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TRENDS IN ETHNOBOTANY AND PHYTOCHEMISTRY RESEARCH

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Trends in Ethnobotany and Phytochemistry Research

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PREFACE

The intricate relationship between humans and plants has been the cornerstone of traditional knowledge systems for millennia. Ethnobotany and phytochemistry, as complementary disciplines, continue to unravel the wisdom of indigenous practices and the chemical intricacies of plant-derived bioactive compounds. The book *"Trends in Ethnobotany and Phytochemistry Research"* brings together recent insights, discoveries, and methodologies that bridge traditional heritage with modern scientific innovation.

This volume highlights the dynamic evolution of ethnobotanical research, emphasizing how communities across the globe preserve and utilize plant resources for food, medicine, and cultural well-being. By integrating traditional ecological knowledge with advanced analytical tools, researchers today are rediscovering the immense therapeutic and economic potential of plant biodiversity. The field of phytochemistry has also advanced remarkably, offering precise techniques for isolating, characterizing, and evaluating phytoconstituents that underpin pharmacological activity and drug discovery.

The chapters in this book collectively explore various dimensions of plant science—from ethnobotanical surveys and ethnopharmacological validation to chemical profiling, bioassay-guided fractionation, and the exploration of novel phytochemicals. This compilation aims to promote interdisciplinary dialogue among botanists, pharmacognosists, chemists, ecologists, and healthcare professionals who share a common goal of sustainable utilization and conservation of natural resources. It also sheds light on ethical considerations, intellectual property rights, and the need to protect traditional knowledge systems from exploitation while fostering equitable benefit-sharing.

We hope this book serves as a valuable reference for academicians, researchers, and students engaged in the fields of plant science, pharmacognosy, and natural product chemistry. It is our belief that the growing confluence of ethnobotany and phytochemistry will continue to inspire new discoveries and strengthen the bridge between tradition and technology for the betterment of human health and environmental sustainability.

- Editors

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ETHNOBOTANICAL PERSPECTIVES ON HEMP IN UTTARAKHAND

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Abstract:

Hemp holds a significant place among the local communities of Uttarakhand through their traditional knowledge systems. This chapter discusses about the ethnobotanical perspectives on hemp by emphasizing its indigenous ecological knowledge, cultural symbolism, and sustainable resource use. The ethnobotanical insights of local people on hemp reveal how the indigenous classification systems, seed selection, and resource management practices are grounded in centuries of experiential learning and collective wisdom that are transmitted through oral traditions. The chapter also delves into the intricate and multifaceted relationship among hemp plant, local peoples, and their environment through the concept of multispecies ethnography. It highlights how the local communities have long relied on hemp for food, oil, fibre, and ethnomedicine. The shifts in legal frameworks, market dynamics, and modernization which have altered the community perceptions and practices related to hemp have also been discussed in this chapter. The chapter concludes with emphasizing the need for documentation and revitalization of traditional ethnobotanical knowledge of hemp in order to promote ecological resilience in Uttarakhand.

Keywords: Ethnobotanical, Hemp, Indigenous, Multispecies, Sustainable

Introduction:

Hemp cultivation is being done since generations by the local communities in Uttarakhand, but in recent years, cultivation of hemp crops has become apparent as a significant socio-economic and ecological phenomenon in Uttarakhand. In the context of a plant like hemp that has diverse applications and cultural significance, multispecies ethnography becomes a conduit for exploring the co-existence and collaboration between hemp and humans. Therefore, this chapter encompasses the concept of multispecies ethnography as it offers a framework that goes beyond human-centric perspectives, enabling a broader comprehension of intricate relationships among human beings, hemp plants and various other species in their shared environments. The cultural significance of hemp is multifaceted that includes historical practices, spiritual associations, economic contributions and contemporary usages. Ranging from the traditional use of hemp in textiles, ropes, paper, medicines, etc. to its ritualistic use in various religious ceremonies and spiritual practices, there is a cultural connection between hemp and human beings. The role of hemp plants in shaping human-plant affinity and co-becoming in Uttarakhand can be explored through the concept of multispecies ethnography.

Socio-Cultural Knowledge on Hemp:

Hemp has been associated with the daily lives of the local communities of Uttarakhand since ancient times in their day-to-day food practices, farming activities, leisure activities, spiritual activities, etc. There were also some social complexities generated by the locals of Uttarakhand themselves, which were based on the cultivation of hemp, manufacturing as well as trading of hemp and hemp-based products. In Uttarakhand, human society and culture have advanced significantly from treating hemp as a curse in antiquity to using it as a revered and life-saving crop now. The emotional connection between hemp and humans that is evocative of parental affection and caring has progressively grown throughout time. Due to its nutritional value and ability to keep the body warm throughout the bitterly cold months, hempseeds were widely used by the locals to make gravy for practically any recipe at any time of the year. There was a saying used by the local pahadi women during cooking- '*Bhang daal la ya bhuttan*', which represents the traditional practice of asking family members before preparing any dish, whether they would like to have bhang or ghee in their food. No more oil needs to be added to the dish if the family members respond that they would prefer to have bhang in their food. To improve the dish's thickness, nutritional value, and flavor, the hemp seeds were crushed into a paste, which was then utilized as the gravy. The gravy was made over charcoal to add a deep flavor after the hemp seeds were mashed into a very fine paste. In the past, local women produced and stored hempseed salt all year long. We appreciated the hempseed salt by using it as a condiment to sprinkle over cucumber and fruits. There is also a song in Uttarakhand which is related to hempseed salt: "*Haaye kakadi jhil maa, loon piso sil maa*, *Mohana tu basi ge che myara dil maa*". The meanings of the words used in this song are as follows: *Kakadi*- cucumber; *Loon*- salt; *Sil*- mortar and pastel; *Mohana*- a name of boy; *Myara*- my; *Dil*-heart.

Earlier the hemp fibre was used only to make clothes and those clothes were used by the locals. The clothes made from hemp fibre were known as '*gareebon ke kapde'* (clothes for the poor), as of the lower sections of the society used to make clothes out of hemp fibres, and those clothes were cheaper for them. The inhabitants of Rath, a little-known neighbourhood in Garhwal, are the Rathis, who were mostly traditional hemp growers in Uttarakhand. These manufacturers controlled the way of life and social status of a wealthy group in pre-colonial Uttarakhand because of their monopoly in the hempen (hemp clothing) sector. However, as the British introduced cheap, stylish machine-made clothing, the demand for hempen cloth declined, and the socioeconomic standing of the hemp farmers suffered (Joshi, 2017). Without regard to social standing, the bhang-producing community divided itself into three separate caste groups based on their individual financial resources (Atkinson, 1882) .i.e. manufacturers, cultivators, and traders. This demonstrates unequivocally that social prejudices were not caused by the hemp plant; rather, it was humans who added societal complexity in the name of hemp. In the Garhwal region, the Pabila were considered the caste that spun hemp and practiced the unique craft of weaving hemp fibres into fabric. Communities in Kuthaliya Bora also used to produce goods manufactured from hemp, including as beds and tiny bags, as well as hemp fibre rope (Fig.1) that was used to tether animals and secure fuelwood. Similarly, it was believed that the Bhotia or Bhotiya tribes of Uttarakhand were the ones who woven hemp.

Traditional uses of hemp:

Hemp is known by its local name 'bhang' in Uttarakhand, but in some places of Uttarakhand, it is referred by other names such as *Ganra bhang, Van bhang, Junglee bhang*, etc. The relationship between hemp plants and human cultures is not limited to the use of hemp plants only for food, clothing and shelter but also includes their use for religious ceremonies, and health care, as follows:

a) For food purposes-

During the summer, hemp seeds that have been soaked overnight are ground into a thin paste or powder and combined with milk, water, or yoghurt to make *bhang sharbat* or *bhang thandai*. This beverage helps to hydrate the body and relieve heat. In the chilly winter months, hemp seeds are roasted and consumed as a snack or mixed with dry fruits and jaggery to create energy-dense, body-warming hempseed ladoos. In addition to thickening the gravy and giving the body warmth and nutrients in colder months, hemp seed paste is frequently added to a variety of vegetable curries in the form of hemp seed milk. Additionally, during the winter, hempseed oil (Fig.3) is used in cooking to increase blood circulation and provide warmth to the body. Some of the traditional hemp recipes made by the locals are *bhang chutney* (Fig.4)*, bhang ke pakore, gaderi bhang ki sabji, bhuna bhang, bhangeera samosa, bhang sarson ka saag, bhang jholi, moli thechwani,* etc. The pahadi *nimbu saan*, a traditional Uttarakhand winter delicacy made by combining hill lemons, hemp seeds, salt, yoghurt, coriander leaves, chillies, and a few seasoning spices, is enjoyed by the locals, especially the women, at noon in the winter months when they congregate in each other's homes, terraces, or fields. The *nimbu saan* is not only flavorful but also high in vitamin C, which boosts immunity against many wintertime illnesses.

b) For medicinal purposes-

In Uttarakhand, local communities utilise hemp to treat a wide range of illnesses after it has been purified. Hemp plant parts are used to cure a variety of conditions, including fever, inflammation, coughing, skin problems, headaches, piles, and diarrhoea. In addition to humans, animals in Uttarakhand also use cannabis as a medicine. Domestic animals, including cows, buffaloes, and others, have their wounds and joint troubles treated with a paste made from hemp leaves.

c) For material purposes-

Many local peoples in Uttarakhand have been processing hemp fibres into hemp garments for generations. This is a labour-intensive technique that involves multiple steps. When fully grown hemp plants are harvested, the stalks are dried and then soaked in water to soften the fibres, a procedure known as retting. The fibres are next straightened and contaminants are eliminated by scutching and hackling. The fibres are then broken up into clumps and prepared for spinning by hand carders, a process known as carding. In order to spin the fibres into hemp textiles, they are lastly pulled out and slightly twisted (Fig.2). Through this process, the textile workers create beautiful products such as scarves, shawls, rugs, and sarees made of hemp fibres.

d) For religious purposes-

In Uttarakhand, hemp has long been connected to a number of spiritual and symbolic values among the native populations. The locals have been consuming *bhang* for generations during their many festivities, particularly during Maha Shivratri and Holi. Hemp-infused beverages are consumed by devotees during the Maha Shivratri celebration that are commonly known as *bhang*, in the form of an offering to Lord Shiva and to facilitate a deeper connection with the divine. Likewise, during the festival of Holi, cannabis-infused drink commonly called *thandai*, and cannabis-infused sweets such as *gujiya* are shared among the local people as a way to enhance the festive spirit and foster a sense of joy and camaraderie. In Uttarakhand, however, no one used to worship Lord Shiva in their homes by offering bhang. Hemp branches are burned in the sacred fire in a number of the villages of Garhwal and Kumaon in order to fend off evil spirits and cleanse the area. It is thought that burning hemp releases a fragrant smoke that purifies the air and chases away any negative energy.

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Figure 1: Hemp rope Figure 2: Hemp yarn spinning

Figure 3: Hempseed oil Figure 4: Hempseed chutney

Interaction between Hemp Plant and Human

There exists a multifaceted interconnectedness between hemp plants and the daily lives of the local people of Uttarakhand. The hemp plant's life cycle is not merely an agricultural one; it is also a cultural rhythm that influences local populations' daily routines, social contacts, and activities. Its life cycle demonstrates a remarkable sensitivity to the seasonal demands of the people as well as the current environmental conditions. The hemp crop is grown in April and May, which is a perfect time to plant seeds because of the rising temperatures and more sunlight, which promotes the best germination. Since hemp seeds don't require a lot of water to germinate, growers won't have to worry as much about summer water shortages. The hemp plants then mature into their vegetative phase during the monsoon season, which lasts from July to August. Because there is less need for further crop irrigation at this time of heavy rainfall, manpower needs are reduced and water resources are preserved. Usually, the harvest takes place in October through November, during the winter season. This timing is ideal for meeting the community's seasonal needs. During the colder months, the carefully gathered harvested seeds are used to make a range of winter treats that offer much-needed warmth and nourishment. At the same time, the remaining dried stalks are immediately useful as an easily accessible winter fire fuel supply, offering warmth and a place to socialise. A sustainable and mutually beneficial relationship is exemplified by the harmonious interaction between the lifetime of the hemp plant and the seasonal needs of the local community. The consumption of hemp seeds varies dramatically with the seasons in Uttarakhand's hilly environment, where the weather varies greatly from season to season. Throughout the year, the natives eat hemp seeds in a variety of dishes, but in Uttarakhand, the use of hemp seeds rises in the winter. Due to its multitude of applications, hemp can be considered 'immortal' plant since, even after harvest, its life cycle continues to have an impact on humankind and promote sustainable development and development. The people use the hemp stalks as a valuable fuel source rather than discarding them as garbage.

