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PERSPECTIVES IN PLANT SCIENCE RESEARCH

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PREFACE

Plants have been at the heart of life on Earth, sustaining ecosystems, shaping civilizations, and inspiring countless scientific endeavors. As our understanding of plant biology deepens, new frontiers emerge, promising to revolutionize agriculture, medicine, environmental conservation, and biotechnology. It is within this dynamic and evolving landscape that Perspectives in Plant Science Research finds its place.

This book brings together a collection of scholarly articles and research studies that reflect the diverse and vibrant spectrum of contemporary plant science. From fundamental studies on plant physiology and genetics to applied research in sustainable agriculture and environmental adaptation, the chapters presented here offer readers a comprehensive view of the latest advances and ongoing challenges in the field.

Our aim with this volume is twofold: to provide a platform for researchers to share innovative ideas, and to serve as a valuable resource for students, academicians, and professionals seeking to expand their knowledge. By showcasing interdisciplinary approaches and emerging trends, we hope to inspire further exploration and collaboration across the scientific community.

We extend our heartfelt thanks to all the contributing authors for their dedication and insightful work. Their contributions have been instrumental in shaping this volume. We are also grateful to the reviewers and editorial team for their meticulous efforts in ensuring the quality and integrity of the content.

As you turn these pages, we invite you to reflect on the remarkable resilience and complexity of the plant world, and to envision new possibilities for research that will continue to benefit both humanity and the environment.

We hope that Perspectives in Plant Science Research will ignite curiosity, foster innovation, and contribute meaningfully to the advancement of plant science.

- Editors

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SUSTAINABLE STRATEGIES FOR THE CONSERVATION AND USE OF MEDICINAL PLANTS

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Abstract:

Medicinal plants are indispensable to traditional medicine, modern pharmaceuticals, and the livelihoods of millions worldwide. However, unsustainable harvesting practices, habitat destruction, and climate change threaten their availability and biodiversity. This article explores sustainable strategies for medicinal plant conservation and use, emphasizing integrated approaches. Key strategies include sustainable harvesting practices, cultivation, community involvement, regulatory frameworks, research and development, raising awareness, and fostering international cooperation. Sustainable harvesting methods, such as selective and rotational harvesting, and the cultivation of medicinal plants through agroforestry and seed banks, are highlighted as practical solutions. Community engagement, benefit-sharing, and training empower local stakeholders, while regulatory measures, such as permits, quotas, and certification schemes, ensure responsible use. Advances in conservation biology, biotechnology, and post-harvest processing support sustainable practices, while public awareness and global cooperation bolster conservation efforts. A holistic, collaborative approach is essential to safeguarding medicinal plants, ensuring their ecological significance and contribution to global health for future generations.

Key words: Biodiversity, Conservation, Ecosystem, Holistic Approach, Sustainability

Introduction:

Medicinal plants have played an indispensable role in human health and well-being for centuries. These natural resources, often considered the cornerstone of traditional medicine systems worldwide, continue to be a vital source of modern pharmaceuticals and herbal remedies. However, the increasing demand for medicinal plants, driven by population growth, urbanization, and a renewed interest in natural health solutions, has placed immense pressure on these valuable resources. Overharvesting, habitat destruction, and climate change are among the key factors contributing to the rapid decline of medicinal plant species, threatening biodiversity and the ecosystems that support them. The need for sustainable strategies to conserve and utilize medicinal plants has never been more urgent. Effective conservation ensures not only the preservation of these critical species but also their availability for future generations. Moreover, sustainable use involves striking a balance between meeting current human needs and maintaining ecological integrity. Various sustainable strategies for the conservation and use of medicinal plants, focusing on integrated approaches that encompass conservation biology, community involvement, policy frameworks, and advancements in scientific research are to be explored. By addressing the challenges and opportunities in this field, it aims to underscore the importance of a collective effort to safeguard these invaluable natural resources for both their ecological significance and their contributions to global health.

Holistic Approach on Medicinal Plant Collection

The collection of medicinal plants plays a crucial role in traditional medicine, modern pharmaceuticals, and the livelihoods of millions of people worldwide. However, unsustainable harvesting practices have placed many medicinal plant species at risk, threatening biodiversity, ecosystem health, and the long-term availability of these valuable resources. To address these challenges, a sustainable approach to the collection of medicinal plants is imperative. This essay examines essential approaches to achieving sustainability, such as responsible harvesting methods, sustainable cultivation, community engagement, regulatory frameworks, innovation through research and development, public awareness efforts, and global collaboration.

1. Adopt Sustainable Harvesting Practices

Sustainable harvesting is fundamental to preserving medicinal plant populations. Several methods can be employed to ensure that plant resources are not depleted:

Selective Harvesting: Collect only mature plants, leaving younger ones to grow and reproduce. This approach ensures the continued survival and regeneration of plant populations.

Partial Harvesting: Harvest specific parts of the plant, such as leaves, bark, or flowers, rather than uprooting or cutting down the entire plant. This method allows the plant to continue growing and producing valuable resources.

Seasonal Collection: Time harvesting activities to coincide with the seasons when the plant is most abundant and resilient. This minimizes stress on the plant and allows for natural regeneration.

Rotational Harvesting: Avoid overharvesting from the same area repeatedly by rotating collection sites. This practice gives ecosystems time to recover and helps maintain plant populations.

2. Promote Cultivation

Cultivating medicinal plants reduces pressure on wild populations while providing a controlled environment for sustainable production. Key strategies include:

Commercial Cultivation: Encouraging farmers and communities to grow medicinal plants on farms or in gardens can help meet market demands while conserving wild resources. This approach also generates income for local populations.

Agroforestry: Integrating medicinal plants with agricultural crops or forests creates a diverse ecosystem that supports biodiversity and reduces the risk of overharvesting in the wild.

Seed Banks and Nurseries: Establishing seed banks and nurseries for medicinal plants ensures a steady supply of planting material. These facilities can also serve as repositories for rare and endangered species.

3. Community Involvement

Engaging local communities is critical to the success of sustainable collection practices. Communities often possess traditional knowledge and a vested interest in preserving local ecosystems. Strategies include:

Training and Education: Providing training on sustainable harvesting techniques and the ecological importance of medicinal plants empowers communities to act as stewards of their natural resources.

Benefit Sharing: Ensuring fair compensation and benefits for indigenous and local communities who manage medicinal plants promotes equitable resource use. Sharing profits and providing incentives encourage conservation efforts.

Monitoring Systems: Involving communities in monitoring plant populations and maintaining records enhances the effectiveness of conservation programs. This participatory approach fosters a sense of ownership and accountability.

4. Regulatory Measures

Strong regulatory frameworks are essential to prevent overharvesting and illegal trade. Effective measures include:

Permits and Quotas: Implementing licensing systems and setting quotas for collection ensures that harvesting activities remain within sustainable limits.

Protected Areas: Designating conservation zones where harvesting is restricted or prohibited protects critical habitats and endangered species.

Certification Schemes: Certification programs for sustainably harvested medicinal plants provide market incentives for responsible practices. Certified products appeal to environmentally conscious consumers, promoting ethical trade.

5. Research and Development

Scientific research supports sustainable practices by providing insights into plant biology, ecology, and alternative solutions. Areas of focus include:

Conservation Biology: Studying plant populations, growth cycles, and ecological interactions helps determine sustainable harvest levels and identify priority species for conservation.

Alternative Sources: Developing synthetic or lab-grown alternatives for medicinal compounds can reduce reliance on wild-harvested plants. Advances in biotechnology offer promising solutions.

Post-Harvest Processing: Investing in efficient processing technologies minimizes waste and maximizes the value of harvested plants. Improved storage and extraction methods extend the usability of plant materials.

6. Raising Awareness

Public awareness campaigns are vital to garnering support for sustainable practices. Efforts should focus on:

Public Campaigns: Using media, workshops, and educational materials to highlight the importance of conserving medicinal plants fosters a culture of responsibility among consumers and collectors.

Cultural Preservation: Documenting and promoting traditional knowledge ensures that indigenous practices align with modern conservation goals. Recognizing the cultural significance of medicinal plants strengthens conservation efforts.

7. Policy and International Cooperation

Global challenges require coordinated action at local, national, and international levels. Key strategies include:

Legal Frameworks: Supporting international agreements like the Convention on Biological Diversity (CBD) ensures that countries commit to sustainable use and equitable benefit sharing.

Funding and Grants: Governments, NGOs, and international organizations should provide financial support for conservation projects. Funding enables research, capacity building, and community initiatives.

Collaboration: Partnerships among NGOs, academic institutions, industries, and governments facilitate the exchange of knowledge and resources. Collaborative efforts drive innovation and implementation of sustainable practices.

Conclusion:

The sustainable collection of medicinal plants is a shared responsibility that requires a multifaceted approach. By adopting sustainable harvesting practices, promoting cultivation, involving communities, enforcing regulatory measures, advancing research, raising awareness, and fostering international cooperation, we can balance the growing demand for medicinal plants with the need to preserve biodiversity and ecosystem health. Collaboration among stakeholders—including governments, researchers, communities, and industries—is essential to ensure the long-term availability of these vital resources for future generations.

References:

1. Chang, L. I. U., Hua, Y. U., & Shi-Lin, C. H. E. N. (2011). Framework for sustainable use of medicinal plants in China. Plant Diversity, 33(01), 65-68.

2. Chen, S. L., Yu, H., Luo, H. M., Wu, Q., Li, C. F., & Steinmetz, A. (2016). Conservation and sustainable use of medicinal plants: problems, progress, and prospects. Chin Med 11, 37. https://doi.org/10.1186/s13020-016-0108-7
3. Hamilton, A. (2003). Medicinal plants and conservation: issues and approaches. International Plants Conservation Unit, WWF-UK, 51.
4. Hamilton, A. C. (2004). Medicinal plants, conservation and livelihoods. Biodiversity & Conservation, 13, 1477-1517.
5. Larsen, H. O., & Olsen, C. S. (2007). Unsustainable collection and unfair trade? Uncovering and assessing assumptions regarding Central Himalayan medicinal plant conservation. Plant conservation and biodiversity, 105-123.
6. Raina, R., Romesh Chand, R. C., & Sharma, Y. P. (2011). Conservation strategies of some important medicinal plants.
7. Schippmann, U., Leaman, D. J., & Cunningham, A. B. (2002). Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues. Biodiversity and the ecosystem approach in agriculture, forestry and fisheries.
8. Schoen, D. J., & Brown, A. H. (2001). The conservation of wild plant species in seed banks: attention to both taxonomic coverage and population biology will improve the role of seed banks as conservation tools. BioScience, 51(11), 960-966.
9. Semwal, D. P., Saradhi, P. P., Nautiyal, B. P., & Bhatt, A. B. (2007). Current status, distribution and conservation of rare and endangered medicinal plants of Kedarnath Wildlife Sanctuary, Central Himalayas, India. Current Science, 1733-1738.
10. Teklehaymanot, T., & Giday, M. (2007). Ethnobotanical study of medicinal plants used by people in Zegie Peninsula, Northwestern Ethiopia. Journal of ethnobiology and Ethnomedicine, 3, 1-11.

NATURE'S ANTIOXIDANTS: EXPLORING THE MEDICINAL POWER OF HERBS

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Abstract:

Oxidative stress, which arises from an overproduction of reactive oxygen species (ROS), plays a significant role in the onset and progression of various chronic diseases such as cancer, cardiovascular conditions, liver disorders, and neurodegenerative diseases. Antioxidants serve as the body's natural defense against oxidative damage by neutralizing free radicals and maintaining cellular equilibrium. While the body has its own antioxidant systems, both enzymatic and non-enzymatic, there is growing interest in utilizing external sources, particularly those derived from diet and herbal medicine, to enhance this protection. This chapter examines the potential of herbs as natural antioxidants, with a particular focus on Indian medicinal plants and their validation through contemporary scientific research. India is home to an immense diversity of plant species—approximately 45,000—that has supported a long-standing tradition of herbal medicine, particularly through the practice of Ayurveda. Plants such as *Aristolochia bracteolata*, *Cissampelos pareira*, *Amaranthus paniculatus*, *Phyllanthus maderaspatensis*, *Phyllanthus virgatus*, and *Gymnema montanum* are well-known for their potent antioxidant properties, as confirmed through various in vitro and in vivo studies. These plants are rich in bioactive compounds like polyphenols, flavonoids, alkaloids, and essential vitamins, which not only scavenge free radicals but also exhibit additional benefits such as anti-inflammatory, liver-protective, and immune-enhancing effects. Among the most notable examples is *Moringa oleifera*, whose polyphenolic leaf extract (MOEF) has demonstrated remarkable antioxidant activity. MOEF has shown a dose-dependent ability to protect against oxidative DNA damage and significantly reduce lipid peroxidation in models of liver toxicity induced by chemicals. Additionally, MOEF restored the activity of key antioxidant enzymes and contained a variety of phenolic acids (including gallic, chlorogenic, ellagic, and ferulic acids) and flavonoids (such as quercetin, rutin, and kaempferol), underscoring its broad therapeutic potential. This chapter emphasizes the dual benefits of these medicinal herbs: their ability to protect health and their practicality as affordable, accessible alternatives to synthetic drugs, especially in resource-limited settings. By combining traditional knowledge with modern scientific validation, the antioxidant potential of these plants is becoming increasingly recognized in the field of

preventive healthcare. Ongoing research in this area continues to reveal new possibilities for developing plant-based therapies that can help manage oxidative stress and improve public health worldwide.

Keywords: Oxidative Stress, *Aristolochia bracteolata*, *Cissampelos pareira*, *Amaranthus paniculatus*, *Phyllanthus maderaspatensis,* Antioxidants

Introduction:

Antioxidants are vital in assisting organisms to counteract oxidative stress, which is caused by the harmful impact of free radicals. Free radicals are unstable chemical molecules with one or more unpaired electrons, making them highly reactive. To achieve stability, these free radicals attempt to steal electrons from other molecules, resulting in cellular harm and contributing to numerous health problems. Antioxidants help to neutralize free radicals, thereby defending the body from oxidative damage and promoting overall well-being. Reactive oxygen species (ROS), such as superoxide anion, hydroxyl radicals, and hydrogen peroxide, are highly reactive and potentially harmful short-lived chemical compounds that are incessantly produced in the body. These molecules are crucial for several essential physiological processes, including energy production, detoxification, chemical signaling, and immune system function. Under normal conditions, ROS levels are tightly regulated by enzymes like superoxide dismutase, glutathione peroxidase, and catalase, which help maintain a balance. However, when ROS are produced in excess—often as a result of external oxidative stressors or failures in the body's natural defense mechanisms—they can cause significant harm to cellular components such as DNA, lipids, and proteins. This oxidative damage plays a key role in the development of more than 30 different diseases, including chronic conditions like cancer, cardiovascular disease, and neurodegenerative disorders.[1,2] All of these conditions are associated with substantial elevations in the specific and insistent lipid peroxidation marker, F2-isoprostane.[3]

Other diseases associated with ROS/RNS include severe vascular conditions such as cardiovascular disease (CVD) and heart failure, alcohol-induced liver disease, ulcerative colitis, and various cancers. Cancer, in particular, arises from multiple factors, with ROS/RNS playing a pivotal role. Valko *et al.* (2007) carried out a thorough review on the impact of free radicals and antioxidants on normal physiological functions and their contribution to disease. The highly reactive hydroxyl radical is recognised to cause damage to all DNA components, including purine and pyrimidine bases and the deoxyribose backbone. Beyond DNA, ROS also affect other crucial cellular structures, such as polyunsaturated fatty acids in phospholipids and amino acid side chains, especially those of cysteine and methionine. This broad cellular damage promotes the progression of various diseases.[4-8]

Remarkably, the human body possesses a sophisticated and layered defense system designed to counteract oxidative stress caused by free radicals. This system includes a range of protective strategies such as preventive mechanisms, cellular repair processes, physical barriers, and a highly organized antioxidant defense network. This antioxidant system is made up of both enzymatic and non-enzymatic components working together to maintain cellular integrity. Among the enzymatic antioxidants, key players include superoxide dismutase, which facilitates the breakdown of superoxide radicals; glutathione peroxidase, which detoxifies hydrogen peroxide and lipid hydroperoxides; and catalase, which converts hydrogen peroxide into harmless water and oxygen, thereby minimizing oxidative damage to cells. In addition to these enzymes, non-enzymatic antioxidants such as ascorbic acid (vitamin C), alpha-tocopherol, glutathione, carotenoids, and various polyphenolic compounds like flavonoids contribute significantly to the body's defense. These molecules operate through several mechanisms, including neutralizing free radicals, regenerating oxidized antioxidants, binding to metal ions that promote oxidation, and quenching reactive oxygen species like singlet oxygen. The protective functions of these antioxidants highlight their critical role in conserving cellular health and inhibiting oxidative damage that can lead to disease. Enhancing the body's own antioxidant capabilities, either by boosting endogenous production or through dietary and supplemental intake of antioxidants, has been linked with a reduced risk of chronic diseases such as cardiovascular ailments, neurodegenerative disorders, and some cancers. As emphasized by Stanner *et al.* (2004), this has led to growing scientific interest in identifying, developing, and applying new antioxidants for therapeutic purposes. The continuing investigation into both natural and synthetic antioxidants is a dynamic and essential area of biomedical research, offering great potential for improving health and preventing disease.[9]

In developing nations such as India, where poverty, malnutrition, and limited access to modern healthcare are major concerns, exploring the therapeutic potential of plant-derived antioxidants offers a practical and cost-effective approach to improving public health. Traditional systems of medicine in India have a deep-rooted history of using herbs for the prevention and treatment of a wide range of illnesses. These natural remedies, many of which have been passed down through generations, are now gaining scientific recognition for their pharmacological benefits—particularly their antioxidant properties. Modern research methodologies have enabled scientists to investigate and validate the efficacy of these traditional herbal formulations. A growing body of experimental studies and reviews has confirmed that a large number of plants used in Indian traditional medicine exhibit significant antioxidant activity. These antioxidants show a vital role in counteracting free radicals, thereby helping to prevent oxidative stress-related diseases such as cardiovascular disorders, neurodegenerative conditions, liver ailments, and various types of cancer. The importance of these findings lies not only in the health benefits but also in the economic advantages, as reliance on locally available herbal resources can significantly reduce dependence on expensive synthetic drugs. Furthermore, these natural compounds often come with fewer side effects compared to conventional pharmaceuticals.[10,11] This chapter compiles and presents a comprehensive overview of the antioxidant potential of medicinal herbs used in Indian traditional practices. It draws from a wide range of scientific literature to underscore the value of these plants in promoting health, preventing disease, and supporting the development of affordable, plant-based therapeutic options for use in both traditional and modern healthcare systems.

Free Radical Scavenging Plants

Plants with free radical scavenging properties are highly valued for their strong antioxidant effects, which help safeguard the body against oxidative hurt triggered by unstable molecules identified as free radicals. These reactive species, generated through normal metabolic activities or triggered by external factors like pollution, radiation, and poor diet, can harm cells, proteins, and DNA. This oxidative stress is closely linked to aging and the progression of chronic illnesses such as heart disease, neurodegenerative conditions, diabetes, and cancer. Such plants are abundant in health-promoting compounds, including polyphenols, flavonoids, tannins, alkaloids, and vital vitamins like C and E, all of which work together to neutralize oxidative threats. Beyond their antioxidant role, these constituents often possess anti-inflammatory, antimicrobial, and immune-enhancing properties. Consequently, these plants are gaining prominence in the development of pharmaceuticals, dietary supplements, and skincare products. Their enduring presence in traditional healing practices, combined with growing scientific validation, underscores their potential in modern health and wellness applications.

India is endowed with extraordinary plant diversity, comprising approximately 45,000 species, which represent nearly 7% of the world’s entire flora. Among these, about 8,000 species are recognized for their medicinal value, accounting for nearly half of India’s higher flowering plants. This rich and varied plant heritage ranks India among the leading nations globally in terms of medicinal and aromatic plant resources. Notably, the country harbors nearly 11% of all medicinal plant species identified worldwide, emphasizing its importance as a key center for botanical and therapeutic knowledge. The use of plants for healing is deeply woven into India’s cultural and historical fabric, particularly through Ayurveda, the country’s ancient system of medicine. Practiced for over 3,500 years, Ayurveda traces its origins to classical texts like the *Charaka Samhita*, written around 600 BC. This time-honored tradition, preserved through generations, has enabled traditional practitioners to make effective use of plant-based remedies in promoting health and treating various ailments. Although many plants with antioxidant properties have already been studied, the following section introduces additional species recognized for their medicinal applications, further enriching our understanding of the natural world’s healing potential.

Worm Killer / Indian Birthwort

Aristolochia bracteolata, also known as *Worm killer / Indian Birthwort*, is a perennial herb commonly used in traditional medicine by indigenous communities and rural populations in the Chittoor District of Andhra Pradesh, India. It is particularly recognized for its effectiveness in accelerating the healing of wounds and cuts. Beyond India, this plant is also found in various regions across Tropical Africa, as well as in countries such as Oman, Saudi Arabia, the United Arab Emirates, Yemen, Pakistan, and Sri Lanka. A number of scientific studies have been conducted to investigate the wound-healing potential of *A. bracteolata*. In these studies, ethanol extracts from the shade-dried leaves of the plant were tested on rats using incision, excision, and dead-space wound models. The results indicated a noticeable improvement in the healing process, with a significant increase in the activity of two key antioxidant enzymes—superoxide dismutase and catalase—in the wound tissue. These enzymes are vital in reducing oxidative stress and aiding the body's natural healing processes. In addition to its positive impact on wound healing, early phytochemical analysis of the plant extract revealed the presence of several bioactive compounds, including alkaloids, saponins, glycosides, steroids, tannins, phenolic compounds, and flavonoid glycosides. These compounds are supposed to be accountable for the plant's medicinal properties, reinforcing its traditional use and offering promising potential for future pharmaceutical applications.[12]

Velvetleaf

The alkaloidal fraction (AFCP) extracted from *Velvetleaf* (*Cissampelos pareira*) roots was thoroughly examined for its in-vitro antioxidant and immunomodulatory effects in mice. The HPTLC analysis of AFCP revealed the presence of berberine. The fraction exhibited strong antioxidant activity, effectively scavenging the DPPH free radical and superoxide ions, and inhibiting lipid peroxidation in rat liver homogenates persuaded by an iron/ADP/ascorbate complex. At lower doses, AFCP showed significant immunosuppressive effects, including a noticeable reduction in humoral antibody titre. However, no such activity was observed at higher doses. Additionally, AFCP was found to significantly suppress the delayed-type hypersensitivity response at a specific dose. In conclusion, the study confirmed that the alkaloidal fraction of *Velvetleaf* roots possesses both potent antioxidant and immunosuppressive properties.[13] The study revealed that the 50% ethanol extract of *Cissampelos pareira* roots demonstrated significant antioxidant activity both in vitro and in vivo. The extract was rich in polyphenols and showed strong reducing capabilities in various tests, effectively neutralizing superoxide, hydrogen peroxide, hydroxyl radicals, and nitric oxide. It also inhibited protein oxidation caused by hydroxyl radicals. In animal models, the extract effectively protected against oxidative damage induced by benzo(a)pyrene-induced gastric toxicity in mice, preserving the gastric mucosa. Additionally, it lowered gastric lipid peroxidation and enhanced the activity of key antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase. This report is the first to report on the antioxidant properties of *Cissampelos pareira* against oxidative stress caused by benzo(a)pyrene.[14]

Amaranth

Amaranthus paniculatus L., commonly referred to as Amaranth, is a member of the Amaranthaceae family and is known for its ability to thrive in disturbed habitats such as wastelands, short grasslands, and shaded forest areas. Although native to Central America, it has become widely cultivated and naturalized around the world. *A. paniculatus* is highly valued for its rich nutritional profile, being a significant source of antioxidants like beta-carotene, ascorbic acid (vitamin C), and folate. Plants within the Amaranthaceae family are recognized for their high levels of carotenoids, vitamin C, and essential amino acids, including lysine and methionine, as well as proteins vital for human nutrition. Studies have confirmed its strong antioxidant properties, demonstrated through a DPPH radical-scavenging assay, which showed its capacity to neutralize free radicals or donate hydrogen, thus supporting its health-promoting benefits.[15,16]

Phyllanthus maderaspatensis

Phyllanthus maderaspatensis is a widely distributed plant found in India, Pakistan, Yemen, Sri Lanka, parts of Tropical Africa, Indonesia, and Australia. Known for its long history of traditional medicinal use, the leaves of this plant are valued for their expectorant and diaphoretic properties, helping with conditions such as strangury (painful urination) and excessive sweating. The seeds, though bitter in taste, are believed to possess carminative, laxative, and liver-tonic qualities. They are also used as a diuretic and to treat a variety of ailments, including bronchitis, earaches, griping pains, ophthalmia (inflammation of the eye), and ascites (fluid accumulation in the abdomen). Scientific research on *P. maderaspatensis* has primarily explored its hepatoprotective (liver-protecting) and choleretic (bile-producing) effects. Studies have tested different extracts of the plant, including n-hexane, alcohol, and water, for their ability to protect the liver and improve bile production. The plant's extracts were found to provide significant protection against liver damage, especially in cases of acetaminophen-induced hepatotoxicity, as shown by changes in liver enzyme markers. The n-hexane extract was particularly potent, showing protective effects even at low doses, and was also found to have choleretic activity. Additionally, it exhibited antioxidant effects, including the scavenging of hydroxyl radicals and inhibition of lipid peroxidation. In comparison, the water and alcohol extracts showed moderate activity in protecting the liver. Traditionally, *P. maderaspatensis* is also used as a remedy for headaches and as a natural laxative and diuretic.[17,18]

Phyllanthus virgatus

Phyllanthus virgatus is mainly distributed across India, Bhutan, and Nepal, and can also be found in various regions of Southeast Asia, Australia, and the Pacific Islands. According to research conducted by Kumaran and Karunakaran (2007), the antioxidant properties of *P. virgatus* are moderate, positioned between the stronger and weaker antioxidant activities of other species within the *Phyllanthus* genus.[18]

Gymnema montanum

Gymnema montanum is a rare and endangered plant species native to India, particularly the Western Ghats, but it is also found in other regions, including parts of West Tropical Africa, Southern Africa, China, Japan, Sri Lanka, Thailand, Vietnam, Indonesia, and Malaysia. The ethanolic leaf extract of this plant has demonstrated both antihyperglycemic and antiperoxidative effects. It contains active compounds such as gymnemagenin and gymnemic acids, which are primarily responsible for its ability to help lower blood sugar levels. Additionally, the extract has been found to reduce lipid peroxides and increase the levels of vital antioxidants, including reduced glutathione, vitamin C, and vitamin E (α-tocopherol). Research by Ananthan *et al.* (2003) highlighted that administering the leaf extract to diabetic rats resulted in a significant boost in their antioxidant levels, suggesting its potential therapeutic benefits for managing oxidative stress in diabetes.[19,20]

Moringa oleifera

The study demonstrated that both ethanolic (E1) and saline (E2) extracts from *Moringa oleifera* flowers, inflorescence rachis, and leaf tissue contain antioxidant compounds. Notably, the ethanolic leaf extract (E1d) showed the most potent radical scavenging capacity, reflecting strong antioxidant activity. In comparison, the saline extracts reacted more slowly with DPPH, indicating lower antioxidant effectiveness. Further analysis confirmed the presence of at least three flavonoids in the E1 extracts of flowers, rachis, stem tissue, and leaves, while the E2 extracts from flowers and leaves contained at least two flavonoids. Overall, the results emphasize the valuable antioxidant and nutritional potential of *M. oleifera* plant parts.[21] The polyphenolic extract derived from *Moringa oleifera* leaves (MOEF) demonstrated exceptional antioxidant activity, outperforming other tested fractions in a variety of in vitro assays. Its protective effect was shown to be dose-dependent, offering increasing defense against oxidative DNA damage with higher concentrations, suggesting a key role in preserving cellular health. In vivo experiments further validated these observations, revealing that MOEF significantly alleviated oxidative stress caused by CCl_4, a well-known inducer of liver damage. This was evident through lower levels of lipid peroxidation and higher concentrations of glutathione, both critical indicators of oxidative status. Moreover, MOEF aided in normalizing the activity of vital antioxidant enzymes, superoxide dismutase and catalase, which were otherwise suppressed by

CCl_4 exposure. High-performance liquid chromatography analysis confirmed the presence of several potent bioactive compounds within MOEF, including phenolic acids such as gallic, chlorogenic, ellagic, and ferulic acid, along with flavonoids like kaempferol, quercetin, and rutin. These results indicate that MOEF exerts its antioxidant effects through a combination of free radical neutralization and metal ion chelation. Collectively, the findings support MOEF as a promising natural source for combating oxidative stress and promoting overall well-being.[22]

Conclusion:

In conclusion, plants with free radical scavenging properties are essential in protecting the body from oxidative stress, a major contributor to chronic diseases like heart disease, cancer, and diabetes. Rich in bioactive compounds such as polyphenols and flavonoids, these plants offer potent antioxidant, anti-inflammatory, antimicrobial, and immune-boosting effects. India's vast plant diversity, with over 45,000 species, has long supported traditional medicine systems like Ayurveda. Plants like *Moringa oleifera* and *Gymnema montanum* demonstrate significant therapeutic potential. Ongoing research highlights their promise as natural, affordable alternatives to synthetic drugs, contributing to global health and wellness.

References:

1. Aruoma, O. I. (1998). Free radicals, oxidative stress, and antioxidants in human health and disease. *Journal of the American Oil Chemists' Society, 75*(2), 199–212. https://doi.org/10.1007/s11746-998-0032-9
2. Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M. M., & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions, 160*(1), 1–40. https://doi.org/10.1016/j.cbi.2005.12.009
3. Greco, A., Minghetti, L., & Levi, G. (2000). Isoprostanes, novel markers of oxidative injury, help understanding the pathogenesis of neurodegenerative diseases. *Neurochemical Research, 25*, 1357–1364. https://doi.org/10.1023/A:1007684108861
4. Jha, P., Flather, M., Lonn, E., Farkouh, M., & Yusuf, S. (1995). The antioxidant vitamins and cardiovascular disease: A critical review of epidemiologic and clinical trial data. *Annals of Internal Medicine, 123*(11), 860–872. https://doi.org/10.7326/0003-4819-123-11-199512010-00009
5. Ramakrishna, B. S., Varghese, R., Jayakumar, S., Mathan, M., & Balasubramanian, K. A. (1997). Circulating antioxidants in ulcerative colitis and their relationship to disease severity and activity. *Journal of Gastroenterology and Hepatology, 12*(7), 490–494. https://doi.org/10.1111/j.1440-1746.1997.tb00591.x
6. Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M. M., & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions, 160*(1), 1–40. https://doi.org/10.1016/j.cbi.2005.12.009

7. Halliwell, B., & Gutteridge, J. M. (2015). *Free radicals in biology and medicine* (5th ed.). Oxford University Press.
8. Stadtman, E. R. (2004). Role of oxidant species in aging. *Current Medicinal Chemistry, 11*(9), 1105–1112. https://doi.org/10.2174/0929867043365341
9. Stanner, S. A., Hughes, J., Kelly, C. N., & Buttriss, J. (2004). A review of the epidemiological evidence for the 'antioxidant hypothesis'. *Public Health Nutrition, 7*(3), 407–422. https://doi.org/10.1079/PHN2003543
10. Scartezzini, P., & Speroni, E. (2000). Review on some plants of Indian traditional medicine with antioxidant activity. *Journal of Ethnopharmacology, 71*(1–2), 23–43. https://doi.org/10.1016/S0378-8741(00)00187-0
11. Govindarajan, R., Rastogi, S., Vijayakumar, M., Shirwaikar, A., Rawat, A. K., Mehrotra, S., & Pushpangadan, P. (2003). Studies on the antioxidant activities of Desmodium gangeticum. *Biological and Pharmaceutical Bulletin, 26*(10), 1424–1427. https://doi.org/10.1248/bpb.26.1424
12. Shirwaikar, A., Somashekar, A. P., Udupa, A. L., Udupa, S. L., & Somashekar, S. (2003). Wound healing studies of Aristolochia bracteolata Lam. with supportive action of antioxidant enzymes. *Phytomedicine, 10*(6–7), 558–562. https://doi.org/10.1078/0944-7113-00282
13. Bafna, A., & Mishra, S. (2010). Antioxidant and immunomodulatory activity of the alkaloidal fraction of Cissampelos pareira Linn. *Scientia Pharmaceutica, 78*(1), 21–32. https://doi.org/10.3797/scipharm.0912-09
14. Amresh, G., Rao, C. V., & Singh, P. N. (2007). Antioxidant activity of Cissampelos pareira on benzo(a)pyrene-induced mucosal injury in mice. *Nutrition Research, 27*(10), 625–632. https://doi.org/10.1016/j.nutres.2007.06.006
15. Amin, I., Norazaidah, Y., & Hainida, K. E. (2006). Antioxidant activity and phenolic content of raw and blanched Amaranthus species. *Food Chemistry, 94*(1), 47–52. https://doi.org/10.1016/j.foodchem.2004.10.049
16. Bhatia, A. L., & Jain, M. (2003). Amaranthus paniculatus (Linn.) improves learning after radiation stress. *Journal of Ethnopharmacology*, *85*(1), 73–79. https://doi.org/10.1016/S0378-8741(02)00357-1
17. Asha, V. V., Akhila, S., Wills, P. J., & Subramoniam, A. (2004). Further studies on the antihepatotoxic activity of Phyllanthus maderaspatensis Linn. *Journal of Ethnopharmacology, 92*(1), 67–70. https://doi.org/10.1016/j.jep.2004.02.014
18. Kumaran, A., & Karunakaran, R. J. (2007). In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. *LWT - Food Science and Technology*, *40*(2), 344–352. https://doi.org/10.1016/j.lwt.2005.09.011

19. Murakami, N., Murakami, T., Kadoya, M., Matsuda, H., Yamahara, J., & Yoshikawa, M. (1996). New hypoglycemic constituents in "gymnemic acid" from Gymnema sylvestre. *Chemical and Pharmaceutical Bulletin, 44*(2), 469–471. https://doi.org/10.1248/cpb.44.469
20. Ananthan, R., Baskar, C., Narmatha Bai, V., Pari, L., Latha, M., & Ramkumar, K. M. (2003). Antidiabetic effect of Gymnema montanum leaves: Effect on lipid peroxidation induced oxidative stress in experimental diabetes. *Pharmaceutical Research, 48*(6), 551–556. https://doi.org/10.1016/S0031-9422(03)00162-5
21. Santos, A. F., Argolo, A. C., Paiva, P. M., & Coelho, L. C. (2012). Antioxidant activity of *Moringa oleifera* tissue extracts. *Phytotherapy Research, 26*(9), 1366–1370. https://doi.org/10.1002/ptr.3744
22. Verma, A. R., Vijayakumar, M., Mathela, C. S., & Rao, C. V. (2009). In vitro and in vivo antioxidant properties of different fractions of *Moringa oleifera* leaves. *Food and Chemical Toxicology, 47*(9), 2196–2201. https://doi.org/10.1016/j.fct.2009.06.026

BIOPROSPECTING OF ENDOPHYTIC FUNGI: A SOURCE OF NOVEL ANTIMICROBIAL METABOLITES

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Abstract:

Bioprospecting of endophytic fungi these days has paid significant attention due to novel bioactive compounds and possible sources of drugs, agriculture and industrial applications. Their living tangentially, endophytic fungi within the tissues of the plant, through their peculiar metabolic routes, have enabled them to manufacture secondary metabolites with antimicrobial, antioxidant, and enzyme-inhibitory properties. Advances in molecular and analytical techniques have facilitated the discovery of structurally diverse and biologically powerful compounds from these fungi. Additionally, their symbiotic relationship with host plants suggests a role in plant health, stress tolerance and immunity, making them valuable for permanent agriculture. This review examines challenges associated with diversity, biotechnology capacity, and bioprospecting endophytic fungi, which highlights their role in drug discovery, biocontrol and enzyme production. Future research should focus on genome mining, fermentation adaptation and a synthetic biology approach to increase the yield and application of fungi metabolites.

Keywords: Bioprospecting, Endophytic Fungi, Secondary Metabolites, Biocontrol, And Enzyme Production.

Introduction:

Endophytic fungi, which colonize the internal tissues of vegetation without causing apparent harm, have emerged as a promising source of novel bioactive compounds with sizeable pharmaceutical, agricultural, and business applications. These fungi set up symbiotic associations with their host flora, contributing to plant health, stress tolerance, and disease resistance (Strobel & Daisy, 2003). Due to their unique metabolic pathways, they can generate structurally diverse secondary metabolites, such as antibiotics, anticancer agents, and enzyme inhibitors, a lot of which have been identified as potential drug leads (Aly *et al.*, 2011). Bioprospecting, the systematic exploration of biological assets for commercially valuable compounds, has driven the discovery of numerous bioactive metabolites from endophytic fungi. Advances in molecular biology, metabolomics, and genome mining have facilitated the identification and characterization of novel fungal metabolites with promising therapeutic and commercial homes (Gupta *et al.,* 2020). The sustainable nature of fungal cultivation makes them an attractive alternative to standardize resources of bioactive compounds, which include plant life and marine organisms. Despite their monstrous capacity, the bioprospecting of endophytic fungi faces demanding situations, which include difficulties in fungal isolation, the influence of subculture conditions on metabolite production, and the need for effective screening techniques to become aware of novel bioactive compounds (Chowdhury *et al.,* 2022). Future research should focus on optimizing cultivation techniques, applying artificial biology processes, and exploring endophytes from extreme environments to unlock their full capacity. This evaluation highlights the significance of endophytic fungi in bioprospecting.

The discovery of endophytes from extreme environments to adapt to farming techniques, implement synthetic biology approaches and unlock their full capacity. This review highlights the importance of endophytic fungi in bioprospecting, emphasizing their various applications and challenges that must be addressed to maximize their benefits.

Their peculiar ecological niche has made them a gold mine of bioactive metabolites and to a large extent untouched sources, many of which have been reported to have antimicrobial, antioxidant, and anti-inflammatory properties (Strobel & Daisy, 2003). There is a long history of the concept of bioprospecting biologically active compounds from natural sources for use in pharmaceuticals, agriculture and biotechnology, and the endophytic fungi are recognized as a promising source in the region (Aly *et al.,* 2011). These metabolites are often separated from those produced by their host plants, providing them an evolutionary benefit and making them valuable for biotechnology and medical applications.

Metabolic Diversity and Applications

One key factor that makes endophytic fungi highly attractive for bioprospecting is their ability to synthesize a wide array of complex secondary metabolites. These metabolites may include alkaloids, terpenoids, polyketides, and phenolic compounds, all with diverse biological activities. Many of these compounds are very interesting because they have various antimicrobial, antioxidant, and antidiuretic properties, which can be used to discover new medical agents (Aly *et al.,* 2011; Toghueo and Boyom 2019). For example, some species of fungi belonging to the genus *Fusarium oxysporum* have been reported to produce various bioactive compounds, some of which have huge anticancer activities (Gupta *et al.,* 2020). The development of antimicrobial agents, including novel antibiotics and antifungals. In addition to their ability, endophytic fungi play an important role in sustainable agriculture. Their ability to synthesize enzymes such as bioactive compounds, such as phytohormones, antifungal agents, and cellulose, makes them valuable for biocontrol applications, promotes plant growth and helps protect crops from pathogens (Verma *et al.*, 2022). In addition, these fungi can enhance the soil microbiome and contribute to organic farming practices by reducing the requirement of chemical pesticides and fertilizers. Many endophytic fungi have been discovered for their ability to produce plant growth-promoting substances (PGPs), including indole acetic acid (IAA) and gibberellins, which promote plant growth, growth and stress flexibility (Bacon and White, 2000).

Challenges in Bioprospecting

While the endophytic fungi are of immense capacity, bioprospecting of these microorganisms is a challenging function. One of the biggest challenges is the separation and identity of endophytic fungal species. Unlike free-living fungi, which are easy to culture and identify, endophytes have special environmental requirements for development, and many of them have not been cultured under these in vitro conditions (Porras-Alfaro and Bayman, 2011). Endophytic fungi are usually present in low numbers in plant tissues, so their secondary metabolites are difficult to separate and characterize. In addition, bioactive compound production in endophytic fungi is highly dependent on factors such as plant species, geographical location, climate and even the developmental phase of the host plant (Zhong *et al.*, 2016). Therefore, optimal conditions for metabolite production are one of the biggest challenges in fungal bioprospecting. Better genomic and metabolomic tools have enabled it to profile fungal microbiomes and metabolites produced by them, but it is a challenge to increase these processes to a commercial level.

Advances in Techniques and Future Directions

Recent advances in technology have extended the scope of fungal bioprospecting, mainly with the introduction of high-throughput sequencing and metabolomic profiling. Genomic sequencing has enabled investigators to find gene clusters involved in the biosynthesis of bioactive compounds, taking into account the invention of hitherto unknown secondary metabolites (Zhao *et al.*, 2010). Furthermore, technologies like transcriptomics and proteomics have given insights into the metabolic pathways of endophytic fungi and the interaction of these fungi with host flowers, taking into consideration the focused manipulation of fungal strains to enhance the production of specific metabolites (Chowdhury *et al.*, 2022). Furthermore, the mixture of synthetic biology and metabolic engineering in fungal research is incredibly promising. By changing fungal biosynthetic pathways or introducing new gene clusters from different organisms, investigators can potentially increase the yield of favoured metabolites or maybe design new compounds with suitable traits (Vyas *et al.*, 2021). This can potentially cover some of the drawbacks of herbal biosynthesis and make the production of valuable fungal metabolites more powerful and cost-efficient. As the technology of fungal ecology and biosynthesis advances, new and formerly unknown endophytic fungi will be found, in particular in under-explored habitats like tropical rainforests, deserts, and extreme environments. These new resources may provide bioactive compounds that can be stronger, specific, or maybe more environmentally pleasant than their synthetic or traditional counterparts.

Review of Literature: Bioprospecting of Endophytic Fungi

1. Introduction to Endophytic Fungi Endophyte fungi are microorganisms that colonize benignly internal plant tissues, symbiotic with their host plants. Their mechanisms have generally rendered plant growth, stress tolerance, and disease protection (Strobel & Daisy, 2003). The gateway to the source endophytes of bioactive metabolites into some of the richest bioprospecting studies has stimulated a breadth of understanding that underlies how best to utilize endophytic resource materials for the development of pharmaceuticals, agriculture, and biotechnology (Aly *et al.*, 2011).

2. Diversity and Ecology of Endophytic Fungi The Ascomycota, Basidiomycota, and Zygomycota groups comprise so many endophytic fungi that the remainder includes all other fungal groups (Rodriguez *et al.,* 2009). Tropical rainforests, arid lands and marine environments are among many ecosystems where many different plants are inhabited by widespread fungal endophytes (Arnold *et al.,* 2000). Fungal endophyte composition differs based on plant species, geographical site, climate and edaphic conditions, according to (Porras-Alfaro & Bayman 2011).

3. Bioactive Compounds from Endophytic Fungi

3.1 Antimicrobial Metabolites of Endophytic fungi There is a broad consensus that pathogenic micro-organisms can be targeted using antibiotics and antifungal substances, which prescriptive endophytic fungi metabolites are said to produce. A good example is Taxol, the well-known anticancer drug that was first extracted from Taxus trees and is now known to be in the endophytic fungus *Pestalotiopsis microspora*. (Strobel *et al.,* 1996). Similarly, endophytic fungi isolated from *Camptotheca acuminata* are known to produce the powerful anticancer compound Camptothecin (Kusari *et al.,* 2013). Furthermore, certain *Fusarium* and *Penicillium* species have demonstrated activities against cancer in already multi-drug-resistant bacterial strains. (Zhong *et al.,* 2016)

3.2 Anticancer and Cytotoxic Compounds Fungal endophytes have produced numerous cytotoxic metabolites with potential cancer therapeutic capabilities. For example, fumigaclavine C produced by *Aspergillus fumigatus* exhibits significant anticancer activity (Aly *et al.,* 2011).

