et al., (2022). In line with Bhatia et al., (2014) piles have been traditionally managed using small quantities of *Mirabilis jalapa* tuber. Roots were used to treat the accumulation of pus or liquid in cavities, cellular tissues and inflamed and enlarged lymph nodes. The roots are effective in treating syphilitic sores (Kirtikar and Basu, 2001). Additionally, a paste made from the roots is applied to reduce inflammation (Sher et al., 2011; Ghatapanadi et al., 2011). For the treatment of snake bites, an oral suspension of tuber paste is administered (Marandi and Britto, 2014). According to Quer, F. and Medicinales, P. (1962) 2-4 grams of root powder dissolved in water is prescribed as a laxative for adults. Because of its cathartic, emetic and purgative properties the roots of *M. jalapa* have been used for a long time in South Africa and Latin America (Zhou et al., 2012). *M. jalapa* is commonly used as an ethnic drug in China and in traditional Chinese medicine for the cure of diabetes (Swarnkar and Katewa, 2008). Due to its nutrient value, the native inhabitants of the Shivalik Hills region in Himachal Pradesh employ the root tubers as a pickle (Parinitha, 2004). Additionally, in the tribal regions of Rajasthan, a paste made from the root tuber is applied to prevent the growth of old tumors. Kusamba et al., (1991) reported that in Latin America the roots were used as a purgative and emetic. In Malagasy the plant was used to treat intestinal pains. In South Africa the roots were used as a purgative (Kusamba et al., 1991).

It is reported that the powdered seed is used as a cosmetic powder by the natives of Japan (Watt and Breyer-Brandwijk, 1962). The seed powder was used in Zaire externally for infected wounds (Kusamba et al., 1991). The Bhadra wildlife sanctuary area in Karnataka employs an external use of fruit paste prepared with coconut oil to relieve headaches in both individuals and pets, as cited by Chetty et al., (2008) *M. jalapa* has a great anti-poison effect and is one of the best herbs for treating animal bites. It is beneficial for treating snake, scorpion and insect bites.

Conclusion

Mirabilis jalapa is a versatile plant with significant ethnobotanical importance

due to its variety of uses, particularly in traditional medicine and ornamental horticulture. Further research is necessary to explore its potential benefits fully, especially regarding its medicinal applications and to document local knowledge related to this plant. The existing literature provides a foundation for understanding its place within both cultural practices and ecological systems.

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Phytochemical screening of Averrhoa species: *Averrhoa carambola* L. and *Averrhoa bilimbi* L.

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Abstract

The genus *Averrhoa* belongs to the family Oxalidaceae. It consists of two species Averrhoa carambola L. and Averrhoa bilimbi L. The main purpose of this study was to evaluate the phytochemical screening of the fruit extract of Averrhoa carambola L. and Averrhoa bilimbi L. The fruits have great nutritional value, traditional medicine, and have numerous health benefits. The fruits are a good source of antioxidants and are used traditionally in mouth ulcers, toothache, nausea, diarrhea, ascites, and asthma. In preliminary phytochemical investigation, Averrhoa carambola leaves and fruit showed the presence of alkaloids, α-amino acid, carbohydrate, reducing sugars, glycosides, phenolic compounds, tannins, flavonoids, saponins, alkaloids, glycosides, phenols, terpenoids, emodols, coumarins, and starch. The ethyl acetate fraction BE underwent phytochemical screening, analyzed using reverse-phase high-performance liquid chromatography (RP-HPLC), 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS. +) radical scavenging activity, and hydroxyl radical scavenging activity. star fruit leaves contain flavonoids apigenin and quercetin as potential anti-inflammatory and anticancer agents. The raw material for the drug in Indonesia is mostly obtained through imports from other countries. To support the independence of traditional medicine raw materials, it is important to standardize the quality of conventional medicine raw materials, in this case is star fruit leaves, by the High-Performance Liquid Chromatography (HPLC) method.

Keywords: *Averrhoa carambola* L. and *Averrhoa bilimbi* L., Qualitative and Quantitative phytochemical analysis, RP-HPLC, HPLC.