Hemp farming in Uttarakhand is more than just an agricultural activity; it is intricately woven into the social and cultural fabric of the local populace. Locals have created creative ways to safeguard hemp plants in response to possible threats from law enforcement, as demonstrated during the field study. In order to protect the hemp plants from the prying eyes of law enforcement, residents frequently grow hemp in isolated, high-altitude areas that are only reachable by hiking. Furthermore, by carefully cultivating creepers around their hemp crops, the natives carefully conceal them. This sense of protection reflects the concern for the well-being of their 'hemp family', mirroring the protective instincts of parents for their children. Their evident worry of authorities damaging their crops is a reflection of the concern parents feel when they think that their loved ones might suffer harm. People in the area tightly control access to their hemp fields, just like parents are wary of strangers approaching their kids. They show their plants the same amount of love and care that they would give to their own family members by protecting them from outside threats like insects, pests, and even parrots. Despite the negative connotations associated with hemp, its production continues for centuries due to a strong emotional bond that goes beyond legal constraints.

Existing Social Constraints on Hemp:

Even while hemp production has great social, cultural, traditional, and religious value in Uttarakhand's communities, it is currently stigmatised by society and subject to legal restrictions.

a) Law enforcement officers' frequent raids and crop devastation have left farmers feeling extremely afraid and insecure financially. This on-going risk of loss has turned hemp farming from a beloved custom into a taxing undertaking, that result in large financial losses of hemp cultivators.

b) Further exacerbating the stigma attached to hemp production is the public humiliation brought on by these raids, which includes penalties and social exclusion. Due to their fear of being viewed as addicts, growers become socially isolated and their neighbours, especially those with children, avoid their homes. This social exclusion results from worries that youngsters may be exposed to hemp at crucial junctures in their development, which could affect their perspectives and raise their chance of substance dependency. The hemp farmers frequently feel pressured to hide their operations from their neighbours out of concern for criticism and possible social rejection. This stigma encompasses even non-narcotic uses of hemp, like traditional handicrafts, textiles, and medical treatments, and goes beyond the act of cultivating itself.

Additionally, the stigma associated with hemp farming has had a major effect on farmers' mental and emotional health. A persistent fear of raids, the possibility of financial loss, and social rejection can cause constant and prolonged anxiety, sadness, and a generalised lack of hope. These local communities may experience more difficulties as a result of this psychological anguish, which can negatively impact both individual and communal well-being.

Unequal Distribution of Hemp Products among Locals:

The cost and benefits of every product become more considerable when they reach to people with more purchasing power in comparison to the cost and benefits of the same products if they reach to people with less purchasing power and this statement seems to be pertinent for all hemp-based products and activities in Uttarakhand. The workers in factories of hemp-based products state that, the business persons and factory owners at first collect the traditionally grown hemp seeds from local fields of the nearby villages and provide them with price lower than the market and there are also cases of zero payment to them. The factory owners state that the women working there do not feel burdened and enjoy their work, while the fact being the women workers there mostly have to do overtime which affects their daily life and household chores as well. The workers are paid less in comparison to their labour and even don’t get paid regularly. Women working there state that it is only the reason of financial condition of their families and lack of other job opportunities nearby, which forces them to get to work there from morning to evening and sometimes overtime with low and irregular income. And when the manufacture of the products is less there due to off seasons or some other reasons, many women are told to take off for few months which in turn impact the financial and job security of those women. It is obvious that all organic products are expensive because of substituting intensive labour and time for chemicals, lower yields, demands overwhelming the supply, high cost of organic manure and fertilizers, organic certification, etc. which all together increase the cost of production of organic products in comparison to the conventional products. Likewise, the hemp seeds are collected from the local villages and processed in this factory by the local workers, but the products are too high-priced to be used by the local peoples, and even the workers working there since years said that they have never used the products. Most of the raw materials are collected from the local areas and most of the workers are women from same or nearby villages. In spite of the increasing demand for eco-friendly clothing products, women working in the handlooms and local peoples having less purchasing power are unable to buy and use those hemp fabrics which come with an expensive price tag.

Conclusion:

A multifaceted strategy including legislative reforms, public education campaigns, community-based initiatives, and a focus on addressing the underlying economic and social causes of substance abuse is needed to address the legal restrictions and social stigma surrounding hemp cultivation in Uttarakhand. By putting these tactics into reality, it will be feasible to bring back the social and cultural significance of hemp in these communities, resuscitate traditional hemp production methods, and build a more sustainable and prosperous future for everybody. In the end, addressing the underlying reasons of substance misuse in these communities is imperative. This necessitates a multidimensional approach that includes access to high-quality education, mental health treatment, and economic prospects. By addressing these underlying issues and creating a more supportive and resilient environment for their young people, communities can reduce their vulnerability to substance misuse. To address these complex issues, a multi-stakeholder forum should be established, involving scientists, entrepreneurs, enforcement agencies, and policymakers.

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COSMETIC APPLICATION OF PHYTOCHEMICALS

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1. Introduction:

Medicinal herbs have been used for decades to treat skin lesions, healing wounds, burns, and skin problems. Natural medicine is considered plant polymers and used as regenerative medicine for dressing wound. The use of plants for beauty and skincare from dates to ancient civilizations such as Egypt, India, China, and Greece. Natural products derived from plants, known as phytochemicals, are increasingly used in modern cosmetic formulations due to their safety, efficacy, and biodegradability. Phytochemicals are used in cosmetics for their antioxidant, anti-inflammatory, and photoprotective properties, helping to mitigate skin aging, repair damage, and treat various skin conditions. They can be used to increase skin elasticity, reduce hyperpigmentation, promote wound healing, and act as natural moisturizers. Common applications include using antioxidants to protect against UV damage and free radicals, which cause wrinkles and photoaging, and employing anti-inflammatory compounds to soothe skin and treat conditions like acne. Phytochemicals include flavonoids, alkaloids, tannins, terpenoids, phenolic acids, and saponins, which possess antioxidant, anti-inflammatory, antimicrobial, and UV-protective properties beneficial for skin and hair care.

2. Phytochemicals: Nature's Bioactive Compounds

Phytochemicals are secondary metabolites secreted by plants for defence mechanism and adaptation. They are categorized into several groups based on their chemical structure and biological activity:

Flavonoids are certainly the most exciting and most widely investigated subclass of phenolic compounds. This class contains more than 5000 ingredients now. Flavonoids have the flavon backbone in common and are classified to 6 main subgroups.

Flavonoids are phytochemicals and are a subgroup of polyphenols with excellent antioxidant properties. Plant based treasurers in medicine, nutrition and cosmetics.

i. Flavanols: A group of Flavanols ex. Catechin, Epicatechin, Gallo-catechin having the properties antioxidant, anti-aging, skin protection.
ii. Terpenoids: A group of Flavanols Kaepferol, quercetin, myricetin Ex. Menthol, Limonene, Squalene having the properties fragrance, soothing and moisturizing.

iii. Alkaloids: A group of Isoflavanols Diadzein, Genistein, Glycitein Ex. Caffeine, Nicotine having the properties to stimulates blood circulation and reduces puffiness.
iv. Phenolic Compounds: A group of anthocyanidins such as Cyanidin, Delphinidin, Petunidin, along with Curcumin and Gallic acid; *properties:* Anti-inflammatory and UV-protective.
v. Anthocyanidins: Compounds such as Cyanidin, Delphinidin, Malvidin, Pelargonidin, Peonidin, and Petunidin are present in red wine, red and blue berries, and red and purple grapes. These are the beautiful red to blue pigments (the color depends on pH) found in most fruits and some vegetables, and they are highly soluble in water and ethanol.
vi. Flavonones such as Eriodictyol, Hesperetin, Naringenin are present in citrus fruits. These molecules have low water solubility but are soluble in alcohol.
vii. Flavones such as Apigenin, Luteolin, Baicalein, Chrysin present in celery, parsley, thyme and pepper. These are highly insoluble in water but partially soluble in hydroalcoholic solvents or in hot alcohol.
viii. Saponins: Ginsenosides, Diosgenin; *properties:* Cleansing and foaming agents
ix. Tannins: Ellagic acid, Catechins; *properties:* Astringent and pore-tightening
x. Essential Oils: Lavender, Tea tree, Rose; *properties:* Fragrance and antimicrobial

3. Mechanisms of Action in Cosmetics

Phytochemicals enhance cosmetic effects through multiple mechanisms:

i. Antioxidant Protection: Free radicals are one of the leading causes of premature skin aging. Phytochemicals such as flavonoids and polyphenols neutralize oxidative stress, preventing collagen breakdown and reducing the appearance of fine lines and wrinkles. Notable antioxidants include Quercetin (CAS 117-39-5) and Resveratrol (CAS 501-36-0), which help protect the skin from environmental damage.
ii. Anti-Inflammatory Benefits: Many plant-based compounds, such as Curcumin (CAS 458-37-7) from turmeric and Hesperidin (CAS 520-26-3) from citrus fruits, exhibit powerful anti-inflammatory effects. Antioxidant Activity: Neutralize free radicals responsible for skin aging. UV Protection: Absorb harmful ultraviolet radiation, preventing photodamage.
iii. Collagen Stimulation: Promote skin elasticity and reduce wrinkles. Many phytochemicals act as natural sunscreens by protecting the skin from UV radiation damage, reducing oxidative stress, and downregulating inflammation.
iv. Skin repair and wound healing: Extracts from plants such as Centella asiatica can promote wound healing and tissue regeneration. These compounds help soothe irritated skin, making them ideal for sensitive and acne-prone skin types.

v. Skin Brightening and Even-Tone Effects: Some phytochemicals, like Kojic Acid (CAS 501-30-4) and Hesperetin (CAS 520-33-2), are natural skin brighteners.
vi. Antimicrobial Effect: This prevent acne, dandruff, and other skin infections. Skin Whitening: This Inhibits tyrosinase enzyme responsible for melanin production.
vii. Moisturizing Effect: This is very important as it enhance hydration and maintain skin barrier function. This helps reduce hyperpigmentation, dark spots, and uneven skin tone, leading to a radiant complexion enhancing the radiance.Moisturization: Certain plant extracts can help the skin retain moisture and repair its protective barrier, which is crucial for maintaining skin health.
viii. Skin whitening and anti-pigmentation: Some phytochemicals, particularly those with anti-tyrosinase activity, can help reduce hyperpigmentation and even skin tone.
ix. Anti-acne and skin conditions: Ingredients from plants like Pisum sativum and red onion have shown benefits for acne and other skin issues due to their antimicrobial and anti-inflammatory properties.
x. Hydration and Moisture Retention: Phytochemicals such as Hyaluronic Acid (plant-derived) and Tannic Acid (CAS 1401-55-4) helping to improve skin hydration. They work by attracting and retaining moisture, keeping the skin plump, supple and soft.
xi. Collagen Boosting and Anti-Aging: Certain plant compounds, including Epigallocatechin Gallate (CAS 989-51-5) from green tea and Diosmin (CAS 520-27-4) from citrus, promote collagen production by recreating elasticity. This leads to firmer skin, improved elasticity, and a more youthful appearance and glow. Phytochemicals like phenolics and flavonoids scavenge free radicals, inhibit enzymes like collagenase that break down skin structure, and can reduce inflammation, thereby fighting wrinkles and loss of elasticity.