3.3 Antioxidant and Anti-inflammatory Metabolites that have antioxidant and anti-inflammatory effects, which could aid in battling oxidative stress-related disorders, are also produced by endophytic fungi (Toghueo *et al.,* 2019). These include flavonoids and phenolic compounds extracted from species of *Colletotrichum* and *Alternaria*

4. Methodology for Bioprospecting of Endophytic Fungi

4.1 Sample Collection

Choose healthy plant tissues (leaves, stems, roots) from various ecological niches. Surface sterilize plant material with ethanol, sodium hypochlorite, and sterile water to eliminate epiphytic contaminants. - Cut sterilized tissues into small pieces and inoculate them on culture media. (Lal *et al.,* 2023)

4.2 Fungal Isolation and Cultivation-

Incubate plant pieces on potato dextrose agar (PDA) or other selective media at optimal temperatures. Monitor and subculture developing fungal colonies to get pure cultures. Morphological and molecular characterization (ITS sequencing) to identify isolates. (Almjalawi *et al.,* 2023; Bankevich *et al.*, 2012).

4.3 Screening for Bioactive Compounds-

Perform preliminary screening using antimicrobial assays against bacterial and fungal pathogens. Extract secondary metabolites using solvent extraction techniques (ethyl acetate, methanol, etc.). Characterize compounds using chromatographic (HPLC, GC-MS) and spectroscopic (NMR, LC-MS) methods. (Fekry, *et al.*, 2022)

4.4 Molecular and Biotechnological Approaches –

Genomic and transcriptomic analysis to identify biosynthetic gene clusters. Metabolomic profiling to identify novel bioactive compounds. Application of synthetic biology for metabolic pathway engineering to improve metabolite production. (Tran, and Kumaravel, 2023).

4.5. Recent Advances in Genomic and Biotechnological Approaches –

Genomics, transcriptomics and metabolomics have accelerated the discovery of novel bioactive compounds from endophytic fungi (Zhao *et al.,* 2010). Genome mining has enabled the identification of biosynthetic gene clusters for secondary metabolite production. Synthetic biology is further increasing the potential of fungal endophytes by engineering metabolic pathways for increased metabolite yields (Vyas *et al.,* 2021).

4.6. Challenges and Future Directions-

Although promising, bioprospecting of endophytic fungi is faced with some challenges. Culturing Challenges: Most endophytes are difficult to culture under laboratory conditions, limiting their study (Porras-Alfaro & Bayman, 2011).

4.7. Metabolite Production Variability-

The production of secondary metabolites is controlled by environmental and physiological factors and is hard to standardize (Gupta *et al.,* 2020).

4.8. Sustainable Harvesting Requirement-

Over-harvesting of endophyte-host plant species can harm biodiversity; hence, sustainable cultivation is required (Kusari *et al.,* 2013). Future research should focus on discovering new extreme environments for new fungal species.

5. Agricultural and Environmental Applications

5.1 Biocontrol Agents As sources of biocontrol agents, various endophytic fungi, such as *Beauveria bassiana*, have brought interest because of their capability to synthesize antifungal and insecticidal compounds. *B. bassiana* is used as an endophyte and biopesticide against insect pests (Vega *et al.* 2008). *Trichoderma* species increase the growth of plants and interfere with soil plant pathogens (Harman *et al.* 2004).

5.2 Plant Growth Promotion Endophytic fungi enhance a plant's growth by synthesizing plant hormones like indole-3-acetic acid (IAA) and gibberellins. These substances assist in root growth, ingestion of nutrients, and a plant's ability to withstand stress (Khan *et al.* 2012).

5.3 Bioremediation Potential Fungal endophytes have destroyed formatted electrons and heavy metals fully including *Aspergillus* species, which are successful in degrading petroleum hydrocarbons in the soil. For instance, *Pestalotiopsis microspora* has been stated to degrade plastic polymers (Russell *et al.*, 2011).

5.4 Advances in Genomic and Biotechnological Approaches The integration of genomics, transcriptomics and metabolomics has accelerated the discovery of novel bioactive compounds from endophytic fungi (Zhao *et al.*, 2010). Genome mining has enabled the identification of biosynthetic gene groups responsible for the production of secondary metabolites. The advances in synthetic biology are further enhancing the capacity of fungal endophytes by engineering metabolic routes for better metabolite yields (Vyas *et al.*, 2021)

5.5 Challenges and Future Directions While promising, bioprospecting of endophytic fungi is hindered by some challenges, Difficulty in Culturing: Endophytes cannot be cultured by utilizing standard laboratory procedures, which is a hindrance in their research (Porras-Alfaro & Bayman, 2011).

5.6 Variability in Metabolite Production: Environmental and physiological factors often influence the production of secondary metabolites, making standardization difficult (Gupta *et al.*, 2020).

5.7 Need for Sustainable Harvesting: Overexploitation of endophyte-rich plant species can threaten biodiversity; therefore, sustainable cultivation techniques are needed (Kusari *et al.*, 2013).

5.8 Variation in Yield of Metabolites: Environmental and physiological conditions are expected to affect the yield of secondary metabolites, rendering standardization challenging (Gupta *et al.*, 2020).

5.9 Requirement of Sustainable Harvesting: Exploitation of endophyte-dense plant species may undermine biodiversity; thus, sustainable farming practices are required (Kusari *et al.*, 2013).

Future studies should focus on the investigation of extreme habitats for new fungal species, optimization of fermentation processes, and the use of synthetic biology tools for the improvement of metabolite yield.

Conclusion:

Endophytic fungi represent a great and in large part untapped reservoir of biologically active compounds with various programs in medicine, agriculture, and industry. While the bioprospecting of these organisms offers significant promise, it also affords challenges regarding fungal isolation, cultivation, and metabolite production. However, advances in genomics, metabolites, and synthetic biology are likely to overcome these hurdles and unleash the full potential of endophytic fungi. Future research must be focused on optimizing fungal cultivation strategies, improving metabolite yields, and exploring new fungal species from diverse ecological niches. By doing so, we will better harness the valuable sources supplied through these notable microorganisms for the advantage of numerous sectors.

Endophytic fungi represent an in large part untapped reservoir of bioactive compounds with promising packages in medicine, agriculture, and enterprise. Advances in genomic technologies and biotechnology are paving the way for more green bioprospecting strategies. Addressing the challenges of fungal isolation, cultivation, and metabolite variability could be important in unlocking the full ability of these microorganisms for sustainable packages. Delivered an in-depth methodology segment on the bioprospecting of endophytic fungi

References:

1. Almjalawi, Abed BS, Chechan RA, AL-Hadedee LT. (2023) Using food residues (Potato peels) as an alternative to potato dextrose agar in the growth of edible food fungi. *Journal of Applied and Natural Science*.15(1):211- 219.
2. Aly, A. H., Debbab, A., & Proksch, P. (2011). Fungal endophytes: Unique plant inhabitants with great promises. *Applied Microbiology and Biotechnology*, 90 (6), 1829–1845.
3. Arnold, A. E., Z. Maynard, G. S. Gilbert, P. D. Coley, and T. A. Kursar. (2000). Are tropical fungal endophytes hyperdiverse *Ecology Letters* 3: 267–274.
4. Bacon, Charles W., and White, James F. (2000). Physiological adaptations in the evolution of endophytism in the Clavicipitaceae. In *Physiological Adaptations in the Evolution of Endophytism in the Clavicipitaceae*.
5. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. (2012). A new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol.* May;19(5):455-77
6. Chowdhury, S., Basu, S. K., & Kader, J. (2022). Advances in endophytic fungi research: Bioprospecting for bioactive metabolites. *Frontiers in Microbiology, 13*, 845321.
7. Fekry, R. A., Kelani, K., Fayez, Y., & Tantawy, M. (2022). Comparative validated chromatographic methods for the simultaneous determination of caffeine, codeine, and

paracetamol along with the related compound "p-aminophenol" in tablets. JPC – *Journal of Planar Chromatography – Modern TLC*, 35, 51-59.

8. Gupta, S., Chaturvedi, P., & Kumar, P. (2020). Bioprospecting of fungal endophytes: An emerging source of bioactive metabolites. *Current Research in Microbial Sciences*, 1, 100005.
9. Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., & Lorito, M. (2004). Trichoderma species—opportunistic, avirulent plant symbionts. *Nature reviews microbiology*, *2*(1), 43-56.
10. Khan, A.L., Hamayun, M., Kang, SM. (2012). Endophytic fungal association via gibberellins and indole acetic acid can improve plant growth under abiotic stress: an example of *Paecilomyces formosus* LHL10. *BMC Microbiol* 12, 3.
11. Kusari, S., Hertweck, C., & Spiteller, M. (2013). Chemical ecology of endophytic fungi: Origins of secondary metabolites. *Chemistry & Biology*, 20 (6), 703–711.
12. Lal, M., Jamwal, M., Sood, Y., Bakshi, P., Sharma, N., Sharma, S. & Kumar, S. (2023). Micropropagation of fruit crops: A review. *Plant Science Today*, 10, 108-117
13. Porras-Alfaro, A., & Bayman, P. (2011). Ecology of fungal endophytes. *Fungal Ecology*, 4 (3), 123–133.
14. Rodriguez RJ, White JF, Arnold AE, Redman RS. (2009) Fungal endophytes: diversity and functional roles. New Phytol. 30*;* 182:314
15. Russell, J. R., Huang, J., Anand, P., Kucera, K., Sandoval, A. G., Dantzler, K. W., Hickman, D., Jee, J., Kimovec, F. M., Koppstein, D., Marks, D. H., Mittermiller, P. A., Núñez, S. J., Santiago, M., Townes, M. A., Vishnevetsky, M., Williams, N. E., Vargas, M. P., Boulanger, L. A., Bascom-Slack, C., Strobel, S. A. (2011). Biodegradation of polyester polyurethane by endophytic fungi. *Applied and environmental microbiology*, *77*(17), 6076–6084
16. Strobel GA, X Yang, J Sears, R Sidhu and WM Hess. (1996). Taxol from *Pestalotiopsis microspora*, an endophytic fungus of *Taxus wallachiana*. *Microbiology* 142: 435–440.
17. Strobel, G., Daisy, B. (2003). Bioprospecting for microbial endophytes and their natural products. *Microbiology and Molecular Biology Reviews*, 67(4), 491–502.
18. Toghueo K.M., Boyom F.F. (2019). Endophytes from ethno-pharmacological plants: Sources of novel antioxidants- A systematic review. *Biocatal. Agric. Biotechnol*. 22:101430.
19. Tran, K. T., & Kumaravel, A. (2023). Impact of the Synthetic Scaffold Strategy on the Metabolic Pathway Engineering. *Biotechnology and Bioprocess Engineering*, 28, 379-385.
20. Vega F. E. (2008). Insect pathology and fungal endophytes. *J. Invertebr. Pathol.* 98 277–279.

21. Verma, A., Shameem, N., Jatav, H. S., Sathyanarayana, E., Parray, J. A., Poczai, P. (2022). Fungal endophytes to combat biotic and abiotic stresses for climate-smart and sustainable agriculture. *Front. Plant Sci*. 13.
22. Vyas, S., Hussain, A., Kumar, M., Dhiman S. S.K., Bhat, R., and Saran, S. (2021). Synthetic biology and metabolic engineering approaches for improving the production of secondary metabolites in fungi. *Applied Microbiology and Biotechnology*, 105 (4), 1435–1452.
23. Zhao J., Zhou L., Wang J., Shan T., Zhong L., Liu X., Gao X. (2010). Endophytic fungi for producing bioactive compounds originally from their host plants. Curr. Res. Technol. Educ. Trop. *Appl. Microbiol. Microb. Biotechnol*. 1:567–576.
24. Zhong L, Niu B, Tang L, Chen F, Zhao G, Zhao J. (2016). Effects of polysaccharide elicitors from endophytic *Fusarium oxysporum* Fat9 on the growth, flavonoid accumulation and antioxidant property of *Fagopyrum tataricum* sprout cultures. *Molecules*. 21:1590.

SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES PREPARED BY LEAF EXTRACTS OF *AZADIRACHTA INDICA* (NEEM)

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1. Introduction:

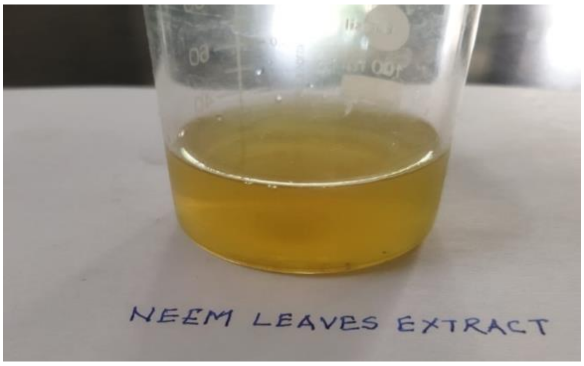
Several methods have been used for the preparation of silver nano-particles which can be either physical, chemical or biological methods. Earlier methods used for the synthesis of silver nano-particles were toxic and hazardous chemicals were used for their synthesis. Thus, the use of eco-friendly processes, for the synthesis of silver nanoparticles is known as ‘‘Green synthesis’’. Green synthesis is preferred over conventional synthesis because it is ecofriendly, cost-effective, single-step method that can be easily scaled up for large scale synthesis and does not require high pressure, temperature, energy and toxic chemicals (1). Many researchers have reported the use of materials such as plant leaf extract, root, stem, bark, leaf, fruit, bud and latex (2), fungi (3), bacteria (4) and enzymes (5) for the synthesis of silver nano-particles. A lot of work has been done on green synthesis of silver nano-particles using microorganisms including bacteria, fungi and plants because of their antioxidant properties capable of reducing metal compounds in their respective nanoparticle.

Plant extracts produce best capping material for the stabilization of silver nanoparticles (6).The present work aims to use the leaf extract of Azadirachta indica (commonly known as neem) a member of the Meliaceae family used for the green synthesis of silver nanoparticles. This is a medicinal plant and is used for the treatment bacterial, fungal, viral and many types of skin ailments since ancient times. The aqueous neem extract is used in the synthesis of various nanoparticles such as gold, zinc oxide, silver, etc. Terpenoids and flavanones are the two important phytochemicals present in neem which play a vital role in stabilizing the nanoparticle and also act as capping and reducing agent (7). Aqueous neem leaf extract reduces silver salt to silver nitrate, this capped nanoparticle with neem extract exhibit antibacterial activity.

2. Methodology:

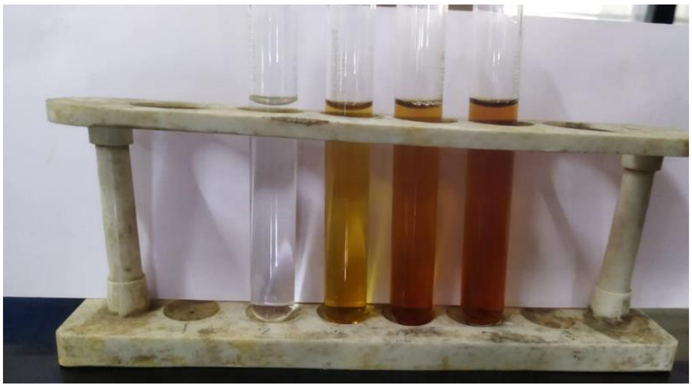
2.1 Preparation of Plant Extract

Preparation of leaf extracts from Azadirachta indica (Neem) leaves Fresh neem leaves were collected from University Campus in the month of February. Leaves were thoroughly washed in running water to remove the dirt and dust on the surface of the leaves. Twenty g of finely chopped neem leaves were added to 100 ml of double-distilled water and boiled for 10 min. The extract was cooled and filtered and store for further use This solution was used for green synthesis of silver nanoparticle (AgNP) or reducing the silver ions.



2.2 Synthesis of Silver Nanoparticles

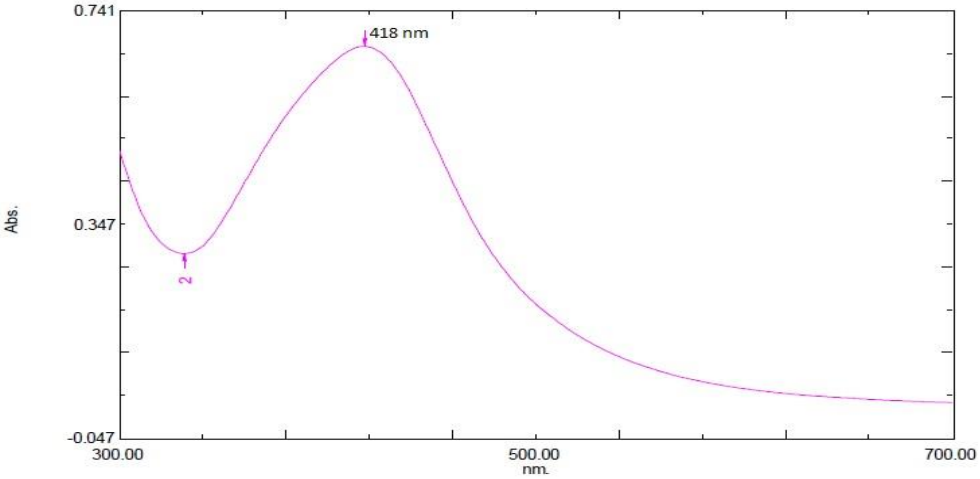
Silver nitrate (LOBA, India) GR was used to prepare 100 ml of 1 mM solution of silver nitrate. The neem extract 5 ml was added to 50 ml of silver nitrate solution with constant stirring. This set up was incubated in dark chamber to minimize photo-activation of silver nitrate at room temperature. The colour change from colourless to dark brown in colour confirms the reduction of silver ions.



3. Analysis and Interpretation Silver Particles

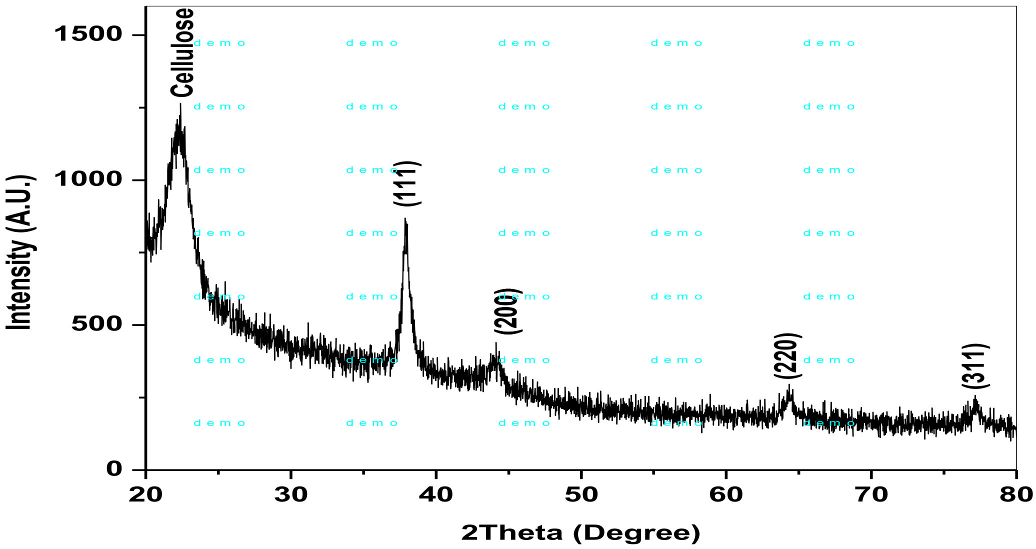
3.1. UV Spectrophotometry

UV–Visible spectroscopy is one of the most common and widely used method to determine the presence of silver nanoparticles in the colloidal solution. Change in the color of the reaction mixture from colorless solution to dark brown solution indicates the synthesis of silver nanoparticles. The sodium borohydride acts as the reducing agent during the synthesis process of silver nanoparticles that turns colorless reaction mixture into dark brown solution. illustrate the UV–visible spectrum for the reaction mixture and presence of surface plasmon resonance (SPR) absorption band with maximum absorbance at 418 nm, signifies the presence of silver nanoparticles in the reaction mixture. A sharp peak indicates the presence of spherical or round shape nanoparticles



3.2 X Ray Diffraction Study

The XRD spectra of the thin coatings on Whatman filter paper irradiated. For this sample coatings, the XRD peaks have appeared at the same 2θ positions at which the characteristic XRD peaks of bulk silver are known to appear. The XRD results, therefore, clearly indicate that, the four distinct XRD peaks at 2θ values of 38.4◦, 44.3◦, 64.4◦ and 77.4◦ represent the (111), (200), (220) and (311) crystalline planes of the face-centered cubic silver [8]. The pattern has three main diffraction features corresponding to (111), (200) and (220) planes and all the three peaks can be indexed to standard cubic phase of silver (JCPDS card No.870598,870720 and 893722). No reflection peaks corresponding to nitrate ions and other impurities were noted in this pattern, indicating the high purity of the final product. The different peak intensity profile was characteristic of cubic structure of AgNPs.

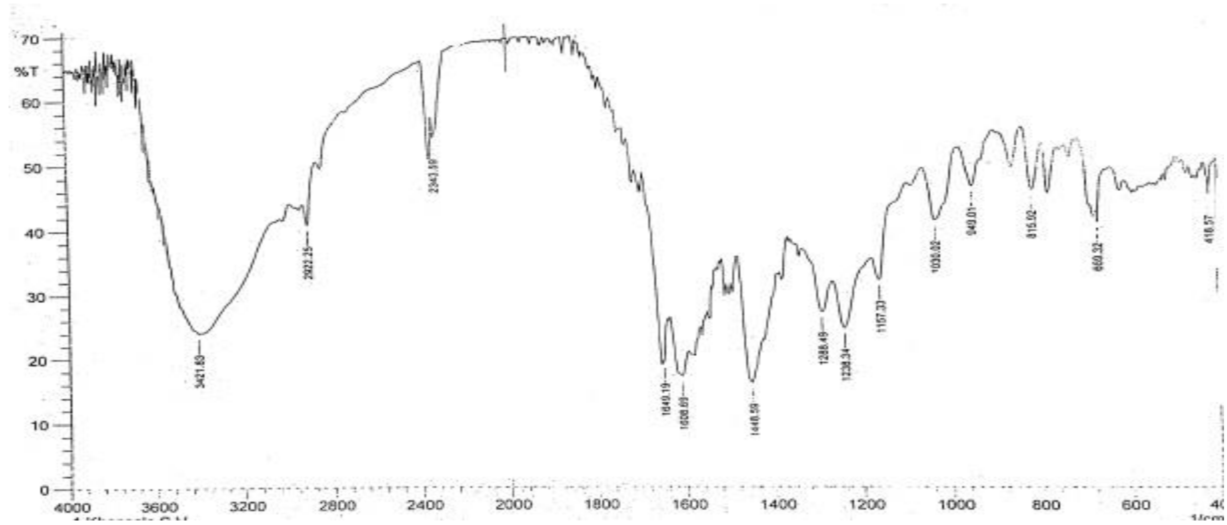


X ray diffraction spectra of AgNPs

(Courtesy: K. A. Bogle, School of physical science SRTMU Nanded)

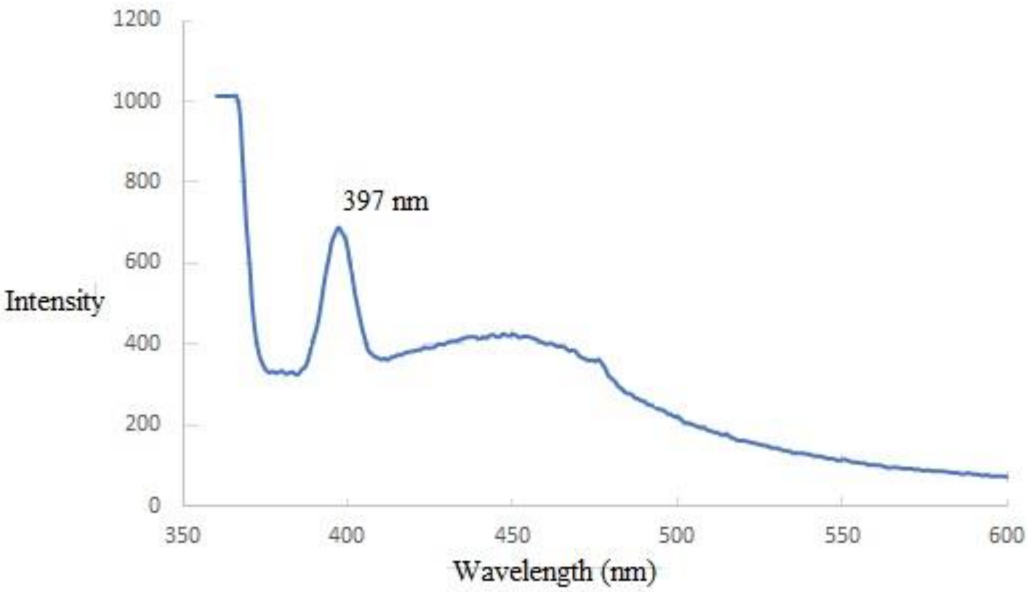
2.3 FTIR Analysis

The dual role of the plant extract as a reducing and capping agent and presence of some functional groups was confirmed by FTIR analysis of silver nanoparticle. A broad band between 3421 cm^{-1} is due to the N-H stretching vibration of group NH_2 and OH the overlapping of the stretching vibration of attributed for water and *A. indica* leaf extract molecules. 29167 cm-1 C-O stretch. The band at 1649 cm^{-1} corresponds to amide C=O stretching and a peak at 2083 cm^{-1} can be assigned to alkyne group present in phytoconstituents of extract. The observed peaks at 1113 cm^{-1} denote -C-OC- linkages, or -C-O- bonds. The observed peaks are mainly attributed to flavanoids and terpenoids excessively present in plants extract [9]. On the other hand, the extract sample prepared shows a wide and strong peak with maximum intensity at 553 cm_1. The results are in good agreement with those found in literature [10]. From FTIR results, it can be concluded that some of the bioorganics compounds from *A. indica* extract formed a strong coating/capping on the nanoparticles



2.4 Photoluminescence Study

Silver nanoparticles are reported to exhibit visible photoluminescence and their fluorescence spectra are shown in Fig. 6. The silver nanoparticles were found to be luminescent with two emissions at 280 and 561 nm for an excitation at 280 nm. When nanoparticles were excited at 300 nm, it showed two excitations at 300 and 600 nm, the excitation at 300 nm is of high intensity in comparison to other one. The luminescence at 280 and 300 nm may be due to presence of biochemical or antioxidants present in plant extract. The nanoparticles synthesized using olive leaf extract are also reported to be luminescent with emission band at 425 nm[11]



Conclusion:

We found the simple, efficient and green method for synthesis of silver nanoparticles. The synthesized silver particle were characterized by using different instruments and results are accordance to the literature values of nano particles.

References:

1. Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2015). Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract. *Journal of Advanced Research*, *6*(1), 105–112. https://doi.org/10.1016/j.jare.2015.02.007
2. Amendola, V., Bakr, O. M., & Stellacci, F. (2010). A study of the surface plasmon resonance of silver nanoparticles by the discrete dipole approximation method: effect of shape and environment. *Plasmonics*, *5*(1), 85–97. https://doi.org/10.1007/s11468-009-9120-4
3. Ankanna, S., Prasad, T. N. V. K. V., Elumalai, E. K., & Savithramma, N. (2010). Production of biogenic silver nanoparticles using *Boswellia ovalifoliolata* stem bark. *Digest Journal of Nanomaterials and Biostructures*, *5*(2), 369–372.
4. Banerjee, P., Satapathy, M., Mukhopahayay, A., & Das, P. (2014). Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresources and Bioprocessing*, *1*(3), 1–10. https://doi.org/10.1186/s40643-014-0003-y
5. Bhainsa, K. C., & D'Souza, S. F. (2006). Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*. *Colloids and Surfaces B: Biointerfaces*, *47*(2), 160–164.

6. Carlson, C., Hussein, S. M., & Schrand, A. M. (2008). Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. *The Journal of Physical Chemistry B*, *112*(43), 13608–13619.
7. Gogoi, S., Gopinath, P., Paul, A., Ramesh, A., Ghosh, S. S., & Chattopadhyay, A. (2006). Green fluorescent protein-expressing *Escherichia coli* as a model system for investigating the antimicrobial activities of silver nanoparticles. *The Journal of Physical Chemistry B*, *110*(14), 7633–7638.
8. Temgire, M. K., & Joshi, S. S. (2004). Optical and structural studies of silver nanoparticles synthesized by microemulsion route. *Radiation Physics and Chemistry*, *71*(6), 1039–1044.
9. Banerjee, P., Satapathy, M., Mukhopahayay, A., & Das, P. (2014). Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresources and Bioprocessing*, *1*(3), 1–10.
10. Mahdi, S., Taghdiri, M., Makari, V., & Rahimi-Nasrabadi, M. (2015). Green synthesis of silver nanoparticles by a novel method: using *Melissa officinalis* leaf extract and their antibacterial properties. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *136*, 1249–1254.
11. Khalil, M. M. H., Ismail, E. H., & El-Magdoub, F. (2012). Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity. *Arabian Journal of Chemistry*, *5*(4), 431–437.

PHYTOCHEMICAL PROFILING OF *MUCUNA PRURIENS* LEAF EXTRACTS FOR QUANTITATIVE ESTIMATION OF POLYPHENOLS AND EVALUATION OF ANTIMICROBIAL POTENCY

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Abstract:

Mucuna pruriens commonly known as velvet beans is a tropical legume belonging to the fabaceae family which is native to tropical. Fabaceae or Leguminosae family is widely known for its nutritional factors and different constituents supporting the therapeutic importance. Extraction of phytoconstituents and standardization of solvents for getting maximum yield is of paramount importance in the world of Natural Therapeutics. We are trying to summarize the overall impact of different solvents on tannin content and the its antimicrobial potential along with Minimum Inhibitory Concentration (MIC) against *Klebsiella pneumoniae*. Tannins are a group of polyphenols that astringent in nature. Tannins possess anti-inflammatory, anti-oxidant, anti-microbial and other activities. In the current paper, we are focusing upon the content of tannins colorimetrically. The Folin-Denis method is used for quantification of tannins. Phenolic hydroxyl groups present in the structure of tannins reduce phosphotungstomolybdic acid to phosphotungstomolybdate in the presence of alkaline reagents like sodium carbonate solution. Tannins act as antimicrobial agents as they inhibit extracellular microbial enzymes which are responsible for microbial metabolism. The organism is specifically chosen as the plant family used is suspected to contain some potential analytes against it. Hence present work also highlights the antimicrobial potential by disc diffusion method and further evaluation of MIC of leaf extracts prepared in different solvents.

Keywords: *Mucuna pruriens*, Tannins, Extraction, Disc Diffusion Method, MIC

Abbreviations: MIC- Minimum Inhibitory Concentration

Introduction:

In India, we have been using drugs of herbal origin as given in our traditional systems of medicines such as Unani and Ayurveda from ancient times. The drugs are derived from different organs of plant or are secondary metabolites or are waste products of the plants such as gum, resins and latex. Many of the developing countries rely on drugs of herbal origin because of the cost of modern medicine and side effects of the same. Green plants synthesize and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations.[1] Many secondary metabolites of plants are commercially important and are used in/as number of pharmaceutical compounds.[1] In some cases, the crude extract of medicinal plants may be used as medicaments. Here we are trying to estimate tannins belonging to the class of polyphenols.

Tannins are naturally occurring plant polyphenols which bind and precipitate proteins. There are different classes of tannins like hydrolysable, condensed and pseudo.

Standardization is the process of implementing and developing technical standards based on the consensus of the standard organization. It helps to maximize compatibility, safety, repeatability and quality. Solvents are used to dissolve or dilute other substances. Standardization is important as it will provide us with the information on the solvent which is most compatible with our analyte of interest. While standardization of the solvents we should keep in mind the ideal properties of solvents.

Depending upon the properties of solvent and analyte recovery we can decide upon which solvent can be used further for the maximum extraction of phytoconstituents. [2,3]

Group of polyphenols (tannins) are first qualitatively estimated using Iron chloride. If tannins are present in the given sample, then they tend to convert Fe^{3+} to Fe^{2+} by reduction. Tannins themselves get oxidized and reduce Fe^{3+} from the $FeCl_3$ solution giving the characteristic colour.

Folin-Dennis method is colorimetric quantification method for tannins. Phenolic group present in tannins is an excellent hydrogen donor that forms strong hydrogen bonds with proteins carboxy group. Folin-Denis reagent is a mixture of phosphomolybdic and phosphotungstic acid and are reduced to molybdenum and tungsten oxides by -OH groups in phenols. Tannins will reduce phosphotungstomolybdic acid in presence of alkaline conditions due to sodium bicarbonate to produce a highly colored blue solution. Intensity of the colour is proportional to concentration of tannins.[4]

Many plants have gained commercial importance because of their antimicrobial traits, which are due to phytochemicals synthesized in the secondary metabolism of the plant. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids, which have been found in vitro to have antimicrobial properties.[5] Agar disc diffusion is one of the methods which is used to evaluate antimicrobial activity of plant extract. This method is based on the principle of antibiotic-impregnated disc, placed on agar previously inoculated with micro-organism, and the disc previously dipped in herbal extracts diffuse radially outward through the agar medium producing an antimicrobial activity. A clear zone or ring is formed around a disc after incubation if the agent inhibits the growth of micro-organisms.[6]

Tannic acid has been reported to present the activity against Influeneza A virus, Papilloma viruses, noroviruses, Herpes simplex virus type 1 and 2, and human immunodeficiency virus (HIV) as well as activity against both Gram-positive and Gram-negative bacteria as *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Listeria innocua*.[7]

The methods for assessing antimicrobial resistance (AMR) of plants by some molecular methods like PCR, DNA microarray, whole-genome sequencing and metagenomics, and matrix-assisted laser desorption ionization-time of flight mass spectrometry.[8] These methods are used for identification of AMR genes. Some non-conventional methods include whole genome sequencing (WGS), matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) spectrometry, Fourier transform infrared (FTIR) spectroscopy, and microfluidics technology.[9]

Inefficiency of medical therapies used in order to cure patients with bacterial infections requires not only to actively look for new therapeutic strategies but also to carefully select antibiotics based on variety of parameters. Minimum Inhibitory Concentration (MIC) defines in-vitro levels of resistance or susceptibility of specific bacterial strains to applied antibiotic. The increasing resistance of bacteria to antibiotics call for searching ways to reduce it and improve the effectiveness of the infection therapies. MIC is the lowest Concentration of an antibacterial agent expressed in ug/mL or mg/L which is assessed under strictly controlled in-vitro strain of an organism. MIC values describe the susceptibility of a specific bacterium to a specific antimicrobial. In general, however the larger the MIC value the lower the susceptibility of the organism.

Materials and Methods:

1. **Collection of Plant material:**
    a. *Mucuna pruriens* fresh sample was collected from rural area of Charkop, Kandivali (West). Plant was washed and sun dried thoroughly.
2. **Preparation of extract:**
    a. Extracts were prepared by maceration of 0.5gm of leaf powder in 20mL water, methanol and Isopropanol respectively. Extracts were further diluted to get the reading in the range of standards. Extracts were prepared in different solvents for involving a range of polarities.
3. **Test for phytoconstituents:**
    a. Qualitative test for Tannins: To 1 ml of extract few drops of 0.1% freshly prepared Iron Chloride was added and was observed for bluish black coloration.
    b. Quantitative test for Tannins: Purified Gallic acid equivalent was used as a standard and quantification was performed by calibration curve method. Standards ranging from 2ug/mL to 10ug/mL from 1mg/mL working stock were

prepared and 1mL of 20% sodium carbonate was added in all the tubes along with 0.5mL of Folin Dennis reagent.

4. **Disc Diffusion test:**
    a. Sterile nutrient agar plate is swabbed with a *K. pneumoniae* suspension and sterile paper discs dipped in all the three extracts are placed in four quadrants along with a control. The results were observed after the incubation at 37^0Celsius for 24hours.
5. **Test for Assessment of MIC:**
    a. Minimum Inhibitory Concentration was carried out adding equal volumes of all the above three extracts and *E.coli* culture suspension in 9ml of Sterile Nutrient broth.
    b. The extracts were further serially diluted upto 10 dilutions each from original concentration of 2,50,00,000 mg/L to $2.4 * 10^{-6}$mg/L.
    c. All the tubes were incubated at 37^0 Celsius for 24hours and evaluated based on the turbidity observed.

Result and Discussion:

Qualitative Confirmation Test for Tannins and Quantification of tannins:

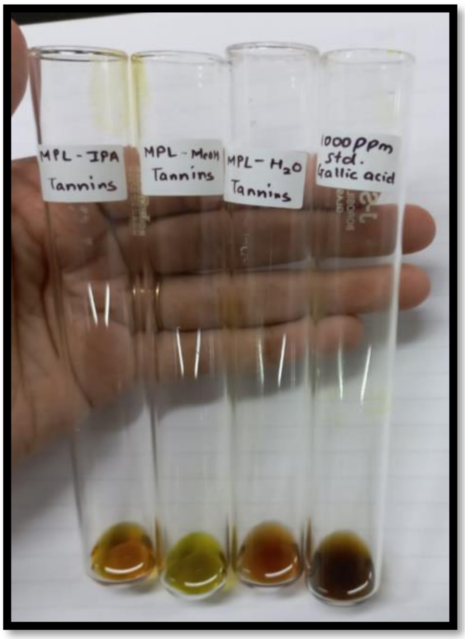


Figure 1: Qualitative $FeCl_3$ test showing the presence of tannins when compared to standard Gallic acid

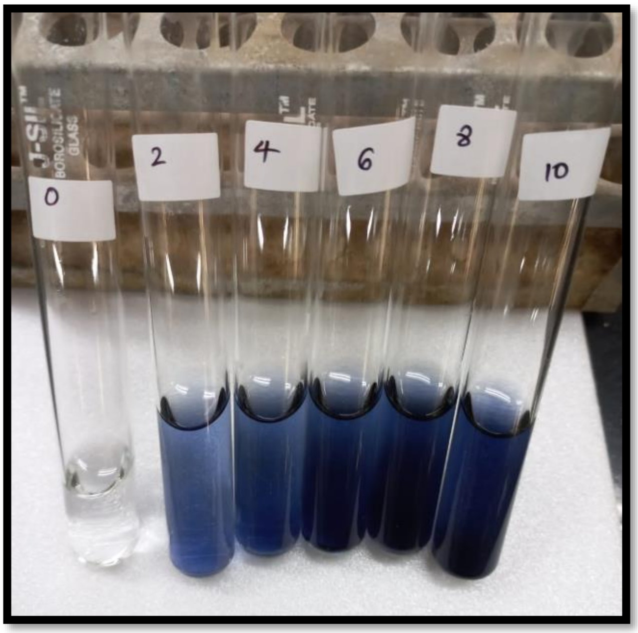
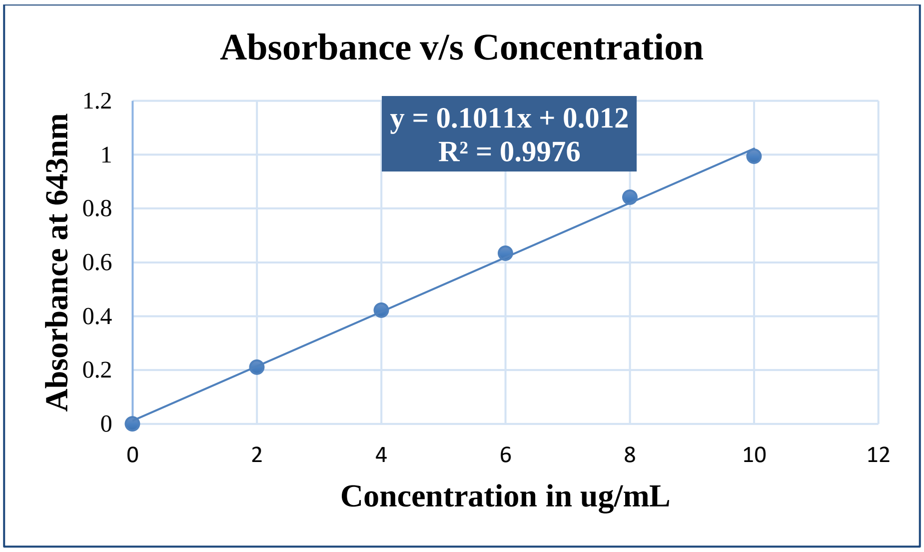


Figure 2: Standard series of Gallic acid ranging from 2ug/mL to 10ug/mL

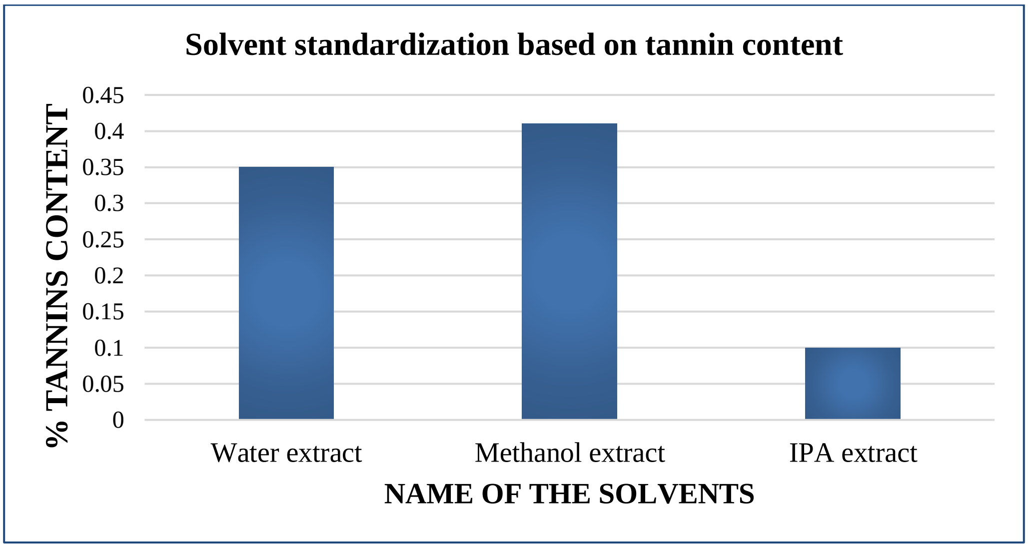


Graph 1: Graph of Absorbance at 643nm v/s concentration in ug/mL

Quantification is performed by calibration curve method quantification. Standards ranging from 2ug/mL to 10ug/mL are showing gradation in the colour proving the Beer-Lambert's law. Hence unknown samples are extrapolated by graphical method.

Table 1: Extractive value of tannins from three different solvents

Name of the solvents used	Tannin concentration (ug/mL)	Tannin content (%)
Water	4.38	0.35
Methanol	5.08	0.41
IPA	1.29	0.1



Graph 2: Bar graph showing extraction yield of solvents

Concentration of tannins was evaluated graphically and % tannis content is calculated based upon the original prepared concentration of all the sample extracts. The above table and graph depict the optimum solvent for extraction.

Disc Diffusion Assay:

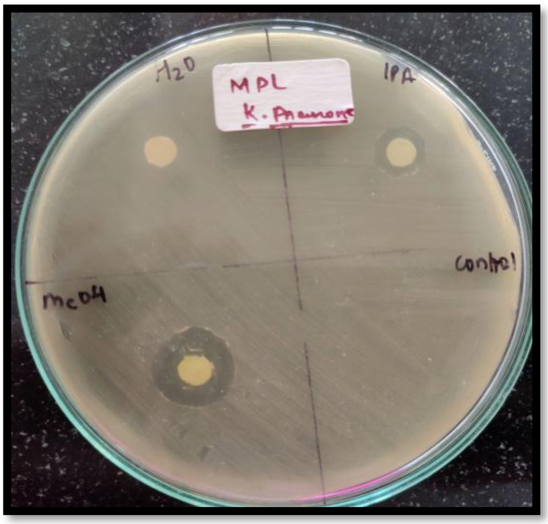


Figure 3: Disc diffusion assay of solvent extract against *K. pneumoniae*

Table 2: Solvent extracts and Zone of inhibition of *K. pneumoniae*

Solvent extract	Average of zones of inhibition (cm)
Water extract	0.0
Methanol extract	1.4
IPA extract	0.8

The plant leaf extract is showing inhibition against the used organism hence proven to be effective against the infection. Zones were measured from 3 different sides and average is reported.