Introduction

The genus Averrhoa belongs to the family Oxalidaceae. It has two species, *Averrhoa carambola* L. and *Averrhoa bilimbi* L. *Averrhoa carambola* L. of Oxalidaceae is native to South-East Asia and cultivated in some parts of India,

and is commonly known in various places as ‘star-fruit’ or ‘kamrakh’. The plant is distributed throughout Myanmar and the warmer regions of the world. The fruits are five-lobbed, fleshy, and yellow-greenish. This fruit is used as traditional medicine for a variety of diseases. It has drooping branches and white wood, which turns reddish. It takes a bushy shape, having many branches producing a broad, rounded crown. The soft leaves are compound in nature, medium-green, and are spirally alternately arranged around the branches. The flowers are lilac or purple-streaked and come up in the axils of leaves at the end of twigs. The attractive fruits are oblong-shaped and in measurement they are longitudinally 5 to 6 angled and 6.35-15 cm long and up to 9 cm wide. The fruits are orange-yellow, having a thin, waxy skin. The fruits are juicy and yellow inside when ripe and have a crisp texture and look like a star when cut in cross-section. Usually, the fruits contain oxalic acid in Odor, which varies from strong to mild depending on the plants. Each fruit may contain up to twelve 6-12.5 mm long seeds, and they are flat, thin shaped and brown. Some cultivated forms produce seedless fruit. (Chakraborty et al.,2012). Quantitative analysis as the determination of the active compounds using high-performance liquid chromatography (HPLC). Star fruit leaves contain major flavonoid compounds such as apigenin and quercetin. Other compounds identified in star fruit leaves are Amarin, rutin, saponin, and tannin. According to the Cabrini report, apigenin compounds in star fruit leaf extracts and fractions can inhibit the formation of edema (redness) on the ear skin of mice and effectively inhibit the migration of leukocytes to the inflammatory. (Araho et al.,2005 and Cabrini et al.,2011). Quercetin-3-O-rhamnoside compound isolated from star fruit extract has high antioxidant activity (5.19 µg/mL) and potential as an anticancer. (Artanti et al., 2006). *A. bilimbi* (common name: Bilimbi) is a medicinal plant belonging to the family Oxalidaceae. The genus Averrhoa was named after an Arab Philosopher, physician, and Islamic Jurist, Ibn Rushd, often known as Averroes (1126-98). *A. bilimbi* is a small tree that grows up to 15 m high with sparsely arranged branches. It has compound leaves with twenty–forty leaflets each and is 5–10 cm long (De Lima et al., 2001). The leaves are hairy with pinnate shapes and form clusters at the end of branches. (Orwa et al., 2009 and Lim, 2012). The tree is cauliflorous with 18–68 flowers in panicles that form on the trunk and other branches. The flowers are heterotrichous with petals 10–30 mm long, yellowish green to reddish purple (Orwa et al., 2009 and Ganders, 1979). The fruits are produced on the bare stem and trunk. The fruits are greenish with a firm and juicy flesh, which becomes soft on ripening (De Lima et al., 2001). The fruit juice is sour and extremely acidic. *A. bilimbi* holds great value in complementary medicine as evidenced by the substantial amount of research on it. Therefore, we aimed to compile an up-to-date and comprehensive review of *A. bilimbi* that covers its traditional and folk medicine uses, phytochemistry, and pharmacology.

Preliminary Phytochemical Investigation of the fruits of *Averrhoa carambola* L. and of *Averrhoa bilimbi* L.

For preliminary phytochemical investigation, the air-dried powders of the fruits were used. Tests for alkaloids, α-amino acids, carbohydrates, starch, reducing sugars, glycosides, phenolic compounds, saponins, tannins and flavonoids were done by using various solvents. These results were carried out according to the (British Pharmacopoeia, 1968; Marrini Bettalo et al., 1981) (Central Council for Research in Unani Medicine, 1987 and Trease and Evans, 2002.)

Test for Alkaloids

The powdered sample (2 g) was boiled with 1%HCL (50 ml) for 20 minutes and filtered off. The filtrate was divided into two portions and tested with Dragendroff's reagent and Wagner's reagent. The precipitates treated with the above-mentioned reagents showed the presence of alkaloid (Central Council for Research in Unani Medicine, 1987).