4. Phytochemicals in Skin Care Products

i. Aloe vera: Botanical name (*Aloe barbadensis* Miller) – Contains aloin, polysaccharides, and vitamins; used in moisturizers and anti-inflammatory creams.
ii. Turmeric: Botanical name (*Curcuma longa*) – Rich in curcumin; has antioxidant and skin-brightening properties. Found in turmeric, it is a potent antioxidant with anti-inflammatory properties.
iii. Green tea: Botanical name (*Camellia sinensis*) – Polyphenols and catechins provide anti-aging and anti-acne benefits.
iv. Neem: Botanical name (*Azadirachta indica*) – Azadirachtin and nimbidin have antibacterial properties; used in acne creams very effection even used in small fraction.
v. Licorice: Botanical name (*Glycyrrhiza glabra)* – Glabridin acts as a natural skin-lightening agent inhibits secretion of melanin pigments

vi. Rose: Botanical name (*Rosa damascena*) – Rose oil hydrates and rejuvenates skin give glowing.

vii. Cucumber: Botanical name (*Cucumis sativus)* – Rich in ascorbic acid; used in soothing facial gels helps to cure acnes

Some of Phytochemicals and Their Uses

- Vitamins: are very essential for elasticity, Vitamins A, C, and E are well-known for their roles as antioxidants and in collagen production.
- Polyphenols: this are large group of antioxidants found in many plant-based products that help protect against oxidative stress.
- Flavonoids: are crucial found in plants like chamomile, they have antioxidant and anti-inflammatory effects and can enhance skin moisturization.
- Terpenoids: These have antioxidant and anti-inflammatory benefits and are found in aromatic plants like rosemary, often used for acnes
- Curcumin: Found in turmeric, it is a potent antioxidant with anti-inflammatory properties.
- Resveratrol: A polyphenol found in grapes, known for its antioxidant and anti-aging properties.

Phytochemicals in Hair Care

Plant Source	**Phytochemicals**	**Cosmetic Benefits**
Henna (*Lawsonia inermis*)	Lawsone	Natural dye, conditioner
Amla (*Phyllanthus emblica*)	Vitamin C, tannins	Strengthens roots, prevents graying
Bhringraj (Eclipta alba)	Wedelolactone	Promotes hair growth
Reetha (*Sapindus mukorossi*)	Saponins	Natural cleanser and foaming agent
Hibiscus (*Hibiscus rosa-sinensis*)	Flavonoids, anthocyanins	Improves hair shine and thickness
Fenugreek (*Trigonella foenum-graecum*)	Mucilage, saponins	Reduces dandruff and hair fall

7. Market and Future Perspectives

The global demand for herbal cosmetics is increasing, driven by consumer preference for natural, chemical-free products. The integration of nanotechnology and biotechnology has improved the delivery and bioavailability of plant-based ingredients. Future trends focus on green formulations, personalized cosmetics, and biodegradable packaging.

Conclusion:

Phytochemicals represent a promising and sustainable alternative to synthetic ingredients in the cosmetic industry. Derived from natural plant sources, these bioactive compounds—such as flavonoids, alkaloids, terpenoids, phenolics, and essential oils—possess remarkable antioxidant, antimicrobial, anti-inflammatory, and anti-aging properties. Their multifunctional nature not only enhances skin health and beauty but also minimizes the risk of adverse reactions commonly associated with synthetic chemicals. The growing consumer preference for herbal and eco-friendly cosmetics has further encouraged research and innovation in phytochemical-based formulations. Modern extraction and analytical techniques have enabled the identification and utilization of plant compounds with high efficacy and stability in creams, lotions, shampoos, and other personal care products. In investigation phytochemicals bridge traditional herbal knowledge with modern cosmetic science, offering safe, effective, and environmentally responsible solutions for skincare and haircare. Continued exploration and standardization of these natural compounds will play a crucial role in developing next-generation cosmeceuticals that combine beauty, health, and sustainability.

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A COMPARATIVE STUDY OF CONVENTIONAL AND ADVANCED EXTRACTION TECHNIQUES WITH CHROMATOGRAPHIC IDENTIFICATION METHODS: INSIGHTS AND FUTURE PERSPECTIVES

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Abstract:

The extraction and identification of bioactive compounds from medicinal plants play a crucial role in modern pharmaceutical and herbal research. Conventional extraction techniques such as maceration, percolation, Soxhlet extraction, and decoction have been widely used due to their simplicity and effectiveness in isolating phytoconstituents. However, these methods often require long processing times and large volumes of solvents. To overcome such limitations, advanced extraction technologies including Supercritical Fluid Extraction (SFE), Microwave-Assisted Extraction (MAE), Ultrasound-Assisted Extraction (UAE), and Solid Phase Extraction (SPE) have emerged as efficient, sustainable, and precise alternatives.Extraction efficiency is influenced by several factors, including solvent type and polarity, extraction temperature, time, and pressure, as well as particle size, solvent-to-solid ratio, agitation, pH, and moisture content. Accurate identification of medicinal plants is equally vital and can be achieved through macroscopic, microscopic, taxonomical, phytochemical, and chromatographic techniques such as Thin Layer Chromatography (TLC), Column Chromatography, High-Performance Thin Layer Chromatography (HPTLC), and High-Performance Liquid Chromatography (HPLC).Looking ahead, the integration of Artificial Intelligence (AI) and Machine Learning (ML) offers promising prospects for optimizing extraction parameters, predicting yields, and enhancing reproducibility. These technologies are expected to revolutionize natural product research by enabling data driven decision making and sustainable process design. Overall, combining traditional knowledge with modern innovations can significantly improve the efficiency, accuracy, and scalability of medicinal plant extraction and identification.

Keywords: Extraction Techniques, Chromatographic Methods, Phytochemical Analysis, Advanced Extraction Technologies, Technological Trends in Extraction.

1. Introduction:

Medicinal plants have been an indispensable source of therapeutic agents for centuries, providing a wide range of bioactive compounds that are crucial in modern drug discovery and healthcare. The growing interest in herbal medicines and natural products has driven extensive research into efficient extraction and identification methods to isolate, characterize, and utilize these phytoconstituents. Extraction is the primary step in obtaining biologically active compounds from plant materials, and the choice of extraction method directly impacts the yield, purity, and efficacy of the final product. Conventional extraction techniques, including maceration, percolation, Soxhlet extraction, and decoction, have long been employed due to their simplicity and cost-effectiveness. Maceration involves soaking plant material in a suitable solvent at room temperature, allowing passive diffusion of bioactive compounds, whereas percolation ensures continuous solvent flow to enhance extraction efficiency. Soxhlet extraction utilizes repeated solvent reflux cycles to extract compounds efficiently, and decoction employs boiling water to extract heat-stable constituents from tough plant parts such as roots and bark. Despite their widespread use, these traditional methods often require prolonged extraction times, large volumes of solvents, and may result in partial degradation of sensitive phytochemicals. To address these limitations, advanced extraction technologies have emerged. Techniques such as Supercritical Fluid Extraction (SFE), Microwave-Assisted Extraction (MAE), Ultrasound-Assisted Extraction (UAE), and Solid Phase Extraction (SPE) offer higher efficiency, reduced solvent usage, and shorter extraction times. SFE uses supercritical fluids like carbon dioxide to selectively extract compounds under controlled temperature and pressure, while MAE and UAE employ energy waves to disrupt plant matrices and facilitate rapid solvent penetration. SPE provides a rapid, selective, and reproducible method for isolating target compounds, especially for analytical and preparative purposes. The efficiency of extraction is influenced by multiple factors, including the choice and polarity of the solvent, extraction time, temperature, and pressure, particle size of the plant material, solvent-to-solid ratio, agitation, pH, and moisture content. Optimizing these parameters is essential for maximizing yield while preserving the integrity of bioactive molecules. After extraction, the identification and characterization of phytochemicals are critical. Traditional macroscopic and microscopic methods, along with taxonomical approaches, provide initial identification, whereas phytochemical screening and chromatographic techniques such as Thin Layer Chromatography (TLC), Column Chromatography, High-Performance Thin Layer Chromatography (HPTLC), and High-Performance Liquid Chromatography (HPLC) allow detailed analysis of plant constituents. These techniques ensure quality control, standardization, and accurate determination of bioactive compounds. The integration of modern technologies, including Artificial Intelligence (AI) and Machine Learning (ML), is now reshaping natural product research. AI and ML can optimize extraction conditions, predict yields, and streamline analytical processes, paving the way for sustainable, reproducible, and cost-effective production of plant-derived therapeutics. Combining traditional knowledge with innovative methodologies offers significant potential to enhance the efficiency, precision, and scalability of medicinal plant research, supporting the development of novel drugs and herbal formulations.

2. Conventional Extraction Techniques

2.1. Maceration Extraction: Maceration is the extraction of bioactive compounds by soaking plant material in a suitable solvent at room temperature. The principle is passive diffusion, where the solvent penetrates plant cells and dissolves the desired compounds without applying heat.

Process: Powdered or chopped plant material is placed in a solvent such as ethanol, methanol, or water. The mixture is left to stand for several hours to days with occasional stirring. After sufficient extraction, the mixture is filtered, and the filtrate is concentrated under reduced pressure to obtain the extract.

Advantages:

a) Simple and cost-effective.
b) Preserves thermolabile compounds due to no heat application.

Disadvantages:

a) Time-consuming; may take several days.
b) Lower extraction efficiency and yield compared to modern methods.

2.2. Percolation Extraction: Percolation is a method of continuous extraction in which solvent slowly passes through plant material to dissolve bioactive compounds. The principle is dynamic solvent flow, which improves extraction efficiency.

Process: Powdered plant material is packed into a percolator. Solvent is poured gradually and allowed to percolate through the material. The extract is collected continuously until the extraction is complete.

Advantages:

a) Faster than maceration.
b) Provides higher yield of active compounds.

Disadvantages:

a) Requires proper control of solvent flow.
b) Not ideal for heat-sensitive compounds if warm solvents are used.

2.3. Soxhlet Extraction: Soxhlet extraction is a method of repeated solvent extraction where the solvent is continuously refluxed over plant material. The principle involves heating and condensation cycles to exhaustively extract bioactive compounds.

Process: Plant powder is placed in a thimble inside the Soxhlet apparatus. Solvent is heated to reflux; its vapor condenses and passes repeatedly through the plant material. After sufficient cycles, the extract is concentrated by evaporating the solvent.

Advantages:

a) Efficient extraction with minimal solvent usage.
b) Suitable for exhaustive extraction of compounds.

Disadvantages:

a) Heat-sensitive compounds may degrade.
b) Longer extraction time compared to modern techniques.

2.4. Decoction: Decoction is the extraction of water-soluble and heat-stable compounds from tough plant materials by boiling in water. The principle is heat-assisted solubilization of compounds.

Process: Coarsely powdered plant parts such as roots, barks, or seeds are added to water and boiled gently for 15–30 minutes. The decoction is then cooled, filtered, and concentrated if needed.

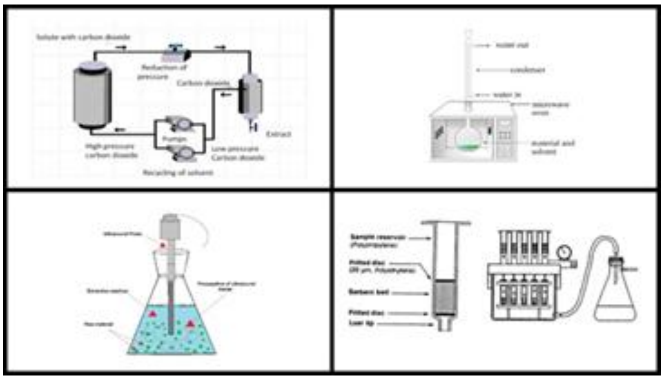
Advantages:

a) Simple and effective for extracting compounds from hard plant materials.
b) Widely used in traditional medicine.

Disadvantages:

a) Heat-sensitive compounds may be destroyed.
b) Extracts may have a short shelf-life

3. Advanced Extraction Techniques



Advanced Extraction Techniques: Supercritical Fluid extraction, Microwave Assisted Extraction, Ultrasound Assisted Extraction and Solid Phase Extraction [12-15].

3.1. Supercritical Fluid Extraction:

Introduction: For the separation of organic molecules, supercritical fluid extraction Procedure (SFE) is an alternative to traditional solvent extraction. The extraction of organic components from herbs and other plants has been accomplished with success using SFE. On-line fractionation and coupling with chromatographic methods, SFE modeling, sample preparation, extraction conditions, collection methods, cleaning up pesticides from herbal plants and other samples, and the main benefits of SFE techniques are all covered. This method offers several advantages over traditional extraction technique including:

1. High extraction efficiency due to superior solvent power of supercritical CO_2.
2. Excellent selectivity, allowing precise extraction of desired compounds.
3. Low solvent consumption, as CO_2 can be easily recycled.
4. No solvent residues, ensuring purity of the extracted products.
5. Mild operating conditions, preserving heat-sensitive phytochemicals and maintaining product integrity.