Evaluation of MIC:

Minimum Inhibitory concentration of 10 serial dilutions of three solvent extracts:

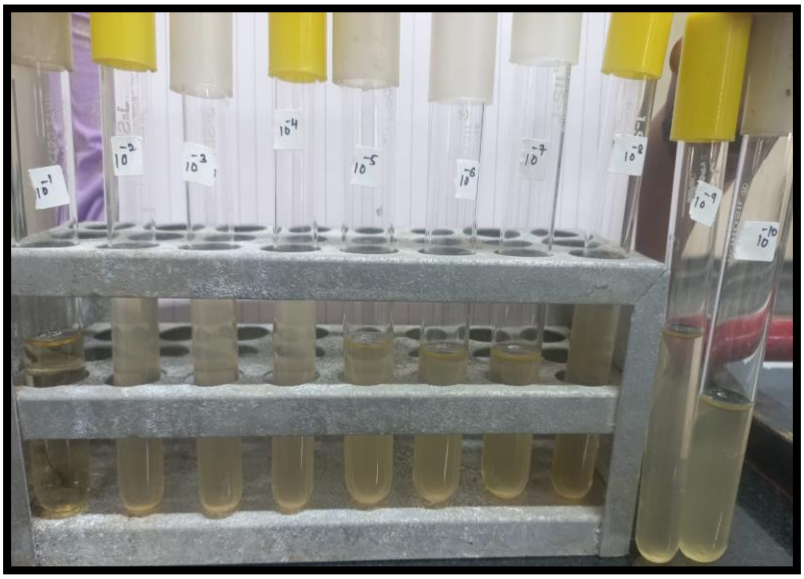


Figure 4: MIC tubes of Water extract

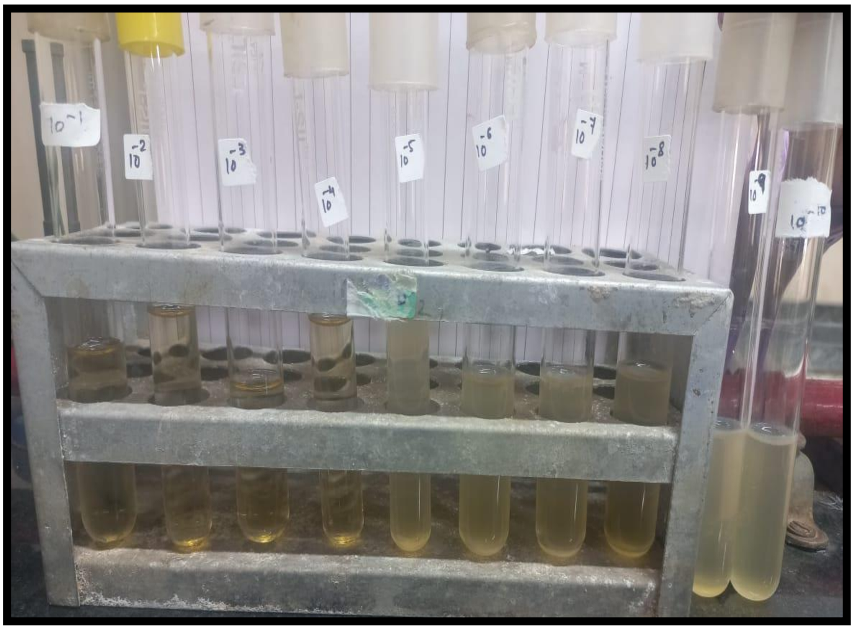


Figure 5: MIC tubes of Methanol extract

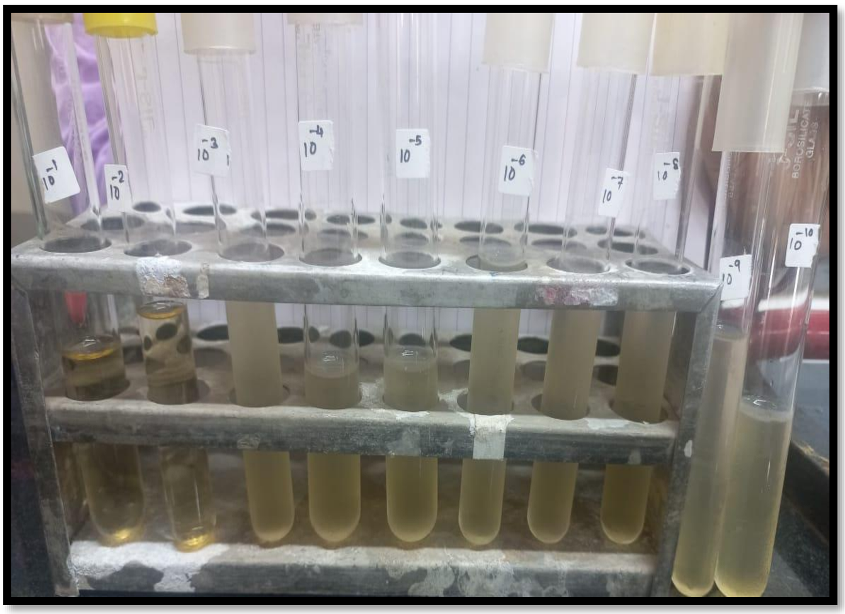


Figure 6: MIC tubes of IPA extract

Table 3: MIC of extracts against *K. pneumoniae*

Solvent extract	**MIC**
Water extract	1250000mg/L
Methanol extract	156.25mg/L
IPA extract	62500mg/L

As the extracts have shown the antibacterial effect against the organism, determination of minimum concentration required for inhibiting the growth and hence the infection is further done by MIC assay. The results of the broth dilution MIC are shown in the images above and the table shows minimum concentrations required in every solvent extract.

Conclusion:

Standardization of solvents was carried out to evaluate (from polar to non-polar) extraction efficiency of polyphenols i.e, tannins from *Mucuna pruriens* leaves powder samples. Concentration of tannins was found highest in methanol extract followed by water followed by IPA. Least concentration of tannins was found in IPA extract. Though methanol being good organic solvent giving the maximum tannin content water can be preferred considering the environment toxicity of methanol. As tannins are water soluble and they contain hydroxyl groups along with non-polar aromatic phenol hence they can be extracted in the mixture of polar (i.e. water) and mid-polar (i.e., methanol). Water is a universal solvent but is not stable for longer periods of storage of extract. Water extracts are easy targets for contaminants and fungus. Also water extracts might not be compatible for further instrumental analysis.

After comparing the zones of inhibition with the control, it was found that methanol extract shows highest antimicrobial activity against the organism (*K. pneumoniae*) followed by IPA and water extract. Methanol is an organic solvent which also contributes to antimicrobial potential. Also considering MIC, methanol extract has shown the most promising effect followed by IPA and water. Tannins present in the plant have proven their potency against the used organism.

As evident from the comparative analysis; methanol is proven to be suitable for extraction as it showed potential promising action against the *K. penumoniae* as compared to IPA and water. Taking into account, the ideal properties of solvents; methanol can be used for further analysis of plant leaf extract. Also the plant has shown the inhibition of *K. pneumoniae* organism proving its activity against the infection. It is clearly evident, that if optimum solvent is used then plant can be used for activity against the said organism. Animal studies can be performed for further verification of action against Pneumonia.

Acknowledgement:

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References:

1. Vaidya, M. (2017). Antimicrobial and antifungal activity of the plant extract of *Euphorbia hirta* L. *World Journal of Pharmaceutical Research*, *6*(8), 2043–2047.
2. Shivakumara, S., Wahengbam, S., Rana, N. K., Kundu, S., Bole, S., & Vedamurthy, A. B. (2014). Phytochemical screening and biological activities of *Impatiens balsamina* L. seeds. *International Journal of Fundamental & Applied Sciences*, *3*(2), 22–26.
3. Revathi, P. L., Jeyaseelan Senthinath, T., & Thirumalaikolundusubramanian, P. (2014–2015). Preliminary phytochemical screening and GC-MS analysis of ethanolic extract of

mangrove plant *Bruguiera cylindrica* (Rhizho). *International Journal of Pharmacognosy and Phytochemical Research*, *6*(4), 729–740.

4. Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature Protocols*, *2*, 875–877. https://doi.org/10.1038/nprot.2007.102
5. Pagare, S., Bhatia, M., Tripathi, N., Pagare, S., & Bansal, Y. K. (2015). Secondary metabolites of plants and their role: Overview. *Current Trends in Biotechnology and Pharmacy*, *9*(3), 293–304.
6. Patel, D., & Desai, S. (2016). Phytochemical screening, in vitro antimicrobial and anti-inflammatory activity of methanolic extract of *Aster lanceolatus* wild leaves. *International Journal of Medicine Research*, *1*(1), 26–30.
7. Kaczmarek, B. (2020). Tannic acid with antiviral and antibacterial activity as a promising component of biomaterials: A mini-review. *Materials (Basel)*, *13*(14), 3224. https://doi.org/10.3390/ma13143224
8. Anjum, M. F., Zankari, E., & Hasman, H. (2017). Molecular methods for detection of antimicrobial resistance. *Microbiology Spectrum*, *5*(6). https://doi.org/10.1128/microbiolspec.ARBA-0036-2017
9. Kaprou, G. D., Bergšpica, I., Alexa, E. A., Alvarez-Ordóñez, A., & Prieto, M. (2021). Rapid methods for antimicrobial resistance diagnostics. *Antibiotics*, *10*(2), 209. https://doi.org/10.3390/antibiotics10020209
10. Nandini, G., Palekar, S., Vaidya, V., & Shinde, M. (2017). Phytochemical profiling of *Wagatea spicata* using GC-MS to reveal the pharmacological significance. *International Journal of Current Research*, *9*(12), 62197–62204.
11. Palekar, S., & Patel, B. (2019). Study of insulin-like proteins from edible leguminous plant: *Dolichos lablab* Linn. *IJRAR - International Journal of Research and Analytical Reviews (IJRAR)*, *6*(2), 576–582.
12. Farooqi, N., Dey, A., Singh, G., Easwari, T., & Pandey, M. (2014). Analytical techniques in quality evaluation of herbal drugs. *Asian Journal of Pharmaceutical Research*, *4*(3), 112–117.
13. Antolovich, M., Prenzler, P., Patsalides, E., McDonald, S., & Robards, K. (2002). Methods for testing antioxidant activity. *Analyst*, *127*, 183–198.
14. Davies, J. (1994). Inactivation of antibiotics and the dissemination of resistance genes. *Science*, *264*, 375–382.
15. Nascimento, G., Locatelli, J., Freitas, P. C., & Silva, G. L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, *31*(4), 247–256.

CALYX SPLITTING IN CARNATIONS: A REVIEW OF PHYSIOLOGICAL DISORDER, THEIR CAUSES, CONTRIBUTING FACTORS, AND A HISTORICAL PERSPECTIVE

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Abstract:

Physiological disorders in plants, such as calyx splitting in carnation, are non-pathological issues caused by various factors. These disorders significantly impact plant growth, development and yield. Calyx splitting in particular is a major concern in carnation cultivation, leading to substantial economic losses due to deformed flowers. This phenomenon is the result of a lack of formation of proper cylindrical calyx tubes that support the base of the petals or of an exceptional increase in petal number or the formation of lateral buds inside the calyx that cause deformed structures and shapes of flowers during the bud opening period when the petals approach their full size. Initially attributed to environmental fluctuations, research has since identified multiple triggers, including genetic, nutritional and cultural practices. Despite this, boron deficiency remains a commonly cited problems, often overshadowing other contributing factors. This chapter offers a comprehensive overview of the causes contributing factors, and historical perspective of calyx splitting in carnations, aiming to broaden our understanding of this complex disorder.

Keywords: Plant Physiology, Boron Deficiency, Mineral Deficiency, Plant Nutrition, Flower Anatomy, Flower Deformation.

1. Introduction:

A non-pathological problem, calyx splitting in carnation (*Dianthus caryophyllus* L.), is a major and complex physiological disorder (Devi *et al.*, 2017). It is a serious phenomenon from an economic point of view, as it is responsible for a considerable decrease in the quality of the flower, thereby reducing its economic value (Skalská, 1983; Yermiyahu and Kafkafi., 2009 and Karrow and Sharma, 2010). When flower bud opening occurs, the petals approach their full size, developing flowers fail to form a cylindrical calyx tube to support the base of the petals (Karrow and Sharma, 2010), resulting in deformed flower structures and shapes and rendering them unsuitable.

Initially, this witch's broom-like effect on carnations was thought to be the result of virus infection or other diseases (Jackson *et al.*, 1959). However, later it was found that environmental conditions affects its incidence and it was considered a sole responsible factors for splitting (Scott, 1899 and Dick, 1915) until different researchers discovered that it can be caused by multiple factors, including soil nutrients (Baur, 1914; Szendel, 1939; Messing, 1958 and Winsor *et al.*, 1970), genetic factors (Ward, 1903; Dorner, 1907 and Batchelor, 1926) and morphological and anatomical factors (Szendel, 1939; Beisland and Kristofferson, 1969 and Holley and Baker, 1992). Thus, for many decades, the actual cause of calyx splitting in carnations has remained unclear and unsettled, and the understanding of this physiological disorder, causes, and contributing factors has changed over time. Nevertheless, owing to the susceptibility of carnations to boron deficiency, calyx splitting is widely and generally considered to be caused by boron deficiency (Jawaharlal *et al.*, 2009), whereas all other possible factors are largely overlooked or not considered fairly in their management in most cases.

Moreover, most of the literature pertaining to calyx splitting in carnations, the causes and factors affecting its incidence is quite old and outdated and thus lacks current literature. Most of the recent studies have focused on boron deficiency and borax application for its management. However, research on other factors influencing calyx splitting is sparse despite their significant influence on splitting. Therefore, the present chapter offers a comprehensive overview of a significant physiological disorder affecting carnation, a highly valued and economically important flower in global floriculture industry.

Picture 1: Different stages of calyx splitting in Chabaud carnation (Picture: Tadar Jamja)

2. Factors Responsible for Calyx Splitting in Carnations

The factors which are reported to affect the incidence of calyx splitting are shown in Figure 1. In the following sections and subsections, these factors which are reported to be the main triggers for calyx splitting in carnations are discussed broadly.

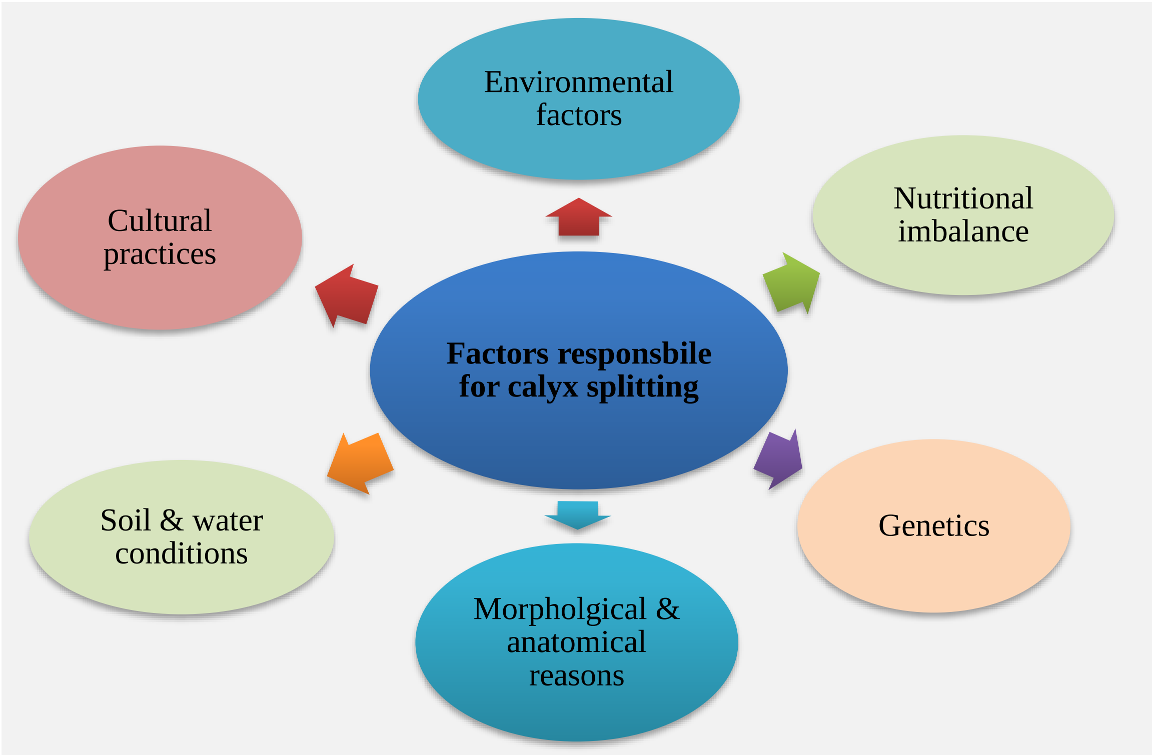

Figure 1: Factors responsible for calyx splitting in carnations

2.1 Environmental Factors

Initially, when calyx splitting was first reported, abiotic factors were considered the main reason. Environmental factors, including temperature and light were considered the main reasons for this physiological disorder.

2.1.1 Temperature

The first environmental factor assumed to cause calyx splitting in carnation was likely fluctuations in temperature. During the late 19^{th} and early 20^{th} centuries, temperature was considered the main reason for calyx splitting in carnation. Scott (1899) and Dick (1915) suggested that sudden fluctuations in temperature caused calyx splitting during carnation, which, for years, was considered to be the main reason. In addition, it was initially believed that only high temperatures cause calyx splitting. However, Szendel (1939), from Cornell University, USA, performed intensive research on the influence of nutrition and temperature on calyx splitting and reported contrasting results that prolonged low-temperature treatment produced a greater percentage of splits.

In line with the findings of Scott (1899) and Dick (1915), one of the earliest reports of a correlation between high temperature and the incidence of calyx splitting was reported by Holley *et al.* (1952). They reported that the higher the temperature was, the greater the amount of calyx splitting in carnation. However, low-temperature treatment during the flower bud initiation stage did not increase calyx splitting, contrary to the findings of Szendel (1939). Nonetheless, Freemand and Langhans (1965), Abou Dahab (1967) and Holley and Baker (1992) later demonstrated that both low and high temperatures during the flower development stage can cause calyx splitting in carnation. Beisland and Kristofferson (1968) also demonstrated that differences in day and night temperatures can also cause calyx splitting, suggesting that this splitting occurred not only because of decreases or increases in temperature but also because of differences in temperature between the day and the night.

Additionally, investigators have recently demonstrated that temperature plays a vital role in triggering calyx splitting in carnations. According to Ebrahimzadeh *et al.* (2008), higher day and night temperatures, especially during the flowering period, cause abnormal flower opening, resulting in calyx splitting, and areas with high light intensities in winter but mild temperatures during summer are best for carnation production. In 2019, Jawaharlal *et al.* (2009) reported similar results: high temperatures during the day and night cause calyx splitting and recommended maintaining the temperature at 15.6°C on cool days and 18.3°C on sunny days for successful cultivation of carnation under polyhouse conditions. Furthermore, they reported that regulating the day temperature to 20–25°C and the night temperature to 12.5–15.5°C is the optimum condition required for carnations. Any alterations or fluctuations in temperature may result in calyx splitting and reduce flower quality (Tah and Mamgain, 2013). The latest reports by Kamachi *et al.* (2023) and Punitha *et al.* (2023) also support that carnation flowers subjected to high temperatures or sudden fluctuations in temperature frequently exhibit partial petalization, consequently causing calyx splitting, which consolidates other findings.

2.1.2 Light

The light conditions and light intensity are crucial for the growth and development of carnations. In 1916, Connor (1916) reported that dull weather and dark light during the bud formation stage led to calyx splitting. Similarly, Allwood (1926) and Weston (1927) reported that inadequate sunshine was the major cause of calyx splitting. Holley (1942) was perhaps the first to establish a correlation between the growth and development of carnation and light conditions and their intensity. Abou Dahab (1967) demonstrated and confirmed that alterations in light conditions cause calyx splitting in carnation. Furthermore, Beisland and Kristofferson (1968) reported that day length can significantly influence the occurrence of calyx splitting. These authors reported that long-day light exposure caused more calyx splitting than short-day light exposure did. However, Singh *et al.* (2006) reported that additional light for 4 hours significantly improved the overall growth and development of the plant but did not influence the incidence of calyx splitting.

2.2 Nutritional Imbalance

Calyx splitting in carnations can be due to nutritional imbalances in the soil (Messing, 1958; Windsor *et al.,* 1970 and Jawaharlal *et al.,* 2009). Excess or deficiency of various soil nutrients is reported to influence calyx splitting. Numerous investigations have been carried out since the discovery of the incidence of calyx splitting to understand the influence of soil nutritional factors. At first, it was thought that an overdose of fertilizer or frequent application of a complete nutrient mixture increased the likelihood of calyx splitting (Baur, 1914 and Szendel, 1939). However, these findings were not consistent with the findings of other researchers seeking to establish a relationship between fertilizer treatments and the incidence of calyx splitting (Dorner *et al.,* 1914 and Pember and Adams, 1920).

2.2.1 Nitrogen Level

Nitrogen is an essential macronutrient for plants, and the primary component of protein, phospholipids, hormones, vitamins, chlorophyll, alkaloids, and hormones contributes to all stages of plant growth and development (O′Brien *et al.,* 2016; Chen *et al.,* 2021; Jiang *et al.,* 2024 and Wang *et al.,* 2024). Thus, it has an irreplaceable role in organ construction and material metabolism (Warner *et al.,* 2004; Liu *et al.,* 2010 and Bai *et al.,* 2016). Nitrogen deficiency severely hinders plant growth and reduces yield and quality (Wang *et al.,* 2024).

The effect of nitrogen application on calyx splitting was first reported during early 20^{th} century. Pember and Adams (1920) reported that high nitrogen levels reduce the likelihood of calyx splitting. Beach (1939) reported similar results and reported that fluctuations in the nitrate level, rather than the quantity itself, resulted in greater calyx splitting. However, Connors (1916) and Biekart (1929) established an interesting relationship between nitrogen and carbohydrate levels. They reported that the tendency of calyx splitting was accentuated by high carbohydrate contents and low nitrogen levels and *vice versa*. Moreover, Post (1949) and Clapp and Folley (1941) reported that high nitrogen levels increased calyx splitting. Nonetheless, the nitrogen level significantly influences splitting. Low nitrogen and high ammoniacal nitrogen increase the chance of calyx splitting; therefore, maintaining the optimum nitrogen level (25–40 ppm) is recommended (Jawaharlal *et al.,* 2009). Arora and Jhon (1978) reported that a nitrogen dose of 40--60 N/m^2 reduced calyx splitting and that the application of split-dose N increased the chance of splitting. Most recently, Punitha *et al.* (2023) reported an increase in calyx splitting with increasing leaf nitrogen content, which coincides with the earlier report of Verma *et al.* (2003), who reported similar observations.

2.2.2 Phosphorus Level

Phosphorus, another essential macronutrient, plays a pivotal role in the growth and development of plants (Khan *et al.,* 2023). It plays fundamental roles in regulating diverse physiological responses (Hawkesford *et al.,* 2023 and Lambers, 2022), including energy metabolism, cell division, DNA synthesis and phospholipid biosynthesis (Isidra-Arellano *et al.*, 2021).

An updated literature pertaining to the effect of phosphorus on calyx splitting is lacking. However, Beach (1939) reported that the level of phosphorus applied had no significant effect on the occurrence of calyx splitting. However, Szendel (1939) and Clapp and Folley (1941) reported that excess phosphorus increased calyx splitting in carnation, whereas low phosphorus reduced calyx splitting. In contrast, Winsor *et al.* (1970) reported that an increase in phosphate fertilization increases the incidence of calyx splitting.

2.2.3 Potassium Level

Potassium is the most abundant inorganic cation and is important for ensuring optimal plant growth (White and Karley, 2010 and Xu *et al.*, 2020). It activates dozens of important enzymes, such as those involved in sugar transport, protein synthesis, and N and C metabolism (Marschner, 2012 and Oosterhuis *et al.*, 2014), which are crucial for cell growth and development (Hepler *et al.*, 2001 and Xu *et al.*, 2020). Potassium has a growth-promoting mechanism that stimulates various other cell functions (Kaiser, 1982 and Hu *et al.*, 2016) and regulates important physiological processes (Xu *et al.*, 2020). However, the direct effect of potassium on calyx splitting is controversial (Yermiyahu and Kafkafi, 2009). Szendel (1939) reported that the amount of potassium available to plants had no significant effect on calyx splitting. On the other hand, Beach (1939) reported that high potassium with high nitrogen content reduced splitting, which was further supported by Clapp and Folley (1941) and Post (1949), who reported that high potassium content causes fewer splits than low potassium content does.

Similarly, different researchers reported different observations between the 1970s and 1980s. Winsor *et al.* (1970) reported that increasing the K concentration increases splitting, whereas Adams *et al.* (1979) reported the opposite result, and Skalskà (1983) reported no effect of the K concentration on calyx splitting. However, Criley *et al.* (1983) demonstrated that calyx splitting occurs when the K content in the leaves of the third flush decreases to less than 4%, indicating that maintaining a certain amount of K in plant cells is necessary to prevent calyx splitting in carnation. Similar observations were also made by Yermiyahu and Kafkafi (2009).

2.2.4 Boron Level

Boron is an essential micronutrient for plants, and its deficiency can lead to impaired growth, development, physiological function, productivity and yield (Wang *et al.*, 2015; Shireen *et al.*, 2018; Pereira *et al.*, 2021 and Vera-Maldonado *et al.*, 2024). It interacts with many other elements that influence various physiological and biochemical processes (Tariq and Mott, 2007). However, the effects of a deficient or excessive supply of boron on mineral uptake and function are not well established (Vera-Maldonado *et al.*, 2024).

Nevertheless, the first confirmation that calyx splitting in carnation occurs as a result of boron deficiency was documented at the Waltham Field Station of the University of Massachusetts in 1954 and reported by Mastalerz *et al.* (1956). They reported that boron deficiency led to a decrease in the normal petal number in carnation flowers, with the decrease being directly correlated with the severity of the deficiency. In addition, at a particular stage of calyx development, some cells are injured, resulting in an epinastic curve, which eventually leads to premature death of flower buds under severe boron deficiency, and the flower/terminal bud is aborted. However, the occasional proliferation of axillary shoot growth below aborted buds continues normally and vigorously until the terminal bud dies, which results in a typical broom-like effect. This study was further supported by the findings of Campbell (1957) and Jackson *et al.* (1959), who reported similar symptoms due to boron deficiency.

Thus, owing to the sensitivity of carnations to boron deficiency (Nayak *et al.,* 2024), when calyx splitting occurs, it is generally characterized by boron deficiency, and it is the first and foremost recommended soil nutrient to correct this physiological disorder. It is now recommended that the boron level in the soil be maintained at least 20–25 ppm (Jawaharlal *et al.*, 2009). The foliar application of borax (0.1%) (Raj *et al.*, 2016; Devi *et al.*, 2017 and Jawaharlal *et al.,* 2009) at fortnightly intervals has been found to significantly reduce calyx splitting (Jawaharlal *et al.,* 2009). Zaman *et al.* (2022) recommended foliar application of boron at a rate of 900 ppm at 50 DAT.

2.2.5 Silicon

Although the metalloid silicon is not considered essential for plant growth and development, an increasing number of studies indicate that it is beneficial to plants, especially under stress conditions (Luyckx *et al.,* 2017). According to Ma and Takahashi (2002), the accumulation of silicon enhances and strengthens cell walls. Nonetheless, the benefits and importance of silicon in plants are controversial (Wang *et al.,* 2021). The role and influence of silicon on calyx splitting have not been widely studied or understood. Nevertheless, some reports have shown that the preharvest application of silicon in the form of K_2SiO_3 @50 ppm significantly enhanced the flower quality and reduced the incidence of calyx splitting (El-Serafy, 2015).

2.2.6 Calcium

Calcium is a macronutrient that is essential for the growth and development of plants. It is not only important for cell wall and membrane stability but also serves as a second messenger in many developmental and physiological processes (Thor, 2019), highlighting its critical role in plant growth and development (Jing *et al.,* 2024). However, similar to reports on silicon, reports on the influence of calcium on calyx splitting remain scarce. El-Serafy (2015) reported that the application of calcium chloride or chelated calcium enhances flower quality and significantly reduces the incidence of calyx splitting.

2.3 Soil and Water Factors

During the early 21st century, Wheeler and Adams (1908) assumed that the application of manure to the soil affects the percentage of calyx splitting. However, Connors (1916) reported that the soil mixture did not affect the percentage of split. In other experiments, Connors (1933 & 1934) and Biekart and Connors (1935) reported that plants grown in sand produced fewer splits than those grown in soil did, which contradicted the findings of Szendel (1939), who reported contrasting results. In another experiment, Weinard and Decker (1934) reported that new soil had greater chances of producing split carnations. There is still a lack of definitive understanding of the effects of different types of soil conditions on calyx splitting.

In terms of water quality and irrigation scheduling, different researchers have reported different results. Some investigators have described calyx splitting with fluctuations in soil moisture conditions, which is directly attributed to the availability of water and irrigation (Qazi *et al.*, 2024). In contrast, Holley *et al.* (1952) reported that high moisture conditions cause more splits than low moisture conditions do. Nonetheless, the earliest reports of Pember and Adams (1920), Beach (1939), and Post (1949) reported that soil moisture conditions had no significant influence on calyx splitting. Studies on water quality and its influence on splits remain scarce. However, according to Motallebifard *et al.* (2003), the quality of water affects calyx splitting, and acidification of irrigation water significantly reduces the chances of calyx splitting. They also reported that an irrigation interval of 9--12 days significantly reduced splitting.

2.4 Morphological and Anatomical Factors

Understandably, calyx splitting occurs due to changes in morphology and anatomy, but such changes are triggered by various factors. To date, there is no clarity as to whether such calyx splitting is independent of other external factors and whether the internal morphological and anatomical structures of flowers or flower buds are solely responsible for it. However, some studies have shown that such morphological and anatomical deformation are influenced by various external factors that cause exceptional increases in petal number or induce the formation of lateral buds inside the calyx (Szendel, 1939; Beisland and Kristoffersen, 1969; Holley and Baker, 1992 and Yermiyahu and Kafkafi, 2009), implying that morphological and anatomical deformation is result of external stimuli.

2.5 Genetics Factors

Various reports indicate that genetic factors influence calyx splitting in carnations such that different genotypes respond differently or their susceptibility to split varies among the genotypes. The influence of genetic factors on splitting was understood from the beginning and found to be relevant even today. In the beginning of the 20th century, splitting was assumed to be entirely hereditary (Ward, 1903 and Dorner, 1907). However, Cornors (1916) assumed that it is affected by both hereditary and environmental factors, whereas Stuart (1912) assumed that it is caused mainly by the formation of adventitious buds in the flower rather than heredity. It was also proposed that the carnation flower form follows Mendel's law of dominance (Norton, 1904, 1907; Stuart, 1912 and Batchelor, 1926) in that the single type is purely recessive, the super double type is completely dominant (always splitting), and the standard or commercial double type is heterozygous. It was recommended that seedlings be taken from plants with good and firm calyces (Ward, 1903) or cuttings from nonsplitting plants (Weinard and Decker, 1934) to avoid splitting.

Such variations in splitting among genotypes have been reported widely even today, implying that the susceptibility of genotypes to splitting varies. Gill and Arora (1988), Karrow and Sharma (2010), Hemanta *et al.* (2012), Tah and Mamgain (2013), Nacho (2016), and Devi *et al.* (2017) reported that the occurrence of calyx splitting in carnations varied significantly among the genotypes used for the study. The most recent study was reported by Sharma *et al.* (2023), who reported genotypic coefficient variation in calyx splitting. They also reported high heritability of calyx splitting.

2.6 Cultural Practices

Some investigators have reported that cultural practices also affect the incidence of calyx splitting in carnation. However, studies pertaining to the effects of cultural practices on calyx splitting are limited to date. It has been reported that planting space and closer spacing encourage splitting (Jawaharlal *et al.*, 2009). Devi *et al.* (2017) concluded that pinching practices influence splitting, and they reported reduced or minimum calyx splitting in double-pinched plants. Other studies have shown that mulching practices and material used also influence the chances of splitting and that the use of green plastic mulching significantly reduces it (Qazi *et al.*, 2024). Nonetheless, the literature on the effects of cultural practices on the occurrence of calyx splitting remains ambiguous and scarce. However, cultural practices such as placing a rubber band around the flower bud to prevent calyx splitting are commonly recommended in commercial carnation cultivation to reduce loss (Jawaharlal *et al.*, 2009).

Conclusion:

In conclusion, calyx splitting in carnation is a complex physiological disorder influenced by multiple factors, including environmental, nutritional, genetics, soil, water and cultural practices. While boron deficiency is often cited as a primary cause, it is clear that this issue cannot be attributed to a single factor. Our understanding of calyx splitting is largely based on outdated research, highlighting the need for contemporary studies that account for the impacts of global warming, and climate change, as environmental factors, fluctuation in temperature and light have been widely debated and reasoned for it. A comprehensive approach, considering all contributing factors, is essential for developing effective management strategies. This review underscores the complexity of calyx splitting and emphasizes the need for further research to inform best practices in carnation cultivation and its management.

References:

1. Abou Dahab, A. M. (1967). Effects of light and temperature on growth and flowering of carnation *(Dianthus caryophyllus L.)*. Ph.D. thesis submitted to the Wagenigen University, The Netherlands.
2. Adams, P., Hart, B.M. and Winsor, G.W. (1979). Some effects of boron, nitrogen and liming on the bloom production and quality of glasshouse carnations. *J. Hort. Sci*., 54(2): 149-154.
3. Allwood, M.C. (1926). Carnations. Country Life. London, England. 59 (1517): https://archive.org/details/sim_country-life_1926-01-30_59_1517/page/n5/mode/2up.
4. Arora, J.S. and Jhon, A.Q. (1978). Effect of different levels of nitrogen, their time of application and plant density on the growth and flowering of carnation (*Dianthus Caryophyllus* Linn.) cv. Marguerite Scarlet. *Indi. J. Horti*., 35(3): 254-259.
5. Bai, L.Q., Deng, H.H., Zhang, X.C., Yu, X.C. and Li, Y.S. (2016). Gibberellin is involved in inhibition of cucumber growth and nitrogen uptake at suboptimal root-zone temperatures. *PLoS One*. 11: e0156188.
6. Batchelor, L.D. (1926). Carnation breeding. Proc. Amer. Breed. Assn., 7: 199-205.
7. Baur, A.F.J. (1914). Carnations. The Standard Cyclopedia of Horticulture. Bailey, L.H., editor. The MacMillan Co., New York. pp. 665-672.
8. Beach, G.A. (1939). Carnation yield and quality as affected by watering and phosphate. *Proceeding in Amer. Soc. Horti. Sci*., 37: 1022-1026.
9. Beisland, A. and Kristoffersen, T. (1968). Some effects of temperature on growth and flowering in the carnation cultivar 'William Sim'. In *Symp. Flwr Regul. Florist Crop.*, 14: 97-108.
10. Biekart and Connors, C.H. (1935). The greenhouse culture of carnations in sand. *New Jersey Agri. Exp. Sta. Bull.*, 588.
11. Biekart, H.K. (1929). Carnation culture. *New Jersey Agri. Exp. Stat. Annu. Rprt*., 33.
12. Campbell, F.J. (1957). Trace element mixtures as a source of boron for carnations. *Mass. Flwr Grow. Assn. Bull*., 44.
13. Chen, H., Zhang, Q., Wang, X., Zhang, J., Ismail, A.M. and Zhang, Z. (2021). Nitrogen form-mediated ethylene signal regulates root-to-shoot K^+ translocation via NRT1.5. *Plant Cell. Environ*. 44: 3806-3818.
14. Clapp, R. and Folley, G.E. (1941). Nutritional symptoms in the carnation. *Proc. American Soci. Horti. Sci*., 38: 673-678.

15. Connors, C. (1916). Factors causing the splitting of carnation calyces. *Rprt, New Jersey Agri. Exp. Stat.*, pp. 83-89.
16. Connors, C.H. (1916). Factors causing splitting of carnation calyces. *New Jersey Agri. Exp. Stat. Annu. Rprt*., pp. 83-96.
17. Connors, C.H. (1933). Carnation culture. *New Jersey Agri. Exp. Stat. Annu. Rprt*. 52.
18. Connors, C.H. (1934). Greenhouse cultivation of carnations. *New Jersey Agri. Exp. Stat. Annu. Rprt*., 54.
19. Criley, R.A., Parvin, P.E., Hori, T.M. and K.W. Leonhardt. (1983). Carnation calyx tip dieback. *II Inter. Symp. Carn. Cul. - ISHS*., 141: 125-131.
20. Devi, M.S., Chawla, S.L., Dodiya, T.P. and Bhatt, D.S. (2017). Response of different varieties of carnation (*Dianthus caryophyllus* L.) to pinching and boron. *J. Pharmacog. Phytochem*., 6(6): 971-974.
21. Dick, J.H. (1915). Commercial carnation culture: a practical guide to modern methods of growing the American carnation for market purposes. *A.T DeLaMare Print. Pub. Co. Ltd*. https://www.loc.gov/item/15017042/.
22. Dorner, F. (1907). Carnation breeding. *Amer. Breed. Asson. Rprt*., 3: 67-71.
23. Dorner, H.B., Muncie, F.W. and Nehrling, A.G. (1914). The use of commercial fertilizer in growing carnations. *Illi. Univ. Agri. Exp. Stat. Bull.*, 176.
24. Ebrahimzadeh, A., Jiménez, S., Da Silva, J.T., Satoh, S. and Lao, M.T. (2008). Postharvest physiology of cut carnation flowers. *Fresh Prod*., 2(2): 56-71.
25. El-Serafy, R.S. (2015). Effect of silicon and calcium on productivity and flower quality of carnation. Ph.D. thesis submitted to the Tanta University, Tanta, Egypt.
26. Freeman, R.N. and Langhans, R.W. (1965). Influence of day and night temperature on carnation. *New York State Flwr. Grow. Bull*., 232: 1-3.
27. Gill, A.P.S. and Arora, J.S. (1988). Performance of Sim carnations under subtropical climatic conditions of Punjab. *Indi. J. Horti.*, 45(3&4): 329-335.
28. Hawkesford, M.J., Cakmak, I., Coskun, D., De Kok, L.J., Lambers, H., Schjoerring, J.K. and White, P.J. (2023). Functions of macronutrients. Marschner's Mineral Nutrition of Plants; Academic Press: Cambridge, MA, USA, 2023; pp. 201-281.
29. Hemanta, L., Jayaprasad, K. V., Kumar, D.P., Manohar, R.K. and Gopinath, G. (2012). Effect of levels of fertigation on yield and flower quality of different varieties of carnation (*Dianthus caryophyllus* L.) under naturally ventilated polyhouse. *Karnataka J. Agri. Sci.*, 46(2): 245-250.
30. Hepler, P.K., Vidali, L. and Cheung, A.Y. (2001). Polarized cell growth in higher plants. *Annu. Rev. Cell. Dev. Biol*., 17: 159-187.

31. Holley, W.D. and Baker, R. (1992). Breeding for better varieties. In: WD Holley, R. Baker, eds. Carnation production. pp. 21-30.
32. Holley, W.D., Wagner, D.L. and Farmer, R.F. (1952). The response of carnation varieties William Sim and White Patrician to various levels of nitrates and soil moisture. *Col. Flwr. Grow. Assn. Bull*., 27.
33. Hu, W., Jiang, N., Yang, J., Meng, Y., Wang, Y., Chen, B., *et al.* (2016). Potassium (K) supply affects K accumulation and photosynthetic physiology in two cotton (*Gossypium hirsutum* L.) cultivars with different K sensitivities. *Field Crop. Res*., 196: 51-63.
34. Isidra-Arellano, M.C., Delaux, P.M. and Valds-Lpez, O. (2021). The phosphate starvation response system: Its role in the regulation of plant–microbe interactions. *Plant Cell Physio*., 62: 392-400.
35. Jackson, C.A., Gartner, G.B. and Cannon, .T.F. (1959). Correcting Boron Deficiency in Carnation. *North Carol. Flwr. Grow. Bull.,* 9: 2-3.
36. Jawaharlal, M., Ganga, M., Padmadevi, K., Jegadeeswari, V. and Karthikeyan, S. (2009). A technical guide on carnation. *Tamil Nadu Agri. Univ. Coimbatore*. pp. 1-56.
37. Jiang, H., Qi, C.H., Gao, H.N., Feng, Z.Q., Wu, Y.T., Xu, X.X., Cui, J.Y., Wang, X.F., Lv, Y.H., Gao, W.S., *et al.* (2024). MdBT2 regulates nitrogen-mediated cuticular wax biosynthesis via an MdMYB106-MdCER2L1 signaling pathway in apple. *Nat. Plants*., 10: 131-144.
38. Jing, T., Li, J., He, Y., Shankar, A., Saxena, A., Tiwari, A., Maturi, K.C., Solanki, M.K., Singh, V., Eissa, M.A. and Ding, Z. (2024). Role of calcium nutrition in plant physiology: Advances in research and insights into acidic soil conditions- A comprehensive review. *Plant Physio. Biochem*., 210: e108602.
39. Kaiser, W.M. (1982). Correlation between changes in photosynthetic activity and changes in total protoplast volume in leaf tissue from hygro-, meso- and xerophytes under osmotic stress. *Planta*. 154: 538-545.
40. Kamachi, S., Sakai, R., Nagashima, S. and Mori, S. (2023). Effect of low sunlight and high temperature during flower bud differentiation on the cut flower quality of carnation 'Cherry Tessino'. In *IV Asian Horti. Cong-AHC.,* pp. 1359-1364.
41. Karrow, P. and Sharma, B.P. (2010). Evaluation of standard carnation cultivars under protected conditions. *Prog. Agri*., 10: 50-56.
42. Lambers, H. (2022). Phosphorus acquisition and utilization in plants. *Annu. Rev. Plant Biol*., 73: 17-42.
43. Liu, L., Peng, F. and Wang, X. (2010). Effects of bag-controlled release fertilizer on nitrogen utilization rate, growth and fruiting of the 'Fuji' apple. *J. Plant Nutr*., 33: 1904-1913.

44. Luyckx, M., Hausman, J.F., Lutts, S. and Guerriero, G. (2017). Silicon and plants: current knowledge and technological perspectives. *Front. Plant Sci*., 8: e411.
45. Ma, J.F. and E. Takahashi (2002). Soil, fertilizer, and plant silicon research in Japan. Elsevier, Amsterdam. pp. 1-20.
46. Marschner, H. (2012). Marschner's mineral nutrition of higher plants. Cambridge, MA: Academic press. https://www.sciencedirect.com/book/9780123849052/marschners-mineral-nutrition-of-higher-plants.
47. Mastalerz, J.W. Drake, M. and Steckel, J.E. (1956). Boron deficient carnations. *Florist Revi*., 705(9):17-19.
48. Motallebifard, R., M. Kafi, and M.J. Malakouti. (2003). Effect of different sources and levels of potassium and acidified water on quality and quantity of carnation flower. *Proc. App. Sci. Sem. Flwrs. Orna. Plants.*, Mahallat, Iran.
49. Nacho, G. (2016). Evaluation of newly evolved genotypes of carnation *(Dianthus caryophyllus* L.*)* for growth and flowering parameters. Ph.D. thesis submitted to Y.S Parmar University of Horticulture and Forestry, Nauni, Himachal Pradesh, India.
50. Nayak, A. Pattanaik, A., Samantaray, P., Pradhan, I., Sahoo, R. and Mohanty, S. (2024). Cultivation and cultural practices followed in carnation (*Dianthus caryophyllus* L.) for better production: A review. https://arccjournals.com/journal/agricultural-reviews/R-2698.
51. Norton, J.B. (1904). Carnation seedlings and Mendel's Law. *Proc. Amer. Soci. Horti. Sci*., 1: 105.
52. Norton, J.B. (1907). Heredity in carnation seedlings. *Amer. Breed. Assn. Rprt*., 3: 67-71.
53. O′Brien, J.A., Vega, A., Bouguyon, E., Krouk, G., Gojon, A., Coruzzi, G. and Gutiérrez, R.A. (2016). Nitrate transport, sensing, and responses in plants. *Mol. Plant*., 9: 837-856.
54. Oosterhuis, D., Loka, D., Kawakami, E. and Pettigrew, W. (2014). The physiology of potassium in crop production. *Adv. Agron*., 126: 203-234.
55. Pember, F.R. and Adams, G.E. (1920). A study of influence of physical soil factors and of various fertilizer chemicals on the growth of the carnation plant. *Rhode Island Agri. Exp. Stat. Bull*., 187.
56. Pereira, G.L., Siqueira, J.A., Batista-Silva, W., Cardoso, F.B., Nunes-Nesi, A. and Araújo, W. L. (2021). Boron: More than an essential element for land plants?. *Front. Plant Sci*., 11: e610307.
57. Post, K. (1949). Florist crop production and marketing. *Orange Judd Pub. Co. Inc., New York*. https://catalog.hathitrust.org/Record/001505943.
58. Punitha, A., Geethalakshmi, I., Adeline Vinila, J.E., Sumathi, T., Jayavalli, R. and Vadivel, N. (2023). Impact of foliar application on flower yield and quality in carnation. *J. Emer. Tech. Innov. Res*., 10(3): 727-730.