Test for α-amino acids

The powdered sample (2 g) was boiled with distilled water (25 ml) for 20 minutes and then filtered. And then a few drops of each filtrate were spotted on a filter paper by using a capillary tube. This paper with filtrate was allowed to dry and sprayed with ninhydrin reagent. It was dried at room temperature and then kept in oven at 110ºC for a few minutes. The colour of spots changes to the pink due to the presence of α-amino acids (Marrini Bettolo et al., 1981).

Test for Carbohydrates

The powdered sample (2 g) was boiled with distilled water (25 ml) for 20 minutes and then filtered. The filtrate was then placed into a test tube and a few drops of 10% α-napthol was added and shaken. The test tube was kept inclined at an angle of 45º and about 1 ml of concentrated sulphuric acid was slowly introduced along the inner side of the test tube. A red ring was formed between the two layers (Trease and Evans, 2002).2nd Myanmar Korea Conference Research Journal 465

Test for Starch

The powdered sample (2 g) was boiled with distilled water (25 ml) for 20 minutes and then filtered. Two drops of iodine solution were added to this filtrate. Bluish black precipitates indicate the presence of starch (Central Council for Research in Unani Medicine, 1987).

Test for Reducing Sugars

The powdered sample (2 g) was boiled with distilled water (25 ml) for 20 minutes and then filtered. A mixture of Benedict's solution was added to this filtrate and boiled on a water bath for a few minutes. Brick red precipitates are

due to the presence of reducing sugars (Trease and Evans, 2002).

Test for Glycosides

The powdered sample (2 g) was boiled with distilled water (25 ml) for 20 minutes and then filtered. To this filtrate, 10% lead acetate solution was added. Yellow precipitates showed the presence of glycosides (Marrini Bettolo et al., 1981).

Test for Phenolic compounds

The powdered sample (2 g) was boiled with 1% hydrochloric acid (25 ml) for about 20 minutes and filtered. The filtrate was treated with 3% ferric chloride solution. The yellow precipitates appeared due to the presence of phenolic compounds (Marrini Bettolo et al., 1981).

Test for Saponins

The powdered sample (2 g) was put into a test tube and some distilled water was added into a test tube. The mixture was vigorously shaken for a few minutes. The frothing appeared due to the presence of saponins (Marrini Bettolo et al., 1981).

Test for Tannins

The powdered sample (2 g) was boiled with distilled water (25 ml) for 20 minutes and then filtered. To this filtrate, 3% ferric chloride solution was added. The observation indicated the greenish brown precipitates because of the presence of tannins (Central Council for Research in Unani Medicine, 1987).

Test for Flavonoids

The powdered sample (2 g) was boiled with 95% ethanol (25 ml) for 20 minutes and filtered. A few drops of concentrated hydrochloric acid and 0.5 g of Mg were added to this filtrate. Pink color appeared due to the presence of flavonoids (Central Council for Research in Unani Medicine, 1987).

Test of protein

4-5 ml of plant extract was taken and added few drops of Melons reagent were added, mixed properly, and heated. We found which precipitate was formed, and the precipitate turns brick red after boiling. (Manda et al.,2012)

Test of coumarins:

A few drops of ammonia were added to a filter paper. A drop of extract was added to it. The fluorescence indicates the presence of coumarins. (Manda et al.,2012)

Test of terpenoids:

To 1 mL of the solvent extract, 2 mL of chloroform and 3 mL of concentrated H2SO4 were added carefully to form a layer. A reddish-brown coloration of the interface indicates the presence of terpenoids. (Manda et al.,2012)

Antioxidant capacity:

1] ABTS. + radical scavenging activity

2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS. +) radical scavenging activity of the BEA extract was conducted using Trolox as a standard [1], and TEAC of the extract was calculated using the standard curve produced by percentage inhibition of Trolox from the following formula:

% Inhibition = [1- A (Test) / A (Control)] x 100

2] Hydroxyl radical scavenging activity

The measured scavenging ability of hydroxyl radicals by the method of Kunchandy and Rao [13] is expressed as an IC50 value using the following formula:

% Inhibition = [1- A (Test) / A (Control)] x 100

Quantitative analysis of apigenin in extract and fraction using high-performance liquid chromatography (HPLC)