Extraction Process:

1. Pre-treatment: Plant material is ground, dried, and prepared for extraction.
2. Extraction vessel: The prepared plant material is loaded into an extraction vessel.
3. Supercritical CO2: CO2 is pumped into the extraction vessel, reaching supercritical conditions.
4. Separation: The extracted compounds are separated from the CO2 using a separator or cyclone.
5. Collection: The extracted compounds are collected and further processed(e.g., drying, purification).

Advantages:

1. No solvent residues: CO2 is non-toxic and non-flammable, leaving no residue
2. Gentle operating conditions: SFE preserves delicate compounds and prevents degradation.
3. Low solvent consumption: SFE uses significantly less solvent than traditional
4. Selectivity: SFE allows for selective extraction of target compounds.
5. High extraction efficiency: SFE can achieve higher extraction yields than traditional methods.

Application:

1. Pharmaceuticals: Active pharmaceutical ingredients (APIs) are extracted.
2. Food industry: Nutrient, taste, and fragrance extraction.
3. Cosmetics: Natural ingredient extraction for hair and skin care.

4. Herbal medicine: the process of obtaining bioactive substances from plants.

Equipment:

1. Extraction vessel: an SFE-specific high-pressure vessel.
2. CO_2 pump: CO_2 delivery pumping system.
3. Heat exchanger: System for controlling temperature.
4. Separator: An apparatus that separates CO_2 from extracted chemicals.
5. Cyclone: An optional tool for extracting chemicals.

Optimization Specifications:

1. Pressure: between 100 and 400 bar
2. 40 to 60 degrees Celsius
3. Flow rate of CO_2: 1–10 mL/min
4. Time needed for extraction: 30 to 120 minutes
5. Size of particle: 0.1–1 mm Problems and Prospects for the

Challenges & Future Direction:

1. Scalability: Increasing the size of SFE procedures for use in industrial settings.
2. Multi-component extraction: Extracting multiple compounds simultaneously.
3. Coupling with other techniques: Combining SFE with other extraction method.

3.2. Microwave Assisted Extraction: Introduction: In order to speed up the extraction of bioactive chemicals, MAE uses microwave energy to heat a mixture of plant material and solvent. Internal heating is caused by two primary processes: ionic conduction, which involves ions moving beneath the electromagnetic field, and dipole rotation, which involves polar molecules aligning with the oscillating field. Cell walls burst, solvent penetration rises, mass transfer occurs, etc.

Principle: It works on the principle of microwave energy interaction with polar molecules in the solvent and plant material. When exposed to microwaves, polar molecules undergo dipole rotation and ionic conduction, generating rapid internal heating. This causes cell wall rupture, allowing the solvent to penetrate easily and dissolve intracellular compounds. The process enhances mass transfer, reduces extraction time, and minimizes solvent use. By optimizing parameters such as microwave power, time, and solvent type, MAE achieves high efficiency while preserving heat-sensitive bioactive compounds.

Advantages:

1. Much shorter extraction times (minutes vs hours).
2. Reduced usage of solvent; often less harmful (preferably green solvents).
3. Higher extraction efficiency / yield; sometimes better selectivity of target compounds.

4. Lower energy consumption (because heating is more direct and rapid).
5. Good for thermolabile compounds if optimized properly (short times, controlled temperature).

Application:

1. Pharmaceuticals: Extraction of active pharmaceutical ingredients (APIs).
2. Food industry: Extraction of flavors, fragrances, and nutrients.
3. Cosmetics: Extraction of natural ingredients for skincare and haircare.
4. Herbal medicine: Extraction of bioactive compounds from herbs.

Equipment:

1. Microwave reactor: Specialized vessel designed for MAE.
2. Microwave generator: Produces microwave energy.
3. Temperature control: Monitors and regulates temperature.
4. Stirring system: ensure uniform heating

Optimization Parameters:

1. Microwave power: 100-1000 W
2. Extraction time: 1-30 minutes
3. Temperature:40-100°C
4. Solvent-to-material ratio: 5:1 to 20:1
5. Frequency: 2450 MHz (standard)

Challenges & Future Directions:

1. Temperature Control: Overheating can degrade heat-sensitive compounds (e.g., vitamins, antioxidants.
2. Solvent Compatibility: Non-polar solvents (e.g., hexane) are poor microwave absorbers; may require co-solvents.
3. Pressure Buildup: Closed systems can build pressure due to rapid heating; risk of explosion if not managed.
4. Selectivity: Less selective than some conventional methods; co-extraction of unwanted materials.

3.3. Ultrasound Assisted Extraction: An essential stage in food science, medicine, nutraceuticals, cosmetics, and other fields is the extraction of bioactive substances (such as phenolics, flavonoids, oils, and proteins) from plants. Conventional extraction techniques, such as steam distillation, maceration, and Soxhlet extraction, frequently call for lengthy processes, high temperatures, and copious volumes of solvent. They may also destroy thermolabile substances. Ultrasound-Assisted Extraction (UAE) is a non-thermal (or mild thermal) method that preserves chemical integrity while increasing extraction efficiency and lowering time, energy, and solvent use.

Extraction Process:

1. Pre-treatment: Plant material is dried, powdered, and made ready for extraction.
2. Ultrasound reactor: An ultrasound reactor is filled with the prepared plant material and solvent.
3. Ultrasound irradiation: Cavitation and mechanical effects are produced by applying ultrasound waves.
4. Extraction: The target compounds are extracted by the solvent and subsequently gathered.
5. Post-extraction processing: Additional processing, such as filtration or purification, may be applied to the extracted substances.

Application:

a) 1.Increased Extraction Efficiency: UAE enhances mass transfer and cell wall disruption, leading to better release of intracellular compounds.
2. Reduced Extraction Time: Compared to conventional methods (like maceration or Soxhlet), UAE significantly reduces the time required.
3. Lower Solvent Usage: Efficient penetration reduces the amount of solvent needed, making the process more environmentally friendly.
4. Energy Efficient: Lower temperature and shorter duration save energy.

Equipment:

1. Ultrasound reactor: Specialized vessel designed for UAE
2. Temperature control: Monitors and regulates temperature.
3. Stirring system: Ensures uniform mixing.
5. Ultrasound generator: Produces ultrasound waves.

Optimization Parameter:

1. Power intensity: 10-100 W/cm^2
2. Frequency: 20-40 kHz
3. Temperature: 20-60°C
4. Extraction time: 10-60 minutes
6. Solvent-to-material ration 5:1 to 20:1

Challenges and Future Directions:

1. Solvent selection: Identifying optimal solvents for UAE.
2. Scalability: Scaling up UAE processes for industrial applications.
3. Mechanistic understanding: Elucidating the underlying mechanisms of UAE

4. Coupling with other techniques: Combining UAE with other extraction methods.

3.4. Solid Phase Extraction:

Introduction: Compounds are separated from a liquid mixture (often plant extract in a solvent) using a process called solid phase extraction (SPE), which involves adsorption to a solid sorbent material and elution with an appropriate solvent. SPE is commonly employed in phytochemical analysis to: Clean up crude extract, Target analytes should be concentrated, eliminate any substances that are interfering.

Principle: SPE is based on the selective adsorption of compounds onto a solid sorbent from a liquid sample. Target analytes are retained on the solid phase while impurities are washed away, and then the desired compounds are eluted using an appropriate solvent. This allows efficient separation, purification, and concentration of analytes.

Advantages:

1. Increased sensitivity: Analyte concentration.
2. Impurity removal reduces matrix interference.
3. Improved selectivity: Analyte retention that is specific.
4. Automation: It is possible to automate SPE.

Application:

1. Pharmaceutical analysis: Drug and metabolite extraction.
2. Environmental monitoring: Pollutant analysis.
3. Food safety: Contamination detection.
4. Clinical research: Biomarker isolation.

Optimization Parameter:

1. Sorbent selection: Selecting the appropriate substance.
2. Sample loading: maximizing the amount of data.
3. Wash solvent: Selecting the right wash solvent.
4. Elution solvent: Choosing the optimal elution solvent.

4. Factors Affecting Extraction Efficiency:

The efficiency of extracting bioactive compounds from plant materials depends on several physical, chemical, and operational parameters. Understanding these factors is essential for optimizing yield, purity, and reproducibility in both laboratory and industrial processes.

4.1 Solvent Type and Polarity: The choice of solvent is one of the most critical factors in extraction. Solvent polarity determines its ability to dissolve specific phytochemicals polar solvents (e.g., water, ethanol) are effective for polar compounds, while non-polar solvents (e.g., hexane, chloroform) are used for non-polar constituents. The solvent's toxicity, volatility, and compatibility also influence the overall extraction efficiency.

4.2. Temperature, Time, and Pressure Conditions: Temperature accelerates mass transfer and improves solubility of compounds, but excessive heat can degrade thermolabile substances. Similarly, extraction time must be optimized too short may yield insufficient extraction, while too long can cause degradation or solvent saturation. In advanced methods like supercritical fluid extraction, pressure plays a crucial role in controlling solvent density and extraction selectivity.

4.3 Particle Size of Plant Material: Smaller particle size increases the surface area for solvent contact, enhancing diffusion and extraction rate. However, excessively fine particles can lead to filtration difficulties or solvent channeling. Therefore, particle size must be carefully controlled to balance extraction efficiency and ease of handling.

4.4 Solvent-to-Solid Ratio: The proportion of solvent to plant material affects the concentration gradient and extraction yield. A higher solvent-to-solid ratio generally increases efficiency by improving solute solubility, though excessive solvent use can lead to higher costs and dilution of extracts.

4.5. Agitation and Extraction Cycles: Agitation improves solvent penetration and mass transfer by minimizing boundary layers around plant particles. Repeated extraction cycles can enhance yield by ensuring complete recovery of active compounds. However, too many cycles may waste time and solvent.

4.6. pH and Moisture Content Effects: The pH of the solvent system influences the ionization and stability of phytochemicals, especially alkaloids, flavonoids, and phenolic compounds. Moisture content of the plant material also affects solvent diffusion overly dry materials may reduce permeability, while high moisture can dilute the solvent and lower extraction efficiency.

5. Different methods of Identifications of Medical Plants

5.1. Macroscopic Method: Initial, surface features, color, texture, refractive qualities, taste, smell, etc. Which sensory traits are employed to identify the plant at the macroscopic level materials.

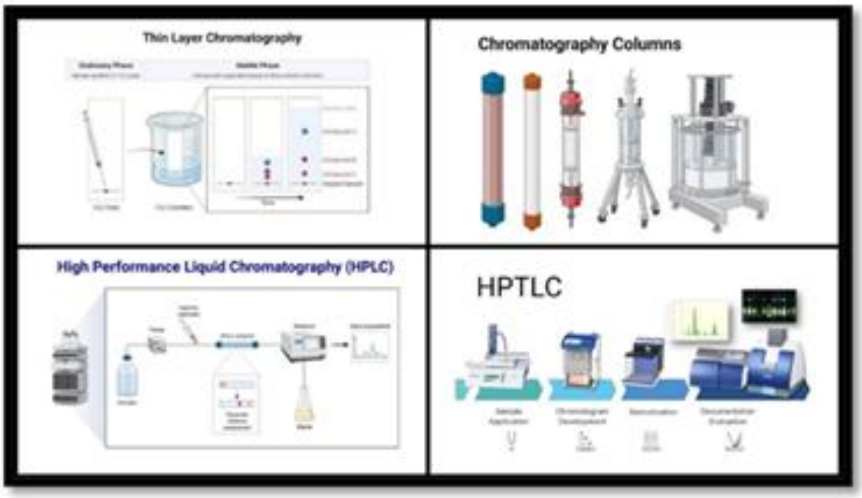
5.2. Phytochemical Techniques: Sulphurated ash, total ash, acid-insoluble ash, and water-soluble ash are the criteria. It is feasible to identify the particular medicines or proprietary remedies by comparing these numbers to the standard values of the Indian Pharmacopoeia.