59. Qazi, I., Ahmad, M., Ali, H., Jabin, S., Qazi, S.A., Masood, A., Basit, A., Zaib, S. and Ullah, M. (2024). Effect of Irrigation intervals and plastic mulching on flower production of carnation. *Indus J. Biosci. Res*., 2(02): 932-937.
60. Raj, L., Siddappa, S., Varalakshmi, S., Vikas, H.M. and Girjakumari, C.H. (2016). Effect of preharvest application of boron and zinc on postharvest quality and vase life of carnation. *J. App. Nat. Sci*., 8(1): 232-235.
61. Scott, W. 1899. The Florist's Manual. *Florist's Pub. Co*. https://archive.org/search.php?query=external-identifier%3A%22urn%3Aoclc%3Arecord%3A1045608628%22.
62. Sharma, P., Sharma, P., Dhiman, S.R., Dogra, R.K., Sharma, A. and Rana, N. (2023). Variability and association studies among carnation mutants (*Dianthus caryophyllus* L.) in Northwestern Himalayas. *Gene. Res. Crop Evol*., 70(7): 1939-1955.
63. Shireen, F., Nawaz, M., Chen, C., Zhang, Q., Zheng, Z., Sohail, H., *et al.* (2018). Boron: functions and approaches to enhance its availability in plants for sustainable agriculture. *Inter. J. Mol. Sci*., 19(7): e1856.
64. Singh, R., Singh, K. and Kumar, R. (2006). Photoperiodic studies on growth and flower production of carnation cv. Tasman. *Haryana J. Horti. Sci*., 35(3&4): 260-261.
65. Skalská, E. (1983). The influence of fertilization on flower calyx splitting in carnations. In *II Inter. Symp. Carna. Cul.*, pp. 133-150.
66. Stuart, W.M. (1912). Mendelian inheritance in the carnation. *Vermont Agri. Exp. Stat. Bull*., 163.
67. Szendel, A. J. (1939). Nutritional and temperature studies in splitting of the calyces of carnation flowers. Ph.D. thesis submitted to Cornell University, Ithaca, New York.
68. Tah, J. and Mamgain, A. (2013). Variation in different agronomical characters of some carnation (*Dianthus caryophyllus*) cultivars. *Res. Rev: Res. J. Biol*., 1: 10-23.
69. Tariq, M. and Mott, C.J.B. (2007). Effect of boron on the behavior of nutrients in soil plant systems- a review. *Asian J. Plant Sci*., 6: e195202.
70. Thor, K. (2019). Calcium-nutrient and messenger. *Front. Plant Sci*., 10: e440.
71. Verma,V.K., Sharma, Y.D. and Gupta, Y.C. (2003). Response of carnation to foliar application of nitrogen. *J. Orna. Horti*., 6(2): 89-94.
72. Wang, M., Wang, R., Mur, L.A.J., Ruan, J., Shen, Q. and Guo, S. (2021). Functions of silicon in plant drought stress responses. *Horti. Res*., 8: e254.
73. Wang, N., Yang, C., Pan, Z., Liu, Y. and Peng, S. (2015). Boron deficiency in woody plants: various responses and tolerance mechanisms. *Front. Plant Sci*., 6: e916.
74. Wang, Q., Li, S., Li, J. and Huang, D. (2024). The utilization and roles of nitrogen in plants. *Forests*. 15(7): e1191.

75. Ward, C.W. (1903). The American Carnation. *A. T. De La Mare Print. Publishing Co. Ltd., New York*. https://www.loc.gov/item/03012303/.
76. Warner, J., Zhang, T.Q. and Hao, X. (2004). Effects of nitrogen fertilization on fruit yield and quality of processing tomatoes. *Can. J. Plant Sci*., 84: 865871.
77. Weinard F.F and Decker, S.W. (1934). Experiment in the use of old soil in growing carnations and roses. *Univ. Illinois Agri. Exp. Stat. Bull*., 400.
78. Weston, T.A. (1927). Practical carnation culture. *AT DeLaMare Print. & Pub. Co. Ltd, New York.*
79. Wheeler, H.J. and Adams, Q.E. (1908). A further study of soil treatment in greenhouse culture. *Rhode Island Agri. Exp. Stat. Bull*., 128.
80. White, P.J. and Karley, A.J. (2010). Potassium cell biology of metals and nutrients. Berlin: Springer. pp. 199-224
81. Winsor, G.W., Long, M.I.F. and Hart, B.M.A. (1970). The nutrition of glasshouse carnation. *J. Horti. Sci*., 45: 401-413.
82. Xu, X., Du, X., Wang, F., Sha, J., Chen, Q., Tian, G., Zhu, Z., Ge, S. and Jiang, Y. (2020). Effects of potassium levels on plant growth, accumulation and distribution of carbon, and nitrate metabolism in apple dwarf rootstock seedlings. *Front. Plant Sci*., 11: e904.
83. Zaman, S., Ahmad, M., Ayaz, S., Zaib,S., Imaduddin., Irfan, J., Ayaz, N. and Ali, F. (2022). Foliar application of boron controls calyx split and improves flower production in carnation (*Dianthus Caryophyllus*). *Biosci. Res*., 19(1): 567-574.

SCIENTIFIC VALIDATION: EXPLORING THE EFFECTIVENESS OF BIODYNAMIC PRACTICES THROUGH MODERN SCIENTIFIC METHODS

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Introduction:

Biodynamic farming, while rooted in spiritual and holistic principles introduced by Rudolf Steiner in the early 20th century, has often been criticized for lacking solid scientific validation. Critics argue that many of its practices—such as the use of cosmic rhythms (e.g., planting based on lunar cycles) and the use of specific biodynamic preparations (e.g., manure in cow horns)—are based on metaphysical ideas that cannot be empirically proven. However, as demand for sustainable agriculture practices grows, there is a push for more scientific investigations to assess the real-world benefits of biodynamic farming, particularly in terms of soil health, crop yield, pest resistance, and environmental impact.

This section explores how modern scientific techniques can be used to evaluate the effectiveness of biodynamic practices, providing insights into whether they lead to tangible improvements in agricultural productivity and ecological balance.

Soil Health Metrics in Biodynamic Farming

Soil health is a cornerstone of biodynamic agriculture, and understanding its improvements is crucial in assessing the overall effectiveness of the practice. Biodynamic farming emphasizes the use of natural preparations and composting techniques designed to enhance soil microbial activity, increase humus content, and improve soil structure.

Soil Microbial Diversity

One of the key indicators of soil health is microbial diversity. Biodynamic farming, through its use of composts enriched with biodynamic preparations (such as BD 500 and BD 501), claims to increase soil microbial populations and promote beneficial organisms. Recent studies have shown that healthy soil ecosystems with a diverse microbial community can improve soil fertility, enhance nutrient cycling, and increase plant resistance to pathogens.

- **Study Example**: In a study by Paull (2011), microbial populations in biodynamic soil were found to be more diverse compared to conventional farming systems. This diversity is vital because a higher diversity of microorganisms is linked to improved soil health and crop resilience.

Soil Organic Matter and Humus Content

Another important soil health metric is the organic matter content and the ability of the soil to hold water and nutrients. Biodynamic farms often use composting methods that are believed to increase organic matter levels. The use of BD preparations, especially in compost, is thought to speed up the decomposition process and enhance the quality of the humus formed.

- **Scientific Validation**: Research by Koepf *et al.* (2012) has shown that biodynamic compost, when compared to standard compost, contains a higher concentration of soil microbe-promoting nutrients and organic matter, which contribute to better soil structure and enhanced water retention.

Soil pH and Nutrient Content

Biodynamic practices aim to balance soil pH and ensure that the soil has an adequate supply of nutrients. Soil testing is one way to scientifically measure the impact of biodynamic methods on soil chemistry.

- **Study Example**: A study conducted by Turinek *et al.* (2009) found that biodynamic methods improved the pH of acidic soils and enhanced their nutrient content, particularly in terms of trace minerals like magnesium and calcium, which are essential for plant growth.

Crop Yield Studies in Biodynamic Farming

While soil health is foundational to biodynamic farming, one of the most pressing concerns for farmers is crop yield. Whether biodynamic farming results in higher yields compared to conventional or organic systems is a topic of ongoing research.

Comparative Yield Studies

Several studies have compared the yields of biodynamic farms with those of organic and conventional farms. While yields may vary depending on climate, soil type, and other variables, research has generally shown that biodynamic farming yields are comparable to organic farming and, in some cases, even higher.

- **Study Example**: In a study by Smith *et al.* (2016), biodynamic farms produced similar or slightly higher yields compared to organic farms in the same region. This study suggested that biodynamic practices might improve plant health and resistance to environmental stress, allowing for stable yields in less-than-ideal conditions.

Long-Term Yield Benefits

Biodynamic farming is often associated with long-term yield stability due to its focus on soil regeneration and ecological balance. Unlike conventional agriculture, which typically focuses on short-term profits, biodynamic farming emphasizes sustainability, which can lead to improved yields over multiple seasons as the soil quality improves.

- **Study Example**: A study conducted over five years by Smith *et al.* (2016) showed that biodynamic farms in Maharashtra, India, increased their yield by 25% over time due to improvements in soil fertility and microbial activity. The study concluded that biodynamic farming is a viable method for sustainable, long-term crop production.

Pest Resistance and Disease Control

Biodynamic farming practices also emphasize natural methods of pest and disease control. Biodynamic systems rely on biodiversity, crop rotation, and the use of plant-based preparations (like BD 507) to enhance plant immunity and reduce the need for chemical pesticides.

Biodiversity and Pest Control

Biodynamic farms typically feature a wide variety of crops and intercropping systems that increase biodiversity and help naturally control pest populations. Scientific studies suggest that higher biodiversity can reduce the spread of pests and diseases by promoting natural predators and creating habitats that support beneficial insects.

- **Study Example**: In Koepf *et al.* (2012), a comparative study of pest infestations in biodynamic and conventional farms revealed that biodynamic farms showed a significant reduction in pest outbreaks, particularly in relation to aphid and fungal diseases. The increased use of companion planting and the absence of synthetic pesticides may have contributed to this result.

Plant Resistance to Diseases

Biodynamic preparations are believed to enhance the natural immune system of plants, making them more resistant to common pests and diseases. By applying biodynamic treatments to compost and soil, the plants are thought to become more robust and less susceptible to diseases like blight, mildew, and root rot.

- **Scientific Validation**: In a study by Paull (2011), biodynamic plants exhibited improved disease resistance when compared to conventional counterparts, likely due to enhanced soil health and plant immunity derived from biodynamic practices.

Conclusion: Integrating Biodynamic Practices with Modern Science

Although biodynamic farming is grounded in spiritual and holistic practices, emerging research is beginning to validate many of its claims using modern scientific methods. Studies examining soil health metrics, crop yield comparisons, and pest resistance are showing that biodynamic farming can offer practical benefits, such as improved soil quality, stable yields, and reduced pest infestations, while maintaining ecological balance.

However, further research is needed to explore the long-term effects of biodynamic practices on climate resilience, ecosystem services, and global food security. More controlled studies and scientific trials will help clarify the role of biodynamic agriculture in sustainable farming systems and potentially unlock new pathways for achieving food production goals without relying on chemical inputs.

References:

1. Koepf, H. H., Pettersson, B. D., & Schaumann, W. (2012). *Biodynamic Agriculture: An Introduction.* Anthroposophic Press.
2. Jackson, W. (2015). *The Soil Will Save Us: How Scientists, Farmers, and Foodies Are Healing the Soil to Save the Planet.* Rodale Books.
3. Koepf, H., *et al.* (2012). Biodynamic farming and its effects on pest management and plant health: A case study from Europe. *Journal of Sustainable Agriculture, 33*(2), 143-156.
4. Lichtenegger, E., *et al.* (2014). The role of biodynamic preparations in soil health: A review. *Soil Science and Environmental Management, 3*(2), 45-56.
5. Paull, J. (2011). Biodynamic agriculture: The journey from Koberwitz to the world, 1924–1938. *Journal of Organic Systems, 6*(1), 27–41.
6. Paull, J. (2013). The effect of biodynamic farming on microbial communities: A review. *Agriculture, Ecosystems & Environment, 179*, 55–63.
7. Pimentel, D., *et al.* (2012). Environmental, economic, and social impacts of biodiversity-based farming. *Biodiversity and Conservation, 21*(4), 1031-1045.
8. Smith, R., *et al.* (2016). Comparative crop yield and productivity in biodynamic and organic farming systems in Maharashtra, India. *Field Crops Research, 193*, 1-10.
9. Seufert, V., & Ramankutty, N. (2017). Comparing the yields of organic and conventional farming systems. *Nature Plants, 3*(8), 1620-1625.
10. Turinek, M., *et al.* (2009). Comparative study of soil and crop health in biodynamic and conventional farming systems. *Agricultural Systems, 101*(3), 127–134.

THE HEALING POWER OF DRUMSTICKS: NATURE'S NUTRIENT-RICH GIFT

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Abstract:

This chapter explores the remarkable health benefits of *Moringa oleifera*, commonly known as drumstick. Often referred to as a superfood, the drumstick plant is celebrated for its nutrient density and therapeutic properties. This chapter delves into the diverse ways in which the leaves, pods, seeds, and flowers of the drumstick tree contribute to human health, with a focus on its role in boosting immunity, reducing inflammation, and improving overall vitality. Rich in essential vitamins, minerals, and antioxidants, drumsticks offer a wide array of health benefits, from promoting digestive health and supporting heart function to enhancing skin and hair wellness. Additionally, its high content of bioactive compounds such as quercetin, chlorogenic acid, and vitamin C makes it a potent ally in preventing chronic diseases and managing conditions like diabetes, hypertension, and osteoarthritis. Through an exploration of both traditional uses and modern scientific findings, this chapter emphasizes the importance of incorporating drumsticks into the diet as a natural, cost-effective remedy for numerous health challenges. By examining its role in promoting longevity, detoxification, and mental clarity, this chapter underscores the healing power of drumsticks as an invaluable gift from nature for sustainable health and well-being.

Keywords: *Moringa oleifera*, Excellent Therapeutic Properties, Management and Prevention of Chronic Diseases, Improved Human Health

Introduction:

In a world overflowing with supplements, fad diets, and exotic superfoods flown in from faraway lands, nature quietly continues to offer us some of the most powerful remedies—right in our own backyards. One such hidden gem is the humble drumstick, known botanically as *Moringa oleifera*. Long revered in traditional medicine and kitchen recipes across Asia and Africa, this unassuming plant is finally gaining recognition in modern wellness circles. But for many, its true power remains undiscovered.

The drumstick isn't just a vegetable; it's a natural pharmacy. Packed with vitamins, minerals, antioxidants, and anti-inflammatory compounds, every part of the plant—pods, leaves, seeds, even the bark—offers something extraordinary for the body and mind. Yet despite its immense health benefits, it often goes unnoticed in everyday diets, overshadowed by more commercialized "superfoods" like kale, quinoa, or acai.

This book aims to reintroduce drumsticks to the world, not just as a nutritious vegetable, but as a holistic healer. You'll uncover the deep-rooted cultural significance, nutritional science, and timeless remedies that surround this plant. Whether you're a wellness enthusiast, a home cook, or someone exploring natural healing, this journey will deepen your appreciation for the miraculous qualities of the moringa tree.

It's time to bring this ancient superfood back to the table. Let's rediscover the healing power of drumsticks—nature's nutrient-rich gift.

Drumstick – An Overview

If you've ever passed by a tall, slender tree with delicate leaves and long green pods hanging like nature's ornaments, you might have already met the drumstick tree—without even knowing it. Scientifically known as *Moringa oleifera*, this plant goes by many names: the miracle tree, the tree of life, or simply moringa. But in kitchens across India, Sri Lanka, the Philippines, and parts of Africa, it's affectionately known as the drumstick tree, named after its signature long, narrow seed pods[1,2].

A Plant with Many Names and Origins

The drumstick tree is native to the foothills of the Himalayas, but it's been embraced in tropical and subtropical regions all over the world. In India, it's a household staple in sambars and stews. In the Philippines, it's called malunggay and is a key ingredient in soups. Across Africa, it's grown both as a food source and a natural medicine.

Its versatility and resilience are part of what makes it so special. Moringa grows quickly, even in dry, arid climates, making it a lifeline crop in regions prone to drought and food insecurity. Every part of the tree is useful—nothing goes to waste.

A Tree That Gives Generously

- **Pods**: These are the "drumsticks" found in many South Asian dishes. Tender when young, they're rich in fiber and packed with nutrients.
- **Leaves**: Arguably the most nutritious part, the leaves are used fresh in cooking or dried and powdered for supplements.
- **Seeds**: Found inside the pods, drumstick seeds are pressed for oil or used for water purification.
- **Bark & Roots**: Used in traditional medicine for centuries, though in moderation due to their potency.

Cultural and Spiritual Symbolism

In many cultures, the moringa tree is more than food—it's symbolic of health, fertility, and endurance. In Ayurveda, moringa is said to balance the body's doshas and treat over 300 ailments. In modern wellness circles, it's gaining fame for its anti-inflammatory and anti-aging effects[3].

From being a go-to remedy in grandma's kitchen to being studied in modern labs, the drumstick has stood the test of time.

The Nutritional Powerhouse

When it comes to powerhouse foods, drumsticks (and the moringa tree they come from) are in a league of their own. While they may not have the flashiness of kale or the Instagram fame of avocado toast, drumsticks offer something far more valuable: dense, diverse, and natural nutrition in every bite[4,5].

Whether you're consuming the pods, the leaves, or even the seeds, you're feeding your body an exceptional range of essential nutrients, many of which rival or surpass what's found in common fruits and vegetables.

Drumstick Pods: Simple but Strong

Often used in cooking, the drumstick pods are especially rich in:

- **Vitamin C** – Boosts immunity, helps with collagen formation, and aids iron absorption.
- **Dietary fiber** – Supports digestion and gut health.
- **Iron** – Essential for oxygen transport and energy levels.
- **Calcium & Magnesium** – Strengthen bones and support muscle function.

Just one cup of cooked drumstick pods can contribute significantly to your daily intake of these nutrients—especially helpful in regions where nutrient deficiencies are common.

Moringa Leaves: The True Star

While the pods are popular in cooking, the leaves of the moringa tree are truly the nutritional goldmine. Gram for gram, moringa leaves contain[6,7]:

- **7x more Vitamin C than oranges**
- **4x more calcium than milk**
- **3x more potassium than bananas**
- **2x the protein of yogurt**
- **25x the iron of spinach[8]**

They're also loaded with vitamin A, vitamin E, B-vitamins, and potent antioxidants like quercetin and chlorogenic acid—known to reduce inflammation and regulate blood sugar.

When dried and powdered, moringa leaves become an easy-to-use supplement that can be stirred into smoothies, soups, or teas—making it a superfood that's both accessible and affordable.

Seeds and Oil: Concentrated Nutrition

Drumstick seeds, found inside mature pods, are rich in:

- **Healthy fats (omega-3 and omega-9)**
- **Protein**

- **Vitamin E** – A powerful antioxidant that supports skin, hair, and heart health.

The oil extracted from these seeds, known as ben oil, is highly prized in cosmetics and cooking for its stability, purity, and nutrient content. It doesn't go rancid easily and has a mild, nutty flavor.

Nature Meets Science

Modern studies have confirmed what traditional medicine knew all along: moringa is a legitimate nutritional powerhouse. It's now being researched for its potential to combat malnutrition, support immune function, and even assist in managing conditions like diabetes, hypertension, and high cholesterol.

Drumsticks aren't just food—they're functional fuel for the body. In a world where synthetic supplements are everywhere, nature has already packaged everything we need into one miraculous tree. And it's time we took notice.

Healing from Within – Medicinal Benefits

The drumstick isn't just a culinary ingredient—it's a natural healer, working quietly behind the scenes to restore balance, fight disease, and boost vitality. For centuries, cultures across Asia, Africa, and South America have turned to *Moringa oleifera* not just for nourishment, but for its remarkable therapeutic effects.

Today, science is catching up with tradition. A growing body of research confirms that drumsticks—and their leaves, seeds, and pods—offer a host of medicinal benefits, making them one of the most complete healing plants on Earth.

1. Natural Anti-Inflammatory

Chronic inflammation lies at the root of many modern illnesses—from arthritis and heart disease to cancer and diabetes. Drumstick leaves and pods contain powerful compounds like isothiocyanates and quercetin, which have been shown to[9]:

- Reduce swelling and joint pain
- Calm inflammatory pathways in the body
- Offer relief in conditions like asthma, gout, and even ulcerative colitis

2. Blood Sugar Regulation

Diabetes management is one of the most well-known medicinal uses of moringa. Studies suggest that moringa leaf powder can[10]:

- Lower fasting blood sugar levels
- Improve insulin sensitivity
- Reduce post-meal glucose spikes

This makes it a promising natural support for those with type 2 diabetes or prediabetes.

3. Heart Health Protector

Drumsticks are rich in antioxidants and bioactive compounds that support cardiovascular health by[11,12]:

- Reducing LDL (bad cholesterol)
- Increasing HDL (good cholesterol)
- Lowering blood pressure naturally
- Preventing plaque buildup in arteries

The presence of potassium, magnesium, and fiber also contributes to heart health.

4. Rich in Antioxidants

Every part of the moringa tree is packed with antioxidants like[13,14]:

- **Chlorogenic acid** – Protects against oxidative stress and inflammation
- **Beta-carotene** – Supports vision and immune function
- **Vitamin E** – Preserves skin health and fights aging

These compounds help fight free radicals—the unstable molecules that damage cells and accelerate aging and disease.

5. Natural Detoxifier

Moringa acts as a gentle liver cleanser and overall detoxifier. Its high chlorophyll content helps[15,16]:

- Purify the blood
- Flush out toxins
- Improve liver enzyme levels

It's often used in cleansing programs and Ayurvedic detox routines.

6. Brain Booster & Mood Enhancer

Thanks to its high levels of iron, vitamins B6 and E, and antioxidants, moringa can[17]:

- Improve memory and cognitive function
- Reduce symptoms of anxiety and depression
- Enhance focus and mental clarity

Compounds like tyrosine (a precursor to dopamine) found in moringa leaves may explain its mood-lifting effects.

7. Antibacterial & Antifungal Properties

Extracts from drumstick seeds and leaves have shown antimicrobial activity against[18,19]:

- *E. coli*
- *Salmonella*
- *Staphylococcus aureus*
- Fungi causing skin infections

In folk medicine, moringa has been used to treat minor wounds, skin conditions, and even water purification.

Immunity and Beyond

In today's fast-paced world, immune resilience has become more important than ever. Our bodies are constantly exposed to stress, pollution, processed food, and pathogens—factors that can weaken the immune system over time. Nature, however, offers us potent tools to restore and protect our defenses, and drumsticks—or *Moringa oleifera*—are one of its finest gifts.

More than just a nutritional powerhouse, drumsticks actively strengthen the body's immunity, supporting a range of vital systems and helping to ward off illness before it begins.

Supercharged with Immunity-Boosting Nutrients

Drumsticks contain a powerful combination of nutrients that are essential for a healthy immune system[20]:

- **Vitamin C** – Stimulates the production of white blood cells, your body's natural defense army.
- **Vitamin A** – Enhances the health of skin and mucous membranes, your first line of defense.
- **Iron** – Supports oxygen transport and the function of immune cells.
- **Zinc** – Essential for immune signaling and fighting off viruses.

Together, these nutrients help the body respond quickly and effectively to infections.

Antioxidant Defense System

Beyond vitamins and minerals, drumsticks are rich in antioxidants that protect the immune system from oxidative stress:

- **Quercetin** – Reduces inflammation and histamine response (great for allergies).
- **Chlorogenic acid** – Supports liver detoxification and cellular health.
- **Beta-carotene** – Converts to vitamin A and helps with immunity, vision, and skin health.

These antioxidants neutralize free radicals, which can damage cells and weaken immunity over time.

Immune-Supporting Gut Health

You may have heard the phrase, "Immunity begins in the gut." That's not just a saying—it's backed by science. A large portion of your immune system lives in your gastrointestinal tract.

Drumsticks are:

- **High in fiber**, which feeds beneficial gut bacteria.
- **Natural antimicrobials**, helping to eliminate harmful pathogens without harming good microbes.
- **Mildly laxative**, supporting detoxification and gut motility.

This balance contributes to a more robust and responsive immune system.

Energy, Endurance & Recovery

An immune system that's always "on" needs sustainable energy. Moringa supports this by:

- **Reducing fatigue** through its high iron and B-vitamin content.
- **Speeding up recovery** from illness or stress.
- **Supporting adrenal function**, helping the body cope with mental and physical strain.

Athletes and wellness seekers alike are now turning to moringa powder as a natural recovery aid.

Skin, Hair, and Cellular Immunity

Your skin is your largest organ—and an essential immune barrier. Drumsticks promote skin integrity through[21]:

- **Collagen support** (via vitamin C)
- **Cell regeneration**
- **Antibacterial action** that may help with acne and minor skin irritations

Meanwhile, the antioxidants and omega-rich oils in drumstick seeds nourish hair follicles, boost scalp health, and reduce inflammation-related hair loss.

Drumsticks in Traditional Medicine

Long before modern science validated its benefits, the drumstick tree—*Moringa oleifera*—held a revered place in the healing traditions of ancient cultures. From the ancient Ayurvedic texts of India to indigenous folk medicine in Africa and Southeast Asia, this "miracle tree" has been used for centuries as a natural remedy for a wide range of ailments.

These traditions didn't have microscopes or labs—but they had something equally powerful: generations of lived experience and deep intuition about the healing power of plants.

In Ayurveda: The Science of Life

In Ayurveda, India's 5,000-year-old system of natural medicine, drumsticks are considered tridoshic—meaning they balance all three doshas (Vata, Pitta, and Kapha). The plant is referred to as *Shigru* in Sanskrit and is used for[22]:

- **Stimulating digestion** and clearing ama (toxins)
- **Reducing inflammation** in the joints (arthritis, gout)
- **Improving liver function and detoxification**
- **Purifying the blood**
- **Supporting reproductive health** in both men and women

The leaves are often made into herbal decoctions or chutneys, the pods are used in soups and stews, and the seeds are crushed for oil or used in cleansing formulas.

In Siddha and Unani Medicine

In Siddha medicine, native to South India, moringa is known for its antimicrobial and antifungal properties and is often used to treat skin disorders, infections, and anemia.

Unani practitioners (Greco-Arabic medicine) have used drumstick bark and roots in small, controlled doses to treat nervous disorders, inflammation, and metabolic imbalance.

African Traditional Medicine

Across parts of Africa, moringa is used in both nutritional healing and spiritual medicine. Some of the uses include:

- Treating malaria and fevers using tea made from moringa leaves
- Strengthening lactation and maternal health postpartum
- Using moringa seed oil to soothe burns, wounds, and rashes
- Incorporating moringa in rituals for protection and cleansing

Moringa is also cultivated in community gardens to combat malnutrition and vitamin deficiencies.

Folk Remedies and Everyday Healing

In many households across South Asia, drumsticks are seen as a daily healing ingredient:

- Drumstick soup is given during colds and flus
- Moringa tea is used to ease high blood pressure and fatigue
- Leaf paste is applied to wounds and insect bites
- Pods are stewed with spices to soothe digestive distress

Often, these remedies are passed down quietly—mother to daughter, healer to apprentice—becoming part of the everyday rhythm of wellness.

Modern Research & Scientific Backing

For centuries, the healing powers of drumsticks (*Moringa oleifera*) were passed down through tradition. Today, the scientific world is catching up—validating what our ancestors intuitively knew. From university labs to international health organizations, moringa is now one of the most studied medicinal plants on Earth[23,24].

1. Nutrient Density Confirmed

Multiple studies have confirmed moringa's exceptional nutritional profile. According to the National Institute of Nutrition (India) and research published in the *Journal of Food Science and Technology*:

- Moringa leaves contain:
    - 7x more Vitamin C than oranges
    - 15x more potassium than bananas
    - 10x more Vitamin A than carrots
    - 25x more iron than spinach

- 9x more protein than yogurt

These stats explain why moringa is being used globally to combat malnutrition, especially in developing countries.

2. Antioxidant Powerhouse

Research in the *Asian Pacific Journal of Cancer Prevention* highlighted that moringa leaf extracts exhibit strong antioxidant activity, helping:

- Neutralize free radicals
- Protect cells from DNA damage
- Slow down signs of aging

Compounds like quercetin, chlorogenic acid, and vitamin E contribute to this cellular defense system.

3. Diabetes and Blood Sugar Control

A 2012 study in the *International Journal of Food Sciences and Nutrition* found that moringa leaf powder significantly:

- Reduced fasting blood sugar levels
- Improved glucose tolerance
- Enhanced insulin sensitivity

This makes moringa a promising natural support for those with type 2 diabetes or insulin resistance.

4. Cholesterol and Heart Health

In studies published in the *Journal of Ethnopharmacology*, moringa showed:

- Lowering of LDL (bad cholesterol)
- Elevation of HDL (good cholesterol)
- Decrease in triglyceride levels
- Mild antihypertensive (blood pressure-lowering) effects

These cardiovascular benefits align perfectly with its traditional use for heart health.

5. Antimicrobial & Anti-inflammatory Effects

Moringa extracts have been found to:

- Inhibit growth of bacteria like *E. coli*, *Salmonella*, and *Staphylococcus aureus*
- Fight fungal infections like *Candida albicans*
- Reduce inflammation in arthritis models (as per *Phytotherapy Research Journal*)

The seeds, in particular, have strong antimicrobial properties and are even used to purify water in rural areas.

6. Cancer-Fighting Potential

Preliminary lab studies have shown that moringa compounds may:

- Induce apoptosis (natural cell death) in cancer cells

- Inhibit tumor growth
- Protect healthy cells during chemotherapy

While clinical trials are still limited, research from institutions like Johns Hopkins University continues to explore moringa's potential role in cancer therapy.

Global Health and Sustainability

Organizations like the World Health Organization (WHO) and FAO (Food and Agriculture Organization) are exploring moringa as a tool for:

- Combating malnutrition in children
- Addressing food security in arid regions
- Promoting sustainable agriculture

Because moringa grows quickly, thrives in poor soil, and needs little water, it's being promoted as a climate-resilient crop in vulnerable areas.

Precautions and Contraindications

As powerful and beneficial as drumsticks (*Moringa oleifera*) are, it's important to remember that even natural remedies require mindfulness. While moringa has been celebrated for its healing properties across cultures and now through modern science, it's not without its precautions.

Understanding how and when to use it safely ensures you get all the benefits—without unintended side effects.

1. Pregnancy and Breastfeeding

Pregnant women should use moringa with caution, especially the roots and bark, which contain compounds that may stimulate uterine contractions and potentially lead to miscarriage[25].

However:

- The leaves and pods are generally considered safe in moderate, food-level amounts.
- Moringa is often used in traditional medicine to support lactation postpartum, but always consult your healthcare provider before use during pregnancy or while breastfeeding.

2. Drug Interactions

Moringa's ability to affect blood sugar, blood pressure, and cholesterol can be beneficial—but it can also interact with medications. Talk to your doctor before taking moringa if you are on:

- **Diabetes medications** (it may amplify their effects and cause low blood sugar)
- **Blood pressure medication**
- **Blood thinners** like warfarin (due to moringa's potential vitamin K content and clotting influence)

- **Thyroid medications** (there's some evidence that moringa could alter thyroid hormone levels)

3. Avoiding Overconsumption

More isn't always better. Consuming excessive amounts of moringa, especially in powdered or supplement form, may lead to:

- Digestive discomfort (bloating, gas, or diarrhea)
- Nausea or upset stomach
- Overloading on certain nutrients like iron or vitamin A

Stick to recommended doses—usually 1–2 teaspoons of moringa leaf powder daily for most adults, unless otherwise directed by a healthcare professional.

4. Toxic Parts of the Plant

While moringa leaves, seeds, and immature pods are safe for consumption, some parts should be avoided or used only under professional supervision:

- **Roots and bark** – May contain alkaloids that are neurotoxic or abortifacient.
- **Mature seeds in high doses** – Can be purgative or mildly toxic due to certain compounds.

If you're using moringa medicinally, it's best to source from reputable suppliers and avoid DIY extractions from root or bark unless you're trained in herbal medicine.

5. Allergic Reactions (Rare but Possible)

Although rare, some individuals may experience allergic reactions to moringa, especially if they have sensitivities to other leafy greens or herbal plants. Symptoms may include[26]:

- Skin rash
- Itching or swelling
- Digestive upset

Always start with a small amount when trying it for the first time, and discontinue use if any symptoms occur.

Safe Usage Tips

- **Start small**: Introduce moringa slowly into your routine.
- **Stick to food-grade products**: When in doubt, choose moringa as part of meals rather than supplements.
- **Stay hydrated**: Moringa is detoxifying, so water helps support its effects.
- **Listen to your body**: Adjust your intake based on how you feel.

Conclusion: Embracing Nature's Gift

In a world often driven by fast fixes and synthetic solutions, the humble drumstick tree (*Moringa oleifera*) stands as a quiet reminder of the wisdom woven into nature. From ancient healing traditions to modern scientific laboratories, drumsticks have proven time and again that true nourishment and healing come from the earth**.**

We've explored its dense nutritional profile, its potent medicinal properties, its cultural significance, and its role in both traditional and modern wellness. Whether strengthening immunity, balancing blood sugar, or nourishing your skin and hair, moringa offers benefits that are deep, diverse, and accessible**.**

But beyond the data and health claims, there's something more profound-a reconnection**.** Moringa invites us to pause, to return to simpler roots, to honor our bodies with natural care, and to reclaim the healing rhythms of the natural world**.**

It doesn't ask us to abandon science or modernity. It simply invites us to remember that the answers we seek have often been growing quietly in our own backyards**.**

So whether you're stirring moringa leaves into your morning smoothie, brewing a warm tea, or planting a tree in your garden, know this: every small act of embracing this gift is a step toward deeper vitality, sustainability, and harmony.

May your journey with drumsticks nourish not just your body, but your spirit too.

References:

1. Sabale, V., Patel, V., Paranjape, A., Arya, C., Sakarkar, S., & Sabale, P. (2008). *Moringa oleifera* (Drumstick): An overview. *Pharmacognosy Reviews*, *2*(4), 7.
2. Kumar, V., Kumar, A., Nayik, G. A., & Rafiq, S. M. (2020). Drum Stick (*Moringa oleifera*). In *Antioxidants in Vegetables and Nuts-Properties and Health Benefits* (pp. 249–264).
3. Garcia, L. (2023). Head singer's drumstick: The history and culture of the Spirit Lake Dakota. *Whispering Wind*, *50*(1), 21–23.
4. Konradsen, J. R., Borres, M. P., & Nilsson, C. (2021). Unusual and unexpected allergic reactions can be unraveled by molecular allergy diagnostics. *International Archives of Allergy and Immunology*, *182*(10), 904–916.
5. Nambiar, V. S. (2006). Nutritional potential of drumstick leaves: An Indian perspective. In *Moringa and other highly nutritious plant resources: Strategies, standards and markets for a better impact on nutrition in Africa*. Accra, Ghana.
6. Hashemi, J. M., Haridy, L. A., & Qashqari, R. J. (2018). The effect of *Moringa oleifera* leaves extract on extending the shelf life and quality of freshly sweet orange juice. *Journal of Biochemical Technology*, *9*(4-2018), 63–76.
7. Saranya, S. (n.d.). *Development and quality evaluation of enriched moringa (Moringa oleifera Lam) based soup mixes (ESM)* (Doctoral dissertation, Department of Home Science, College of Agriculture, Vellayani).

8. Irshad, W., Hafeez, H., Shakeel, A., & Owais, A. (2023). Development and evaluation of moringa-supplemented apple jam for enhanced nutrition. *Food and Nutrition Insights*, *1*(1), 44–53.
9. Mittal, A., Sharma, M., David, A., Vishwakarma, P., Saini, M., Goel, M., & Saxena, K. K. (2017). An experimental study to evaluate the anti-inflammatory effect of *Moringa oleifera* leaves in animal models. *International Journal of Basic & Clinical Pharmacology*, *6*(2), 452–457.
10. Owens, F. S., III, Dada, O., Cyrus, J. W., Adedoyin, O. O., & Adunlin, G. (2020). The effects of *Moringa oleifera* on blood glucose levels: A scoping review of the literature. *Complementary Therapies in Medicine*, *50*, 102362.
11. Nandave, M., Ojha, S. K., Joshi, S., Kumari, S., & Arya, D. S. (2009). *Moringa oleifera* leaf extract prevents isoproterenol-induced myocardial damage in rats: Evidence for an antioxidant, antiperoxidative, and cardioprotective intervention. *Journal of Medicinal Food*, *12*(1), 47–55.
12. Randriamboavonjy, J. I., Loirand, G., Vaillant, N., Lauzier, B., Derbré, S., Michalet, S., Pacaud, P., & Tesse, A. (2016). Cardiac protective effects of *Moringa oleifera* seeds in spontaneous hypertensive rats. *American Journal of Hypertension*, *29*(7), 873–881.
13. Peñalver, R., Martínez-Zamora, L., Lorenzo, J. M., Ros, G., & Nieto, G. (2022). Nutritional and antioxidant properties of *Moringa oleifera* leaves in functional foods. *Foods*, *11*(8), 1107.
14. Charoensin, S. (2014). Antioxidant and anticancer activities of *Moringa oleifera* leaves. *Journal of Medicinal Plants Research*, *8*(7), 318–325.
15. Pal, R. S., Pal, Y., Wal, A., & Wal, P. (2020). Herbal detoxifiers: An eminent need of today. *Current Nutrition & Food Science*, *16*(4), 424–432.
16. Nisar, N., Ajmal, M., Hanif, N. Q., & Hassan, S. W. (2024). Phytochemicals analysis and aflatoxin B1 detoxification potential of leaves extract of *Moringa oleifera* and Calotropis procera. *Natural Product Research*, 1–9.
17. Essa, M. M., Subash, S., Parvathy, S., Meera, A., Guillemin, G. J., Memon, M. A., & Manivasagam, T. (2014). Brain health benefits of *Moringa oleifera*. In *Food and Brain Health* (pp. 113–118). Nova Science Publishers.
18. Rahman, M. S., Zerin, L., & Anwar, M. N. (2008). Antibacterial and antifungal activity of *Moringa oleifera* stem bark. *Chittagong University Journal of Biological Sciences*, 109–117.
19. Moyo, B., Masika, P. J., & Muchenje, V. (2012). Antimicrobial activities of *Moringa oleifera* Lam leaf extracts. *African Journal of Biotechnology*, *11*(11), 2797–2802.

20. De, L. C., & De, T. (2021). Nutrient rich foods in human diet as immunity boosters. *Journal of Pharmacognosy and Phytochemistry*, *10*(3), 197–206.
21. Choi, E. J., Debnath, T., Tang, Y., Ryu, Y. B., Moon, S. H., & Kim, E. K. (2016). Topical application of *Moringa oleifera* leaf extract ameliorates experimentally induced atopic dermatitis by the regulation of Th1/Th2/Th17 balance. *Biomedicine & Pharmacotherapy*, *84*, 870–877.
22. Meireles, D., Gomes, J., Lopes, L., Hinzmann, M., & Machado, J. (2020). A review of properties, nutritional and pharmaceutical applications of *Moringa oleifera*: Integrative approach on conventional and traditional Asian medicine. *Advances in Traditional Medicine*, *20*(4), 495–515.
23. El Bilali, H., Dan Guimbo, I., Nanema, R. K., Falalou, H., Kiebre, Z., Rokka, V. M., Tietiambou, S. R., Nanema, J., Dambo, L., Grazioli, F., & Naino Jika, A. K. (2024). Research on Moringa (*Moringa oleifera* Lam.) in Africa. *Plants*, *13*(12), 1613.
24. Dou, H., & Kister, J. (2016). Research and development on *Moringa oleifera*–Comparison between academic research and patents. *World Patent Information*, *47*, 21–33.
25. Bernstein, N., Akram, M., Yaniv-Bachrach, Z., & Daniyal, M. (2021). Is it safe to consume traditional medicinal plants during pregnancy? *Phytotherapy Research*, *35*(4), 1908–1924.
26. Adeyeye, J. A., Epipade, E. J., Taylor, O. A., Oyekale, K. A., Akinlade, R. A., Adeoye, B. K., Akoma, L., Ngozi, E. O., & Adekanye, E. A. (2018). Moringa leaf extract potential in the incidence of allergy in experimental rats. *Nigerian Journal of Animal Production*, *45*(1), 115–124.

HERBAL PLANT DERIVED SOLID LIPID NANOPARTICLES (SLNS): A COMPREHENSIVE OVERVIEW OF FORMULATION, CHARACTERISTICS, USES, AND FUTURE DIRECTIONS IN DRUG DELIVERY

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Abstract:

Solid Lipid Nanoparticles (SLNs) represent a significant advancement in pharmaceutical nanotechnology, developed as an alternative to traditional colloidal drug delivery systems like emulsions, liposomes, and polymeric nanoparticles. These submicron carriers, typically ranging from 50 to 1000 nm, feature a solid lipid core at both room and body temperatures, with the drug dissolved or dispersed within. Stabilized by surfactants in an aqueous phase, SLNs aim to combine the benefits of solid matrices, such as controlled release and drug protection, with the biocompatibility of lipids. Key advantages include biocompatibility, biodegradability, controlled/sustained drug release, protection of sensitive molecules, improved bioavailability for poorly water-soluble drugs, targeting potential, and ease of scale-up. However, limitations exist, such as limited drug loading capacity, particularly for water-soluble drugs, and potential drug expulsion during storage due to lipid crystallization. Preparation methods for SLNs include high-pressure homogenization, microemulsion technique, solvent-based methods, and phase inversion methods, with high-pressure homogenization being widely used for industrial scale-up. Characterization involves assessing particle size, polydispersity index, zeta potential, surface morphology, crystallinity, drug entrapment efficiency, and in vitro drug release. SLNs demonstrate diverse applications in oral, parenteral, dermal, ocular, pulmonary, and nasal drug delivery, as well as lymphatic targeting, gene delivery, and cosmetics. To address the limitations of SLNs, Nanostructured Lipid Carriers (NLCs) were developed, incorporating liquid lipids into the solid matrix to enhance drug loading and stability. Advancements continue with lipid-drug conjugates and polymer-lipid hybrid nanoparticles. Herbal plant-derived compounds like curcumin and neem oil have been successfully formulated into SLNs, showing improved delivery and therapeutic potential.

Keywords: Solid Lipid Nanoparticles (SLNs), Drug Delivery, Nanotechnology, Lipid-based Nanocarriers.

1. Introduction:

Over recent decades, the field of pharmaceutical nanotechnology has seen significant growth, driven by the goal of creating innovative drug delivery systems (DDS) to overcome the limitations of traditional dosage forms. Standard treatments often face challenges such as poor drug solubility, low absorption into the bloodstream, lack of specific targeting, rapid breakdown, and toxicity related to dosage. Nanoparticulate systems, typically sized between 1 and 1000 nanometers, offer unique physical and chemical properties that can help address these issues. Among the various nanocarriers investigated, including liposomes, polymeric nanoparticles, dendrimers, and micelles, lipid-based nanoparticles have attracted considerable interest due to their natural compatibility with biological systems and ability to degrade safely. (1)

Solid Lipid Nanoparticles (SLNs), first introduced in the early 1990s, represent a notable advancement in lipid-based drug delivery systems. They were developed as an alternative to conventional colloidal systems like emulsions (which tend to be unstable), liposomes (which have stability issues and poor encapsulation of fat-soluble drugs), and polymeric nanoparticles (some of which raise concerns regarding potential toxicity and lack of biodegradability). SLNs are submicron colloidal carriers, typically ranging from 50 to 1000 nm, where the liquid oil component of an oil-in-water emulsion is substituted with a solid lipid or a mixture of solid lipids. This means the core structure is solid at both room and body temperatures. (2) Drugs are typically dissolved or dispersed within this solid lipid core, which is stabilized by surfactants in an aqueous phase.