The apigenin standard calibration curve was done by plotting six concentrations, i.e., 10, 20, 40, 60, 80, and 100 ppm. Standards solution and samples (extract and fraction) were analyzed using HPLC (Waters), with a 4.6 × 150 mm Sun Fire C18 column size, 1.0 mL/min flow rate, 20 µL injection volume, and Photodiode Array (PDA) detector at 340 nm. The mobile phase used isocratic system, comprising acetonitrile and distilled water (45:55, v/v). 9

Quantitative analysis of quercetin in extract and fraction using high-performance liquid chromatography (HPLC)

The quercetin standard calibration curve was done by plotting six concentrations, i.e., 10, 20, 40, 60, 80, and 100 ppm. Standards solution and samples (extract and fraction) were analyzed using HPLC (Waters), with a 4.6 × 150 mm Sun Fire C18 column size, 1.0 mL/min flow rate, 20 µL injection volume, and Photodiode Array (PDA) detector at 273 nm. The mobile phase used isocratic system, comprising acetonitrile: water: acetic acid (10:90:0.2, v/v). 10

High-performance liquid chromatography Averrhoa bilimbi L. (HPLC)

A BDS HYPERSIL C-18 column (150×4.6 mm, 5 µm particle size) equipped with PDA/ultraviolet detector with 310 nm as the detecting wavelength in room temperature (27°C) enabled chromatic separations under the following conditions: 1 ml min−1; solvent A, 10% acetic acid in water; and solvent B, 15% methanol in water starting from 0 to 20 min (40–52% A), 20–40 min (52–80%

A), and 40–60 min (80% A).

Result:

The fruits of Averrhoa carambola have been investigated from the point of view of medicinal aspects in this research. In preliminary phytochemical investigation, the presence of fruits showed that alkaloids, α-amino acid, carbohydrate, reducing sugars, glycoside, phenolic compound, tannin, flavonoids, saponin, and starch. The phytochemical analysis of the Averrhoa bilimbi fruit extract showed the presence of carbohydrates, flavonoids, phenols, glycosides, protein, and amino acids. ABTS. + Radical scavenging activity, the percentage inhibition of BE was dose dependent, wherein as the concentration of extract increased, the percentage inhibition increased. The ABTS. + Radical scavenging activity of BE was found to be 11.5 µM TEAC. Hydroxyl radical scavenging activity was measured by the inhibition of degradation of deoxyribose by the free radicals generated by the Fenton reaction. The BE inhibited to production of hydroxyl radicals, wherein the IC50 value of the extract and Trolox was 69.42 µg/ml and 45.58 µg/ml, respectively. HPLC After running the extract in HPLC, chromatographic separations showed prominent peaks at 23, 27, and 37 min under 310 nm HPLC spectrum of apigenin compounds in the extract, ethyl acetate fraction and water fraction has a retention time 4.055 minutes with a mobile phase acetonitrile water (45:55, v/v) is similar with retention time of apigenin standard. The hexane fraction did not show any apigenin spectrum. HPLC spectrum of quercetin only appears in the extract and ethyl acetate fraction, has a retention time of 6.116 minutes with a mobile phase of acetonitrile, water, acetic acid (10:90:0.2, v/v) is similar to the retention time of quercetin standard. While in water and hexane fractions, HPLC spectra did not show any quercetin.

Conclusion

Preliminary phytochemical analysis of Averrhoa carambola L. and Averrhoa bilimbi L. revealed the presence of alkaloids, α-amino acid, carbohydrate, reducing sugars, glycoside, phenolic compound, tannin, flavonoids, saponin, starch, carbohydrates, flavonoids, phenols, protein, and glycosides. Averrhoa carambola L. and Averrhoa bilimbi L. both have the highest antioxidant properties, with a very high vitamin C content. The results of apigenin and quercetin assays the highest content of apigenin was found in the ethyl acetate fraction. While the hexane fraction did not contain apigenin compound, which is proven by zero apigenin levels, the highest quercetin level was found in the ethyl acetate fraction. While water and hexane fractions did not contain any quercetin compound, it is proven by zero quercetin level, in HPLC spectra.

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