5.3. Chromatographical Techniques: Capillary for liquid chromatography with good performance: The two most popular analytical techniques for herbal substances are thin-layer chromatography and electrophoresis. The chemical analysis of herbal remedies relies heavily on the examination of volatile compounds using gas chromatography

5.4. Taxonomical Technique: To categorize, identify, name, and characterize plants according to their morphological, anatomical, biochemical, and genetic traits, taxonomical procedures are methodical approaches. Plant taxonomy, the study of naming and grouping plants into a hierarchical structure (Kingdom → Division → Class → Order → Family → Genus → Species), relies heavily on the chromatograph

5.5. Microscopic Method: A microscope can be used to determine the interior tissue characteristics, cell structure, and plant structure. It is widely used to identify and distinguish between two types of medications. This method is well-liked, rapid, simple to apply, and suitable for patented drugs. One plant that can be recognized by microscopic methods is star anise. (Hook's v. Illicium). Once found only in southern China, the fruit known as star anise has a flavor resembling a star. Since then, though, it has spread throughout the tropical and subtropical regions of East Asia. In China and India, the fruit is used as a tasty flavoring in food and desserts. Its medicinal properties are well known in Chinese medicine. Back pain, hernias, and rheumatism.

6. Chromatography Techniques



Different Chromatography Techniques (a) TLC, (b) Column Chromatography, (c) HPLC, (d) HPTLC [20]

6.1 TLC (Thin Layer Chromatography): TLC is a simple, quick method to separate and identify components in a mixture. A small sample is placed on a coated plate (usually silica gel), then the plate is placed in a solvent. As the solvent moves up, different compounds travel at different speeds, creating separate spots. These spots can be seen under UV light or by using chemicals like iodine. It helps in analyzing plant extracts, drugs, and other substances.

6.2. Column Chromatography: Column chromatography is a technique used to separate and purify individual components from a mixture by passing it through a column packed with a stationary phase, while a solvent (mobile phase) flows through. Different compounds travel at different rates, allowing separation based on their interaction with the stationary phase

6.3. HPLC (High-Performance Liquid Chromatography): It is an analytical technique used to separate, identify, and quantify each component in a mixture. It works by passing a liquid sample through a column packed with solid adsorbent material under high pressure. Different compounds in the sample move at different speeds, allowing for their separation and analysis.

6.4. HPTLC (High-Performance Thin Layer Chromatography): HPTLC is an advanced form of thin layer chromatography that allows for the separation, identification, and quantification of components in a mixture using high-resolution plates, automated sample application, and densitometry analysis. It offers better reproducibility, sensitivity, and accuracy compared to conventional TLC.

7. Future Perspectives and Technological Trends in Extraction:

7.1. Integration of Artificial Intelligence (AI): Artificial Intelligence (AI) is playing an increasingly vital role in modern extraction processes for plant-based compounds. By analyzing large sets of experimental data, AI systems can identify the most efficient combinations of extraction parameters such as solvent type, temperature, pressure, and extraction time. This helps achieve maximum yield, purity, and selectivity of bioactive compounds. AI-driven models can also automate process control, allow real-time adjustments and minimizing human error. In addition, AI supports the development of green and sustainable extraction methods—such as supercritical fluid extraction, ultrasound-assisted extraction, and microwave-assisted extraction—by predicting the most energy-efficient and environmentally friendly operating conditions. Overall, AI integration ensures precision, consistency, and reproducibility in both research and industrial-scale applications.

7.2. Machine Learning (ML) for Process Optimization: Machine Learning (ML), a key component of AI, is increasingly being adopted to optimize and predict extraction performance. ML algorithms learn from experimental data to build predictive models that can determine the best solvent ratios, temperature profiles, and extraction durations. These models not only enhance efficiency but also reduce the number of experimental trials needed, saving time and resources.

Furthermore, ML assists in scaling up laboratory findings to industrial production by maintaining uniform quality and yield. It can detect patterns and correlations within complex datasets, helping researchers understand how different parameters affect extraction outcomes. By integrating ML into extraction systems, industries can achieve higher process efficiency, lower costs, and more standardized herbal formulations.

Conclusion:

Medicinal plants continue to be a vital source of bioactive compounds for therapeutic applications. Efficient extraction and precise identification are essential for maximizing their potential. Conventional methods like maceration, percolation, Soxhlet extraction, and decoction provide foundational approaches but often face limitations in time, solvent usage, and compound stability. Advanced techniques, including Supercritical Fluid Extraction, Microwave-Assisted Extraction, Ultrasound-Assisted Extraction, and Solid Phase Extraction, offer improved efficiency, selectivity, and sustainability. Extraction efficiency depends on factors such as solvent type, temperature, time, particle size, and solvent-to-solid ratio, while identification relies on macroscopic, microscopic, taxonomical, phytochemical, and chromatographic methods. The integration of Artificial Intelligence and Machine Learning further enhances optimization, reproducibility, and scalability. Combining traditional knowledge with modern technologies provides a comprehensive strategy to harness medicinal plants effectively, ensuring high-quality, sustainable, and efficient production of plant-derived therapeutics.

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INTEGRATIVE ETHNOBOTANY AND PHYTOCHEMISTRY: PHYTOCHEMICAL PROFILING AND ETHNOBOTANICAL INSIGHTS INTO NATURE'S PHARMACY FOR EFFECTIVE AND SAFER THERAPEUTICS

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Abstract:

Medicinal plants have been an integral part of human healthcare for millennia, serving as a primary source of therapeutic agents and a foundation for traditional medicine systems worldwide. The convergence of ethnobotany and phytochemistry provides a multidisciplinary approach to explore and validate the therapeutic potential of plants used in traditional healing practices. Ethnobotany documents the knowledge, beliefs, and practices of indigenous communities regarding plant use, offering valuable insights into species selection, preparation methods, and therapeutic applications. Phytochemistry complements this by identifying and characterizing bioactive compounds responsible for the observed medicinal effects, thereby bridging traditional wisdom with modern scientific validation

Introduction:

1.1: Background and Historical Perspective of Medicinal Plants:

Medicinal plants have been a cornerstone of healthcare since ancient times, forming the foundation of traditional medicine systems such as Ayurveda, Traditional Chinese Medicine, and Indigenous healing practices. Globally, these natural resources continue to serve as a primary source of therapeutic agents, contributing significantly to modern drug discovery and the development of novel pharmacological interventions. The continued interest in plantbased therapeutics stems from their perceived efficacy, lower incidence of adverse effects, and accessibility compared to synthetic drugs.

1.2: Importance of Ethnobotanical Research in Drug Discovery

Ethnobotany, the scientific study of the relationships between people and plants, provides invaluable insights into the therapeutic potential of medicinal plants. By documenting indigenous knowledge regarding plant identification, preparation methods, dosage, and administration, ethnobotany captures centuries of empirical evidence that can guide contemporary pharmacological research. However, while traditional use offers important clues, Phytochemistry complements ethnobotany by exploring the chemical composition of plants, focusing on the isolation, identification, and characterization of bioactive secondary metabolites. These compounds—such as alkaloids, flavonoids, phenolics, terpenoids, glycosides, and saponins—often given the biological activity observed in traditional uses. Modern analytical techniques, including chromatography, spectroscopy, mass spectrometry and XRD, enable researchers to validate traditional claims, uncover novel therapeutic agents, and understand mechanisms of action at the molecular level.

The integration of ethnobotanical knowledge with phytochemical research offers a holistic approach to natural product discovery by scientifically substantiating traditional claims, and ensure safety through comprehensive toxicological evaluation. Moreover, such an integrative strategy supports the sustainable use of biodiversity and ethical acknowledgment of indigenous knowledge systems. This chapter explores the dynamic interface between ethnobotany and phytochemistry, revealing how traditional knowledge of medicinal plants offers valuable therapeutic insights, while emphasizing that the inherent use of natural remedies does not automatically ensure their safety or efficacy without rigorous scientific validation.

Ethnobotany: Understanding and Exploring Nature's Pharmacy

Ethnobotany, the study of the interactions between humans and plants, provides a systematic approach to understanding how indigenous and local communities utilize plant resources for food, medicine, and cultural practices. The field bridges traditional knowledge with modern scientific inquiry, offering insights into plant selection, preparation, and therapeutic applications. Medicinal plants documented through ethnobotanical surveys often represent a rich repository of pharmacologically active compounds, reflecting centuries of empirical use and observations.

Scope and Significance

The scope of ethnobotany extends beyond mere documentation of plant use. It encompasses the study of cultural practices, ecological knowledge, and the socio-economic significance of plants within communities. Traditional knowledge about medicinal plants can guide researchers in selecting species with high therapeutic potential, reducing the time and resources required for preliminary screening in drug discovery programs. Moreover, ethnobotanical research supports the conservation of plant biodiversity, promotes sustainable use, and safeguards indigenous intellectual property rights.

Methods of Ethnobotanical Research

Ethnobotanical data is typically collected through structured and semi-structured interviews, participant observations, and field surveys. Key methods include:

Methods	Details	Application
Informant interviews	Engaging with traditional healers, elders, and local practitioners to document plant use, preparation, and dosage.	To gather firsthand ethnobotanical knowledge and traditional practices.
Participatory observation	Observing and recording plant collection, processing, and administration practices in natural settings.	To understand practical applications and techniques used by local communities.
Herbarium specimen collection	Properly collecting and preserving plant samples for botanical identification and further phytochemical study.	To ensure accurate identification and provide reference material for research.
Quantitative approaches	Techniques such as Use Value (UV), Relative Frequency of Citation (RFC), and Informant Consensus Factor (ICF).	To prioritize plants with high medicinal relevance and evaluate consensus among informants.

Ethnobotanical Relevance in Medicine

Plants documented through ethnobotanical studies often provide leads for pharmacological investigations. Several studies highlight the importance of ethnobotany in guiding drug discovery:

Ethnobotanically significant plants, their traditional uses, active compounds, and validated biological activities, illustrates the rich diversity of plants utilized in traditional medicine systems worldwide, highlighting their potential in modern drug discovery.

Plant Species	Traditional Use	Active Compounds	Validated Biological Activity
Tinospora cordifolia	Immune booster	Alkaloids, Glycosides	Antioxidant, Antiinflammatory
Azadirachta indica	Skin infections	Limonoids, Flavonoids	Antimicrobial, Hepatoprotective
Gymnema sylvestre	Diabetes	Gymnemic acids	Hypoglycemic
Momordica charantia	Diabetes	Charantin, Polypeptide-P	Blood sugar regulation
Trigonella foenumgraecum	Diabetes, digestive issues	Trigonelline, Saponins	Hypoglycemic, Lipidlowering
Harpagophytum procumbens	Arthritis, pain relief	Harpagoside (iridoid glycosides)	Anti-inflammatory, Analgesic
Curcuma longa	Inflammation, wounds	Curcumin	Anti-inflammatory, Antioxidant, Antimicrobial
Withania somnifera	Stress, vitality	Withanolides	Adaptogenic, Antiinflammatory, Immunomodulatory
Ginkgo biloba	Memory enhancement	Flavonoids, Terpenoids	Neuroprotective, Antioxidant, Circulatory support
Panax ginseng	Fatigue, immunity	Ginsenosides	Immunomodulatory, Antifatigue, Antiinflammatory
Ocimum sanctum	Respiratory disorders, stress	Eugenol, Ursolic acid	Anti-inflammatory, Antimicrobial, Adaptogenic
Allium sativum	Cardiovascular health	Allicin, Sulfur compounds	Antimicrobial, Hypolipidemic, Antioxidant
Silybum marianum	Liver disorders	Silymarin (Flavonolignans)	Hepatoprotective, Antioxidant

Aloe vera	Wound healing, skin disorders	Polysaccharides, Anthraquinones	Wound healing, Antiinflammatory, Antimicrobial
Glycyrrhiza glabra	Respiratory ailments, ulcers	Glycyrrhizin	Anti-inflammatory, Antiulcer, Antiviral
Zingiber officinale	Nausea, inflammation	Gingerols, Shogaols	Anti-inflammatory, Antiemetic, Antioxidant
Camellia sinensis	General health, infections	Catechins (EGCG)	Antioxidant, Antimicrobial, Anticancer potential
Aegle marmelos	Gastrointestinal disorders	Marmelosin, Tannins	Antidiarrheal, Antimicrobial, Hepatoprotective
Artemisia annua	Malaria	Artemisinin	Antimalarial
Tabernaemontana divaricata	Cancer, malaria	Tabernaemontanine (alkaloid)	Anticancer, Antimalarial
Datura stramonium	Anesthesia, asthma	Atropine, Scopolamine	Anticholinergic, Anesthetic
Brugmansia spp.	Spiritual rituals, anesthesia	Atropine, Scopolamine	Anticholinergic, Anesthetic
Centaurea damascena	Antioxidant, antibacterial	Phenolic compounds	Antioxidant, Antibacterial
Crataegus monogyna	Cardiovascular health	Flavonoids, Tannins	Antioxidant, Cardioprotective
Psidium guajava	Digestive disorders	Flavonoids, Tannins	Antioxidant, Antidiarrheal
Althaea officinalis	Respiratory issues	Mucilage, Flavonoids	Demulcent, Antiinflammatory
Tilia cordata	Respiratory issues	Flavonoids, Tannins	Antioxidant, Antibacterial
Hawthorn (Crataegus spp.)	Cardiovascular health	Flavonoids, Proanthocyanidins	Antioxidant, Cardioprotective

Commiphora wightii	Hypolipidemic agent	Guggulsterones	Hypolipidemic, Antiinflammatory
Picrorhiza kurroa	Hepatoprotective	Picroside	Hepatoprotective
Bacopa monnieri	Cognitive enhancer	Bacosides	Cognitive enhancer, Antioxidant
Curculigo capitulata	Antioxidant, antiinflammatory	Phenols, Flavonoids	Antioxidant, Antiinflammatory

Through ethnobotanical knowledge, researchers can prioritize plants with high therapeutic potential, reducing resource-intensive trial-and-error approaches and enhancing the likelihood of discovering effective natural therapeutics. By linking traditional use with phytochemical investigation, ethnobotany serves as the first critical step in harnessing nature's pharmacy for modern medicine.