The main reason for developing SLNs was to combine the benefits of various traditional systems while minimizing their drawbacks. They leverage the advantages of solid matrices, such as controlled release and drug protection, with the biocompatibility of lipids.

Key advantages associated with SLNs include:

- **Biocompatibility and Biodegradability:** They are made from physiological lipids, which minimizes toxicity concerns.
- **Controlled/Sustained Release:** The solid matrix slows down drug diffusion, allowing for extended-release profiles. (3)
- **Drug Protection:** Encapsulating sensitive molecules within the solid lipid core shields them from chemical and enzymatic breakdown.
- **Improved Bioavailability:** Especially for drugs that don't dissolve well in water (BCS Class II/IV), SLNs can enhance oral absorption, potentially through lymphatic pathways and by increasing how quickly the drug dissolves due to their small size.
- **Targeting Potential:** The surface can be modified to allow for passive targeting (via the EPR effect) or active targeting to specific tissues or cells.
- **Ease of Scale-up and Sterilization:** Standard production methods like high-pressure

homogenization can be adapted for large-scale manufacturing, and SLNs can often withstand sterilization techniques such as autoclaving or gamma irradiation, depending on the lipid used.

- **Occlusive Properties:** This is beneficial for skin applications, improving skin hydration and drug penetration.

This chapter aims to provide a comprehensive overview of Solid Lipid Nanoparticles, covering their composition, various preparation techniques, essential methods for characterization and quality control, diverse therapeutic uses across different administration routes, and a critical discussion of their advantages and limitations. Furthermore, it will touch upon the progression towards next-generation lipid nanoparticles and outline future directions in this rapidly developing area.

2. Composition of Solid Lipid Nanoparticles

SLNs are typically formulated as dispersions in water. The main components are the solid lipid(s) that form the matrix and the surfactant(s) needed for stabilization.

2.1. Solid Lipids

The choice of lipid is crucial as it determines the nanoparticle's structure, how much drug it can hold, how the drug is released, and its stability. The lipids used must be solid at both room and body temperature. Ideally, they should be biodegradable, biocompatible, and generally recognized as safe (GRAS).

Commonly used solid lipids can be broadly categorized as:

- **Triglycerides:** These are compounds formed from glycerol and three fatty acids (e.g., tristearin, tripalmitin, trilaurin). Commercially available mixtures like the Dynasan® and Witepsol® series are widely used. Their composition affects their melting point and crystalline structure.
- **Partial Glycerides:** These include mono-, di-, or mixtures of glycerides (e.g., glyceryl monostearate (GMS), glyceryl behenate (Compritol® 888 ATO), glyceryl palmitostearate (Precirol® ATO 5)). These often have some surfactant-like properties themselves. (4) Compritol® 888 ATO is particularly popular due to its relatively high melting point (around 70°C) and good tolerability.
- **Fatty Acids:** Long-chain fatty acids such as stearic acid, palmitic acid, behenic acid, and decanoic acid have been used. Their properties depend significantly on their chain length and saturation.
- **Steroids:** Cholesterol and its esters can be included, though they are less commonly used as the primary matrix lipid.
- **Waxes:** Solid waxes like cetyl palmitate, carnauba wax, and beeswax can also form the SLN matrix. Cetyl palmitate is frequently used due to its defined melting point and

crystalline nature. (5)

The criteria for selection include:

- **Melting Point:** It should be high enough to remain solid at body temperature but suitable for the chosen preparation method (e.g., melt emulsification requires melting).
- **Crystallinity and Polymorphism:** Lipids can exist in different crystal forms. The more stable form has a highly ordered structure, which can lead to lower drug loading and potential drug expulsion if the crystal form changes during storage. Less ordered structures or using lipid blends can create imperfections, increasing the space for drug accommodation.
- **Drug Solubility/Miscibility:** The drug should dissolve or disperse well in the molten lipid to achieve sufficient loading.
- **Degradation Rate:** How easily the lipid is broken down by enzymes (like lipases) affects the rate at which the drug is released in the body. (6)

2.2. Surfactants (Emulsifiers/Stabilizers)

Surfactants are essential for reducing the surface tension between the melted lipid and the aqueous phase during production and for maintaining the physical stability of the nanoparticle dispersion by preventing clumping. They create a protective layer around the nanoparticles. The choice and amount of surfactant significantly influence particle size, surface charge (zeta potential), stability, drug loading, and how the nanoparticles behave in the body.

Common surfactants include:

- **Non-ionic Surfactants:** These are generally preferred because they are less toxic than ionic surfactants. Examples include:
  - Polysorbates (Tweens®): Tween® 80, Tween® 20.
  - Sorbitan Esters (Spans®): Span® 80, Span® 60 (often used with other surfactants).
  - Polyoxyethylene Stearates (Myrj® series).
  - Poloxamers (Pluronics®): E.g., Pluronic® F68, Pluronic® F127. These block copolymers can also provide steric stabilization.
  - Lecithins (Phospholipids): E.g., soy lecithin, egg lecithin. These are often used, especially in formulations given by injection, due to their excellent biocompatibility.
- **Ionic Surfactants:** These are used less frequently for delivery into the body's circulation due to a higher potential for irritation or toxicity, but they can be used for specific routes or to achieve a higher zeta potential for stability.
  - Anionic: Sodium dodecyl sulfate (SDS), sodium cholate, sodium glycocholate.
  - Cationic: Quaternary ammonium compounds like cetyltrimethylammonium bromide (CTAB), benzalkonium chloride. Cationic surfactants can help interact with negatively charged cell membranes or genetic material for gene delivery.

- **Zwitterionic Surfactants:** These are less common but include certain phospholipids like phosphatidylcholine.

Often, a combination of surfactants (e.g., a primary emulsifier like Tween® 80 and a co-surfactant/stabilizer like soy lecithin or Pluronic® F68) is used to achieve the best particle size reduction and long-term stability through both electrostatic and steric mechanisms. The Hydrophilic-Lipophilic Balance (HLB) value is an important factor when choosing surfactants for emulsification.

3. Preparation Methods for Solid Lipid Nanoparticles

Several techniques have been developed to produce SLNs, adapted from methods used to prepare emulsions. The chosen method depends on the properties of the drug and lipids, the desired particle characteristics, the required production scale, and the need to avoid potentially harmful organic solvents. (7)

3.1. High-Pressure Homogenization (HPH)

This is one of the most widely used methods and is suitable for industrial scale-up. It can be performed using either hot or cold homogenization.

- **Hot Homogenization:** The drug is dissolved or dispersed in the lipid melted at 5-10°C above its melting point. This lipid phase is then mixed into a hot aqueous surfactant solution at the same temperature using a high-shear mixer to form a coarse pre-emulsion. This pre-emulsion is then passed through a high-pressure homogenizer (typically 100-1500 bar, sometimes up to 2000 bar) for several cycles. The high shear stress and cavitation forces break down the droplets into the nanometer range. The resulting hot nanoemulsion is then cooled, causing the lipid to solidify and form SLNs. This method is suitable for drugs and lipids that are stable at high temperatures.
- **Cold Homogenization:** This method was developed to address issues with drugs that are sensitive to heat and the possibility of the drug moving into the water phase during hot homogenization. The drug-loaded melted lipid is rapidly cooled to solidify it. This solid lipid mass is then ground into microparticles (typically 50-100 µm). These lipid microparticles are dispersed in a cold aqueous surfactant solution and then passed through the high-pressure homogenizer at or below room temperature. The high pressure breaks down the microparticles into nanoparticles. This technique avoids heat stress but requires an extra grinding step and may be less effective at reducing particle size compared to the hot method.

3.2. Microemulsion Technique

This method involves creating thermodynamically stable, clear microemulsions. A warm microemulsion is formed by mixing the molten lipid (containing the drug), surfactant(s), possibly co-surfactant(s), and water with gentle stirring. This warm microemulsion is then dispersed into a large volume of cold water (typically 2-4°C) with mild agitation. The rapid drop in temperature causes the lipid to precipitate, forming SLNs, while the surfactants diffuse into the cold water, stabilizing the new nanoparticles. This method is relatively simple, avoids high shear, and can produce very small particles, but it often requires high concentrations of surfactants/co-surfactants and involves dilution, which might be a disadvantage.

3.3. Solvent-Based Methods

These methods involve dissolving the lipid and drug in an organic solvent, followed by emulsification and removal of the solvent.

- **Solvent Emulsification/Evaporation:** The lipid and drug are dissolved in an organic solvent that does not mix with water (e.g., dichloromethane, chloroform, cyclohexane). This organic phase is then emulsified in an aqueous surfactant solution using high-speed homogenization or sonication to form an oil-in-water emulsion. The organic solvent is then removed by evaporation under reduced pressure or by continuous stirring at room temperature, leading to the lipid solidifying and forming SLNs. Key challenges include ensuring complete solvent removal (due to potential toxicity) and possible drug leakage during emulsification.
- **Solvent Emulsification/Diffusion:** Similar to the above, but uses an organic solvent that mixes with water (e.g., ethanol, acetone). The drug and lipid are dissolved in the solvent, which is then added to an aqueous surfactant solution while stirring. The rapid diffusion of the solvent into the water causes the lipid to precipitate and form SLNs. This method avoids the need for evaporation but requires careful selection of a solvent that dissolves the lipid well but diffuses easily into water.
- **Solvent Injection:** The lipid and drug are dissolved in an organic solvent or solvent mixture that mixes with water (e.g., ethanol, acetone, isopropanol). This solution is then quickly injected through a fine needle into an aqueous surfactant solution with moderate stirring. The rapid dispersion and diffusion of the solvent cause the lipid to precipitate as nanoparticles. This method is simple and does not require high energy input, but it may result in larger or less uniformly sized particles.

3.4. Methods Based on Phase Inversion

- **Phase Inversion Temperature (PIT) Method:** This method is primarily used with non-ionic surfactants whose hydration changes significantly with temperature. A mixture of lipid, drug, surfactant, and water is heated. As the temperature rises, the surfactant becomes more soluble in the lipid, leading to a phase inversion from an oil-in-water to a water-in-oil emulsion. Rapid cooling then reverses this process, forming finely dispersed oil-in-water nano emulsions that solidify into SLNs.

3.5. Ultrasound/Sonication Method

High-power ultrasound can be used as an alternative or in addition to high-speed stirring or HPH for emulsification. The cavitation forces produced by ultrasound effectively reduce droplet size. It can be used in both hot and cold processes, but scaling up can be difficult, and potential degradation of components due to sonication needs to be assessed.

3.6. Spray Drying

While mainly a drying technique, spray drying can be adapted to produce SLNs. A hot nano emulsion (prepared by HPH or other methods) is sprayed into a stream of hot air. The rapid evaporation of water leads to the formation of solid lipid particles, often embedded within a carrier matrix if included, resulting in a dry powder suitable for reconstitution or direct use. Controlling particle size solely through spray drying can be challenging.

The choice of method depends significantly on the properties of the drug (solubility, stability), the characteristics of the lipid, the desired particle size range, and the production scale. HPH remains the most relevant method for industrial production due to its scalability and not requiring organic solvents in its hot or cold variations.

4. Characterization of Solid Lipid Nanoparticles

Thorough characterization is essential to ensure the quality, stability, effectiveness, and safety of SLN formulations. Key parameters include:

4.1. Particle Size and Polydispersity Index (PDI)

Particle size is a crucial factor influencing stability (clumping), how fast the drug is released, how well it's absorbed, how cells take it up, and where it goes in the body. PDI measures the uniformity of the particle size distribution; a value below 0.3 generally indicates a narrow, uniform population, which is desirable.

- **Methods:** Dynamic Light Scattering (DLS) is the most common technique. Laser Diffraction (LD) is suitable for larger particles or aggregates. Microscopic techniques (SEM, TEM) provide direct images but analyze a smaller sample size.

4.2. Zeta Potential (ZP)

ZP measures the magnitude of the electrical charge on the surface of the nanoparticle, indicating how much repulsion there is between nearby particles and predicting the physical stability of the colloidal dispersion against clumping. A minimum ZP of ±20 mV (for electrostatic stabilization) or ±30 mV is generally considered necessary for good stability, although stabilizers like PEGylated surfactants or Pluronics® can ensure stability even at lower ZP values.

- **Method:** Measured using Laser Doppler Velocimetry (LDV) or Electrophoretic Light Scattering (ELS), often part of DLS instruments.

4.3. Surface Morphology

Visualizing the shape and surface characteristics of SLNs.

- **Methods:** Scanning Electron Microscopy (SEM) provides information on the surface topography (requires drying the sample). Transmission Electron Microscopy (TEM) allows visualization of internal structure and shape (often requires staining or cryo-TEM for better contrast). Atomic Force Microscopy (AFM) can provide high-resolution 3D surface images.

4.4. Crystallinity and Polymorphism

The crystalline state of the lipid matrix affects how much drug can be loaded, whether the drug is expelled, and the release profile. Lipids can change their crystal form during production or storage.

- **Methods:** Differential Scanning Calorimetry (DSC) measures thermal transitions (melting point, enthalpy), indicating changes in crystallinity and interactions between the drug and lipid. A shift or broadening of the lipid melting peak in the SLN compared to the bulk lipid often suggests reduced crystallinity or amorphization, which can be beneficial for drug loading. X-ray Diffraction (XRD), including Small-Angle (SAXS) and Wide-Angle (WAXS), provides information on the crystal lattice structure and polymorphic form.

4.5. Drug Entrapment Efficiency (EE) and Drug Loading (DL)

These parameters quantify the amount of drug successfully incorporated into the nanoparticles.

- **EE (%) = (Total Drug - Free Drug) / Total Drug × 100**
- **DL (%) = (Total Drug - Free Drug) / Total Nanoparticle Weight × 100**
- **Method:** Requires separating the free drug from the SLNs. This is typically done by ultrafiltration, centrifugation, or dialysis. The amount of free drug or the drug within the SLNs is then measured using a suitable analytical method. EE and DL are strongly influenced by the drug's solubility in the melted lipid, the structure of the lipid matrix, and the preparation method.

4.6. In Vitro Drug Release

Studying the rate and mechanism of drug release from SLNs under simulated physiological conditions.

- **Methods:** The dialysis bag method is common, where the SLN dispersion is placed inside a dialysis membrane bag immersed in a release medium (e.g., phosphate buffer simulating body fluids), and the diffusion of the drug into the medium is monitored over time. Sample-and-separate methods or flow-through cell systems can also be used. Release profiles are affected by particle size, lipid matrix properties, where the drug is located within the SLN, and surfactant concentration.

4.7. Stability Studies

Assessing the physical and chemical stability of the SLN formulation under various storage conditions over time. Parameters typically monitored include particle size, PDI, ZP, EE, and visual appearance. Changes in crystal form that affect drug release should also be monitored. (8)

4.8. In Vivo Studies

Evaluating how the drug is absorbed, distributed, metabolized, and excreted (ADME), and its therapeutic effects in animal models after administration. Studies using labeled SLNs can track where they go in the body. Toxicity assessments are also crucial.

5. Applications of Solid Lipid Nanoparticles

SLNs have shown promise in delivering a wide variety of therapeutic agents through numerous routes.

5.1. Oral Drug Delivery

This is a major area of application, particularly for improving the absorption of drugs that don't dissolve well in water.

- **Mechanisms:** Enhanced dissolution due to small size, protection from the harsh conditions in the stomach and intestines, increased time spent in the GI tract, potential adherence to the gut lining, and importantly, possible absorption through the intestinal lymphatic system, bypassing the liver's first-pass metabolism.
- **Examples:** Delivery of cyclosporine A, coenzyme Q10, paclitaxel, amphotericin B, various antiretrovirals.

5.2. Parenteral Drug Delivery (Intravenous, Intramuscular, Subcutaneous)

SLNs offer advantages for intravenous delivery of poorly soluble drugs, providing sustained release and potentially reducing toxicity associated with direct injections or harsh solubilizers. Surface modification can extend circulation time and allow passive targeting to tumors via the Enhanced Permeability and Retention (EPR) effect.

- **Examples:** Delivery of anticancer drugs (doxorubicin, paclitaxel), antifungal agents, imaging agents.

5.3. Dermal and Transdermal Delivery

SLNs are attractive for topical application because the occlusive effect of lipids hydrates the skin's outer layer and can improve drug penetration. Their solid nature provides controlled release into the skin layers.

- **Benefits:** Improved penetration of active ingredients, reduced systemic absorption (for a local effect), protection of unstable actives, sustained release, skin hydration. (9)
- **Examples:** Delivery of anti-inflammatory drugs, sunscreens, retinoids, antifungal agents.

5.4. Ocular Drug Delivery

SLNs can improve how well drugs are absorbed in the eye by increasing the time they stay on the eye's surface and enhancing penetration into the cornea compared to conventional eye drops. They can provide sustained release, reducing the frequency of dosing.

- **Examples:** Delivery of pilocarpine (for glaucoma), cyclosporine A (for dry eye), flurbiprofen (anti-inflammatory). Cationic SLNs may show enhanced interaction with the cornea.

5.5. Pulmonary Drug Delivery

Delivery by inhalation allows direct access to the lungs for local treatment or systemic absorption. SLNs can be formulated into dry powders or nebulized dispersions suitable for inhalation, offering controlled release within the lungs.

- **Examples:** Delivery of corticosteroids, bronchodilators, antitubercular drugs.

5.6. Nasal Drug Delivery

The nasal cavity offers a route for rapid systemic absorption and direct delivery to the brain, bypassing the blood-brain barrier. SLNs that stick to the nasal lining can prolong contact time.

- **Examples:** Delivery of peptides/proteins, vaccines, drugs targeting central nervous system disorders.

5.7. Lymphatic Targeting

Oral administration of lipid nanoparticles can favor absorption into the lymphatic system, which is beneficial for bypassing the liver's first-pass metabolism and potentially targeting lymphatic tissues.

5.8. Gene Delivery

Cationic SLNs can bind and condense negatively charged nucleic acids, protecting them from degradation and facilitating cellular uptake for gene therapy.

5.9. Cosmetics

SLNs are used in cosmetic formulations to encapsulate and deliver active ingredients, improving their stability, penetration, and providing controlled release and skin hydration benefits.

6. Advantages and Disadvantages of SLNs

6.1. Advantages

- **Biocompatibility/Biodegradability:** Use of physiological lipids minimizes toxicity.
- **Controlled/Sustained Release:** The solid matrix allows for prolonged drug release.
- **Drug Protection:** Protects unstable drugs from degradation.
- **Improved Bioavailability:** Especially for fat-soluble drugs taken orally.
- **Targeting Possibilities:** Surface modification enables targeted delivery.

- **Scalability:** Methods like HPH are suitable for industrial production.
- **Sterilization Feasibility:** Can often be sterilized by autoclaving or other methods.
- **Occlusion:** Beneficial for dermal delivery.
- **Avoidance of Organic Solvents:** HPH methods are solvent-free.

6.2. Disadvantages

- **Limited Drug Loading:** Particularly for drugs that dissolve well in water. Drug solubility in the melted lipid is a key factor. Highly ordered crystalline structures provide less space for drug accommodation compared to less ordered structures.
- **Potential Drug Expulsion:** During storage, lipid crystallization and changes in crystal form can occur, leading to the expulsion of the incorporated drug, especially if the drug was located in areas with structural defects. This reduces entrapment efficiency and alters release profiles.
- **Particle Growth:** Potential for clumping during storage, although generally more stable than emulsions.
- **Water Content:** Aqueous dispersions have a high-water content, which might be limiting for certain applications. (10)
- **Complexity of Physical State:** The solid nature and potential for different crystal forms make characterization and predicting long-term behavior more complex than liquid systems.

7. Nanostructured Lipid Carriers (NLCs) and Other Advancements

To overcome the limitations of SLNs, especially low drug loading and drug expulsion, second-generation lipid nanoparticles called Nanostructured Lipid Carriers (NLCs) were developed. NLCs incorporate a mixture of solid lipid(s) and a liquid lipid within the solid matrix. This creates imperfections and a less ordered structure, offering more space to accommodate drug molecules, thereby increasing drug loading capacity and reducing drug expulsion during storage.

Three main types of NLC structures are proposed:

- **Imperfect Type:** Blending solid lipids with different chain lengths creates defects in the crystal structure.
- **Amorphous Type:** Mixing lipids (e.g., with specific surfactants) prevents crystallization, forming an amorphous matrix.
- **Multiple Type:** Small oil compartments are dispersed within the solid lipid matrix.

NLCs generally show higher drug loading and better long-term stability in retaining the drug compared to SLNs, while keeping the advantages of lipid nanoparticles.

Other advancements include:

- **Lipid-Drug Conjugates (LDCs):** Covalently linking the drug to a lipid, then formulating nanoparticles.

- **Polymer-Lipid Hybrid Nanoparticles:** Combining lipids with polymers to leverage the benefits of both material types.
- **Functionalized/Targeted SLNs/NLCs:** Surface modification with ligands for active targeting or with polymers like PEG for stealth properties.

9. Example of Some Herbal Plant Derived Solid Lipid Nanoparticles.

Curcumin-loaded solid lipid nanoparticles represent a promising drug delivery system that utilizes solid lipids as a matrix to encapsulate the herbal active compound. These nanoparticles are typically in the size range of 50 to 1000 nm and remain solid at both room and body temperatures. The solid lipid matrix offers advantages such as improved stability of the encapsulated compound, controlled release kinetics, and enhanced cellular uptake compared to free curcumin. Curcumin-loaded SLNs prepared using a high-pressure homogenization method with cholesterol as the lipid carrier and Tween 80 as a surfactant. The optimized SLNs exhibited a particle size suitable for drug delivery and demonstrated a sustained release profile for curcumin over 48 hours. Morphological analysis confirmed the spherical shape of the nanoparticles. This research highlights the potential of formulating herbal compounds like curcumin into solid lipid nanoparticles to overcome their inherent limitations and improve their therapeutic efficacy. (11)

Neem oil contains various bioactive compounds known for their therapeutic properties, including potential antibacterial and anti-inflammatory effects. However, the direct application or formulation of Neem oil can be challenging due to its lipophilic nature and potential for degradation. Solid lipid nanoparticles offer a colloidal drug delivery system composed of a solid lipid matrix that can effectively entrap lipophilic substances like Neem oil. Formulation of Neem oil-loaded solid lipid nanoparticles using methods such as double emulsification. These nanoparticles are characterized by their small particle size, which enhances skin penetration for topical applications, and high encapsulation efficiency, ensuring a significant amount of Neem oil is loaded. The solid matrix of the SLNs also contributes to the sustained release of the encapsulated Neem oil, potentially prolonging its therapeutic effect. Such formulations hold promise for improving the delivery and efficacy of Neem oil for various biomedical applications, such as the treatment of acne. (12)

SLNs and their successors, NLCs, continue to be an exciting area of research with considerable potential to improve therapeutic outcomes of herbal plant by optimizing drug delivery, enhancing efficacy, and reducing side effects across a wide range of medical and cosmetic applications. Continued innovation in material science, formulation design, and targeted delivery strategies will further unlock the potential of these lipid-based nano systems.

References:

1. Bunjes, H. (2010). Lipid nanoparticles: formulation, characterization, and applications. *Advances in Polymer Science*, 233, 1-36.
2. Cavalli, R., Gasco, M. R., Chetoni, P., Burgalassi, S., & Saettone, M. F. (1995). Solid lipid nanoparticles (SLN) as ocular delivery system for tobramycin. *International Journal of Pharmaceutics*, 117(2), 243-245.
3. Mehnert, W., & Mader, K. (2001). Solid lipid nanoparticles: Production, characterization and applications. *Advanced Drug Delivery Reviews*, 47(2-3), 165-196.
4. Mehnert, W., & Mader, K. (2012). Solid lipid nanoparticles: concepts, procedures, and formulation aspects for drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, 82(2), 245-261.
5. Muller, R. H., Mader, K., & Gohla, S. (2000). Solid lipid nanoparticles (SLN) for controlled drug delivery–a review of the state of the art. *European Journal of Pharmaceutics and Biopharmaceutics*, 50(1), 161-177.
6. Müller, R. H., Radtke, M., & Wissing, S. A. (2002). Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Advanced Drug Delivery Reviews*, 54 (Suppl 1), S131-S155.
7. Pardeike, J., Hommoss, A., & Müller, R. H. (2009). Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. *International Journal of Pharmaceutics*,366(1-2), 170-184.
8. Souto, E. B., & Muller, R. H. (2008). SLN and NLC for topical delivery of ketoconazole. *Journal of Microencapsulation*, 25(1), 51-59.
9. Wissing, S. A., Kayser, O., & Muller, R. H. (2004). Solid lipid nanoparticles for parenteral drug delivery. *Advanced Drug Delivery Reviews*, 56(9), 1257-1272.
10. Zur Muhlen, A., Schwarz, C., & Mehnert, W. (1998). Solid lipid nanoparticles (SLN) for controlled drug delivery – drug release and release mechanism. *European Journal of Pharmaceutics and Biopharmaceutics*, 45(2), 149-155.
11. Naseri N, Valizadeh H, Zakeri-Milani P. Sustained release curcumin loaded Solid Lipid Nanoparticles. DARU J Pharm Sci. 2015;23(1):56.
12. Adel MM, Abd El-Naby SSI, Abdel-Rheim KHM, Salem N. Formulation and characterization of solid lipid nanoparticles loaded Neem oil for topical treatment of acne. J Chem. 2022;65(12):59-71.

ETHNOMEDICINAL PLANT FOR TREATMENT OF JAUNDICE

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Abstract:

Medicinal plants are regarded as the gift of nature to humans. Various parts of medicinal plants, including herbs, shrubs, and trees, are used for curing jaundice and diseases like neurodegenerative, inflammatory, anthelmintic, diaphoretic, diuretic, etc. According to WHO (World Health Organization), "medicinal plant is a plant, within which one or more of its parts contains the substances, which can be further used for various therapeutic purposes, and serves as a precursor for chemo-pharmaceutical semi-synthesis. The current study may be useful for the development of new medicines for the treatment of jaundice. The study carried out in Junnar and Ambegaon taluka during 2023 to 2025. Jaundice is one of the very common diseases affecting general people of world. Traditionally used medicinal plants are found to be very much effective for the treatment of various types of diseases including Jaundice. This article describes various ethnomedicinal plant species used to cure jaundice by the people of Junnar and Ambegaon. Information of plant species, plant parts used, local name and mode of utilization of the plant parts which are traditionally used against jaundice was recorded. The main objective of the present study was to investigate the medicinal plants used for the management of jaundice. The common people of both tehsils are mostly dependent on herbal medicines for the management of jaundice.

Jaundice is a life-threatening disease affects human liver, the symptoms manifested in the form of extreme weakness, constipation, nausea, yellowing of the eyes, tongue, skin and urine; dull ache in the liver region of stomach and possible itching all over the body. The yellow colour first appears in the cornea of eyes and then spreads to the whole skin. The excess of bile pigments circulating in the blood give yellow colour to the eye and skin. There are several conditions that may interrupt the elimination of bilirubin from the blood and cause jaundice. The present work is focused on medicinal plants used to treat jaundice and hepatitis. The most frequent used plant parts are fruits (37%); leaves (28%); roots (14%); Bark and whole plant (11%). Part of the plant used, dosage, duration, restriction on intake of food etc. has been reported.

Keywords: Jaundice, Medicinal plant, Maharshtra, Junnar, Ambegaon.

Introduction:

Plant Based Remedies for Jaundice

From literature survey of medicinal plant of western ghat of India it has been observed that many plants are used for the remedial purpose of jaundice directly or indirectly. Out of these some plants which have been enlisted based on personal interview, questionnaire and reports of medicinal claims of these areas are presented in Table 1. However, most plants have similar mode of administration but having different results and dosing regimen. It has been observed that all plants are not 100% efficient in curing but are having one or other way of action towards liver improvement. jaundice is one of the most common medical condition affecting infants, children, and adults. Jaundice is not a disease, but it is a visible sign and symptom of liver disease which occurs when there is an increase in the amount of bilirubin circulating in the blood due to the abnormal metabolism and excretion in the urine. Medicinal plants are being used long ago by our ancestors for the treatment of jaundice. At the present era, consumption of these herbal medicines is increasing at a high speed, due to its less or no side effect and cost-effectiveness as compared to synthetic medicines. The data was collected from different kinds of literature survey from the year 1993 to 2025. This review has been conferred in a systematic way which includes plant's vernacular name, botanical name along with its family, part used and ethnomedicinal uses in a tabulated form and the description of scientific evidence behind the folklore uses of some medicinal plants along with their mechanism of action which have been reported in different kinds of literature.

Further investigations are required to explore the biological activity of different active ingredients of traditionally used medicinal plants.

According to the pathophysiology of jaundice, it is mainly caused due to increased level of bilirubin and its overproduction in the liver, which may occur due to many reasons like acute or minor liver inflammation, obstruction of the bile duct, Gilbert’s syndrome, cholestasis, and haemolytic anaemia. Jaundice is usually found much more effective and serious in adults rather than in new-born children (neonates), and sometimes it causes even death of the adult individual. Jaundice shows three different stages or types based on its pathophysiology:

Prehepatic Jaundice

That is caused due to the haemolysis of red blood cells, also called erythrocytes. Hepatic jaundice occurs due to the abnormal metabolism of the liver or dysfunction of the liver, and post-hepatic jaundice is caused due to less liver functioning or any obstruction in the bile duct; which can spread through contaminated water and food-related items or due to poor sanitization conditions or through several other diseases, such as hepatitis A, hepatitis B, hepatitis C, hepatitis D, liver cancer etc. which damage liver. It has been estimated that more than two billion people worldwide are infected annually with the hepatitis B virus. The history of jaundice is very long and shown in ancient Ayurveda and the Indian traditional system of medicines. Jaundice is also known as Hariman disease in Rigveda (8000 BC). Herbal treatment is prescribed for jaundice because medicinal plants show a faster rate of reduction in cases when compared with western medicines. This disease shows different kinds of symptoms like weakness, high fever, nausea, loss of appetite, vomiting, and the main symptom shown by this disease is the dark urine colour. Sometimes, it also leads to serious conditions like coma, a sudden attack of illness or epileptic fits, psychosis (like having a severe mental disorder), and finally, death of the patient. Precautions or prevention for jaundice generally requires a low-fat diet, high water intake as much body requires, and mainly a healthy diet routine and proper nutrition. The medicinal property of plants is mainly because of the formation or stimulation of various chemical compounds that occur naturally in the plants and hence used to cure jaundice and various other diseases. So, this review describes various phytochemical constituents present in the ethnomedicinal plants used by people of Himachal Pradesh for curing jaundice, and the data is taken from different sources. The plants are further used for the development of antimicrobial and antioxidant drugs thus proving their medicinal worth. Plant like *Justicia adhatoda* consists of various organic and bioactive compounds which possess numerous biological activities, such as antitussive, abortifacient, cardiovascular protection, anti-inflammatory, and antimicrobial. *Berberi's lyceum* plant shows the presence of tannins, terpenoids, fats, resins, and many active alkaloids. The roots are the foremost important part of the Berberis species as they contain a variety of alkaloids, and the most prominent one is berberine. It has been found that the inhibitory activity of *Berberis lyceum* is shown by the components present in its root extract. Like this, many other plants are used in the treatment of jaundice, possessing various phytochemical constituents the word jaundice comes from the French word jaune, which means yellow. When the yellow Jaundice is one of the most common diseases affecting a large portion of citizens throughout the whole world. It is very much common disease especially in the developing countries. Jaundice is some sign and symptoms which have occurred due to various other diseases. During Jaundice the concentration of bilirubin in the blood becomes very high which may be due to haemolysis[1]. Yellowish eyes and vomiting with yellowish fluid are commonly found to be the initial symptoms of Jaundice. Bilirubin is biosynthesized in the body. Liver cells produce bilirubin through a process called hemolysis. Depending on the bilirubin concentration the skin colours are different. Jaundice is also very much common among newborn babies. The immature liver of newborn babies cannot control the amount of bilirubin like an adult.

Medicinal plants are found to have great role in the healthcare systems. Human beings are depended on nature for different purposes throughout the ages. In the development of human society plants have played an important role. Mainly there are two types of use of medicinal plants; those are used for traditional herbal folk medicines by local physicians, and plants can be used by various pharmaceutical industries for the development of modern medicines from their active ingredients. Plants have potential use as traditional medicine. Many important drugs, used in modern medicinal practice, have been isolated or derived from medicinal plants. Herbal plants have a great role around traditional healing systems. Plants have been found to be the source of many traditional medicine systems throughout the whole world[1]. In Maharashtra many people depend on herbal folk medicines to fight against jaundice. The present investigation was carried out in some of the Talukas of Pune District. The aim to document the information of traditionally used medicinal plant resources used for the treatment of jaundice.

Herbal medicines have been used for the treatment and management of jaundice and currently these are considered as an alternative therapy for jaundice treatment. Plants have always been considered as important source of drugs. Medicinal plants have yielded directly or indirectly many important medicines in the past. Junnar And Ambegaon is found to be a land of very rich biodiversity. Different plants commonly used in jaundice treatment are reported to be found in Pune District state Maharshtra. Many of these medicinal plants have not yet been completely explored experimentally.

Some Remedies.

1. ***Aloe vera*****:** This Ayurvedic herb is a gentle cleanser that detoxifies the kidneys and liver of the toxins accumulated in the system. It improves the overall digestive functions of the body and is a great tonic for the digestive system. It can be consumed in the form of juice, or its pulp can be taken with water.
2. **Bhuiavala (*Phyllanthus niruri*):** A comprehensive liver tonic, bhumyalaki is effective in all kinds of liver disorders. It gives relief in case of all the symptoms of jaundice. Being anti-inflammatory, anti-bacterial, and anti-spasmodic, it cures the liver of infections and treats the dysfunction.
3. **Yakrit Plihantak Churn:** A mixture of beneficial herbs such as kalmegh, katuki, bhringaraj, Bhumi amla and others, this churn is a complete medicine for liver disorders. It reduces inflammation and flushes out the toxins accumulated in the body. It also restores the health of the liver by boosting the regeneration of liver cells.
4. **Changeri (Indian Sorrel):** This is helpful in curing symptoms of low appetite and poor digestion. It stimulates digestion and helps in proper bowel movement.
5. **Chichinda (Snake Gourd):** Effective in detoxification and improving the digestive system, chichinda is a rich source of nutrients needed by the human body. It boosts the immune system and removes worms from the intestines. A natural diuretic, it increases urine secretion, thereby, helping the body get rid of toxins. It can be consumed in the form of juice or taken in mashed form after boiling.

6. **Karela (bitter gourd):** A natural cleanser, karela improves the filtration functions of the liver. Being an antioxidant, it also restores the immunity of the body, thereby, helping it recovers fast. Ayurveda believes that jaundice or pilia is caused by pitta dosha in the human body and all these remedies work to correct this dosha. Along with these natural remedies, Ayurveda also prescribes plenty of water intake and fluids and abstinence from oily and spicy food as they are known to aggravate the pitta dosha. So, one should follow these instructions and also, take the advice of an Ayurvedic practitioner before taking these remedies In relation to the condition of the patients these preparations are used once or twice daily for a week to months till the problem is cured.

Table 1: Plants used for the management and cure of jaundice by the local communities in the study areas.

Sl. No.	Botanical Name & Family	Local Name	Habit	Common Name	Part Used	Mode of Utilization of the Herbal Medicines
1	*Aloe barbadensis* (Asphodelacae)	Korfad	Shrub	Barbados aloe	Leaf	Leaves paste is used
2	*Andrographis paniculata* (Acanthaceae)	Kalmegh	Herb	King of Bitters	Whole plant	Aqueous extracts of the whole plant at a dose of 2-3 tea spoon in empty stomach
3	*Argemone mexican* (Papavaraceae)	Ghaypat	Herb	Prickly poppy	Roots, seeds	Seed and root powder is taken with water
4	*Ananus comosus* (Bromeliaceae)	Ananas	Herb	Pineapple	Leaf	Leaves juice is taken
5	*Averrhoa carambola* (Oxalidaceae)	Kardoi	Tree	Carambola	Fruit	Ripe fruits are eaten
6	*Baliospermum solanifolium* (Euphorbiaceae))	Donti, Phuljelon	Shrub	Red nut, Wild castor	Root	The root powder is used
7	*Bryophyllum pinnatum* (Crassulaceae)	Panfuti	Herb	Air plant	Leaf	Water extract of fresh leaf is given orally

8	*Boerhaavia diffusa* (Nyctaginaceae)	Punarnava	Herb	Nyctaginaceae	Whole plant	A decoction of the plant is consumed in empty stomach
9	*Cajanus cajan* (Fabaceae)	Rahar-mah	Shrub	Pigeon pea	Leaf	A decoction of leaves is used
10	*Costus speciosus* (Zingiberaceae)	Insulin plant	Herb	Crapeginger	Leaf	Leaf consumed raw
11	*Carica papaya* (Caricaceae)	Papai	Woody herb	Pepita	Fruit	Ripe fruits are eaten
12	*Curculigo orchioides* (Amaryllidaceae)	Kali Musali	herbs	Black Musali	Rhizome	Rhizome paste taken orally
13	Cyperus rotundus (Cyperaceae)	Lavala	Herb	Coco-grass	Whole plant	Plant juice is taken orally
14	*Cynodon dactylon* (Poaceae)	Harali	Herb	Green couch grass	Whole plant	Decoction are used
15	*Centella asiatica* (Apiaceae)	Bor-manimoni	Herb	Indian pennwort	Whole plant	Boiled plant parts are eaten
16	*Eclipta alba* (Compositae)	Maka	Herb	False daisy	Leaf, root	Leaf, root juice taken orally
17	*Euphorbia nerifolia* (Euphorbiaceae)	Dudhani	Shrub	Common milk hedge	Leaf	Young Leaf juice
18	*Ficus religiosa* (Moraceae)	Pimpal	Tree	Peepal tree	Stem bark	Powder of stem bark is taken with water
19	*Glycosmis pentaphylla* (Rutaceae)	Chauldhowa	Shrub	Orange berry	Leaf	Leaf juice with cow's milk taken orally
20	*Hemidesmus indicus* (Asclepiadaceae)	Anantmool	Shrub	Indian Sarsaparilla	Root	Juice of fresh roots were used orally

21	*Houttuynia cordata* (Saururaceae)	Mesondori	Herb	Lizard tail	Whole plant	Boiled extract of the plant
22	*Morus alba* (Moraceae)	Tuti	Tree	White mulberry	Fruit	Fruit juice is used
23	*Mentha arvensis* (Lamiaceae)	Pudina	Herb	Field mint	Whole plant	Used as vegetable
24	*Moringa oleifera* (Moringaceae)	Shevaga	Tree	Drumstick tree	Leaf, flower, fruit	Fresh plant parts are used as vegetable
25	*Oxalis corymbosa* (Oxalidaceae)	Bor-tengechi	Herb	Wood-sorrels	Whole plant	Boiled extract are used
26	*Oroxylum indicum vent* (Bignoniaceae)	Bhatghilla	Tree	Broken Bones Tree	Bark	Dried bark powder taken with honey.
27	*Punica gratum* (Punicaceae)	Dalimb	Shrub	pomegranate	Fruit	Ripe fruits are eaten
28	*Piper nigrum* (Piperceae)	Kali miri	Climbing vine	Black peper	Fruit	Boiled extract is consumed orally
29	*Terminalia citrine* (Combretaceae)	Bibava	Tree	Black chuglam	Fruit	Fresh or dry fruit extracts
30	*Tinospora cordifolia* (Menispermaceae)	Gulvel	Climber	Gulancha tinospora	Stem bark, leaf	Boiled extract of stem bark and leaf are taken

Conclusion:

Ethnomedicinal knowledge is respected by rural people and has been shown to be useful in the treatment of various diseases and the production of medicines in the Western Himalaya from time to time. Traditional or folk-based plant medicines have shown great potential to form the basis of jaundice-curing drugs. The purpose of the present study was to record the ethnomedicinal knowledge of plants used for the treatment of jaundice by the rural and tribal communities of Himachal Pradesh in western Himalaya. The other aims of this research were to discuss the different important phytochemicals, and active compounds present in these plants and to discuss the different in vivo studies performed in support of their medicinal uses, with specific reference to the treatment of jaundice. The outcome of this research showed that the rural people of Junnar and Ambegaon used different plant species to treat jaundice and contribute to healthcare. These plants demonstrated the presence of several phytochemicals in them and displayed phenolic and flavonoid compounds with hepatoprotective properties in most of the experimental studies (in vivo) performed with these plants. With antioxidant potential, the phenolic and flavonoid compounds are recognized, and due to this property, these plants have been shown to be important in curing jaundice. *Aloe vera, Bauhinia variegata, Berberis aristata, Emblica officinalis, and Terminalia chebula* are some of these herbs, which suggest the ethnopharmacological approach to treating jaundice with the hepatoprotective operation. There is a lot of knowledge in the latest literature on the use of various plants for treating jaundice. Nevertheless, very few studies are carried out on the scientific validation of medicinal plants by means of biochemical, clinical, and pharmacological screening to validate the jaundice healing folklore medicine. In the future, it is, therefore, very important to pursue steps that do not deviate from shifting the view of tribal people toward their indigenous belief in the treatment of jaundice to develop successful drugs or to discover new potential sources of drugs. In addition, nano-formulation of plant extracts also improves their therapeutic significance, and it is also possible to use nano-formulation of herbal plants as an alternative and refining conventional knowledge for the potential cure of jaundice.

Referrances:

1. Roy, A., Bhoumik, D., Sahu, R. K., & Dwivedi, J. (2014). Medicinal plants used in liver protection: A review. *UK Journal of Pharmaceutical and Biosciences*, 2, 23–33.
2. Verma, R. (2018). A review on hepatoprotective activity of medicinal plants. *Journal of Medicinal Plants Studies*, 6, 188–190.
3. Pandit, A., Sachdeva, T., & Bafna, P. (2012). Drug-induced hepatotoxicity: A review. *Journal of Applied Pharmaceutical Science*, 2, 233–243.
4. Muhammad, W. A., Shamshad, T., Muhammad, A. A., & Rukhsar, J. (2016). Jaundice: A basic review. *International Journal of Research in Medical Sciences*, 4, 1313–1319.

5. Tewari, D., Mocan, A., Parvanov, E. D., Sah, A. N., Nabavi, S. M., Huminiecki, L., et al. (2017). Ethnopharmacological approaches for therapy of jaundice: Part I. *Frontiers in Pharmacology*, 8, 1–18.
6. Jose, J. K., & Kuttan, R. (2000). Hepatoprotective activity of *Emblica officinalis* and Chyavanaprash. *Journal of Ethnopharmacology*, 72, 135–140.
7. Senthil, K. K., & Senthil, K. K. L. (2010). Hepatoprotective effects of 50% ethanolic extract of *Mimosa pudica* against CCl4 induced hepatotoxicity in rats. *Pharma Letters*, 2, 261–264.
8. Purkayastha, A., Chakravarty, P., & Dewan, B. (2016). Evaluation of hepatoprotective activity of the ethanolic extract of leaves of *Mimosa pudica* Linn. in carbon tetrachloride induced hepatic injury in albino rats. *International Journal of Basic and Clinical Pharmacology*, 5, 496–501.
9. Suneetha, B., Pavan, K. P., Prasad, K. V. S. R. G., Vidyadhara, S., & Rao, K. R. S. S. (2011). Hepatoprotective and antioxidant activities of methanolic extract of *Mimosa pudica* roots against carbon tetrachloride-induced hepatotoxicity in albino rats. *International Journal of Pharmacy*, 1, 46–53.
10. Sattar, M. A., Iqbal, M. M., Asad, B. S., & Ibrahim, M. (2015). Pharmacological evaluation of *Pamarus* seeds and *Leucas aspera* leaves for its hepatoprotective and nephroprotective activities. *Research Journal of Pharmacy and Biological and Chemical Sciences*, 6, 1017–1025.
11. Pramyothin, P., Ngamtin, C., Poungshompoo, S., & Chaichantipyuth, C. (2007). Hepatoprotective activity of *Phyllanthus amarus* Schum & Thonn. extract in ethanol-treated rats: In vitro and in vivo studies. *Journal of Ethnopharmacology*, 114, 169–173.
12. Harish, R., & Shivanandappa, T. (2006). Antioxidant activity and hepatoprotective potential of *Phyllanthus niruri*. *Food Chemistry*, 95, 180–185.
13. Ramamurthy, V., & Abarna. (2015). Evaluation of ethanolic leaf extract of *Phyllanthus niruri* and its effect on carbon tetrachloride intoxicated hepatotoxicity in albino rats. *International Journal of Innovative Research in Science, Engineering and Technology*, 4, 6255–6261.
14. Chatterjee, M., & Sil, P. C. (2006). Hepatoprotective effect of aqueous extract of *Phyllanthus niruri* on Nimesulide-induced oxidative stress in vivo. *Indian Journal of Biochemistry & Biophysics*, 43, 299–305.
15. Singh, B., Saxena, A. K., Chandan, B. K., Agarwal, S. G., Bhatia, M. S., & Anand, K. K. (2001). In vivo hepatoprotective activity of active fraction from ethanolic extract of *Eclipta alba* leaves. *Indian Journal of Physiology and Pharmacology*, 45, 435–441.

16. Krishna, M. G., Pallavi, E., Ravi, K. B., Ramesh, M., & Venkatesh, S. (2007). Hepatoprotective activity of *Ficus carica* Linn. leaf extract against carbon tetrachloride-induced hepatotoxicity in rats. *Daru*, 15, 162–166.
17. Ashaa, S., Tajub, G., & Jayanthi, M. (2011). Study of hepatoprotective effect of *Hemidesmus indicus* on paracetamol induced liver damage in rats. *Journal of Pharmacy Research*, 4, 624–626.
18. Baheti, J. R., Goyal, R. K., & Shah, G. B. (2006). Hepatoprotective activity of *Hemidesmus indicus* R.Br. in rats. *Indian Journal of Experimental Biology*, 44, 399–402.
19. Murali, A., Ashok, P., & Madhavan, V. (2012). Protective effects of *Hemidesmus indicus* var. *pubescens* root extract on paracetamol induced hepatic damage. *Spatula DD*, 2, 51–58.
20. Ravishah, S., Manjula, S. N., Mruthunjaya, K., Krishnanand, P., Chakravarthy, P. K. N., Madhu, R. M., et al. (2012). Hepatoprotective activity of roots of *Lawsonia inermis* against paracetamol and anti-tubercular drugs induced hepatotoxicity in rats. *International Journal of Pharmacy*, 2, 306–316.
21. Mohamed, M. A., Imad, M. T. E., Abd, E. H. M., & Hozeifa, M. H. (2016). Effects of *Lawsonia inermis* L. (Henna) leaves' methanolic extract on carbon tetrachloride-induced hepatotoxicity in rats. *Journal of Intercultural Ethnopharmacology*, 5, 22–26.
22. Hossain, C. M., Maji, H. S., & Chakraborty, P. (2011). Hepatoprotective activity of *Lawsonia inermis* Linn, warm aqueous extract in carbon tetrachloride induced hepatic injury in Wistar rats. *Asian Journal of Pharmaceutical and Clinical Research, 4*, 106–109.
23. Kumar, M., Kaur, P., Chandel, M., Singh, A. P., Jain, A., Kaur, S., Singh, B., Saxena, A. K., Chandan, B. K., Agarwal, S. G., Bhatia, M. S., & Anand, K. K. (1993). Hepatoprotective effect of ethanolic extract of *Eclipta alba* on experimental liver damage in rats and mice. *Phytotherapy Research, 7*, 154–158.
24. Singh, B., Saxena, A. K., Chandan, B. K., Agarwal, S. G., Bhatia, M. S., & Anand, K. K. (2001). In vivo hepatoprotective activity of active fraction from ethanolic extract of *Eclipta alba* leaves. *Indian Journal of Physiology and Pharmacology, 45*, 435–441.
25. Krishna, M. G., Pallavi, E., Ravi, K. B., Ramesh, M., & Venkatesh, S. (2007). Hepatoprotective activity of *Ficus carica* Linn. leaf extract against carbon tetrachloride-induced hepatotoxicity in rats. *Daru, 15*, 162–166.
26. Ashaa, S., Tajub, G., & Jayanthi, M. (2011). Study of hepatoprotective effect of *Hemidesmus indicus* on paracetamol induced liver damage in rats. *Journal of Pharmacy Research, 4*, 624–626.
27. Baheti, J. R., Goyal, R. K., & Shah, G. B. (2006). Hepatoprotective activity of *Hemidesmus indicus* R.Br. in rats. *Indian Journal of Experimental Biology, 44*, 399–402.
28. Murali, A., Ashok, P., & Madhavan, V. (2012). Protective effects of *Hemidesmus indicus* var. pubescens root extract on paracetamol induced hepatic damage. *Spatula DD, 2*, 51–58.

29. Ravishah, S., Manjula, S. N., Mruthunjaya, K., Krishnanand, P., Chakravarthy, P. K. N., Madhu, R. M., et al. (2012). Hepatoprotective activity of roots of *Lawsonia inermis* against paracetamol and anti-tubercular drugs induced hepatotoxicity in rats. *International Journal of Pharmacy, 2*, 306–316.
30. Hossain, C. M., Maji, H. S., & Chakraborty, P. (2011). Hepatoprotective activity of *Lawsonia inermis* Linn, warm aqueous extract in carbon tetrachloride induced hepatic injury in Wistar rats. *Asian Journal of Pharmaceutical and Clinical Research, 4*, 106–109.
31. Mohan, V. R., & Janardhanan, K. (1995). Chemical analysis and nutritional assessment of lesser known pulses of the genus, *Mucuna*. *Food Chemistry, 52*(33), 275–280.
32. Rain-tree.com. (n.d.). Retrieved from http://www.rain-tree.com/abrus.htm#.V86nBvl9600
33. Sultana, N. (2000). *Phytochemical and structural studies on the chemical constituents of Saligna, Adhatoda vasica, Sarcococca and Skimmia laureola* (Doctoral dissertation, pp. 1–267).
34. Singh, P. T., Okram, M., & Singh, H. B. (2011). *Adhatoda vasica* Nees: Phytochemical and pharmacological profile. *The Natural Products Journal, 1*, 29–39.
35. Pankti, K., Payal, G., Manodeep, C., & Jagadish, K. (2012). A phytopharmacological review of *Alstonia scholaris*: A panoramic herbal medicine. *International Journal of Research in Ayurveda and Pharmacy, 3*(3), 367–371.
36. Kulyal, P., Tiwari, U. K., Shukla, A., & Gaur, A. K. (2010). Chemical constituents isolated from *Andrographis paniculata*. *Indian Journal of Chemistry, 49B*, 356–359.
37. Zhongguo, Z. Y. Z. (2006). Chemical constituents from roots of *Ardisia punctata*. *Journal of Chinese Medicine, 31*(7), 562–565.
38. Bose, B. C., Vijayvargiya, R., Saifi, A. Q., & Sharma, S. K. (1963). Chemical and pharmacological studies on *Argemone mexicana*. *Indian Journal of Medical Research, 52*(12), 1172–1175.
39. Iwashina, T., & Matsumoto, S. (2011). Flavonoid properties of six *Asplenium* species in Vanuatu and New Caledonia, and distribution of flavonoid and related compounds in *Asplenium*. *Bulletin of the National Museum of Natural Science, 37*(3), 133–139.
40. Wu, X. Y., Chao, Z. M., Wang, C., Tan, Z. G., & Sun, W. (2012). Chemical constituents contained in fatty oil from seeds of *Cucumis sativus*. *Zhonghua Zhong Yao Za Zhi, 37*(21), 3252–3256.
41. Irshad, S., Singh, J., Jain, S. P., & Khanuja, S. P. S. (2006). *Curculigo orchioides* Gaertn. (Kali Musali): An endangered medicinal plant of commercial value. *Natural Product Radiance, 5*, 373–376.
42. Bizuneh, A. (2012). Textile effluent treatment and decolorization techniques – a review. *Chemistry: Bulgarian Journal of Science Education, 21*(3), 434–456.

43. Leela, N. K., Tava, A., Shafi, P. M., John, S. P., & Chempakam, B. (2002). Chemical composition of essential oils of turmeric (*Curcuma longa* L.). *Acta Pharmaceutica, 52*, 137–142.
44. Richard, L., Prabhu, K. S., Shirwaikar, A., & Shirwaikar, A. (2009). *Curcuma zedoaria* Rosc. (White turmeric): A review of its chemical, pharmacological and ethnomedicinal properties. *Journal of Pharmacy and Pharmacology, 61*, 13–21.
45. Bharathi, R., Ravi Shankar, K., & Geetha, K. (2014). In vitro antioxidant activity and in vivo hepatoprotective activity of ethanolic whole plant extracts of *Nymphoides hydrophylla* in CCl4 induced liver damage in albino rats. *International Journal of Research in Ayurveda and Pharmacy, 5*(6), 667–672.
46. Madhavan, V., Arora, S., Murali, A., & Yoganarasimhan, S. N. (2009). Anti-convulsant activity of aqueous and alcohol extracts of roots and rhizomes of *Nymphoides indica* (L.) Kuntze in Swiss albino mice. *Journal of Natural Remedies, 9*, 68–73.
47. Harminder, Singh, V., & Chaudhary, A. K. (2011). A review on the taxonomy, ethnobotany, chemistry and pharmacology of *Oroxylum indicum* Vent. *Indian Journal of Pharmaceutical Sciences, 73*(5), 483–490.
48. Poornima, N., Uma Rajan, K. M., & Babu, K. (2009). Studies on anatomical and phytochemical analysis of *Oxystelma esculentum* (L.f.) R.br. ex Schltes. *Botany Research International, 2*(4), 239–243.
49. Chouhan, H. S., & Singh, S. K. (2011). A review of plants of genus *Leucas*. *Journal of Pharmacognosy and Phytotherapy, 3*(3), 13–26.
50. Barik, A., Bhattacharya, B., Laskar, S., & Banerjee, T. C. (2004). The determination of n-alkanes in the cuticular wax of leaves of *Ludwigia adscendens* L. *Phytochemical Analysis, 15*(2), 109–111.
51. Ali, A., Islam, M. T., Sultana, I., Mahmood, A., Hossain, J. A., Homa, Z., et al. (2012). Pharmacological and phytochemical screening of ethanol extract of *Litsea monopetala* (Roxb.) Pers. *IOSR Journal of Pharmacy, 2*(3), 398–403.
52. Mondal, A., Maity, T. K., Pal, D. K., & Santanu, S. (2010). HPTLC determination of phenolic compounds and antioxidant activity of the aerial parts of *Melothria heterophylla* (Lour.) Cogn. *International Journal of Drug Development & Research, 2*, 750–757.
53. Shodhganga. (n.d.). Retrieved from http://shodhganga.inflibnet.ac.in/bitstream/10603/29060/1/11_chapter2.pdf
54. Naeem, M., Tariq, A., Idrees, M., Singh, M., Akbar, A., Masroor, M., et al. (2016). Modulation of physiological activities, active constituents and essential oil production of *Mentha arvensis* L. by concomitant application of depolymerised carrageenan, triacontanol and 28-homobrassinolide. *Journal of Essential Oil Research*.

GREEN ALGORITHMS:
OPTIMIZING CODE FOR ENERGY-EFFICIENT COMPUTING

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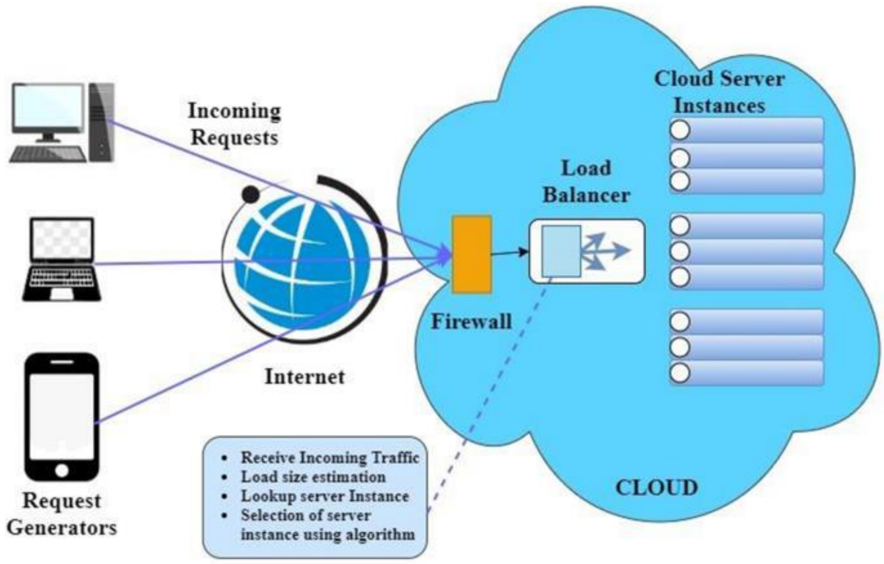
Introduction:

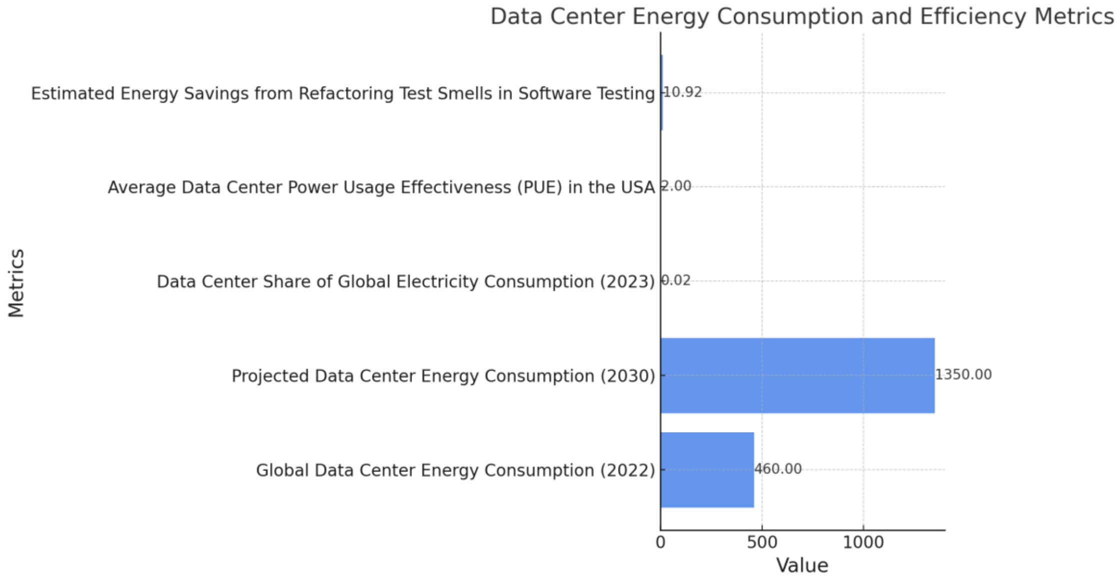
Our digital world keeps growing, and as it does, using energy wisely in computing is becoming a real necessity. Data keeps piling up and forces us to crank up computing power, which naturally eats up more energy and boosts our environmental footprint – data centers, in particular, end up being pretty big players in carbon emissions (Fern Gández *et al.*, 2016). This mix of needing top-notch performance while also being sustainable really pushes us toward creative fixes, like green algorithms that tweak our code just enough to cut down on energy use without messing things up. Cloud computing and big data have, in most cases, sped up this need, making us look twice at how power is managed in these systems (Mittal *et al.*, 2014). In a lot of situations, taking a broad, all-around approach is key – one that blends smarter software choices with better hardware tweaks. A schematic of the main parts in a Green Data Center show, quite clearly, that combining many ideas into one comprehensive strategy is essential for truly sustainable computing practices.

Overview of the Importance of Energy Efficiency in Computing

Computational power keeps climbing these days, and energy efficiency in computing has suddenly become a big deal for tech teams and companies alike. Data centers are growing just to keep up with our nonstop processing needs, and that means they're using more power than ever—something we really can't ignore. It's not just about cutting costs; reworking code to use less energy can help make our digital practices more sustainable across many fields. Developers, for example, often spot what some call Energy Code Smells—those clunky, inefficient coding patterns that waste energy—and by retooling them, they can boost overall energy performance without hurting how software runs or feels (Ardito *et al.*, 2013). In most cases, adopting smarter power management strategies in these centers goes a long way in shrinking their huge energy footprint and even trimming down greenhouse gas emissions (Mittal *et al.*, 2014). Ideas like switching to renewable energy sources, choosing more energy-efficient hardware, and rethinking cooling systems all add up. Embracing such energy-smart programming practices is key to setting up a greener and more sustainable computing scene. Overall, this mix of rapid tech growth and a rising call for environmental care shows just how much skilled professionals and fresh, innovative thinking matter in steering us toward an eco-friendlier future in computing.



Cloud Computing Architecture for Load Management



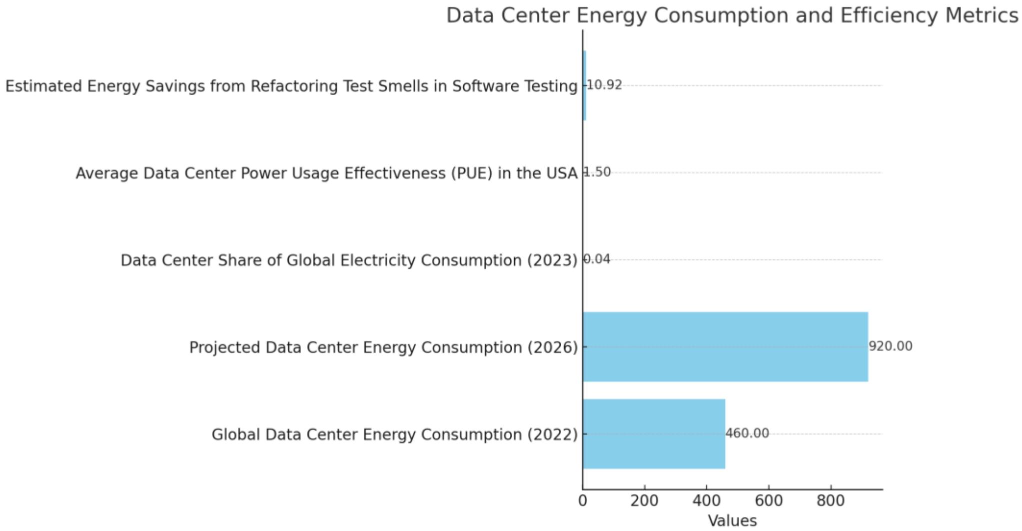
The bar chart presents data on various metrics related to data center energy consumption and efficiency. It highlights the significant increase in global data center energy consumption projected for 2030 compared to 2022, the minuscule share of total global electricity consumption in 2023, the average power usage effectiveness in the USA, and estimated energy savings that could be achieved through software optimization practices. This information emphasizes the urgent need to address the growing energy demands of data centers.

Understanding Green Algorithms

Algorithm efficiency is grabbing attention nowadays because our tech isn't just about raw speed—it's increasingly about keeping our planet in check. Energy use keeps climbing, making it almost mandatory to rework our algorithms so they're greener and kinder to the environment. Clever programming tricks can retool these routines to sip resources rather than gulp them, without sacrificing performance. "Energy has become a leading design constraint for computing devices," many experts note, as hardware engineers and system designers are constantly hunting for fresh ways to cut down energy consumption. In most cases, mixing smart efficiency with sustainable practices isn't only about easing climate concerns—it actually boosts how well our systems run. Take data centers, for instance; when they adopt these eco-friendly tweaks, as illustrated in, they show just how much looser resource management can slash energy use and trim waste.

Definition and Principles of Green Algorithms in Software Development

Energy-conscious software begins with ideas from green algorithms, which essentially help cut down energy use while running computer tasks. Instead of just tweaking code for speed, these methods also mix in sustainable practices for building software. Sometimes, they borrow from nature—ways of thinking like those found in Digital Ecosystems—to allow real-time adjustments based on what users need and what the environment is throwing at us (Briscoe *et al.*, 2011). It's also interesting to note that green algorithms often lean on market-based resource management ideas, making sure that computing power is spread out in a smart way (C Vecchiola *et al.*, 2009). Generally speaking, this broad view equips developers not only to produce applications that work well but also to take care of our surroundings. Plus, a snapshot of a Green Data Center—highlighting things like resource optimization and monitoring systems—can really clarify how these ideas connect in practice.



The bar chart illustrates various metrics related to data center energy consumption and efficiency. It displays the current global data center energy consumption for the year 2022, projected consumption for 2026, the share of global electricity consumption attributed to data centers in 2023, the average power usage effectiveness (PUE) in the USA, and potential energy savings from optimizing software testing practices. This data highlights the increasing energy demands of data centers and emphasizes the importance of efficiency improvements in software development.

Techniques for Optimizing Code

Chasing energy-efficient computing means keeping performance and power use on a dizzying balance—it's like walking a tightrope. A neat trick is to use quantization-aware training in neural networks; this method lets a standard convolutional network morph into a spiking one with hardly any drop in its game, all while grabbing energy perks from the sparse computation setup (Bortone *et al.,* 2020). You might even spend some time cleaning up your code to ditch those so-called Energy Code Smells, which can really make a difference in how lean your software runs. Recent studies suggest, generally speaking, that tweaking software for better energy use is a hurdle both researchers and industry folks will likely wrestle with in the near future (Ardito *et al.,* 2013). These subtle refinements can chop power consumption down by a surprising margin—a real step toward greener, more sustainable coding practices. And a broader look shared elsewhere underlines, in most cases, that mixing various tactics is key to really hitting these energy-saving targets.

Optimization Technique	Performance Improvement	Source	
Approximate Computing	Significant performance and energy savings	LDRD Annual Report	
Conjugate Gradient with Iterative Refinement	Time-to-solution reduced by a factor of 10 or more compared to non-banded methods	Energy consumed is just 6% of that used by non-banded methods for the same problem	Changing computing paradigms towards power efficiency
GPU-Only Dense LU and Cholesky Factorizations	Achieves 90+% of the performance of optimized hybrid solutions	Improves energy efficiency by 50% compared to hybrid solutions	Analysis and design techniques towards high-performance and energy-efficient dense linear solvers on GPUs

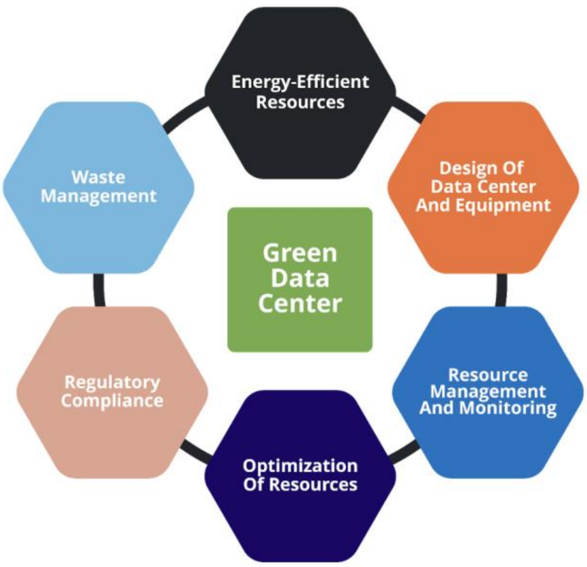
Energy Efficiency Gains from Code Optimization Techniques

Strategies for Reducing Energy Consumption in Algorithm Design

Smart algorithm design is at the heart of cutting energy use in computing—it's really the key to making our systems more energy-savvy. Instead of following a cookie-cutter approach, we can opt for techniques like workload consolidation, which, in most cases, spreads processing tasks across available hardware and helps shrink a data center's total energy footprint. Sometimes, it feels like all the parts of data handling get wedded together when intuitive management systems step in, coordinating bits here and there to bump up overall efficiency. Data centers tend to do better when they're built with energy awareness woven right into their architectural blueprints (see (Fern Gández *et al.*, 2016) and (Mittal *et al.*, 2014) for details). Interestingly, a diagram in , even hints at how these interconnected systems sometimes team up in unexpected ways to form what many call a Green Data Center—reminding us that, generally speaking, a bit of teamwork goes a long way in energy management.

Conclusion:

Digital progress is booming, and with it comes a real need to rethink how we compute. Green algorithms now pop up as a cool way to tweak our code so it sips energy rather than guzzling it, a move that also cuts down on carbon waste. Data centers are sprouting everywhere—each one chowing down on power—and that makes it clear we must sort out smarter ways to manage energy. You might even catch a few clever building strategies for saving power in (Mittal *et al.*, 2014); these tips lend a hand to making systems more energy-wise. Then there's this shift from our usual convolutional neural networks to spiking neural networks, which—surprisingly enough—seems to trim energy use while still keeping things running smoothly, as (Bortone *et al.*, 2020) points out. At the end of the day, if we really want to ease computing's strain on our planet, we've gotta lean into these green algorithms, showing that tech and nature can, in most cases, work together pretty well.



Key Components of a Green Data Center

The future of green algorithms and their impact on sustainable computing practices

Computing demands keep rising, pushing us toward a greener way of doing things. Green algorithms now play a major role—they help cut energy usage and trim carbon footprints while also making software run smoother. Data centers, which churn out a lot of emissions, seem to benefit from these methods, fitting into a broader sustainable data management vibe that leans on smart, somewhat intuitive solutions ((Fern Gández *et al.*, 2016)). It's also interesting that tracking down and reworking what many call Energy Code Smells exposes certain coding habits that end up wasting power, opening the door for real software improvements ((Ardito *et al.*, 2013)). All in all, rolling out these techniques goes hand in hand with fresh energy efficiency tactics, as depicted in , and generally underscores that the tech sector really must lean into greener practices if we're to secure a more sustainable digital future.

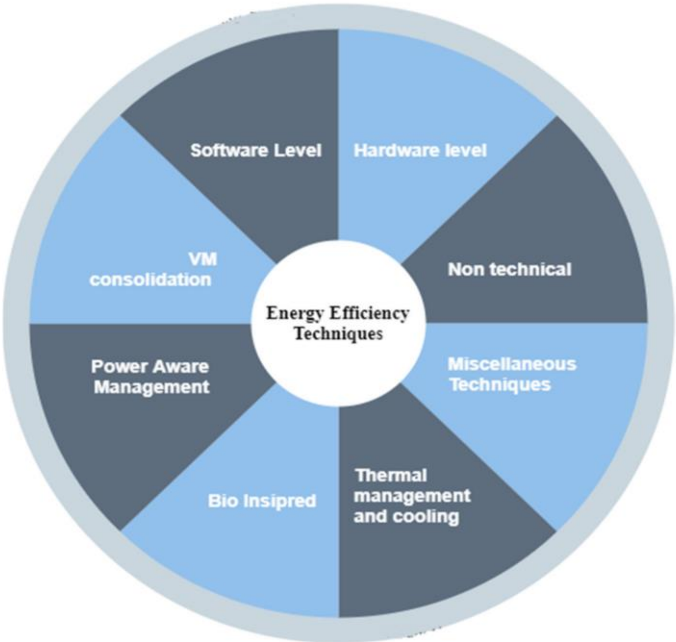


Diagram of Energy Efficiency Techniques in Computing

References:

1. Ardito, L., Morisio, M., Procaccianti, G., Vetro', A., et al. (2013). *Definition, implementation and validation of energy code smells: An exploratory study on an embedded system*. https://core.ac.uk/download/pdf/11432461.pdf
2. Bortone, M., Liu, Q., Sheik, S., Sorbaro, S., et al. (2020). *Optimizing the energy consumption of spiking neural networks for neuromorphic applications*. http://arxiv.org/abs/1912.01268
3. Bortone, M., Liu, Q., Sheik, S., Sorbaro, S., et al. (2020). *Optimizing the energy consumption of spiking neural networks for neuromorphic applications*. http://arxiv.org/abs/1912.01268
4. Ardito, L., Morisio, M., Procaccianti, G., Vetro', A., et al. (2013). *Definition, implementation and validation of energy code smells: An exploratory study on an embedded system*. https://core.ac.uk/download/pdf/11432461.pdf
5. Guitart Fernández, J. (2016). *Toward sustainable data centers: A comprehensive energy management strategy*. https://core.ac.uk/download/81578280.pdf

6. Mittal, S. (2014). *Power management techniques for data centers: A survey*. http://arxiv.org/abs/1404.6681
7. Reiher, M., Simm, G. N., & Vaucher, A. C. (2018). *Exploration of reaction pathways and chemical transformation networks*. http://arxiv.org/abs/1810.07490
8. Weiss, A., Vecchiola, C., Kleinrock, L., Barham, P., Buyya, R., Buyya, R., Buyya, R., et al. (2010). *InterCloud: Utility-oriented federation of cloud computing environments for scaling of application services*. http://arxiv.org/abs/1003.3920
9. Ardito, L., Morisio, M., Procaccianti, G., Vetro', A., et al. (2013). *Definition, implementation and validation of energy code smells: An exploratory study on an embedded system*. https://core.ac.uk/download/pdf/11432461.pdf
10. Mittal, S. (2014). *Power management techniques for data centers: A survey*. http://arxiv.org/abs/1404.6681
11. Briscoe, G., De Wilde, P., & Sadedin, S. (2011). *Digital ecosystems: Ecosystem-oriented architectures*. http://arxiv.org/abs/1112.0204
12. Vecchiola, C., Assunção, D., Thain, D., Erwin, D. W., Foster, I., Broberg, J., Kim, K. H., et al. (2009). *Cloudbus Toolkit for market-oriented cloud computing*. http://arxiv.org/abs/0910.1974
13. Guitart Fernández, J. (2016). *Toward sustainable data centers: A comprehensive energy management strategy*. https://core.ac.uk/download/81578280.pdf
14. Mittal, S. (2014). *Power management techniques for data centers: A survey*. http://arxiv.org/abs/1404.6681
15. Guitart Fernández, J. (2016). *Toward sustainable data centers: A comprehensive energy management strategy*. https://core.ac.uk/download/81578280.pdf
16. Ardito, L., Morisio, M., Procaccianti, G., Vetro', A., et al. (2013). *Definition, implementation and validation of energy code smells: An exploratory study on an embedded system*. https://core.ac.uk/download/pdf/11432461.pdf

Image References

1. Key components of a green data center. (2025). Retrieved from https://www.device42.com/wp-content/uploads/2023/06/Screenshot-2023-06-19-at-19.23.58.png
2. Diagram of energy efficiency techniques in computing (2025). Retrieved from https://www.mdpi.com/sustainability/sustainability-14-06256/article_deploy/html/images/sustainability-14-06256-g011.png
3. Cloud computing architecture for load management (2025). Retrieved from https://media.springernature.com/lw685/springer-static/image/art%3A10.1007%2Fs10586-021-03431-z/MediaObjects/10586_2021_3431_Fig8_HTML.png

AN OVERVIEW OF CAROTENOIDS

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Abstract:

Carotenoids are a diverse group of natural pigments found in abundance across bacteria, plants, algae, fungi, and. These fat-soluble compounds are accountable for the bright red, yellow, and orange hues seen in numerous fruits, vegetables, and other organisms. Structurally, they are divided into two primary groups: carotenes, which consist solely of hydrocarbons, and xanthophylls, which contain oxygen. In plants, carotenoids are essential for photosynthesis, aiding in light absorption and protecting against oxidative stress caused by reactive oxygen species. In humans, they function as crucial dietary antioxidants and serve as precursors to vitamin A, playing a key role in maintaining vision, immune function, and overall health. Moreover, carotenoids like lutein, zeaxanthin, lycopene, and astaxanthin have been linked to a lower risk of chronic disorders such as heart disease, cancer, and age-linked macular degeneration. Their potent reducing and anti-inflammatory actions further enhance their potential therapeutic value. This review explores the biosynthesis, functions, dietary sources, and health benefits of carotenoids, emphasizing their vital part in nutrition and preventing diseases.

Keywords: Carotenoids, Antioxidant, Antidiabetic, Cardiovascular Health.

Introduction:

Carotenoids are pigments found naturally in a wide variety of plants; their colors range from yellow to red. They occur in high concentrations in plants, animals, and people. Plants and some bacteria can produce carotenoids on their own, but animals and humans can only get them through food. But once in the body, they might undergo structural changes. Carotenoids are involved in a wide variety of biological processes and perform important physiological roles. The majority of carotenoids found in nature are chiral compounds, with an average of one to six chiral centers. There are mainly two groups of carotenoids that are defined by their structural similarities:

- Carotenes are carotenoids that rely on hydrocarbons, which include β-carotene and α-carotene.
- Xanthophylls are derivatives of plants that contain oxygen, such as lutein, zeaxanthin, and astaxanthin.

In order to reduce the risk of acquiring chronic diseases such as heart disease, cancer, and age-related macular degeneration, carotenoids are crucial. Their primary protective impact is the fact that they are powerful antioxidants. Antioxidants neutralize harmful free radicals, reduce oxidative stress, and protect cells from harm.

In addition to their antioxidant benefits, carotenoids have powerful anti-inflammatory properties that contribute to disease prevention and overall well-being. Since chronic inflammation has a substantial impact on the development of numerous health disorders, carotenoids may help reduce this risk by modulating inflammatory pathways and lowering inflammation markers in the body.

Among the most well-known carotenoids are zeaxanthin, beta-carotene, lycopene, and lutein, each providing unique health benefits. Beta-carotene serves as a precursor to vitamin A, which is essential for preserving eyesight, supporting immunity, and promoting skin health. Lycopene, widely present in tomatoes, has been related to a lesser threat of prostate cancer and improved cardiac health. Lutein and zeaxanthin, predominantly found in leafy green vegetables, vital to supporting healthy vision by safeguarding against age-related macular degeneration. They help protect the retina by filtering dangerous blue light and minimizing oxidative injury. [1-3]

Carotenoids are naturally occurring pigments noticed in numerous plant-based foods and some animal sources, acting as powerful antioxidants and precursors to vitamin A. They have a prominent role in supporting human health. Carrots, sweet potatoes, pumpkin, and butternut squash are some of the foods that are rich in carotenoids, which are compounds that give them their vibrant colors. Additionally, lutein and zeaxanthin, which are crucial for eye health, are abundant in dark leafy greens like collard greens, spinach, and kale. Tomatoes, watermelons, and pink grapefruit are good sources of lycopene, an antioxidant that has been associated to a reduced risk of chronic diseases. Another important carotenoid, beta-cryptoxanthin, is present in oranges, papaya, red peppers, and peaches, aiding in vitamin A synthesis. Additionally, astaxanthin, a powerful antioxidant carotenoid, is naturally occurring in marine sources such as salmon, shrimp, and krill. Including a diverse selection of these carotenoid-rich foods in the diet helps combat oxidative stress, strengthens the immune system, and promotes overall well-being. [4]

Cardiovascular Diseases

CVD persists as the top global cause of disease and death. Key contributors to coronary disease comprise smoking, elevated cholesterol, and high blood pressure. Atherosclerosis, the primary cause of CVDs, leads to blood vessel damage and structural changes, impairing circulation to the heart and brain. Hypertension has a chief role in the development of strokes, heart attacks, kidney collapse, and other serious conditions. Experiments have shown that enhanced carotenoid consumption is associated to a decreased danger of CVDs [5-7]. Specifically, lycopene consumption has been shown to significantly reduce systolic blood pressure (SBP), particularly in persons with a preliminary SBP of 130 mmHg. Additionally, research from the USA and Finland indicates that a daily lycopene intake averaging 9.81 mg can significantly decrease the possibility of having stroke. As a cardiac shielding nutrient, lycopene has been found to lower blood pressure and reduce the likelihood of major cardiovascular complications [8]. Epidemiological studies strongly support lycopene's protective effects against cardiovascular disease. It helps neutralize potent oxidants associated with atherosclerosis and reduces cholesterol oxidation. Lycopene supplementation has been shown to increase blood lycopene concentrations, decrease markers of oxidative stress, and improve overall antioxidant capacity. Furthermore, research indicates that lycopene safeguards transplanted arteries by regulating the generation of essential proteins involved in arteriosclerosis progress. The expression of ROCK1, Ki-67, ICAM-1, and ROCK2 was notably lessened, whereas eNOS stimulation in implanted arteries and plasma cGMP levels showed an increase. Lycopene was also found to diminish monocyte–endothelial cell interactions and suppress TNF-alpha-induced NF-κB activation. Furthermore, serum lycopene levels exhibited a negative correlation with VCAM-1 and LDL. Lycopene consumption has been shown in pre-clinical research to support better endothelial function. Its antioxidant effects contribute to increased NO bioavailability, improved endothelium-mediated vasodilation, reduced damage to proteins, DNA, and lipids, and better mitochondrial function [9].

Antioxidant Properties

Carotenoids' antioxidant properties help alleviate oxidative stress, guard against oxidative DNA injury, and decrease the risk of reactive species formation. The antioxidant ability of plasma is vital for organisms, predominantly the relative levels of antioxidant substances and their synergistic interactions. Studies have used different oxidative stress markers to establish the connection between carotenoid consumption, blood levels, and the development of chronic diseases and oxidative stress markers. Intervention trial results have been variable, showing either no changes or only slight improvements in oxidative stress markers in healthy individuals. In contrast, carotenoid supplementation has been found to positively affect oxidative stress markers in those with chronic illnesses. However, more studies are required on individuals with chronic conditions who experience higher oxidative stress and reduced antioxidant capacity [10].

The health benefits of carotenoids may be attributed to their mechanisms of action, such as antioxidant effects, either directly or through transcription factors like Nrf2 and NF-κB. In addition, the metabolites of carotenoids can activate nuclear hormone receptor pathways, such as RAR, RXR, and PPARs. Animal and in vitro studies have shown that carotenoids can reduce oxidative stress when Nrf2, a transcription factor that activates antioxidant response elements, is present. The transcription factor NF-κB is involved in regulating numerous genes, influencing both physical and pathophysiological practises, such as embryonic progress, skin, bone, and CNS function. It also plays a crucial role in metabolic inflammation, cellular proliferation, apoptosis, and tumor formation. Lycopene has been shown to significantly affect NF-κB signaling in adipocytes. Meanwhile, RAR and RXR receptors are accountable for regulating the expression of genes related to cellular development and immune system function [11,12].

Photoprotective Properties

Carotenoids offer a range of photoprotective benefits. As noted earlier, their antioxidant properties help neutralize free radicals, preventing oxidative injury to cellular constructions, comprising DNA, and reducing the danger of UV-facilitated cellular injury. The skin and eyes, which are highly subjected to ultraviolet (UV) radiation, are prone to photo-oxidative damage. UV exposure and singlet oxygen trigger a series of gene activations in vitro, including metalloproteinase 1 (MMP-1), heme oxygenase 1 (HO-1), collagenase, and other genes linked to oxidative stress [13]. These photo-oxidative processes cause skin damage by degrading collagen, leading to photoaging and photodermatoses. Carotenoids can support skin health by moderating gene expression, enhancing the cellular defense against UV-facilitated stress. Shielding from UV damage can be achieved with low doses of carotenoids, such as lycopene, when paired with vitamins C and E. Supplementing with 24 mg of a carotenoid blend (containing 8 mg each of lutein, lycopene, and β-carotene) for 12 weeks has been shown to reduce UV-induced skin damage [14].

The photoprotective effects are thought to be associated with intercellular signaling and gap junction communication (GJC). In vitro research has demonstrated that lycopene, when used in conjunction with 1,25-dihydroxyvitamin D3, hinders tumor cell progression. Lycopene's ability to inhibit cell proliferation is linked to a reduction in IGF-I receptor signaling and, seemingly, a lag in cell cycle progression. Additionally, carotenoids can prevent the transformation of carcinogen-introduced fibroblasts in cell cultures reversibly, a process associated with an increase in gap junction communication (GJC) [15].

Type 2 Diabetes

Type 2 diabetes (T2D) is a long-term metabolic condition regarded as by excessive blood sugar readings, which can cause severe harm to the cardiovascular, renal, respiratory, and other systems. The worldwide prevalence of diabetes and the associated healthcare costs are on the rise, with projections suggesting that 700 million people will be affected and USD 776 billion will be spent by 2045. Adopting an active lifestyle, especially a diet abundant in fruits and vegetables, is crucial for controlling blood sugar levels and preventing T2D [16]. There have been recent meta-analyses looking into the link between carotenoid consumption, blood levels, and the risk of type 2 diabetes (T2D). Lower risk of type 2 diabetes has been associated with increased consumption and blood levels of entire carotenoids, especially β-carotene. Besides, lycopene consumption appears to have a potential effect in reducing fasting blood glucose (FBG) levels. Still, despite these encouraging outcomes, the researchers caution that additional researches are necessary to approve connection and to further examine the part of carotenoid-rich foods in preventing T2D [16,17].

It is still debatable and limited how much lycopene is used as an additional treatment for type 2 diabetes. In a study involving 87 type 2 diabetic patients and 122 healthy people, Leh *et al.* examined the impact of lycopene intake on antioxidant capability and glycemic management, which was measured by fasting blood glucose and glycated hemoglobin. In type 2 diabetics, they discovered a correlation between lycopene intake and peripheral antioxidant levels. Alternatively, fasting blood glucose levels and glycated hemoglobin were significantly lower in those whose lycopene consumption was higher. Lycopene may ameliorate the pathogenesis of Type 2 diabetes (T2D) and lower oxidative stress, according to this case-control study [18].

Influence on the cellular mechanisms that regulate signaling pathways

A broad range of in vitro and in vivo projects have established the numerous purposes of carotenoids. They participate in multiple cellular mechanism and exhibit adaptability in impacting diverse pathways inside cells. They also help regulate cell growth and proliferation by downregulating insulin-like growth factor 1 (IGF-1) activity. Also, carotenoids have the potential to act as anti-inflammatory mediators, since studies on animals have shown that they can suppress iNOS and COX-2 [19,20]. Carotenoids also increase the cytotoxicity of NK cells, which helps the immune system to identify and destroy aberrant cells [21, 22]. They also contribute to the blocking of androgen receptors, introduction of cell cycle arrest at various stages, overpowering of telomerase action, and instigation of caspases 3, 8, and 9. These actions play a significant role in regulating cell survival and apoptosis, as demonstrated in numerous cell culture and animal studies [23, 24]. Moreover, studies in cell cultures have demonstrated that carotenoids suppress the expression of the anti-apoptotic protein BCL2, decrease cyclin D levels, and inhibit the secretion of vascular endothelial growth factor (VEGF). These effects underscore their vital role in curbing essential cellular procedures that support overall well-being and homeostasis [25-27].

Cancer

The potential benefits of carotenoids in reducing cancer progression and metastasis have been suggested by a number of studies in the clinical, preclinical, and epidemiological domains [28]. However, there is insufficient or insufficient evidence to support the claim that eating foods high in carotenoids reduces the risk of colon cancer, according to a recent specialized evaluation by the World Cancer Research Fund (WCRF) and the American Institute for Cancer Research (AICR) [29]. Furthermore, there is data that indicates a strong inverse association between the consumption of carotenoids and the risk of ER-negative breast cancer. Higher carotenoid consumption, especially lycopene from tomatoes, may be associated with a reduced threat of developing prostate cancer, according to a review of six cohort studies, eleven case-control studies, three cross-sectional studies, and two organized clinical trials that investigated the effect of carotenoids on prostate cancer occurrence [30]. A significant link between β-carotene consumption and increased breast cancer survival was found in a thorough review and meta-analysis that comprised eight cohort studies, one pooling study, one clinical trial, and data from 19,450 breast cancer patients. On the other hand, other carotenoids that aren't pro-vitamin A had no discernible positive effects on health [31].

Skin and Eye Ailments

Due to their UV-protective actions, they are commonly incorporated into cosmetics. In addition, they may contribute to improved skin health [32]. For as long as 16 weeks, taking a supplement containing 3–6 mg of astaxanthin daily enhanced skin look by decreasing wrinkles and boosting moisture levels, according to a comprehensive analysis of 11 clinical trials [33]. The fovea and inner plexiform layer of the human retina accumulate xanthophylls such as lutein, zeaxanthin, and meso-zeaxanthin, which form macular pigment. While improving contrast and sharpness of vision, this pigment protects the retinal membrane from the damaging effects of intense, short-wavelength light. Dietary lutein and zeaxanthin consumption is critical in reducing the risk of age-associated macular degeneration, according to epidemiological and clinical evidence [34].