Phytochemistry of Medicinal Plants

Phytochemistry is the branch of chemistry concerned with the study of bioactive compounds produced by plants. These secondary metabolites, while not directly involved in primary metabolic processes, play a crucial role in plant defense and survival, and are largely responsible for the medicinal properties of plants. By understanding the phytochemical composition of ethnobotanically significant plants, researchers can scientifically validate traditional uses and identify novel therapeutic agents.

Overview of Phytochemicals

Phytochemicals are broadly classified into several major groups based on their chemical structures and biosynthetic pathways:

- **Alkaloids:** Nitrogen-containing compounds with diverse pharmacological activities, including analgesic, antimalarial, and anticancer properties. Examples include morphine from *Papaver somniferum* and berberine from *Berberis aristata*.
- **Flavonoids:** Polyphenolic compounds known for antioxidant, anti-inflammatory, and cardioprotective effects. Examples include quercetin and kaempferol.
- **Phenolics:** Simple phenols and polyphenols contribute to antioxidant and antimicrobial activity. Examples include gallic acid and tannins.
- **Terpenoids:** Structurally diverse compounds with anti-inflammatory, anticancer, and antimicrobial activities. Examples include limonene, artemisinin, and ginsenosides.
- **Glycosides:** Compounds where a sugar moiety is bound to a non-sugar moiety; cardiac glycosides like digoxin are well-known for their therapeutic significance.

- **Saponins and Steroids:** Known for immunomodulatory, anticancer, and lipidlowering effects.

These metabolites and many more are often responsible for the pharmacological effects observed in traditional medicine and serve as lead compounds for drug discovery.

Analytical Techniques for Phytochemical Profiling

Modern phytochemical research employs advanced analytical tools to isolate, identify, and quantify bioactive compounds:

- **Chromatography:**
  - **Thin Layer Chromatography (TLC):** Rapid screening of plant extracts for specific classes of compounds.
  - **High-Performance Liquid Chromatography (HPLC):** Precise quantification of phytochemicals, widely used in standardization of herbal formulations.
  - **Gas Chromatography (GC):** Suitable for volatile compounds such as essential oils.
- **Spectroscopy:**
  - **UV-Vis Spectroscopy:** Preliminary identification and quantification of phenolics and flavonoids.
  - **Infrared (IR) Spectroscopy:** Functional group identification.
  - **Nuclear Magnetic Resonance (NMR) Spectroscopy:** Structural elucidation of isolated compounds.
- **Mass Spectrometry (MS):** Highly sensitive method for molecular weight determination and structural analysis, often coupled with chromatography (LC-MS, GC-MS).
- **XRD:** X-ray diffraction (XRD) is an analytical technique used to determine the crystalline structure of bioactive compounds. It helps determine the purity, polymorphism, and crystallinity of natural products, which is essential for understanding their stability, solubility, and bioavailability. XRD also aids in confirming the structural identity of isolated compounds and supports the standardization of herbal formulations and nutraceuticals.

Linking Phytochemistry with Ethnobotany

The integration of ethnobotanical knowledge and phytochemistry enhances the discovery of effective therapeutics. Plants selected based on traditional/ethnobotanical use are subjected to phytochemical screening, which identifies bioactive constituents responsible for observed biological activities.

Selected Ethnobotanical Plants and Their Phytochemical Constituents

Plant Species	Ethnobotanical	Major Phytochemicals	Biological Activity Use
Momordica charantia	Diabetes	Charantin, Polypeptide-P	Hypoglycemic
Azadirachta indica	Skin infections	Limonoids, Flavonoids	Antimicrobial, Antiinflammatory
Tinospora cordifolia	Immunity booster	Alkaloids, Glycosides	Antioxidant, Immunomodulatory
Gymnema sylvestre	Diabetes	Gymnemic acids	Hypoglycemic, Anti-obesity
Ocimum sanctum	Respiratory ailments	Eugenol, Ursolic acid, Flavonoids	Anti-inflammatory, Immunomodulatory
Curcuma longa	Inflammation, Wounds	Curcumin, Demethoxycurcumin	Anti-inflammatory, Antioxidant
Withania somnifera	Stress, Weakness	Withanolides	Adaptogenic, Anti-stress, Immunomodulatory
Aloe vera	Skin burns, Digestion	Anthraquinones, Polysaccharides	Wound healing, Laxative, Antioxidant
Piper nigrum	Digestive issues	Piperine	Bioavailability enhancer, Antioxidant
Terminalia chebula	Digestive health	Tannins, Chebulagic acid	Antioxidant, Antimicrobial
Zingiber officinale	Nausea, Inflammation	Gingerols, Shogaols	Anti-inflammatory, Antiemetic
Eclipta alba	Hair loss, Liver ailments	Wedelolactone, Coumestans	Hepatoprotective, Hair growth stimulant
Turnera diffusa	Anxiolytic, Aphrodisiac	Apigenin, Acacetin, βsitosterol	Anxiolytic, Aphrodisiac
Senna auriculata	Diabetes, Antioxidant	Anthraquinones, Flavonoids	Antidiabetic, Antioxidant
Aristotelia chilensis	Antioxidant, Antiinflammatory	Anthocyanins, Alkaloids	Antioxidant, Antiinflammatory
Crataegus azarolus	Cardiovascular health	Kaempferol, Catechin, Gallic acid	Antioxidant, Antimicrobial

Curculigo capitulata	Diabetes, Blood tonic	Curculigoside, βsitosterol	Antidiabetic, Antioxidant
Hydnocarpus macrocarpus	Skin diseases, Antibacterial	Hydnocarpic acid, Flavonoids	Antibacterial, Antifungal
Horsfieldia iryaghedhi	Antibacterial	Essential oils, Triterpenoids	Antibacterial
Suillus sp.	Antioxidant, Antimicrobial	Phenolic compounds, βcarotene	Antioxidant, Antimicrobial
Senna alata	Skin infections	Anthraquinones, Flavonoids	Antifungal, Antimicrobial
Cinnamomum verum	Antioxidant, Antimicrobial	Cinnamaldehyde, Eugenol	Antioxidant, Antimicrobial
Allium sativum	Cardiovascular health	Allicin, S-allyl cysteine	Antihypertensive, Antimicrobial
Coriandrum sativum	Digestive aid, Antioxidant	Linalool, α-pinene	Antioxidant, Digestive aid
Mentha piperita	Digestive aid, Headache relief	Menthol, Menthone	Analgesic, Antispasmodic
Cucumis sativus	Skin hydration	Cucurbitacin, Vitamin C	Antioxidant, Skin hydrating
Carthamus tinctorius	Skin care, Antioxidant	Safflower oil, Flavonoids	Antioxidant, Skin care
Nigella sativa	Immune support	Thymoquinone, Nigellidine	Immunomodulatory, Antioxidant
Boswellia serrata	Anti-inflammatory	Boswellic acids	Anti-inflammatory
Commiphora wightii	Anti-inflammatory	Guggulsterone, Steroids	Anti-inflammatory
Glycyrrhiza glabra	Respiratory health	Glycyrrhizin, Flavonoids	Antitussive, Antiinflammatory

Phytochemical profiling not only validates the therapeutic claims of traditional medicine but also facilitates the discovery of novel drugs. By identifying the bioactive constituents responsible for specific pharmacological effects, researchers can develop standardized, effective, and safer herbal therapeutics. The integration of phytochemistry with ethnobotanical knowledge ensures that the selection of plants for further research is guided by empirical evidence, enhancing efficiency and success rates in natural product-based drug discovery.

Integrative Approaches: Linking Ethnobotany and Phytochemistry

The integration of ethnobotanical knowledge with phytochemical research represents a holistic approach to natural product discovery, bridging traditional wisdom with modern scientific validation. While ethnobotany provides a framework for understanding how plants are utilized for therapeutic purposes, phytochemistry elucidates the bioactive compounds responsible for these effects. Combining these two disciplines allows researchers to systematically prioritize, study, and develop plant-based therapeutics that are both effective and safe.

Methodology of Integration

The integrative approach generally follows a structured workflow:

1. Ethnobotanical Survey and Data Collection:

- Conduct surveys and interviews with traditional healers, elders, and local practitioners to document medicinal plant use. o Collect information on plant parts used, preparation methods, dosage, and therapeutic indications.
- Quantitative tools such as Use Value (UV) and Informant Consensus Factor (ICF) help in identifying plants with high ethnomedicinal relevance.

2. Botanical Identification and Authentication:

- Accurate taxonomic identification using herbarium specimens and botanical keys ensures reproducibility of research.
- Voucher specimens are deposited in herbarium collections for future reference.

3. Phytochemical Screening and Profiling:

- Extract plant material using appropriate solvents.
- Screen for bioactive secondary metabolites using qualitative and quantitative methods (TLC, HPLC, GC, UV-Vis, NMR, MS).
- Identify compounds responsible for the observed therapeutic effects reported in traditional knowledge.

4. Biological Activity Assessment:

- Conduct in vitro assays (antioxidant, antimicrobial, anti-inflammatory, enzyme inhibition) to validate traditional claims.
- In vivo studies may follow to assess efficacy and toxicity in animal models.

5. Prioritization for Drug Development:

- Plants with high ethnobotanical use and confirmed bioactive compounds are prioritized for further pharmacological and clinical research. o Safety, standardization, and reproducibility are key considerations in developing plant-based therapeutics.

Benefits of the Integrative Approach

- **Scientific Validation:** Ethnobotanical leads are confirmed through phytochemical and pharmacological studies, establishing credibility for traditional medicine.
- **Efficiency:** Reduces the need for random plant screening, focusing resources on species with demonstrated traditional relevance.
- **Discovery of Novel Compounds:** Secondary metabolites identified may serve as lead compounds for drug development.
- **Sustainability and Conservation:** Prioritizing plants based on ethnobotanical significance supports targeted conservation and sustainable use.
- **Ethical Recognition:** Integrates community knowledge and promotes benefit-sharing with indigenous practitioners.

Case Examples

- *Gymnema sylvestre*: Traditionally used for diabetes, its bioactive gymnemic acids were identified through phytochemical studies and are now incorporated into standardized supplements.
- *Harpagophytum procumbens* (Devil's Claw): Ethnobotanical use for arthritis guided phytochemical analysis revealing iridoid glycosides responsible for anti-inflammatory effects.
- *Curcuma longa*: Extensive traditional use for wound healing and inflammation led to the isolation of curcumin, which is now widely studied for its therapeutic applications.

Summary Table: Integrative Workflow

Step	Description	Outcome
Ethnobotanical Survey	Documentation of traditional plant use	Identification of high-use plants
Botanical Authentication	Taxonomic verification and herbarium deposition	Accurate plant identification
Phytochemical Profiling	Extraction and analysis of secondary metabolites	Identification of bioactive compounds
Biological Validation	In vitro and in vivo assays	Confirmation of therapeutic activity
Prioritization & Development	Standardization and safety evaluation	Selection for drug development

By linking ethnobotanical observations with phytochemical evidence, researchers can maximize the success of natural product discovery while ensuring the safety and efficacy of plant-based therapeutics. This integrative strategy not only advances scientific knowledge but also preserves cultural heritage and promotes ethical use of biodiversity.

Safety and Toxicity Evaluation

The perception that natural products are inherently safe often leads to their misuse. However, the therapeutic potential of medicinal plants must always be balanced with their safety profile. Safety evaluation is critical to ensure that herbal formulations are free from toxic constituents and safe for human consumption.

Importance of Safety Profiling in Natural Therapeutics

Safety profiling includes both preclinical and clinical evaluation of plant-based formulations. It ensures that the pharmacologically active compounds do not cause adverse reactions, mutagenicity, or organ toxicity. The WHO emphasizes standardization, safety, and efficacy assessment before commercialization of herbal products.

In Vitro and In Vivo Toxicity Assessments

In vitro tests such as MTT assay, brine shrimp lethality bioassay, and cell line cytotoxicity studies are used to screen for cellular toxicity. In vivo models—like acute, subacute, and chronic toxicity studies in rodents—help determine the lethal dose (LD_{50}) and establish safe dosage ranges. Histopathological and biochemical analyses further confirm tissue-level safety.

Potential Herb–Drug Interactions

Many herbal constituents can alter the pharmacokinetics of synthetic drugs by affecting cytochrome P450 enzymes or drug transporters. For instance, St. John's Wort reduces the efficacy of warfarin and cyclosporine, while *Ginkgo biloba* can increase bleeding risk when combined with antiplatelet agents. Awareness and pharmacovigilance are essential in preventing such adverse interactions.

- Herb–drug interactions may alter drug absorption, metabolism, or excretion, leading to therapeutic failure or toxicity.
- Most interactions involve modulation of cytochrome P450 enzymes (especially CYP3A4, CYP2C9) and P-glycoprotein transporters.
- Caution is advised in polypharmacy or in patients using long-term herbal supplements alongside prescription medicines.

Examples of Potential Herb–Drug Interactions

Herbal Plant / Product	Major Active Constituent(s)	Interacting Drug(s)	Mechanism of Interaction	Clinical Implication / Outcome
St. John's Wort (*Hypericum perforatum*)	Hyperforin, hypericin	Warfarin, cyclosporine, oral contraceptives, digoxin	Induces CYP3A4 and Pglycoprotein enzymes	Reduced plasma drug levels; decreased efficacy (e.g., transplant rejection, contraceptive failure)
Ginkgo (*Ginkgo biloba*)	Ginkgolides, bilobalide	Aspirin, warfarin, NSAIDs	Antiplatelet activity; CYP2C19 inhibition	Increased risk of bleeding
Garlic (*Allium sativum*)	Allicin, S-allyl cysteine	Warfarin, saquinavir	Antiplatelet and CYP3A4 induction	Enhanced bleeding risk; reduced antiviral efficacy
Ginseng (*Panax ginseng*)	Ginsenosides	Warfarin, insulin, hypoglycemics	Alters coagulation and blood glucose	Decreased anticoagulant effect; hypoglycemia
Licorice (*Glycyrrhiza glabra*)	Glycyrrhizin	Digoxin, corticosteroids, diuretics	Inhibits 11βhydroxysteroi d dehydrogenas e; potassium loss	Hypokalemia, hypertension, arrhythmias
Grapefruit (*Citrus paradisi*)	Furanocoumari ns (bergamottin)	Statins, calcium channel blockers, benzodiazepines	Inhibits CYP3A4 in intestinal wall	Increased drug plasma levels; toxicity risk
Kava (*Piper methysticum*)	Kavalactones	CNS depressants (benzodiazepines, barbiturates)	Potentiation of GABAergic effects	Enhanced CNS depression, hepatotoxicity
Echinacea (*Echinacea purpurea*)	Caffeic acid derivatives, alkamides	Immunosuppressan ts (cyclosporine, tacrolimus)	Modulates immune response	Reduced immunosuppressi ve effect
Aloe vera	Aloin, polysaccharides	Hypoglycemics, digoxin	Hypoglycemic potentiation, electrolyte imbalance	Hypoglycemia, digoxin toxicity due to hypokalemia
Turmeric (*Curcuma longa*)	Curcumin	Anticoagulants, antiplatelets, NSAIDs	Additive anticoagulant effect	Increased bleeding risk

Regulatory and Ethical Considerations

Herbal products are regulated under frameworks such as AYUSH (India), EMA (Europe), and FDA (U.S.) dietary supplement guidelines. Ethical considerations include informed consent for ethnobotanical surveys and fair benefit-sharing with indigenous communities.

Summary of Safety Studies on Commonly Used Medicinal Plants: Herbal dose ranges and toxic thresholds vary by extract type, standardization, formulation, route, species and study quality —the numbers below as *guidance only*, not absolute "safe limits

Examples of Safety studies, adverse effects, and dose/limit data

Plant Name	Traditional uses	Reported toxicity / adverse effects	Toxic dose /NOAEL / commonly used dose (human or animal data)	Precautions / notes
Neem (*Azadirachta indica*)	Antimicrobial, antiparasitic, wound care	Reports of acute poisoning (esp. neem oil in children): vomiting, metabolic acidosis, hepatic injury, encephalopathy; animal target organs: liver, kidney, testis.	Acute oral LD_{50} (neem oil, rat): ~~31.95 g/kg reported; subacute studies noted effects at high doses (e.g., target-organ changes up to 1,600 mg/kg/day in rats). Human poisoning cases reported at household exposures (no universal "daily dose").	Avoid concentrated neem oil internally in children; use caution in pregnancy; standardized commercial preparations vary greatly.
Ashwagand ha (*Withania somnifera*)	Adaptogen, anxiety, stress, CNS	Generally well tolerated in trials but increasing case reports of herbinduced liver injury; GI upset, drowsiness reported.	Typical clinical extracts: 300–600 mg twice daily (varies); animal subacute studies show no toxicity at several 100s mg/kg; repeated-dose rat studies used up to 800 mg/kg without major toxicity in some reports. Longterm safety data limited.	Avoid if preexisting liver disease; monitor liver enzymes for prolonged use; potential interactions with sedatives, thyroid and immunosuppressants.

Turmeric / Curcumin *(Curcuma longa*)	Anti-inflammatory, antioxidant	Very low toxicity in humans; mild GI upset at higher doses; interactions (antiplatelet, with some drugs) possible.	Clinical safety data support doses up to 4–8 g/day curcumin being tolerated in trials; FDA GRAS assessments and clinical reports show tolerability up to ~8 g/day in shortterm studies.	Use caution with anticoagulants; ensure standardized extract; bioavailability enhancers (piperine) may alter effects.
Bitter melon (*Momordica charantia*)	Antidiabetic, hypoglycemic agent	Hypoglycemia reported (including severe hypoglycemia/convul sions in children in case reports), GI upset; animal studies generally show low acute toxicity but effects vary by extract/seed vs fruit.	Animal acute/subchronic studies: no consistent LD_{50} across studies; human trials use variable dosages (extracts or juices). Clinical caution: hypoglycemic effect — dose should be titrated and monitored.	Not recommended to combine with antidiabetic drugs without monitoring; caution in children and pregnancy.
Gymnema (*Gymnema sylvestre*)	Antidiabetic (reduces sweetness perception)	Generally well tolerated; rare hepatotoxicity case reports exist; hypoglycemia risk if combined with other antidiabetics.	Typical clinical dosing in studies: 200–400 mg/day (some use up to several grams); LiverTox notes typical recommended 400 mg TID in marketed products but standardization varies.	Monitor blood glucose closely when used with insulin/oral hypoglycemics; pregnancy safety not established.
Tinospora / Guduchi (*Tinospora cordifolia*)	Immunomodulator, antipyretic	Increasing case reports of herbinduced liver injury (HILI) since widespread use; many preparations not standardized.	No universally accepted human "toxic dose" — case reports describe liver injury after typical supplemental intakes. Animal data previously suggested low toxicity but human hepatotoxicity reports changed safety perspective.	Use caution in patients with liver disease; stop and test LFTs if symptoms of liver injury appear.

Garlic (*Allium sativum*)	Cardiovascular, antimicrobial	Generally low toxicity. High doses: GI upset, bleeding risk (antiplatelet effect). Rare allergic reactions.	Many trials use ~400 mg powdered garlic/day or variable fresh garlic amounts; no consistent LD_{50} in humans — considered lowtoxicity food/ supplement.	Use cautiously with anticoagulants (warfarin/antiplatelet agents); watch for GI side effects at high doses.
Amla / Indian gooseberry (*Emblica officinalis*)	Antioxidant, nutraceutical	Animal studies: low toxicity in acute/subchronic testing; no major adverse signals in many studies.	Reported NOAEL in some rat studies: ~1000 mg/kg/day; LD_{50} >2000 mg/kg in certain reports (animal data). Human dosing in supplements varies (e.g., 500–1000 mg/day typical).	Considered low risk in usual doses; standardization and extract solvent can change profile.
Ginger (*Zingiber officinale*)	Nausea, digestion, antiinflammatory	Mild GI effects, heartburn, loose stool; rare anaphylaxis cases reported.	Clinical studies commonly use 0.5–2 g/day; many sources state up to 3–4 g/day is generally safe for adults; expert panels show absence of toxicity in several animal studies up to g/kg levels.	Pregnancy: often used for nausea but some authorities advise limiting dose (many trials used ~1 g/day); avoid very high doses and monitor for bleeding risk if on anticoagulants.
Aloe vera (oral) (*Aloe barbadensis / Aloe vera*)	Laxative (latex), topical uses for skin	Whole-leaf extracts / latex contain anthraquinones (aloin) linked to laxative effects, genotoxicity in vitro and carcinogenicity signals in high-dose animal studies; oral whole-leaf extract has been associated with adverse effects.	Industry standard for orally consumed decolorized wholeleaf extract: aloin <10 ppm; some EU rules limit aloin/hydroxyanthra cene derivatives in foods. Toxicity concerns mainly with non-decolorized latex; genotoxicity evidence and animal carcinogenicity for whole-leaf extracts.	Use oral aloe products only if processed to remove latex anthraquinones; avoid long-term oral use; monitor for electrolyte disturbance with laxative use.

Short Interpretation and Practical Points

- **Numeric values:** most *definitive* numbers (LD_{50}, NOAEL) come from animal studies; human "toxic doses" are rarely precisely established because of variability in preparations and exposures. Animal LD_{50}/NOAEL where good data exist (neem, amla) and common clinical dosing where trials give guidance (turmeric, ginger, gymnema).
- **Hepatotoxicity watch list:** several herbs classically considered "safe" (e.g., ashwagandha, Tinospora) now have published cases of herb-induced liver injury (HILI) — monitor LFTs if used long-term or in patients with liver disease.
- **Drug interactions & special populations:** herbs that affect blood sugar (gymnema, bitter melon), clotting (garlic, high-dose turmeric/curcumin), or liver metabolism warrant careful co-use with prescription medicines, pregnancy avoidance or medical supervision.

Therapeutic Applications of Ethnobotanically-Informed Phytochemical and Translational Case Studies

Curcumin, derived from *Curcuma longa*, transitioned from traditional use to clinical relevance due to its validated anti-inflammatory and anticancer effects. Similarly, artemisinin from *Artemisia annua* became a WHO-recommended antimalarial agent.