The antioxidant lycopene boosts the skin's natural defenses by increasing synthesis of cell membrane components phospholipids and prostaglandins [35]. Acute skin damage from UVB rays can be lessened or prevented with its help. Lycopene lessens photodamage by lowering DNA damage, regulating inflammatory reactions, promoting regular cell proliferation, and blocking epidermal ornithine decarboxylase [36]. Multiple investigations have pointed to elevated plasma lycopene isomers as a potential risk factor for the onset of Alzheimer's disease (AD) [37]. These isomers are believed to affect the activation of signaling pathways involving nuclear hormone receptors.

Bone Ailments

A diet high in carotenoids and other antioxidants may delay the age-related decline in physical function and muscle mass. Muscle strength and walking speed are two metrics that older persons (average age 61 years) can improve by increasing their intake of total carotenoids, lutein/zeaxanthin, and lycopene. In addition, lycopene has a wide range of cellular and molecular effects on human osteoblasts and osteoclasts. Without affecting cell survival or density, it decreases osteoclast differentiation and calcium phosphate resorption [38]. Additionally, lycopene promoted osteoblast development and proliferation (with a decrease in cell death) [39].

Supplementing with lycopene in ovariectomized (OVX) mice reduced the increase in bone turnover through influencing the following markers: serum osteocalcin, bone metabolism, serum N-terminal pro-peptide of type 1 collagen, and urine deoxypyridinoline. In addition, the treatment with lycopene enhanced the activities of osteoblasts, GPx, CAT, and SOD, while inhibiting the activity and differentiation of osteoclasts. By protecting bone tissue from oxidative stress, postmenopausal women who take lycopene daily reduce their risk of bone resorption [40].

Potential Negative Side-Effects

Carotenoids generally promote good health, but there are some potential side effects to be aware of. Unlike high levels of preformed vitamin A, increased beta-carotene intake doesn't cause the same issues. However, excessive beta-carotene ingestion can yeild in a yellow-orange tint to the skin, which is a harmless condition that fades once the intake of beta-carotene is lowered. However, randomized controlled trials have found that high-dose beta-carotene supplementation in those who smoke, have smoked in the past, or have been exposed to asbestos are more likely to get lung cancer and die from the disease [41]. Additionally, some experimental studies suggest that excessive carotenoid intake, when not adequately detoxified, can mount up in mitochondria, causing oxidative stress and mitochondrial dysfunction [42].

Canthaxanthin is an innately existing carotenoid frequently used as a food and dye colorant, as well as a skin tanning agent. A significant health concern associated with canthaxanthin is canthaxanthin retinopathy, a condition where birefringent yellow-to-red crystals accumulate around the macula. In general, patients do not show symptoms. However, some case reports have highlighted persistent visual changes. The retinopathy typically improves after stopping canthaxanthin intake, though the time taken for the crystals to vanish can differ, with some cases taking up to 20 years [43,44].

It is important to note that taking excessive carotenoid supplements, as opposed to getting them from food, may raise the danger of adverse effects. A diet which is balanced with a variety of fruits and vegetables is usually harmless and promotes well-being.

Furthermore, acceptable daily intake (ADI) levels have been established for some carotenoids, although there is ongoing debate about specific carotenoids. Bohm *et al.* examined existing information on carotenoid ingestion, blood plasma/serum levels, and tissue concentrations, emphasizing how this information can be used to develop dietary approvals for carotenoids. They also reviewed existing guidelines and offered practical dietary suggestions [45].

Conclusion:

This review article offers a thorough summary of recent research on carotenoids, focusing on their structural properties, antioxidant potential, and the health benefits associated with dietary consumption. Carotenoids, a varied group of pigments present mainly in fruits and vegetables, are vital for human health because of their capability to nullify free radicals and mitigate oxidative stress. Although plant-based diets are the primary sources of carotenoids, some animal-derived foods, such as egg yolks, salmon, and shrimp, can also be rich in these compounds. Moreover, recent studies suggest that alternative sources of carotenoids, such as macroalgae and edible insects, could offer promising solutions for sustainable nutrition and enrichment with bioactive compounds. In addition to the presence of these bioactive components in diet, their bioavailability plays a critical role in their beneficial effects on the body. This bioavailability is significantly predisposed by the molecular construction of the carotenoids, along with their interactions with other nutrients (e.g., proteins, lipids, and fibers) and dietetic phytochemicals. Carotenoid ingestion and absorption are well-known for their ability to combat oxidative stress and lessen the risk of various long-lasting illnesses, such as diseases affecting the heart and nervous system, type 2 diabetes, and several cancers. Extensive research in animal models, cell cultures, and epidemiological studies has demonstrated the biological effects of lycopene. These results have encouraged further research into the role of lycopene and its metabolites in the development of long-lasting conditions. In conclusion, this chapter serves as an important starting point for guiding future investigation on carotenoids as potential components of foods and nutraceuticals, with possible applications in both the medical and cosmetic fields.

References:

1. Britton G. Structure and properties of carotenoids in relation to function. FASEB J. 1995;9(15):1551-8.
2. Krinsky NI, Johnson EJ. Carotenoid actions and their relation to health and disease. Mol Aspects Med. 2005;26(6):459-516.
3. Rao AV, Rao LG. Carotenoids and human health. Pharmacol Res. 2007;55(3):207-16.
4. Jiří B, Lenka V, Josef S, Věra K. Exploring carotenoids: Metabolism, antioxidants, and impacts on human health. J Funct Foods. 2024;118:106284.
5. Shanely RA, Zwetsloot JJ, Jurrissen TJ, Hannan LC, Zwetsloot KA, Needle AR, *et al.* Daily watermelon consumption decreases plasma sVCAM-1 levels in overweight and obese postmenopausal women. Nutr Res. 2020;76:9-19.
6. Asbaghi O, Nazarian B, Naeini F, Kaviani M, Moradi S, Askari G, *et al.* Lycopene supplementation and blood pressure: Systematic review and meta-analyses of randomized trials. J Herb Med. 2022;31:100521.
7. Cheng HM, Koutsidis G, Lodge JK, Ashor AW, Siervo M, Lara J. Lycopene and tomato and risk of cardiovascular diseases: A systematic review and meta-analysis of epidemiological evidence. Crit Rev Food Sci Nutr. 2019;59:141-58.

8. Casas R, Castro-Barquero S, Estruch R, Sacanella E. Nutrition and cardiovascular health. Int J Mol Sci. 2018;19:3988.
9. Sanajou D, Haghjo AG, Argani H, Aslani S. AGE-RAGE axis blockade in diabetic nephropathy: Current status and future directions. Eur J Pharmacol. 2018;833:158-64.
10. Bohn T. Carotenoids and markers of oxidative stress in human observational studies and intervention trials: Implications for chronic diseases. Antioxidants (Basel). 2019;8(6):179.
11. Savory JG, Edey C, Hess B, Mears AJ, Lohnes D. Identification of novel retinoic acid target genes. Dev Biol. 2014;395(2):199-208.
12. Bonet ML, Canas JA, Ribot J, Palou A. Carotenoids and their conversion products in the control of adipocyte function, adiposity and obesity. Arch Biochem Biophys. 2015;572:112-25.
13. Basu-Modak S, Tyrrell RM. Singlet oxygen: A primary effector in the ultraviolet A/near-visible light induction of the human heme oxygenase gene. Cancer Res. 1993;53(19):4505-10.
14. Heinrich U, Gartner C, Wiebusch M, Eichler O, Sies H, Tronnier H, *et al.* Supplementation with beta-carotene or a similar amount of mixed carotenoids protects humans from UV-induced erythema. J Nutr. 2003;133(1):98-101.
15. Black HS, Boehm F, Edge R, Truscott TG. The benefits and risks of certain dietary carotenoids that exhibit both anti- and pro-oxidative mechanisms – A comprehensive review. Antioxidants (Basel). 2020;9(3):264.
16. Inoue T, Yoshida K, Sasaki E, Aizawa K, Kamioka H. Effect of lycopene intake on fasting blood glucose level: A systematic review with meta-analysis. Nutrients. 2023;15:122.
17. Jiang YW, Sun ZH, Tong WW, Yang K, Guo KQ, Liu G, *et al.* Dietary intake and circulating concentrations of carotenoids and risk of type 2 diabetes: A dose-response meta-analysis of prospective observational studies. Adv Nutr. 2021;12:1723-33.
18. Leh HE, Mohd Sopian M, Abu Bakar MH, Lee LK. The role of lycopene for the amelioration of glycaemic status and peripheral antioxidant capacity among Type II diabetes mellitus patients: A case–control study. Ann Med. 2021;53:1060-6.
19. Firdous AP, Kuttan G, Kuttan R. Anti-inflammatory potential of carotenoid meso-zeaxanthin and its mode of action. Pharm Biol. 2015;53(7):961-7.
20. Soontornchaiboon W, Joo SS, Kim SM. Anti-inflammatory effects of violaxanthin isolated from microalga Chlorella ellipsoidea in RAW 264.7 macrophages. Biol Pharm Bull. 2012;35(7):1137-44.
21. Li Q, Huyan T, Ye LJ, Ren HL, Shi JL, Huang QS. Lycopene prolongs the lifespan and enhances the cytotoxicity of NK cells after Ex vivo expansion. Bioeng Biosci. 2014;2(3):23-30.

22. Wood SM, Beckham C, Yosioka A, Darban H, Watson RR. Beta Carotene and selenium supplementation enhances immune response in aged humans. Integr Med. 2000;2(2):85-92.
23. He Q, Zhou W, Xiong C, Tan G, Chen M. Lycopene attenuates inflammation and apoptosis in post-myocardial infarction remodeling by inhibiting the nuclear factor-κB signaling pathway. Mol Med Rep. 2015;11(1):374-8.
24. Cui SX, Qu XJ, Xie YY, Zhou L, Nakata M, Makuuchi M, Tang W. Curcumin inhibits telomerase activity in human cancer cell lines. Int J Mol Med. 2006;18(2):227-31.
25. Nahum A, Hirsch K, Danilenko M, Watts CK, Prall OW, Levy J, Sharoni Y. Lycopene inhibition of cell cycle progression in breast and endometrial cancer cells is associated with reduction in cyclin D levels and retention of p27(Kip1) in the cyclin E-cdk2 complexes. Oncogene. 2001;20(26):3428-36.
26. Palozza P, Serini S, Maggiano N, Angelini M, Boninsegna A, Di Nicuolo F, Ranelletti FO, Calviello G. Induction of cell cycle arrest and apoptosis in human colon adenocarcinoma cell lines by beta-carotene through down-regulation of cyclin A and Bcl-2 family proteins. Carcinogenesis. 2002;23(1):11-8.
27. Chen HY, Huang SM, Yang CM, Hu ML. Diverse effects of β-carotene on secretion and expression of VEGF in human hepatocarcinoma and prostate tumor cells. Molecules. 2012;17(4):3981-8.
28. Fakhri S, Abbaszadeh F, Dargahi L, Jorjani M. Astaxanthin: A mechanistic review on its biological activities and health benefits. Pharmacol Res. 2018;136:1-20.
29. Ahmadkelayeh S, Hawboldt K. Extraction of lipids and astaxanthin from crustacean by-products: A review on supercritical CO2 extraction. Trends Food Sci Technol. 2020;103:94-108.
30. Dulińska-Litewka J, Sharoni Y, Hałubiec P, Łazarczyk A, Szafrański O, McCubrey JA, Bohn T. Recent progress in discovering the role of carotenoids and their metabolites in prostatic physiology and pathology with a focus on prostate cancer—A review—Part I: Molecular mechanisms of carotenoid action. Antioxidants. 2021;10(4):585.
31. He J, Gu Y, Zhang S. Vitamin A and breast cancer survival: A systematic review and meta-analysis. Clin Breast Cancer. 2018;18(6):e1389-e1400.
32. Donoso A, González-Durán J, Muñoz AA, González PA, Agurto-Munoz C. Therapeutic uses of natural astaxanthin: An evidence-based review focused on human clinical trials. Pharmacol Res. 2021;166:105479.
33. Ng QX, De Deyn MLZQ, Loke W, Foo NX, Chan HW, Yeo WS. Effects of astaxanthin supplementation on skin health: A systematic review of clinical studies. J Diet Suppl. 2021;18(2):169-82.

34. Arunkumar R, Gorusupudi A, Bernstein PS. The macular carotenoids: A biochemical overview. Biochim Biophys Acta Mol Cell Biol Lipids. 2020;1865(11):158617.
35. Liu TY, Chen SB. Sarcandra glabra combined with lycopene protects rats from lipopolysaccharide-induced acute lung injury via reducing inflammatory response. Biomed Pharmacother. 2016;84:34-41.
36. Ascenso A, Pinho S, Eleutério C, Praça F, Bentley MV, Oliveira H, Simões S. Lycopene from tomatoes: Vesicular nanocarrier formulations for dermal delivery. J Agric Food Chem. 2013;61(30):7284-93.
37. Lucas R, Mihály J, Lowe GM, Graham DL, Szklenar M, Szegedi A, Rühl R. Reduced carotenoid and retinoid concentrations and altered lycopene isomer ratio in plasma of atopic dermatitis patients. Nutrients. 2018;10(10):1390.
38. Sahni S, Dufour AB, Fielding RA, Newman AB, Kiel DP, Hannan MT, Jacques PF. Total carotenoid intake is associated with reduced loss of grip strength and gait speed over time in adults: The Framingham Offspring Study. Am J Clin Nutr. 2021;113(2):437-45.
39. Costa-Rodrigues J, Fernandes MH, Pinho O, Monteiro PR. Modulation of human osteoclastogenesis and osteoblastogenesis by lycopene. J Nutr Biochem. 2018;57:26-34.
40. Ardawi MSM, Badawoud MH, Hassan SM, Rouzi AA, Ardawi JM, AlNosani NM, Mousa SA. Lycopene treatment against loss of bone mass, microarchitecture and strength in relation to regulatory mechanisms in a postmenopausal osteoporosis model. Bone. 2016;83:127-40.
41. Kordiak J, Bielec F, Jabłonski S, Pastuszak-Lewandoska D. Role of beta-carotene in lung cancer primary chemoprevention: A systematic review with meta-analysis and meta-regression. Nutrients. 2022;14(7):1361.
42. Amengual J, Lobo GP, Golczak M, *et al.* A mitochondrial enzyme degrades carotenoids and protects against oxidative stress. FASEB J. 2011;25(3):948-59.
43. Challiol CF, Bastien A, Giambruni JM, Fernandez Meijide N. Retinal toxicity due to canthaxanthin. Case series. Arch Soc Esp Oftalmol. 2018;93(8):411-5.
44. Hueber A, Rosentreter A, Severin M. Canthaxanthin retinopathy: Long-term observations. Ophthalmic Res. 2011;46(2):103-6.
45. Bohm V, Lietz G, Olmedilla-Alonso B, Phelan D, Reboul E, Bánati D, *et al.* From carotenoid intake to carotenoid blood and tissue concentrations - implications for dietary intake recommendations. Nutr Rev. 2021;79(5):544–73.

BOTANICAL INSIGHTS: TECHNIQUES FOR ANALYZING HERBAL MATERIALS

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Abstract:

From more archaic ethno-botanical methods to more modern analytical approaches that probe the molecular complexity of phytochemicals, herbal plant science has come a long way. To emphasize this change, the metaphorical movement "from leaf to lab" is used to validate therapeutic characteristics, standardize herbal compositions, and guarantee their safety and effectiveness through the incorporation of current scientific methodologies. Major analytical techniques utilized in the field, including as chromatography, spectroscopy, and mass spectrometry, are reviewed in this chapter. We look at methods including HPLC, GC-MS, TLC, and NMR spectroscopy (Nuclear Magnetic Resonance). The purpose of this chapter is to shed light on the main analytical methods used in herbal plant research by outlining these methods' guiding principles, potential uses, and constraints. By highlighting their function in phytochemical analysis and quality control, this chapter connects conventional herbal wisdom with modern pharmaceutical practices.

Keywords: Herbal Plants, Analytical methods, Chromatography, Spectroscopy

Introduction:

The field of herbal plant science has progressed from relying on simple botanical observation to conducting in-depth analyses of phytochemicals using complex analytical approaches. The symbolic and literal leap "from leaf to lab" represents the validation of medicinal characteristics, standardization of formulations, and assurance of safety and efficacy, as well as a figurative trip. The principles, applications, benefits, and limits of the most common analytical methods employed in herbal plant research are covered in detail in this chapter. The field of herbal plant science has experienced a tremendous shift, moving away from ethnobotany's more conventional methods of observation and toward the application of modern analytical tools. The scientific community's ongoing effort to understand the intricate phytochemistry of medicinal plants and provide proof of their therapeutic effectiveness is mirrored in this development. "From leaf to lab" is a great way to describe the process that begins with collecting and identifying plants in the field and ends with their thorough study in cutting-edge labs. To standardize herbal compositions and guarantee their safety and medicinal efficacy, such advancement is essential.

Chromatography, spectroscopy, and mass spectrometry are only a few of the high-tech tools used in modern phytochemical investigation. Bioactive chemicals in plant matrices can be better identified, quantified, and characterized with the help of these instruments. Analyzing complicated plant extracts has never been easier than with techniques like High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), and Nuclear Magnetic Resonance (NMR) spectroscopy. Nevertheless, the phytochemical profile and the research or industrial goals dictate the benefits and drawbacks of each approach. The purpose of this chapter is to shed light on the main analytical methods used in herbal plant research by outlining these methods' guiding principles, potential uses, and constraints. Enhancing the credibility of plant-based treatments in global healthcare systems, these methodologies when used to herbal research not only help with quality control but also close the gap between traditional medicine and modern pharmacological standards [1-4].

1. Sample Preparation, Extraction and Phytochemical Screening

Plant materials must be appropriately treated before analytical techniques can be used. Drying, grinding, and homogenizing the sample are the steps before extraction. The amount and composition of phytochemicals recovered are highly sensitive to the solvent used (e.g., ethanol, methanol, water, etc.). Supercritical fluid extraction (SFE), ultrasound-assisted extraction (UAE), maceration, and Soxhlet extraction are some of the most used techniques [5]. Herbal research begins with phytochemical screening, which seeks to identify the different classes of bioactive chemicals found in plants. Some examples of common phytochemicals are glycosides, alkaloids, tannins, and flavonoids. As an example of a qualitative test, the Shinoda assay for flavonoids, the Dragendorff assay for alkaloids, and the ferric chloride assay for phenols are all examples of color reactions and precipitation tests. For quantitative estimations, spectrophotometric tests are commonly employed; examples include the Folin-Ciocalteu method for total phenolics and the aluminum chloride colorimetric method for flavonoids [6].

2. Chromatographic Techniques

Herbal analysis relies on chromatography because of its sensitivity and resolution.

2.1 High-Performance Liquid Chromatography (HPLC)

When it comes to quantifying phenolic chemicals, alkaloids, flavonoids, and flavonoids, HPLC is the method of choice. By combining it with mass spectrometry (MS) or diode-array detectors (DAD), it enables precise phytoconstituent identification and quantification [7]. It is perfect for quality control because it is reproducible. Due to its excellent sensitivity and resolution, high-performance liquid chromatography (HPLC) is extensively utilized to quantify phytoconstituents [8]. One common analytical method for separating, identifying, and quantifying components in liquid samples is High-Performance Liquid Chromatography, or HPLC. Because of its accuracy, precision, and capacity to manage complicated combinations, high-performance liquid chromatography (HPLC) is vital in many areas, such as clinical research, environmental monitoring, food safety, and medicines [9]. A high-performance liquid chromatography (HPLC) system works by separating substances according to how they react with a stationary phase, which is a column filled with solid particles, and a mobile phase, which can be a liquid solvent or various solvent mixtures. The mobile phase is responsible for transporting liquid samples from the injection point to the column. variable parts of the sample have variable retention durations because of how they interact with the stationary phase. The compounds can be separated due to these peculiarities [10,11]. The separated components are identified and quantified using detection devices that respond specifically to their elution from the column. These systems can be mass spectrometers, UV-vis detectors, or refractive index detectors [12].

2.2 Gas Chromatography-Mass Spectrometry (GC-MS)

Essential oils and terpenes are examples of volatile substances that are commonly analyzed using GC-MS. To help identify compounds, even in complicated combinations, the MS detector gives structural information [13]. Works wonders with small molecular weight chemicals and volatile oils. Frequently used in conjunction with mass spectrometry to identify compounds. One potent analytical tool for separating, identifying, and quantifying volatile chemicals in a mixture is gas chromatography (GC). Many industries rely on it, including those dealing with environmental analysis, forensic science, medicines, and food testing. Because of its great sensitivity, resolution, and capacity to examine complicated mixes with little sample preparation, the method is very valuable [14].

Analytes are separated in gas chromatography (GC) by a stationary phase, which is a coated liquid or solid substance inside a column, and a mobile phase, which is an inert carrier gas such as helium or nitrogen. The vaporized sample is transported through the column by the mobile phase when it is injected into the GC apparatus. Because of their unique interactions with the stationary phase, the sample's constituent chemicals elute at varying rates and travel at varying velocities. They can be separated and detected due to the difference in retention time [15,16].

A chromatogram, useful for both qualitative and quantitative analysis, is produced when a detector at the column's end (e.g., a mass spectrometer or flame ionization detector) records the presence of each component as it departs the column [17].

2.3 Thin Layer Chromatography (TLC)

TLC is an affordable and fast way to screen for potential issues. Field laboratories or places with limited resources can benefit from its affordability and ease of use [18]. A quick and easy way to take fingerprints and do preliminary screenings. The identification of marker chemicals is a common application of TLC. An analytical method that is often utilized in herbal plant research for the first screening and identification of bioactive substances is thin layer chromatography (TLC). It is quick, easy, and inexpensive. Due to the complex phytochemical combinations found in herbal goods, thin-layer chromatography (TLC) is a crucial initial step in phytochemical profiling, quality control, and plant material authentication [19]. A mobile phase influences a stationary phase differentially, which is the basis of thin-layer chromatography (TLC). A thin layer of stationary phase (usually silica gel or alumina) is put on a flat, inert plate with a tiny quantity of herbal extract in this method. After that, the mobile phase, which is a solvent or solvent mixture, is added to the developing chamber with the plate. various chemicals in the sample migrate at various rates when the solvent ascends the sample via capillary action, based on their affinity for the mobile and stationary phases [20,21]. Utilizing ultraviolet light or chemical reagents, separated compounds can be observed after development, and their migration can be measured by determining the retention factor (Rf) value. Scientists are able to quickly profile samples for target phytochemicals and use that information to direct subsequent HPLC or purification studies [22].

3. Spectroscopic Techniques

3.1 UV-Visible Spectroscopy

Using colorimetric tests, this approach determines the total phenolic and flavonoid contents. For regular screenings, it is easy, quick, and effective [23]. Polyphenols and flavonoids are typical compounds to be detected with this method. When it comes to studying herbal plants, ultraviolet (UV) spectroscopy is a go-to method for both quantitative and qualitative phytochemical investigation. It is a quick and non-destructive way to check if plant extracts include conjugated systems including alkaloids, flavonoids, phenolic acids, and other bioactive chemicals [24].

When molecules absorb light, whether it's visible or ultraviolet, it causes electronic transitions between their orbitals, which is the basis of ultraviolet spectroscopy. Depending on the molecular structure of the chemical, especially if there are chromophores or auxochromes present, certain wavelengths of ultraviolet or visible light (usually between 200 and 800 nm) are absorbed by the sample when it is mixed with a solvent. Absorbance is a measure of the strength of absorption; it is directly proportional to concentration according to Beer-Lambert's law [25]. Standardization of extracts, routine screening, and monitoring of specific markers are prominent uses of ultraviolet spectroscopy in herbal research. For thorough phytochemical profiling and quality control, it is commonly used in conjunction with other methods like HPLC or TLC [26].

3.2 Fourier-Transform Infrared Spectroscopy (FTIR)

Beneficial for chemical fingerprinting and functional group identification. Infrared spectroscopy provides a "fingerprint" of a plant's phytochemical profile by revealing the presence of functional groups in its components. Identifying pollutants and adulterants is another use for it [27]. When it comes to herbal plant science, one of the most common analytical tools used for phytochemical identification and characterization is Fourier Transform Infrared Spectroscopy, or FTIR Spectroscopy. It is very useful for chemotaxonomic studies of medicinal herbs, quality control, and authenticity since it may give extensive information about the functional groups found in complex plant matrices [28].

The fundamental idea behind Fourier transform infrared spectroscopy is that chemical bonds in a sample absorb infrared radiation. Vibrational transitions of chemical bonds, such as C-H, O-H, N-H, and C=O, determine which wavelengths of infrared light are absorbed by a sample. The infrared absorption spectra that result from these vibrations at specific frequencies constitute a chemical "fingerprint" of the material under study [29].

The Fourier transform is employed in Fourier transform infrared spectroscopy (FTIR) to transform the simultaneously collected frequencies into a spectral format that can be understood. With little sample preparation required, this method enables sensitive, non-destructive, and quick examination of solid and liquid plant extracts [30]. Finding important components in plant materials and formulations, evaluating sample purity, detecting functional groups, and keeping an eye out for adulteration are all areas where FTIR shines in the field of herbal plant science [31].

3.3 Nuclear Magnetic Resonance (NMR) Spectroscopy

Natural product research continues to rely on it, despite its high price tag and sample purity requirements [32]. A powerful and non-destructive analytical tool, Nuclear Magnetic Resonance Spectroscopy (NMR) is extensively used in the field of herbal plant science to identify and quantify bioactive chemicals, as well as to better understand their structures. Since herbal formulations and crude extracts typically contain a diverse array of phytochemicals, NMR offers a thorough and objective view into their molecular structure, allowing for in-depth metabolic profiling and evaluation of quality [33].

The nuclear magnetic resonance (NMR) spectra of specific atomic nuclei form the basis of this technique. Nuclear spin states can be changed when hydrogen-1 (^1H) or carbon-13 (^13C) nuclei are exposed to a powerful external magnetic field and absorb certain radiofrequency (RF) energy. Upon removal of the RF pulse, the nuclei revert to their initial states and release energy, which is then measured and converted into a spectrum [34].

A distinct chemical shift occurs because the magnetic environment around each molecule's nucleus is somewhat different. Information on the number of nuclei, their chemical surroundings, and the connectivity between atoms inside a molecule can be gleaned from the resulting nuclear magnetic resonance (NMR) spectrum. Dereplication, isomer detection, and establishing the authenticity of isolated natural compounds are three areas where nuclear magnetic resonance (NMR) shines in herbal analysis [35].

An indispensable tool in contemporary phytochemistry and metabolism, NMR enables the direct examination of crude extracts, in contrast to methods that necessitate chromatographic separation [36].

3.4 Mass Spectrometry (MS)

Combined with chromatography, MS becomes a powerful tool for metabolite profiling and structural elucidation. There has been a recent uptick in the utilization of HRMS and MS/MS for metabolomic profiling, expanding the scope of analysis beyond GC-MS and LC-MS. In order to identify minute components of therapeutic value, these approaches offer great sensitivity and specificity [37]. For the purpose of identifying, structurally elucidating, and quantifying phytochemicals, even at trace quantities, mass spectrometry (MS) is a very sensitive and accurate analytical tool that finds extensive application in herbal plant science. Its exceptional specificity in analyzing complicated mixtures makes it a priceless tool for biochemical profiling, quality control, metabolism, and the identification of new bioactive chemicals in medicinal plants [38]. The mass-to-charge ratio (m/z) is the basis for ion formation, separation, and detection in mass spectrometry. The first step in a conventional MS analysis is to ionize the sample, which is done by converting molecules into charged particles using an ion source. After that, an electric or magnetic field is used to accelerate the ions into a mass analyzer, where their m/z values are used for separation. In order to determine the analyte's molecular weight and structure, a mass spectrometer is created by recording the intensity of each ion using a detector [39]. Chromatographic methods like Gas Chromatography (GC-MS) or Liquid Chromatography (LC-MS) are frequently used in conjunction with mass spectrometry to improve the separation and identification of individual chemicals in herbal matrices that are complicated. Researchers are able to more accurately discover the chemical fingerprints of therapeutic plants because to this integration, which enables focused and untargeted metabolite analysis [40].

4. Microscopic and Macroscopic Analysis

Color, texture, and smell are examples of morphological features that make up macroscopic evaluation. Among the first and most important processes in verifying the authenticity and quality of herbal raw materials is a macroscopic examination. Herbal plant science relies on sensory analysis and visual examination of basic plant components (e.g., seeds, roots, stems, flowers, and leaves) to determine important morphological traits. If you want to tell the difference between real plant materials and imposters, this method is essential.

Color, size, form, surface texture, crack features, and odor are some of the most important criteria examined under a microscope. These anatomical features are detailed in botanical and pharmacopoeia references, and they are frequently species-specific. This technique is highly advantageous in traditional medicine, raw material gathering, and fieldwork due to its non-destructive nature, speed, and low cost. Even though it's basic, macroscopic evaluation is the bedrock of more complex analytical methods because it filters out incorrectly detected material before moving on to testing [41].

Identification of trichomes, stomata, and vessels—essential cellular features for authentication—involves microscopic examination. Herbal plant science relies on microscopic analysis as a primary tool for medicinal plant material identification, authenticity, and quality control [42]. Light microscopy, compound microscopy, and scanning electron microscopy are some of the instruments used in this approach, which aims to examine structures at the cellular and tissue levels. Researchers can identify adulterants or contaminants in herbal preparations and differentiate closely related species by observing crucial morphological traits such trichomes, stomata, vascular bundles, and cell types. Prior to moving on to more sophisticated analytical methods like chromatography or spectroscopy, microscopy is an essential step in standardization protocols for verifying the authenticity of plant-based goods. Microscopic methods are still a dependable and affordable way to do early-stage botanical analysis and pharmacognostic evaluations, which is becoming more important as herbal medicine becomes more popular around the world [43].

5. Chemometric and Data Analysis Tools

Chemometrics is used to decipher multivariate data since herbal matrices are so complicated. To distinguish between different species of plants, their places of origin, and the impacts of treatments, researchers utilize principal component analysis (PCA), hierarchical cluster analysis (HCA), and partial least squares-discriminant analysis (PLS-DA) [44].

6. DNA Barcoding and Molecular Techniques

The use of DNA barcoding has grown in importance as a method of plant species authentication alongside chemical examination. Conserved genomic areas (such as rbcL and matK) allow researchers to identify botanicals and identify adulterants [45].

Conclusion:

A major shift from antiquated methods to contemporary, evidence-based approaches has occurred with the use of sophisticated analytical tools into herbal plant research. This change, which has been aptly dubbed the "from leaf to lab" process, has completely altered the methods used to discover, define, and verify curative plants. Researchers can now properly profile complicated phytochemical compositions using sophisticated methods such as mass spectrometry, chromatography, and spectroscopy. This ensures that herbal preparations are of high quality, safe, and effective. Accurate chemical fingerprinting is made possible by methods such as high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC–MS), nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy (FTIR), and ultraviolet–visible spectroscopy (UV–Vis spectroscopy). Microscopic and macroscopic investigations aid in early-stage authenticity and quality evaluation. The use of statistical chemometric approaches and molecular tools such as DNA barcoding allows for even more exact species identification and data interpretation. The scientific legitimacy and worldwide acceptance of plant-based medicines are both boosted by these improvements, which help to close the gap between traditional knowledge and modern pharmacological standards. In order to ensure consistency, conformity with regulations, and therapeutic dependability, these analytical approaches must be applied rigorously as herbal treatments become more prominent in integrative healthcare systems. A more thorough and evidence-based use of medicinal plants in healthcare and research is ultimately promoted by this merging of traditional herbal knowledge with modern analytical science.

References:

1. Efferth, T., & Greten, H. J. (2012). Quality control and standardization of herbal medicine: Evidence-based clinical pharmacology. *Current Drug Targets, 13*(14), 1779–1791.
2. Zhang, A., Sun, H., & Wang, X. (2013). Recent advances in natural products from plants for treatment of liver diseases. *European Journal of Medicinal Chemistry, 63*, 570–577.
3. Sarker, S. D., & Nahar, L. (2007). *Chemistry for pharmacy students: General, organic and natural product chemistry*. Chichester: Wiley.
4. Devi, V. K., Jain, N., & Valli, K. S. (2010). Importance of novel drug delivery systems in herbal medicines. *Pharmacognosy Reviews, 4*(7), 27–31.
5. Mandal, V., Mohan, Y., & Hemalatha, S. (2007). Microwave assisted extraction—An innovative and promising extraction tool for medicinal plant research. *Pharmacognosy Reviews, 1*(1), 7–18.
6. Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). London: Chapman and Hall.
7. Bansal, V., Malviya, N., & Malviya, S. (2016). High performance liquid chromatography: A short review. *Journal of Global Pharma Technology, 8*(7), 1–5.
8. Waksmundzka-Hajnos, M., Sherma, J., & Kowalska, T. (2008). *Thin layer chromatography in phytochemistry*. Boca Raton: CRC Press.
9. Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2010). *Introduction to modern liquid chromatography* (3rd ed.). Hoboken, NJ: Wiley-Interscience.
10. Dong, M. W. (2006). *Modern HPLC for practicing scientists*. Hoboken, NJ: Wiley-Interscience.
11. Swartz, M. E., & Krull, I. S. (1997). *Analytical method development and validation*. New York: Marcel Dekker.
12. Meyer, V. R. (2010). *Practical high-performance liquid chromatography* (5th ed.). Hoboken, NJ: Wiley.
13. Shellie, R., Marriott, P., & Morrison, P. (2001). Concepts and preliminary observations on the triple-dimensional analysis of essential oils using GC×GC–MS. *Journal of Separation Science, 24*(10–11), 823–830.

14. McNair, H. M., & Miller, J. M. (2009). *Basic gas chromatography* (2nd ed.). Hoboken, NJ: Wiley-Interscience.
15. Poole, C. F. (2012). *Gas chromatography* (2nd ed.). Amsterdam: Elsevier.
16. Sparkman, O. D., Penton, Z., & Kitson, F. G. (2011). *Gas chromatography and mass spectrometry: A practical guide* (2nd ed.). Burlington, MA: Academic Press.
17. Smith, R. M. (2008). *Understanding gas chromatography*. Cambridge: Royal Society of Chemistry.
18. Wagner, H., & Bladt, S. (1996). *Plant drug analysis: A thin layer chromatography atlas* (2nd ed.). Berlin: Springer.
19. Stahl, E. (1969). *Thin-layer chromatography: A laboratory handbook* (2nd ed.). Berlin: Springer-Verlag.
20. Wagner, H., & Bladt, S. (1996). *Plant drug analysis: A thin layer chromatography atlas* (2nd ed.). Berlin: Springer-Verlag.
21. Wallis, T. E. (2005). *Textbook of pharmacognosy* (5th ed.). New Delhi: CBS Publishers.
22. Mukherjee, P. K. (2019). *Quality control of herbal drugs: An approach to evaluation of botanicals* (2nd ed.). New Delhi: Business Horizons.
23. Singleton, V. L., & Rossi, J. A., Jr. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*(3), 144–158.
24. Beckett, A. H., & Stenlake, J. B. (2005). *Practical pharmaceutical chemistry: Part II* (4th ed.). New Delhi: CBS Publishers & Distributors.
25. Skoog, D. A., Holler, F. J., & Crouch, S. R. (2007). *Principles of instrumental analysis* (6th ed.). Belmont, CA: Thomson Brooks/Cole.
26. Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: A review. *International Pharmaceutical Science,* 1(1), 98–106.
27. Naumann, D. (2000). Infrared spectroscopy in microbiology. In R. A. Meyers (Ed.), *Encyclopedia of analytical chemistry* (pp. 102–131). Chichester: Wiley.
28. Smith, B. C. (1999). *Infrared spectral interpretation: A systematic approach*. Boca Raton, FL: CRC Press.
29. Stuart, B. (2004). *Infrared spectroscopy: Fundamentals and applications*. Chichester, UK: John Wiley & Sons.
30. Mukherjee, P. K. (2019). *Quality control of herbal drugs: An approach to evaluation of botanicals* (2nd ed.). New Delhi: Business Horizons.
31. Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: A review. *International Pharmaceutical Science,* 1(1), 98–106.

32. Pauli, G. F., Gödecke, T., Jaki, B. U., & Lankin, D. C. (2012). Quantitative 1H NMR: Development and potential of a method for natural products analysis. *Journal of Natural Products*, 75(4), 834–851.
33. Claridge, T. D. W. (2016). *High-resolution NMR techniques in organic chemistry* (3rd ed.). Oxford: Elsevier.
34. Lindon, J. C., Tranter, G. E., & Holmes, J. L. (2010). *Encyclopedia of spectroscopy and spectrometry* (2nd ed.). Oxford: Academic Press.
35. Mukherjee, P. K. (2019). *Quality control of herbal drugs: An approach to evaluation of botanicals* (2nd ed.). New Delhi: Business Horizons.
36. Kim, H. K., Choi, Y. H., & Verpoorte, R. (2010). NMR-based metabolomic analysis of plants. *Nature Protocols*, 5(3), 536–549.
37. Wolfender, J. L., Marti, G., Thomas, A., & Bertrand, S. (2015). Current approaches and challenges for the metabolite profiling of complex natural extracts. *Journal of Chromatography A*, 1382, 136–164.
38. Hoffmann, E., & Stroobant, V. (2007). *Mass spectrometry: Principles and applications* (3rd ed.). Chichester, UK: John Wiley & Sons.
39. Gross, J. H. (2017). *Mass spectrometry: A textbook* (3rd ed.). Cham, CH: Springer International Publishing.
40. Wolfender, J. L., Queiroz, E. F., & Hostettmann, K. (2005). Phytochemistry in the microgram domain – A LC-MS-based approach for the phytochemical profiling of botanical extracts. *Phytochemical Reviews*, 4(3), 431–443.
41. Kokate, C. K., Purohit, A. P., & Gokhale, S. B. (2021). *Pharmacognosy* (49th ed.). Pune: Nirali Prakashan.
42. Trease, G. E., & Evans, W. C. (2002). *Pharmacognosy* (15th ed.). London: Saunders.
43. World Health Organization. (1998). *Quality control methods for medicinal plant materials*. Geneva: World Health Organization.
44. Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., et al. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, 117(4), 426–436.
45. Newmaster, S. G., Grguric, M., Shanmughanandhan, D., Ramalingam, S., & Ragupathy, S. (2013). DNA barcoding detects contamination and substitution in North American herbal products. *BMC Medicine*, 11, 222.

HERBS IN DIGESTIVE HEALTH

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Abstract:

Nutrient absorption, metabolism, and immunological support are three of the digestive system's most important functions in maintaining general health. Many medicinal herbs have carminative, antispasmodic, laxative, anti-inflammatory, antibacterial, hepatoprotective, and demulcent effects; as a result, there has been a recent uptick in interest in their use to improve digestive health. Various digestive herbs are analyzed in this chapter according to their medicinal properties. These plants include milk thistle, peppermint, ginger, chamomile, fennel, licorice, and slippery elm. In addition to providing antioxidant and antibacterial effects, these herbs stimulate digestive secretions, calm mucous membranes, and modulate gastrointestinal motility. Ayurveda, TCM, and Western herbalism are just a few of the traditional systems that have long made use of these herbs. Now, thanks to current clinical research, we know that they can help with a variety of diseases, including stomach inflammation, irritable bowel syndrome (IBS), nausea, and bloating. Despite its usefulness, safety must be considered at all times, but particularly during long-term use or in conjunction with traditional medicine. The effectiveness and safety of herbal therapies must be confirmed through scientific validation and well-designed clinical trials. In areas where conventional medical treatment is scarce, herbal remedies show potential as an alternative that is easy to obtain, inexpensive, and holistic for the management of gastrointestinal problems.

Keywords: Digestive Health, Herbs, Ginger, Peppermint, Chamomile, Fennel

Introduction:

Keeping one's digestive system in good working order is vital to one's health in general because of the significant roles it plays in nutrient absorption, metabolism, and immunological function. Over the last several decades, there has been a surge in interest in natural therapies as a means to preserve or restore digestive function. Medicinal plant usage is one example of these alternative treatments. Since ancient times, the medicinal properties of herbs have been acknowledged by various traditional medical systems, including Ayurveda, TCM, and Western herbalism, as valuable assets in promoting GI tract health. These plants may have a variety of medicinal uses, including relaxing the muscles of the digestive tract, preventing spasms, reducing inflammation, killing germs, and protecting the liver. Additionally, it has anti-inflammatory, depressive, and antibacterial effects, among other medical uses. [1]

Classification of Digestive Herbs

Herbs used for digestive health can be classified based on their primary therapeutic actions:

- **Carminatives**: By softening sphincters, aromatic digestives (carminatives) improve blood flow to the intestines and facilitate wind release. Although they might make reflux worse, they are helpful for many common gastrointestinal issues. Many herbs have carminative effects, such as angelica (also bitter), caraway, fennel, turmeric, cardamom, chamomile, peppermint, thyme, ginger, lemon balm, and lemon balm. The most effective of these are taken as hot tea before meals.
- **Bitters**: A digestive system "tonic" is what these are. They promote an increase in hunger and motility in the digestive tract by stimulating all digestive secretions and increasing blood flow to the gut. Some common bitter plants are peppermint, dandelion root, and hops. When taken half an hour before eating, they get the digestive system ready for food, increasing the likelihood of good digestion and decreasing the likelihood of issues afterwards.
- **Laxatives and Purgatives**: Senna pods are the herbal laxative most commonly associated with the phrase. These pods include substances called anthraquinones, which can cause long-term issues due to their powerful stimulating action on the intestines. Bulking agents like slippery elm and linseed are gentler alternatives. It may also be beneficial to use herbs that stimulate bile flow in the liver. Encourage defecation (e.g., aloe vera, senna, cascara).
- **Antispasmodics**: Along with the carminatives, these calming herbs have many practical uses. Herbal relaxants can alleviate symptoms of irritable bowel syndrome (IBS) by calming the digestive tract, which is often tense due to the condition. Chamomile, peppermint, lemon balm, and valerian are some of the most common herbs used as relaxants. Some herbs, like peppermint and chamomile, can ease stomach cramps and spasms.
- **Astringents**: The tannins in it have an immediate and beneficial effect on the intestinal lining, which is why it is utilized. As a stopgap solution, they trigger reactions that "harden" the tissue, which in turn decreases secretions and provides protection from irritations. When administered for a short period of time, they can alleviate diarrhea that

is not caused by an infection. Astringent properties are found in black tea, raspberries, and blackberry leaves. Because of its tannin content, slippery elm is an effective remedy for both constipation and diarrhea.

- **Hepatoprotective**: Bile, which the liver produces, promotes fat digestion and has many other positive benefits on the digestive system. Artichoke leaf and the famous milk thistle seed are two examples of plants that mainly protect the liver. Others, like peppermint and dandelion root (and artichoke leaf again), encourage the generation and/or flow of bile.
- **Anti-inflammatory:** For those who suffer from gastrointestinal inflammation—caused by things like irritated intestines or more significant medical issues—these can be a lifesaver. Along with its anti-inflammatory properties, liquorice encourages the formation of the protective mucous layer in the digestive tract. Another benefit of meadowsweet is that it acts as a natural "antacid." Other soothing herbs include chamomile and marigold.
- **Demulcents:** The gut lining is soothed and protected from irritations that can cause diarrhea by these. One very helpful demulcent plant is powdered slippery elm. When consumed with plenty of water, it forms a slimy mucilaginous barrier that protects the lining of the gut. However, because it absorbs a lot of water in the intestines, it will also work as a bulking agent to ease constipation. Slippery elm also has probiotic components, which are consumed by healthy bacteria in the digestive tract and contribute to overall gut health. Additional demulcents that are commonly used include linseed, marshmallow, and bladderwrack.
- **Mucous membrane tonics:** In a broader sense, these help maintain healthy tissue tone and function. The plantain is a typical example of a plant that grows on garbage. Most gastrointestinal diseases involve some degree of poor tissue tone, therefore these medications help alleviate the associated inflammation, excess secretions, and speed up the healing process.
- **Anti-infective herbs:** There are a lot of herbs that help the immune system or reduce gastrointestinal infections. Cinnamon, for instance, inhibits the growth of Helicobacter pylori, while bilberry and barberry have broader anti-infective properties. [2]

Mechanisms of Action

Various metabolic pathways are utilized by herbal medicines to produce their effects. Example: substances with a bitter taste stimulate the tongue's taste receptors, which in turn stimulate the production of gastric juice, bile, and saliva. Carminative herbs' volatile oils have antibacterial and anti-inflammatory qualities, and they relax smooth muscle to relieve bloating and gas. Many herbs contain flavonoids and polyphenols, which have antioxidant and anti-inflammatory properties, and mucilage-containing herbs cover and calm irritated mucous membranes. [1,3-5]

Common Herbs in Digestive Health

- **Ginger (*Zingiber officinale*)**: Ginger has been revered for its flavor and medicinal qualities for countless generations, making it a popular culinary spice and traditional medicinal herb. Concentrations of bioactive chemicals found in gingerols and shogaols, such as [6]-gingerol and [6]-shogaol, vary with the origin and processing of the rhizome. Although many of the results are based on observation rather than mechanism, research on ginger's potential for illness prevention has been growing in recent years. The pharmacokinetics, effects of long-term ingestion, and molecular targets of ginger's components require further in-depth research. Another issue is that ginger supplements aren't standardized, so it's not certain how safe it is to consume high doses of separated components. One of ginger's traditional uses is to alleviate nausea, and it may also help prevent colon cancer due to the substances it contains that tend to collect in the gastrointestinal tract. It has limited in vivo evidence and unclear mechanisms, yet it has strong antioxidant characteristics in vitro. Although research on ginger's effectiveness in alleviating arthritic pain and swelling is inconsistent, it may decrease inflammation by blocking COX-2 and associated pathways. It has no known side effects and is most commonly used in clinical settings to reduce nausea and vomiting caused by chemotherapy, surgery, and pregnancy. It is often as effective as vitamin B6 in this regard. Ginger may also help lessen the risk of cardiovascular disease and diabetes by improving lipid metabolism and decreasing cholesterol. More evidence that ginger may be an effective anticancer drug has come from recent studies that pinpoint a protein target in colon cancer. But there are still major holes in our understanding of the mechanisms involved and the need for regulated dosing. [6,7]
- **Peppermint (*Mentha piperita*)**: Many people utilize peppermint (Mentha piperita L.) for a variety of medicinal reasons; the leaves and essential oil are also popular ingredients in herbal teas. The leaves have flavonoids such as hesperidin, eriocitrin, and luteolin in addition to phenolic substances like rosmarinic acid. Many volatile components, including menthone and menthol, are present in the essential oil. The antibacterial, antiviral, antioxidant, anticancer, and antiallergenic properties of peppermint have been proven in vitro by scientific studies. Furthermore, research on animals has shown that it can relax the tissues of the gastrointestinal tract, give analgesic and anesthetic effects, modify the immune system, and even prevent cancer. Peppermint oil has alleviated pain, gastrointestinal issues, and respiratory issues in humans. Evidence from multiple randomized controlled trials points to its usefulness in reducing IBS symptoms. Nevertheless, there is a dearth of clinical trials investigating peppermint tea, and there is a dearth of human studies concentrating on peppermint leaves. Although peppermint tea

has not been linked to any negative consequences, anyone with GERD, a hiatal hernia, or kidney stones should exercise caution when using peppermint oil. In general, peppermint shows potential therapeutic effects, particularly when consumed as an essential oil, although additional studies on humans are required, especially with peppermint tea. [8,9]

- **Chamomile (*Matricaria chamomilla*)**: Traditional uses of chamomile for digestive health date back to ancient Greece, Rome, and Egypt. Chamomile is a member of the chamomile plant family, which also includes Chamaemelum nobile (L.) All. (CN). When it comes to relieving indigestion, cramps, and other gastrointestinal problems, it is highly regarded. The broad variety of bioactive components found in chamomile—including organic acids, polysaccharides, coumarins, terpenes, volatile oils, and flavonoids—contributes to its medicinal potential. Chamomile is beneficial to digestive health because it has antispasmodic, anti-inflammatory, and antioxidant characteristics, which ease gas, cramps, and bloating. In addition to supporting digestive function, its modest calming effects may alleviate stress-related disruptions to the digestive system. Because of their antibacterial and anti-inflammatory properties, chamomile chemicals have shown promise in pharmacological investigations for the treatment and prevention of gastrointestinal infections and ulcers. To prove its safety, particularly with long-term or high-dose use, more toxicological studies are necessary, despite its widespread use and largely positive safety reviews. More thorough scientific studies are needed to confirm the traditional uses of chamomile and to guarantee safe, standardized uses in contemporary medicine, but it has great potential for gastrointestinal health. As a whole, chamomile shows a lot of promise as a future medicinal herb, and it is already a popular choice for digestive health. [10,11]
- **Fennel (*Foeniculum vulgare*)**: A well-known medicinal herb traditionally used to enhance digestive health, Foeniculum vulgare (fennel) is a member of the Apiaceae family. For those who suffer from indigestion, gas, bloating, or flatulence, fennel is a common carminative and digestive aid. Because of their calming effects on the gastrointestinal tract, the seeds of this plant find widespread use in traditional medicine and cuisine. One reason fennel is good for your digestive system is that it includes important phytochemicals like phenols, phenolic glycosides, and volatile compounds like trans-anethole, estragole, and fenchone. These chemicals ease gas in the intestines, speed up digestion, and calm the muscles in the digestive tract. Scientific research has shown that this plant can help keep the digestive tract healthy by reducing inflammation and harmful bacteria. What's more, it has antioxidant and antibacterial properties that have been validated in both laboratory and animal experiments. Its essential oils improve its flavor profile and carminative qualities, while its phenolic components are primarily

responsible for its antioxidant activity. Indirectly supporting digestive function, fennel's therapeutic effects—which include hepatoprotective and antithrombotic properties—maintain metabolic and liver health. [12,13]

- **Licorice (*Glycyrrhiza glabra*)**: Licorice, scientifically known as *Glycyrrhiza glabra* Linn., is a Leguminosae family medicinal herb that has a rich history of usage in traditional medicine, especially for gastrointestinal health. Its medicinal qualities are due in part to the many bioactive components it contains, such as glycyrrhizinic acid, isoliquiritin, glycyrrhizic acid, and glycyrrhizin. Because of its anti-inflammatory, antispasmodic, and antibacterial properties, licorice is very important for digestive health. These effects alleviate gastritis, ulcer, indigestion, bloating, and stomach cramp symptoms while also soothing the gastrointestinal tract. Its antibacterial and antispasmodic characteristics make it useful in the fight against gastrointestinal infections and in the relief of gastrointestinal pain. In addition, licorice helps keep the digestive system healthy by supporting liver function through its hepatoprotective qualities. Potentially useful in the treatment of acid reflux and peptic ulcers, it may also aid in the regulation of stomach secretions and the promotion of mucosal repair. Side effects, such as elevated blood pressure and electrolyte imbalances, can occur from using licorice for an extended period of time or in excess, despite the fact that it has several systemic and digestive health benefits. Hence, it is important to keep an eye on its usage. At its best, licorice is a helpful herb for the digestive system when used properly and with supervision. [14,15]
- **Milk Thistle (*Silybum marianum*)**: Aquaculture studies with young Pangasianodon hypophthalmus have demonstrated encouraging results for digestive health when administered milk thistle extract (MTE), a derivative of Silybum marianum. Over a 60-day period, MTE significantly improved feed intake, weight increase, and specific growth rate in fish when added to diets at varying concentrations (0.1%, 0.2%, and 0.3%) compared to a control group. Improvements in digestive efficiency and intestinal health were noticeable, although there was no statistical change in body composition, survival rate, or feed conversion ratio. Intestinal histomorphometry, including villus height, breadth, and crypt depth, were improved by MTE supplementation, and digestive enzyme activity, including protease, lipase, and amylase, was enhanced. These alterations point to better digestion and absorption of nutrients. The levels of oxidative stress marker malondialdehyde dropped, but those of liver function enzymes and antioxidant markers (CAT and SOD) were enhanced, indicating better hepatic health. There was a dose-dependent rise in immunological indicators, such as total immunoglobulin levels and serum lysozyme activity, which indicate an improved immune response. By preserving

the natural structure of the liver and intestines, histological examination proved that MTE did not cause any tissue damage. [16,17]

- **Slippery Elm (*Ulmus rubra*)**: It helps with gastritis and acid reflux by coating the digestive system with mucilage, which reduces inflammation and speeds up the healing process. Irritable bowel syndrome (IBS), diarrhea, constipation, indigestion, and reflux are some of the symptoms of gastrointestinal (GI) disorders that impact around half of the Western population. An evaluation of the Nutrition Care (NC) Gut Relief Formula, a combination of peppermint oil, glutamine, slippery elm, guar gum, pectin, and curcumin, was conducted over the course of 16 weeks on persons suffering from gastrointestinal issues. Throughout the 4-week intervention phase, participants were given 5g of the formula daily, followed by 10g. Validated gastrointestinal symptom questionnaires, intestinal permeability testing, and gut microbiota analysis all showed substantial improvements in the evaluations. Heartburn, nausea, bloating, abdominal pain, constipation, and diarrhea were among the upper and lower gastrointestinal symptoms that the formulation alleviated by 60-80%. In addition, it improved indicators of quality of life, such as vitality, disposition, rest, and bodily function. The majority of subjects, including those with irregular stool consistency, had their intestinal permeability normalized by the formula, and the gut microbiota was enhanced by an increase in good bacteria such Lactobacillus, Clostridium, and Faecalibacterium prausnitzii. Half of the people who took proton pump inhibitors (PPIs) for heartburn were able to stop taking them, and a third could eat the items that made their symptoms worse without experiencing any worsening. [18,19]

Clinical Applications and Evidence

Digesting food, absorbing nutrients, metabolizing it, cleansing the body of toxins, and protecting the immune system are all fundamental functions of the human gastrointestinal (GI) tract and liver. It is crucial for general health and wellness to keep these organs functioning at their best. Diarrhea, stomach discomfort, bloating, constipation, nausea, and vomiting are just a few examples of the gastrointestinal (GI) problems that are incredibly common and put a huge strain on healthcare systems around the world. Although there are several choices for gastrointestinal (GI) symptom relief in modern pharmacotherapy, these treatments are typically only symptomatic and not curative. Additionally, traditional medicine has the potential to have side effects or limited effectiveness when used for an extended period of time. This has led to a rise in the popularity of complementary and alternative medicine approaches, especially those with roots in traditional medicine. Herbal therapy is one of the most popular methods for treating hepatic and gastrointestinal issues, whether they are acute or chronic. With an emphasis on clinical applications backed by systematic reviews, meta-analyses, and evidence-based trials, this chapter offers a practical reference to the function of herbal medicines in gastrointestinal and liver health. Ayurveda, TCM, and Western herbalism are just a few of the ancient medical systems that have relied on herbal medicines for ages to treat a variety of gastrointestinal issues. Herbal remedies have seen a comeback in popularity in the last several decades, in both developing and industrialized countries. A combination of discontent with the shortcomings of conventional treatments and the widespread belief that herbal remedies are safer and more "natural" is fueling their increasing popularity. Scientific confirmation through rigorous clinical trials is still lacking for a number of plants, despite the abundance of anecdotal and historical evidence supporting the use of numerous botanicals. Numerous herbal products also have a lack of study on their safety profile, standardization, dosage, pharmacokinetics, and interactions with other drugs. Herbal remedies still carry some degree of danger, even with these encouraging results. There are valid worries about possible toxicological consequences, contamination, active ingredient fluctuation, and the absence of regulatory supervision. For this reason, more funding for ethnopharmacology is desperately needed. This funding should go toward conducting standardized evaluations and classifications of herbal products, as well as pharmacovigilance systems and controlled clinical trials. The ideal system for evaluating and controlling herbal medications would include the same comprehensive criteria as those for synthetic pharmaceuticals, including but not limited to: indications, mechanisms of action, dose forms, effectiveness, safety, and adverse effects. [4,20-22]

Safety and Considerations

Over 80% of the global population uses herbal medications and supplements for primary healthcare in some way, a number that has increased dramatically over the last 30 years. There are a lot of herbal products that have showed promise, but they haven't all been evaluated, and their use is either not supervised or isn't up to par. As a result of this neglect, there is a lack of information regarding how they work, possible side effects, when they are not appropriate to use, and how they interact with traditional medications and functional foods. The usage of herbal treatments is still fraught with serious safety concerns. As a result, the appropriate regulatory agencies must take action to guarantee the security, excellence, and effectiveness of herbal remedies. The review emphasizes the serious safety concerns and issues connected to toxicity that are linked to herbal product use. To guarantee their reasonable and safe use in healthcare, it also highlights the difficulties of adequately monitoring the safety of these goods. References [1,23]

Conclusion:

Worldwide, digestive system disorders are a major cause of illness and death. Because people in rural areas are less likely to know how to properly clean themselves and avoid getting sick, the issue is much worse there. Medicinal plants are still used as a first aid remedy and illness preventative by people in low- and middle-income nations. Research into the biological activity of plant extracts with carminative, antispasmodic, intestinal transit-delaying, gut motility-affecting, adsorption-stimulating, or electrolyte secretion-reducing properties has corroborated the traditional use of the medicinal plant in the treatment of gastrointestinal system disorders. As long as some populations lack access to contemporary medical care, they will keep using traditional remedies for gastrointestinal issues. Validating traditional plant usage and discovering active components of extracts and other preparations are the primary goals of current scientific research of medicinal herbs. Toxic effects may occur in rare cases while using some herbal remedies or medicinal plant active components. Hence, traditional plant medicines must undergo ongoing research to determine their safety, effectiveness, and quality, and to build a scientific foundation for their use. The effectiveness of herbal medicines can only be shown with the help of well-designed clinical research. Traditional and herbal remedies for gastrointestinal problems have only been the subject of a small number of safety and tolerability studies, the results of which have shown that these treatments have few, if any, negative side effects. Recent research on traditional plant-based remedies has shown promising results, which bodes well for the future of digestive disease treatment options.

References:

1. Cheema, H. S., & Singh, M. P. (2021). The use of medicinal plants in digestive system related disorders—a systematic review. *Journal of Ayurvedic and Herbal Medicine*, *7*(3), 182–187.
2. Herbs for Healing. (n.d.). Classes of herbs for good digestive health. Retrieved from https://herbsforhealing.co.uk/classes-herbs-good-digestive-health/
3. Kmail, A. (2024, April 18). Mitigating digestive disorders: Action mechanisms of Mediterranean herbal active compounds. *Open Life Sciences*, *19*(1), 20220857.
4. Yao, Y., Habib, M., Bajwa, H. F., Qureshi, A., Fareed, R., Altaf, R., Ilyas, U., Duan, Y., & Abbas, M. (2022, October 5). Herbal therapies in gastrointestinal and hepatic disorders: An evidence-based clinical review. *Frontiers in Pharmacology*, *13*, 962095.
5. Czigle, S., Bittner Fialova, S., Tóth, J., Mučaji, P., & Nagy, M. (2022, April 30). Treatment of gastrointestinal disorders—Plants and potential mechanisms of action of their constituents. *Molecules*, *27*(9), 2881.
6. Bode, A. M., & Dong, Z. (2011, March 28). The amazing and mighty ginger. In *Herbal Medicine: Biomolecular and Clinical Aspects* (2nd ed.).
7. Mao, Q. Q., Xu, X. Y., Cao, S. Y., Gan, R. Y., Corke, H., Beta, T., & Li, H. B. (2019, May 30). Bioactive compounds and bioactivities of ginger (Zingiber officinale Roscoe). *Foods*, *8*(6), 185.

8. McKay, D. L., & Blumberg, J. B. (2006, August). A review of the bioactivity and potential health benefits of peppermint tea (Mentha piperita L.). *Phytotherapy Research*, *20*(8), 619–633.
9. Gholamipourfard, K., Salehi, M., & Banchio, E. (2021, September 1). Mentha piperita phytochemicals in agriculture, food industry and medicine: Features and applications. *South African Journal of Botany*, *141*, 183–195.
10. Dai, Y. L., Li, Y., Wang, Q., Niu, F. J., Li, K. W., Wang, Y. Y., Wang, J., Zhou, C. Z., & Gao, L. N. (2023, January). Chamomile: A review of its traditional uses, chemical constituents, pharmacological activities and quality control studies. *Molecules*, *28*(1), 133.
11. El Mihyaoui, A., Esteves da Silva, J. C., Charfi, S., Candela Castillo, M. E., Lamarti, A., & Arnao, M. B. (2022, March 25). Chamomile (Matricaria chamomilla L.): A review of ethnomedicinal use, phytochemistry and pharmacological uses. *Life*, *12*(4), 479.
12. Badgujar, S. B., Patel, V. V., & Bandivdekar, A. H. (2014). Foeniculum vulgare Mill: A review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. *BioMed Research International*, *2014*, 842674.
13. Rather, M. A., Dar, B. A., Sofi, S. N., Bhat, B. A., & Qurishi, M. A. (2016, November 1). Foeniculum vulgare: A comprehensive review of its traditional use, phytochemistry, pharmacology, and safety. *Arabian Journal of Chemistry*, *9*, S1574–S1583.
14. Noreen, S., Mubarik, F., Farooq, F., Khan, M., Khan, A. U., & Pane, Y. S. (2021, November 27). Medicinal uses of licorice (Glycyrrhiza glabra L.): A comprehensive review. *Open Access Macedonian Journal of Medical Sciences*, *9*(F), 668–675.
15. Hasan, M. K., Ara, I., Mondal, M. S., & Kabir, Y. (2021, June 1). Phytochemistry, pharmacological activity, and potential health benefits of Glycyrrhiza glabra. *Heliyon*, *7*(6), e07135.
16. Abdel-Latif, H. M., Shukry, M., Noreldin, A. E., Ahmed, H. A., El-Bahrawy, A., Ghetas, H. A., & Khalifa, E. (2023, January 15). Milk thistle (Silybum marianum) extract improves growth, immunity, serum biochemical indices, antioxidant state, hepatic histoarchitecture, and intestinal histomorphometry of striped catfish, Pangasianodon hypophthalmus. *Aquaculture*, *562*, 738761.
17. National Center for Complementary and Integrative Health. (n.d.). Milk thistle. Retrieved from https://www.nccih.nih.gov/health/milk-thistle
18. Healthline. (n.d.). Slippery elm bark. Retrieved from https://www.healthline.com/health/food-nutrition/slippery-elm-bark
19. Ried, K., Travica, N., Dorairaj, R., & Sali, A. (2020, April 1). Herbal formula improves upper and lower gastrointestinal symptoms and gut health in Australian adults with digestive disorders. *Nutrition Research*, *76*, 37–51.

20. Kim, Y. S., Kim, J. W., Ha, N. Y., Kim, J., & Ryu, H. S. (2020). Herbal therapies in functional gastrointestinal disorders: A narrative review and clinical implication. *Frontiers in Psychiatry*, *11*, 601.
21. Langmead, L., & Rampton, D. S. (2001). Review article: Herbal treatment in gastrointestinal and liver disease—benefits and dangers. *Alimentary Pharmacology & Therapeutics*, *15*(9), 1239–1252.
22. Quan, N. V., Dang Xuan, T., & Teschke, R. (2020). Potential hepatotoxins found in herbal medicinal products: A systematic review. *International Journal of Molecular Sciences*, *21*(14), 5011.
23. Ekor, M. (2014, January 10). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, *4*, 177.

PHYTOCHEMICAL AGENTS AS POTENTIAL THERAPEUTICS FOR BIOFILM INHIBITION: AN OVERVIEW

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Abstract:

Biofilms significantly contribute to antimicrobial resistance (AMR) by creating protective environments that enhance bacterial survival against antibiotics. These structured communities, encased in a self-produced extracellular matrix, can be 10 to 1,000 times more resistant than planktonic bacteria. Their resilience stems from physical barriers, altered metabolism, and genetic adaptations, making biofilm-associated infections particularly difficult to treat. Natural biofilm disruptors work at the molecular level through several mechanisms like inhibition of initial adhesion, inhibition of quorum sensing , disruption of EPS Matrix to prevent biofilm formation or disrupt existing biofilms. Natural agents offer structural and functional diversity compared to conventional antibiotics, showing promise in overcoming drug-resistant biofilms. Quorum quenching molecules and EPS-degrading enzymes from natural sources may also provide a novel strategy for biofilm destruction, particularly for species-specific targeting in medical settings.

Keywords: Antibiofilm Agent, Biofilms, Phytoconsistuents, Natural Biofilm Disruptors

Introduction:

Biofilms play a major role in antimicrobial resistance (AMR) by forming protective environments that allow bacteria to survive antibiotic treatments more effectively. These organized bacterial communities are embedded in a self-generated extracellular matrix and can be 10 to 1,000 times more resistant than individual, free-floating (planktonic) bacteria. A combination of physical barriers, metabolic changes, and genetic adaptations helps biofilms endure and thrive in chronic infections, making them especially challenging to treat. The development of a biofilm is a dynamic and multi-stage process, beginning when microorganisms attach to surfaces and gradually evolve into structured, resilient communities.[1,2]

Table 1: Stages of biofilm Formation

Initial Attachment	Planktonic bacteria encounter a surface and weakly adhere via physical forces	Reversible attachment - No structural changes in cells
Irreversible Attachment	Bacteria firmly attach using adhesins and start secreting EPS.	Irreversible binding - Start of EPS production - Gene expression changes
Maturation I	Microcolonies form; EPS production increases, creating a matrix.	Microcolony formation - Quorum sensing begins - Increased resistance
Maturation II	Biofilm develops complex 3D structures with nutrient/waste channels.	-Mature architecture - Differentiated cell types - Strong environmental resistance
Dispersion	Cells detach to spread and start new biofilms elsewhere	- Matrix degradation - Active/passive release - Cycle restarts

Contribution of biofilm in Chronic Infections

- **Physical Barrier from the Extracellular Matrix of biofilm in antibiotic resistance:**

The extracellular matrix (ECM) is a vital component of biofilms that plays a key role in antibiotic resistance by serving as a physical barrier. Biofilms consist of organized microbial communities surrounded by a self-produced matrix made up of polysaccharides, proteins, extracellular DNA (eDNA), and lipids. This matrix not only maintains the structural stability of the biofilm but also functions as a defensive layer that hinders the effective penetration of antibiotics.

- **Limited Antibiotic Penetration and creation of antibiotic gradients:**

The extracellular matrix (ECM) in biofilms restricts antibiotic penetration, creating sub-lethal drug exposure that allows bacteria to survive and adapt. Alongside the ECM, biofilms use other defense strategies like efflux pumps, persister cells, stress responses, and altered gene expression. Notable examples include *Pseudomonas aeruginosa* in cystic fibrosis and *Staphylococcus aureus* biofilms, both of which resist antibiotic treatment through ECM-related mechanisms. [3,4]

- **Protection of Dormant or Slow-Growing Cells:**

The matrix also restricts nutrient and oxygen flow, leading to the formation of metabolically inactive zones within the biofilm. These cells are less susceptible to antibiotics that target active cell processes (synthesis of cell membrane, protein formation).[5]

- **Matrix Composition-Specific Resistance:**

Matrix composition-specific resistance is influenced by different bacterial species, with Staphylococcus aureus biofilms, rich in polysaccharide intercellular adhesin (PIA), being a prime example. For example, the ECM in Staphylococcus aureus biofilms, rich in polysaccharide intercellular adhesin (PIA), can inhibit vancomycin diffusion. [6]

Genetic Adaptations in Biofilms Leading to Antibiotic Resistance

- **Differential Gene Expression**

Bacteria within biofilms undergo changes in gene expression that influence metabolism, stress adaptation, and resistance to antibiotics. For instance, efflux pump systems like mexAB-oprM in Pseudomonas aeruginosa are frequently upregulated, enabling cells to expel antibiotics more efficiently. [7] Additionally, genes responsible for producing antibiotic-degrading enzymes, such as beta-lactamases, can be activated or expressed at higher levels in response to the biofilm environment.

- **Stress-Induced Responses and Elevated Mutation Rates**

The harsh conditions inside biofilms—such as limited oxygen and nutrients—activate various stress response pathways, including the SOS response. This can lead to increased mutation rates, promoting the emergence of antibiotic-resistant variants.[8] Furthermore, oxidative stress within the biofilm can damage DNA, further driving genetic variation and resistance evolution.

- **Horizontal Gene Transfer (HGT)**

Biofilms provide an optimal setting for genetic exchange, facilitating the spread of resistance genes. Close cell proximity supports conjugation, while transformation involves uptake of extracellular DNA, and transduction enables gene transfer via bacteriophages. These mechanisms collectively drive the rapid dissemination of resistance traits within biofilm communities. [9]

- **Quorum Sensing and Regulation of Resistance**

A bacterial communication system known as quorum sensing (QS) is crucial for coordinating both the formation of biofilms and the activation of resistance strategies. In *Pseudomonas aeruginosa*, QS networks such as the Las and Rhl systems control the expression of genes linked to virulence and biofilm formation, thereby indirectly contributing to the bacteria's antibiotic resistance. [10]

Natural biofilm disruptors work at the molecular level through several mechanisms to prevent biofilm formation or disrupt existing biofilms. Here's a breakdown of their molecular actions

Molecular Mechanisms of Natural Biofilm Disruptors. [11,12]

Natural antibiofilm agents work through multiple mechanisms, including:

Antibiofilm action of Phytochemicals	Mechanism
Inhibition of Initial Adhesion	Prevent bacteria from attaching to surfaces, which is the first step in biofilm formation
Disruption of EPS Matrix	Degrades or alters the matrix to weaken the structural integrity of the biofilm.
Inhibition of Quorum Sensing (QS)	Blocks signaling molecules (e.g., AHLs in Gram-negative bacteria), preventing coordinated biofilm development.
Bactericidal/Bacteriostatic Effects Within Biofilm	Directly kill or inhibit the growth of bacteria within biofilms, which are often more resistant than planktonic cells.
Induction of Biofilm Dispersal	Stimulates bacteria to leave the biofilm and revert to planktonic (free-swimming) state, where they are more susceptible to antibiotics.

Phytochemicals as Biofilm agents:

Cumin Oil: it is reported that Cumin Oil can disrupt the structural integrity of oral biofilms by decreasing EPS production, thereby hindering the development biofilms. They can suppress bacterial growth, limit acid production, and inhibit glucosyltransferase activity, which reduces glucan formation in the EPS. This, in turn, prevents bacterial adhesion to teeth and weakens biofilm stability. Also, it has been reported that Cumin oil suppress biofilm development and the reduction of certain virulence traits regulated by the quorum sensing system in *Chromobacterium violaceum* and *Pseudomonas aeruginosa* strains. [13,14]

Epigallocatechin gallate (EGCG): Epigallocatechin gallate (EGCG), a polyphenol found in green tea, exhibits significant antibiofilm activity against diverse bacterial pathogens through multiple mechanisms. It can cleave peptidoglycan in the biofilm matrix. It reduces biofilm biomass of *Vibrio mimicus* by 80% at 256 μg/ml via membrane damage and reactive oxygen species. Also, reduced bacterial swimming and autoaggregation, critical for biofilm formation. [15,16]

Curcumin: The application of curcumin compound at 0.25% b/v results in noticeable shrinkage, disruption of the EPS matrix, and cell lysis. This effect is likely due to increased cell membrane permeability, which facilitates the breakdown of mature biofilms by targeting and destroying the cells embedded within them. Additionally, noted that curcumin can inhibit yeast cell adhesion. Curcumin has also been shown to disrupt the polymicrobial EPS biofilm matrix on catheters and reduce the density of biofilm-associated cells.[17] Furthermore, it significantly decreases the biomass of *Candida albicans* biofilms also, it influences gene expression affecting biofilm formation. Curcumin exhibits antibacterial and antibiofilm effects against a *P. aeruginosa* clinical isolate when used at 10-30 µg/ml and 1.5-3 µg/ml, respectively.[18]

Quercetin: Quercetin's antibiofilm concentrations typically range from about 25 µg/ml to 500 µg/ml depending on the bacterial strain and whether it is used alone or synergistically with antibiotics. Quercetin act as antibiofilm component by decreasing the hydrophobicity of bacterial cells, quercetin hampers their initial adhesion to surfaces—a critical early step in biofilm formation. In *Staphylococcus epidermidis*, quercetin suppresses the production of polysaccharide intercellulalar adhesion and disrupts quorum sensing systems that bacteria use to coordinate biofilm development and regulate virulence factor expression, ultimately reducing biofilm resilience.[19,20] An gene level it binds to key biofilm-related proteins such as *icaB* and *icaC* in *Staphylococcus aureus*, interfering with the formation of the biofilm matrix.[21]

Berberine: Found in Oregon grape root and goldenseal, it inhibits bacterial growth and disrupts biofilms. Berberine's antibiofilm efficacy is achieved at sub-MIC (≤128 µg/mL) levels for many pathogens, primarily by disrupting biofilm structural components and enhancing antibiotic penetration. Its ability to target virulence factors (e.g., PSMs) without direct bactericidal action makes it a promising adjunct therapy against drug-resistant biofilms. [22-24]

Apigenin: Apigenin, a naturally occurring flavonoid, exhibits strong antibiofilm activity through multiple mechanisms. It interferes with the early stages of biofilm formation by preventing bacterial adhesion and surface colonization. A key mode of action is its ability to disrupt quorum sensing (QS), particularly in *Pseudomonas aeruginosa*, where it targets QS-regulated genes and signaling pathways such as the *las* and *rhl* systems. This disruption reduces both biofilm maturation and the production of virulence factors. Additionally, apigenin significantly lowers the synthesis of extracellular polymeric substances (EPS), which are essential for maintaining biofilm structure and protection. In *Staphylococcus aureus*, apigenin downregulates biofilm-associated genes including *icaA*, *icaD*, and *sarA*, which are involved in the production of polysaccharide intercellular adhesin (PIA), further weakening the biofilm matrix. At higher concentrations, apigenin can also induce membrane damage and generate reactive oxygen species (ROS), leading to oxidative stress and disruption of bacterial cell integrity. In vitro studies have demonstrated that apigenin inhibits biofilm formation in *S. aureus* at concentrations ranging from 32 to 128 µg/mL, in *P. aeruginosa* at 50 to 200 µg/mL, and in *Escherichia coli* and other species at 64 to 256 µg/mL, typically in a dose-dependent manner. These findings highlight apigenin's potential as a multifunctional antibiofilm agent. [25,26]

Oregano Essential oil: Oregano essential oil (OEO), rich in active compounds like carvacrol and thymol, has demonstrated strong antibiofilm activity against various bacterial pathogens. It disrupts bacterial cell membranes, interferes with quorum sensing, and reduces extracellular polymeric substance (EPS) production, all of which weaken biofilm formation and maintenance. These combined effects make OEO an effective natural agent against biofilm-forming bacteria.32 Effective at low concentrations, OEO shows MIC values ranging from 0.02% to 0.19% v/v depending on the species. It inhibits biofilm formation in *S. aureus* and *E. coli* at 0.19% v/v. It also eradicates biofilms of *Pseudomonas aeruginosa* and MRSA at 1.0 mg/mL and 0.4 mg/mL, respectively. [27,28]

Eugenol: Eugenol, a phenolic compound predominantly found in clove oil, exhibits significant antibiofilm activity against various bacterial pathogens, including *Staphylococcus aureus, Helicobacter pylori* (25–50 µg/mL) , *Vibrio parahaemolyticus* (0.1% to 0.6%), and *Escherichia coli* O157:H7 (0.005% (v/v). Eugenol compromises bacterial cell membranes by increasing their permeability, leading to leakage of intracellular contents and cell death .It interferes with quorum sensing (QS) systems and decreases the production of extracellular polymeric substances (EPS), weakening the structural integrity of biofilms. [29,30] It disrupts cell-to-cell connections within biofilms, and downregulates genes (fimA, hagA, hagB, rgpA, rgpB, kgp) associated with biofilm formation and virulence, further inhibiting biofilm development. [31,32]

Naringin: Naringin, a citrus flavonoids typically effective within the range of 50–200 µg/mL. At these concentrations, naringin does not inhibit planktonic bacterial proliferation but specifically targets biofilm formation and quorum sensing pathways. For instance, in Pseudomonas aeruginosa, treatment with 100 µg/mL of naringin resulted in a reduction of biofilm biomass by up to 60%. [33] Similarly, in Aeromonas hydrophila, quorum sensing gene expression was significantly downregulated at 75 µg/mL, indicating a strong interference with bacterial communication systems essential for virulence and colonization. It disrupts quorum sensing by blocking LuxR-type regulators and reducing virulence gene expression. It weakens biofilm structure by decreasing exopolysaccharide production (40–60%) and interfering with alginate synthesis. Additionally, naringin impairs bacterial adhesion and mobility by lowering cell surface hydrophobicity and inhibiting swarming and swimming motility. [34,35]

Gallic Acid: Gallic acid exhibits potent antibiofilm activity through multiple mechanisms. It disrupts biofilm integrity in Staphylococcus aureus by downregulating key genes like icaAD and sarA, thereby inhibiting polysaccharide intercellular adhesion (PIA) synthesis. In Pseudomonas aeruginosa, it interferes with quorum sensing by reducing acyl homoserine lactones (AHLs) and altering membrane permeability. Additionally, it compromises bacterial membranes by inducing calcium and potassium ion leakage and chelating essential metals such as iron, impairing bacterial metabolism. GA also prevents bacterial adhesion by modifying cell surface hydrophobicity and effectively inhibits early biofilm formation in Escherichia coli and Streptococcus mutans at 8 mg/mL. Effective concentrations vary by strain, with a minimum inhibitory concentration (MIC) of 32 µg/mL against planktonic MRSA and 8 µg/mL for biofilm inhibition. In P. aeruginosa, mature biofilms are significantly reduced at 5 mg/mL. Gallic acid also shows synergy with compounds like carvacrol and β-lactam antibiotics, enhancing their efficacy.[36,37] Notably, Gallic acid demonstrates dual strain-specific effects—exhibiting antibiofilm activity against pathogens like S. aureus and P. aeruginosa, while promoting exopolysaccharide production in beneficial strains like Streptococcus thermophilus. [38]

Conclusion:

The rise of biofilm-based infections and their antimicrobial resistance is a major concern in medicine and human health. Biofilms contribute to chronic infections in areas like bones, dental implants, eye lenses, and breast implants, where infection sites are difficult to detect. Natural agents offer structural and functional diversity compared to conventional antibiotics, showing promise in overcoming drug-resistant biofilms.

Despite promising results in laboratory settings, no FDA-approved drugs have emerged from natural anti-biofilm agents, largely due to challenges in human efficacy during clinical trials, particularly in phases II and III. Combining natural agents with antibiotics may offer a solution, and future research is needed to optimize such combination therapies. Quorum quenching molecules and EPS-degrading enzymes from natural sources may also provide a novel strategy for biofilm destruction, particularly for species-specific targeting in medical settings.

References:

1. Nadar S, Khan T, Patching SG, Omri A. Development of Antibiofilm Therapeutics Strategies to Overcome Antimicrobial Drug Resistance. Microorganisms. 2022 Jan 27;10(2):303. doi: 10.3390/microorganisms10020303. PMID: 35208758; PMCID: PMC8879831.
2. Hussaini IM, Oyewole OA, Sulaiman MA, Dabban AI, Sulaiman AN, Tarek R. Microbial anti-biofilms: types and mechanism of action. Research in Microbiology. 2024 Mar 1;175(3):104111.
3. Tseng BS, Zhang W, Harrison JJ, Quach TP, Song JL, Penterman J et al. The extracellular matrix protects P seudomonas aeruginosa biofilms by limiting the penetration of tobramycin. Environmental microbiology. 2013 Oct;15(10):2865-78.
4. Stewart PS. Mechanisms of antibiotic resistance in bacterial biofilms. International Journal of medical microbiology. 2002 Jan 1;292(2):107-13.
5. Mah TF, OToole GA. Mechanisms of biofilm resistance to antimicrobial agents. Trends in microbiology. 2001 Jan 1;9(1):34-9.
6. Otto M. Staphylococcal biofilms. Bacterial biofilms. 2008 Jan 1:207-28.
7. De Kievit TR, Iglewski BH. Bacterial quorum sensing in pathogenic relationships. Infection and immunity. 2000 Sep 1;68(9):4839-49.

8. Boles BR, Singh PK. Endogenous oxidative stress produces diversity and adaptability in biofilm communities. Proceedings of the National Academy of Sciences. 2008 Aug 26;105(34):12503-8.
9. Madsen JS, Burmølle M, Hansen LH, Sørensen SJ. The interconnection between biofilm formation and horizontal gene transfer. FEMS Immunology & Medical Microbiology. 2012 Jul 1;65(2):183-95.
10. Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP. The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science. 1998 Apr 10;280(5361):295-8.
11. Asadi A, Razavi S, Talebi M, Gholami M. A review on anti-adhesion therapies of bacterial diseases. Infection. 2019;47:13–23.
12. Ghilini F, Pissinis DE, Minan A, Schilardi PL, Diaz C. How functionalized surfaces can inhibit bacterial adhesion and viability. ACS Biomater. Sci. Eng. 2019;5:4920–4936.
13. Jo A, Kim HE. Antibacterial Effects of Black Cumin Seed Oil on Oral Microcosm Biofilms. Microorganisms. 2024 Oct 20;12(10):2098.
14. Ghannay S, Aouadi K, Kadri A, Snoussi M. In Vitro and In Silico Screening of Anti-Vibrio spp., Antibiofilm, Antioxidant and Anti-Quorum Sensing Activities of Cuminum cyminum L. Volatile Oil. Plants (Basel). 2022 Aug 29;11(17):2236.
15. Knidel C, Pereira MF, Barcelos DHF, Gomes DCO, Guimaraes MCC, Schuenck RP. Epigallocatechin gallate has antibacterial and antibiofilm activity in methicillin resistant and susceptible Staphylococcus aureus of different lineages in non-cytotoxic concentrations. Nat Prod Res. 2021 Nov;35(22):4643-4647.
16. Xue P, Sang R, Li N, Du S, Kong X, Tai M, Jiang Z, Chen Y. A new approach to overcoming antibiotic-resistant bacteria: Traditional Chinese medicine therapy based on the gut microbiota. Front Cell Infect Microbiol. 2023 Apr 6;13:1119037.
17. Memariani H, Memariani M, Ghasemian A. An overview on anti-biofilm properties of quercetin against bacterial pathogens. World J Microbiol Biotechnol. 2019 Sep 6;35(9):143
18. Mu Y, Zeng H, Chen W. Quercetin inhibits biofilm formation by decreasing the production of EPS and altering the composition of EPS in Staphylococcus epidermidis. Frontiers in Microbiology. 2021 Mar 4;12:631058.
19. Shastri T, Binsuwaidan R, Siddiqui AJ, Badraoui R, Jahan S, et al. Quercetin Exhibits Broad-Spectrum Antibiofilm and Antiquorum Sensing Activities Against Gram-Negative Bacteria: In Vitro and In Silico Investigation Targeting Antimicrobial Therapy. Canadian Journal of Infectious Diseases and Medical Microbiology. 2025;2025(1):2333207.

20. Hamzah H, Hertiani T, Pratiwi SU, Nuryastuti T, Murti YB. The biofilm inhibition and eradication activity of curcumin againts polymicrobial biofilm. InBIO Web of Conferences 2020;28: 04001.
21. Moshe M, Lellouche J, Banin E. Curcumin: a natural antibiofilm agent. InScience and Technology against microbial pathogens: research, development and evaluation 2011 (pp. 89-93).
22. Chu M, Zhang Mb, Liu Yc. et al. Role of Berberine in the Treatment of Methicillin-Resistant Staphylococcus aureus Infections. Sci Rep 6, 24748 (2016). https://doi.org/10.1038/srep24748
23. Lin Q, Li Y, Sheng M, Xu J, Xu X, Lee J, et al. Antibiofilm effects of berberine-loaded chitosan nanoparticles against Candida albicans biofilm. LWT. 2023 Jan 1;173:114237.
24. Sun L, Yu Q, Peng F, Sun C, Wang D, Pu L, et al. The antibacterial activity of berberine against Cutibacterium acnes: its therapeutic potential in inflammatory acne. Frontiers in Microbiology. 2024 Jan 5;14:1276383.
25. Pei ZJ, Li C, Dai W, Lou Z, Sun X, Wang H., et al. The Anti-Biofilm Activity and Mechanism of Apigenin-7-O-Glucoside Against Staphylococcus aureus and Escherichia coli. Infect Drug Resist. 2023 Apr 11;16:2129-2140. doi: 10.2147/IDR.S387157. PMID: 37070126; PMCID: PMC10105580.
26. Fydrych D, Jeziurska J, Wełna J, Kwiecińska-Piróg J. Potential Use of Selected Natural Compounds with Anti-Biofilm Activity. International Journal of Molecular Sciences. 2025 Jan 13;26(2):607.
27. Cai T, Li Z, Guo P, Guo J, Wang R, Guo D, et al. Antimicrobial and antibiofilm efficacy and mechanism of oregano essential oil against Shigella flexneri. Foodborne Pathogens and Disease. 2023 Jun 1;20(6):209-21.
28. Zhan X, Tan Y, Lv Y, Fang J, Zhou Y, Gao X, et al. The antimicrobial and antibiofilm activity of oregano essential oil against Enterococcus faecalis and its application in chicken breast. Foods. 2022 Aug 1;11(15):2296.
29. Tahric A, Kolic H, Lavic A, Latinovic D, Pramenkovic E. Antibacterial Activity of Oregano Essential Oil and Its Effect on Biofilm Formation. J. Pure Appl. Microbiol. 2023 Jun 1;17:1205-13.
30. Ribeiro TA, Dos Santos GA, Dos Santos CT, Soares DC, Saraiva MF, Leal DH, et al. Eugenol as a promising antibiofilm and anti-quorum sensing agent: a systematic review. Microbial Pathogenesis. 2024 Sep 16:106937.
31. Zhang Y, Wang Y, Zhu X, Cao P, Wei S, Lu Y. Antibacterial and antibiofilm activities of eugenol from essential oil of Syzygium aromaticum (L.) Merr. & LM Perry (clove) leaf

against periodontal pathogen Porphyromonas gingivalis. Microbial pathogenesis. 2017 Dec 1;113:396-402.

32. Lahiri D, Nag M, Dutta B, Dey S, Mukherjee D et al. Antibiofilm and anti-quorum sensing activities of eugenol and linalool from Ocimum tenuiflorum against Pseudomonas aeruginosa biofilm. Journal of Applied Microbiology. 2021 Dec 1;131(6):2821-37.
33. Husain FM, Perveen K, Qais FA, Ahmad I, Alfarhan AH, El-Sheikh MA. Naringin inhibits the biofilms of metallo-β-lactamases (MβLs) producing Pseudomonas species isolated from camel meat. Saudi J Biol Sci. 2021 Jan;28(1):333-341.
34. Wen QH, Wang R, Zhao SQ, Chen BR, Zeng XA. Inhibition of Biofilm Formation of Foodborne Staphylococcus aureus by the Citrus Flavonoid Naringenin. Foods. 2021 Oct 28;10(11):2614.
35. Srinivasan R, Devi KR, Santhakumari S, Kannappan A, Chen X, Ravi AV, Lin X. Anti-quorum sensing and protective efficacies of naringin against Aeromonas hydrophila infection in Danio rerio. Frontiers in Microbiology. 2020 ;11:600622.
36. Borges, A., Saavedra, M. J., & Simoes, M. (2012). The activity of ferulic and gallic acids in biofilm prevention and control of pathogenic bacteria. Biofouling. 2012; 28(7):755–767.
37. Rajan PP, Kumar P, Mini M, Jayakumar D, Vaikkathillam P, Asha S, et al. Antibiofilm potential of gallic acid against Klebsiella pneumoniae and Enterobacter hormaechei: in-vitro and in-silico analysis. Biofouling. 2023;39:948-961.
38. Badra B, Soungalo DM, Hafidha KH, Rimmibtiri S, Muhammad AS, Aly SA. Antimicrobial, antibiofilm, and probiofilm effects of gallic acid on exopolysaccharide-dependent and-independent biofilm of model strains Streptococcus thermophilus Cnrz 447 and Staphylococcus aureus Atcc 43300. Journal of microbiology, biotechnology and food sciences. 2022 Feb 8:e1781-.

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