Phytochemical and Translational Case Studies illustrating how traditional plant knowledge led to modern therapeutic or nutraceutical development

Phytochemical Compound	Plant Source	Traditional Use (Ethnobotanical Origin)	Scientific Validation /Pharmacological Evidence	Translational Outcome (Clinical/ Commercial Application)
Artemisinin	*Artemisia annua*	Used in Chinese medicine for fevers	Identified as potent antimalarial compound active against *Plasmodium falciparum*	Development of artemisinin-based combination therapies (ACTs); WHO-approved first-line malaria treatment
Paclitaxel (Taxol)	*Taxus brevifolia* (Pacific yew)	Used in Native American traditional medicine for wounds	Isolated as a potent microtubule-stabilizing anticancer agent	Commercialized as Taxol®; frontline therapy for breast and ovarian cancers

Reserpine	*Rauwolfia serpentina*	Used in Ayurveda for hypertension and mental disorders	Demonstrated antihypertensive and tranquilizing properties	Led to development of the first modern antihypertensive drug (Serpasil®)
Morphine	*Papaver somniferum*	Traditional analgesic and sedative	Identified as opiate alkaloid with CNS depressant effects	Foundation for modern opioid analgesics
Aspirin (Salicylic acid derivative)	*Salix alba* (Willow bark)	Pain, fever, and inflammation relief	Isolation of salicin → acetylation to acetylsalicylic acid	Development of Aspirin® (Bayer); global OTC and prescription drug
Digoxin	*Digitalis purpurea*	Cardiac remedy in European folk medicine	Shown to enhance cardiac contractility by inhibiting Na^{+}/K^{+}ATPase	Used clinically in congestive heart failure and arrhythmias
Quinine	*Cinchona officinalis*	Traditional remedy for fevers	Confirmed antimalarial alkaloid activity	Precursor for synthetic antimalarial drugs; still used in resistant malaria cases
Epigallocatechin gallate (EGCG)	*Camellia sinensis*	Used in Asian medicine for detoxification	Demonstrated antioxidant, anticancer, and cardioprotective effects	Incorporated into nutraceuticals, antiaging cosmetics, and functional beverages
Curcumin	*Curcuma longa*	Anti-inflammatory, wound healing	Validated for antiinflammatory, antioxidant, anticancer activity	Investigational agent in clinical trials; marketed in standardized extracts and nanoformulations
Silymarin	*Silybum marianum*	Used for liver protection	Validated hepatoprotective, antioxidant, and antifibrotic activity	Developed into standardized formulations (e.g., Legalon®) for liver diseases

Capsaicin	*Capsicum annuum*	Stimulating and analgesic use	Demonstrated TRPV1 receptor activation causing desensitization of pain fibers	Approved topical analgesic in creams and patches (e.g., Qutenza®)
Cannabidiol (CBD)	*Cannabis sativa*	Analgesic, anticonvulsant	Demonstrated antiepileptic, anxiolytic, and anti-inflammatory effects	FDA-approved drug (Epidiolex®) for epilepsy
Forskolin	*Coleus forskohlii*	Used in Ayurveda for heart diseases	Activates adenylate cyclase → increases cAMP levels	Used in ophthalmic (glaucoma) and metabolic formulations
Boswellic acids	*Boswellia serrata*	Traditional antiarthritic	Validated as 5lipoxygenase inhibitors	Developed as standardized Boswellia extracts (e.g., H15®) for arthritis management
Guggulsterone	*Commiphor a mukul*	Used in Ayurveda for lipid disorders	Demonstrated hypolipidemic and antiinflammatory effects	Marketed as natural lipidlowering nutraceutical
Vincristine / Vinblastine	*Catharanth us roseus*	Folk remedy for diabetes and infections	Found to inhibit mitosis by disrupting microtubules	Widely used chemotherapeutic agents for leukemia and lymphoma
Andrographolide	*Andrograp his paniculata*	Used in Ayurveda and Chinese medicine for infections	Exhibits immunomodulatory and hepatoprotective effects	Used in standardized extracts (e.g., KalmCold®) for respiratory infections
Emodin	*Rheum emodi*, *Aloe vera*	Laxative and detoxifying agent	Demonstrated anticancer and antiviral potential	Investigational phytoconstituent in anticancer therapy
Ginsenosides	*Panax ginseng*	Tonic, revitalizing agent	Demonstrated adaptogenic, immunomodulatory effects	Marketed globally as energy and cognitiveenhancing supplement

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Summary of Translational Significance These examples demonstrate **ethnopharmacological documentation → phytochemical isolation → mechanistic validation → clinical translation and commercialization** which explains

- The scientific merit of traditional knowledge,
- The need for standardization and quality assurance,
- The ethical obligation to recognize indigenous intellectual property, and
- The potential for developing evidence-based herbal drugs and nutraceuticals.

Selected Phytochemicals, Their Plant Sources, Traditional Uses, and Validated Pharmacological Actions

Phytochemical	Plant Source	Traditional Use:	Validated Pharmacological Action
Curcumin	*Curcuma longa*	Inflammation, wound healing	Anti-inflammatory, antioxidant, anticancer
Allicin	*Allium sativum*	Infections, hypertension	Antimicrobial, antihypertensive, cardioprotective
Withanolides	*Withania somnifera*	Stress, vitality, rejuvenation	Adaptogenic, anti-stress, neuroprotective
Catechins	*Camellia sinensis*	General health, antioxidant	Cardioprotective, neuroprotective, anticarcinogenic
Gymnemic acids	*Gymnema sylvestre*	Diabetes	Hypoglycemic, antihyperlipidemic
Saponins	*Tribulus terrestris*	Reproductive health, vitality	Aphrodisiac, androgenic, cardioprotective
Ginsenosides	*Panax ginseng*	Fatigue, weakness	Adaptogenic, immunomodulatory, neuroprotective
Berberine	*Berberis aristata* / *Coptis chinensis*	Diarrhea, infections	Antimicrobial, antidiabetic, antiinflammatory
Flavonoids (Quercetin)	*Onion (Allium cepa)*, *Ficus species*	Respiratory ailments, allergy	Antioxidant, antiinflammatory, antihistaminic
Resveratrol	*Vitis vinifera* (Grapes)	Cardiovascular wellness	Antioxidant, cardioprotective, antiaging
Gingerols	*Zingiber officinale*	Digestive disorders, nausea	Anti-inflammatory, antiemetic, antioxidant
Tannins (Gallotannins, Ellagitannins)	*Terminalia chebula*, *Quercus infectoria*	Diarrhea, wound healing	Astringent, antimicrobial, antioxidant

Boswellic acids	*Boswellia serrata*	Arthritis, inflammation	Anti-inflammatory, antiarthritic
Piperine	*Piper nigrum*	Digestive aid	Bioavailability enhancer, antioxidant, antiinflammatory
Silymarin	*Silybum marianum*	Liver ailments	Hepatoprotective, antioxidant, antifibrotic
Azadirachtin	*Azadirachta indica*	Skin infections, pest control	Insecticidal, antimicrobial, antiparasitic
Ellagic acid	*Punica granatum* (Pomegranate)	Antioxidant, heart health	Antioxidant, anticancer, cardioprotective
Phyllanthin	*Phyllanthus amarus*	Liver protection	Hepatoprotective, antiviral (anti-HBV)
Hesperidin	*Citrus aurantium* / *Citrus sinensis*	Circulatory support	Antioxidant, vasoprotective, antiinflammatory
Aloin / Aloin A & B	*Aloe vera* (latex)	Laxative	Purgative, wound-healing (leaf gel)
Baicalin	*Scutellaria baicalensis*	Fever, infections	Anti-inflammatory, antiviral, antioxidant
Eugenol	*Syzygium aromaticum* (Clove)	Dental pain, infections	Analgesic, antiseptic, antioxidant
Rosmarinic acid	*Rosmarinus officinalis*	Respiratory and inflammatory disorders	Anti-inflammatory, neuroprotective, antioxidant
Lupeol	*Aloe vera*, *Mango bark*, *Betula spp.*	Skin diseases, wounds	Anti-inflammatory, anticancer, hepatoprotective

Conservation, Sustainability, and Intellectual Property

Biodiversity Conservation

Overharvesting of medicinal plants threatens biodiversity and ecosystem balance. Conservation strategies involve *in situ* (protected areas, sacred groves) and *ex situ* (botanical gardens, seed banks) preservation.

Sustainable Harvesting and Cultivation

Adopting Good Agricultural and Collection Practices (GACP) ensures sustainability and quality. Cultivation of high-demand species like *Rauvolfia serpentina* prevents extinction threats.

7.3 Intellectual Property Rights (IPR) and Benefit-Sharing

Protection of indigenous knowledge under TRIPS, Nagoya Protocol, and Traditional Knowledge Digital Library (TKDL) ensures fair benefit-sharing and prevents biopiracy.

7.4 Community Participation

Involving local communities in documentation and conservation initiatives promotes awareness, ethical research, and economic empowerment.

Future Perspectives

- **Integration in Drug Discovery:** Combining ethnobotanical leads with modern drug discovery tools shortens the path from traditional use to clinical application.
- **Modern Analytical Tools:** Metabolomics, chemoinformatics, and AI-driven pattern recognition are revolutionizing natural product research by identifying bioactive markers efficiently.
- **Challenges and Opportunities:** Challenges include lack of standardization, regulatory hurdles, and variability in plant composition. Opportunities lie in multidisciplinary collaborations and advanced validation models.
- **Personalized Herbal Medicine:** Integrating ethnobotanical evidence with genomics can pave the way for personalized phytomedicine, aligning natural therapeutics with individual health profiles.

Conclusion:

The integration of ethnobotany and phytochemistry serves as a crucial interface between traditional knowledge systems and contemporary scientific analysis. By systematically linking ethnobotanical applications with phytochemical profiles and pharmacological validation, researchers can identify and develop natural therapeutics that are both safe and effective. This interdisciplinary approach enhances scientific rigor, promotes the sustainable use of plant resources, and upholds the ethical recognition and preservation of indigenous knowledge and biodiversity.

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COMPUTATIONAL PHYTOCHEMISTRY IN DRUG DISCOVERY: DATABASES AND TOOLS

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Abstract:

Computational phytochemistry has become a paradigm shift in the integration of the traditional ethnobotanical knowledge into the modern drug discovery. It incorporates bioinformatics, cheminformatics and molecular modeling to investigate plant-derived compounds that have therapeutic development potential. The rapid identification and optimization of bioactive phytoconstituents is possible due to innovation in high-throughput screening, virtual docking, and in silico ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis. Phytochemical databases like IMPPAT, KNApSAcK and PhytoHub contain detailed information about plant metabolites, biological activities and molecular structures which are useful in predicting pharmacological targets. In addition, computational packages such as AutoDock, Discovery Studio and SwissADME facilitate the ligand screening and drug-likeness screening process. By combining ethnopharmacological information with computational analysis, the reliability of drug discovery pipelines can be strengthened in that traditional uses are validated using scientific modeling. This multidisciplinary strategy, in addition to saving time and cost of the experimental screening, has been used in the rational design of lead molecules of natural origin. Finally, the computational phytochemistry is more of a paradigm shift, as it allows the sustainable and evidence-based discovery of the chemical diversity that nature offers to tackle the current issues in healthcare.

1. Introduction:

Natural products have played a long history as an excellent source of bioactive compounds of drug development. Ethnobotany Ethnobotany, which consists of studying the relationship between plants and people, is a source of important information about the medicinal properties of traditional flora [1]. As computational technologies have emerged, phytochemistry has progressed to a new stage called computational phytochemistry which uses in silico tools to comprehend, project, and improve plant-based molecules. The combination of conventional expertise and online analysis makes researchers discover drugs at a faster rate and reduce the cost of experimentation and ethical limitations [2].

The area of computational phytochemistry is an interdisciplinary field, combining pharmacognosy, cheminformatics, molecular biology and bioinformatics. By artificial methods, including molecular docking, virtual screening and quantitative structure-activity relationship (QSAR), it is possible to systematically consider the biological relevance of phytochemicals, without necessarily having laboratory validation [3]. Due to changes in global health demands, this is a most efficient route to finding new therapeutics in the broad range of chemicals available in plants, since it provides a sustainable and efficient way to access new therapeutics.

2. Ethnobotany and Its Application in Drug Discovery.

Using ethnobotanical knowledge, drug discoveries have been conducted over the centuries. About a quarter to a third of the contemporary medicine is made using plants that had been used in the ancient systems of healing [4]. Quinine (Cinchona bark), artemisinin (Artemisia annua) and paclitaxel (Taxus brevifolia) are examples. These findings affirm the significance of maintaining native knowledge systems which in most cases are well tried solutions.

When ethnobotany is combined with computational phytochemistry, scientists are able to focus on plants that have already been reported to have therapeutic effects to be analyzed molecularly. Conventional use gives context - such as disease targets and preparation methods - whereas computational models give mechanistic understanding. The combination of them forms a logical system of choosing bioactive leads [5]. As an example, structure-activity relationships towards ethnomedical relevance of Ayurvedic plant compounds can be seen by molecular docking known plant compounds with viral or cancer targets.

Computational Methods inPhytochemistry.

Computation techniques have become an essential part of natural product studies. The significant methods are:

- Molecular Docking: It predicts the interaction of phytochemicals with the binding sites on the proteins. The tools such as Glide and AutoDock are normally employed [6].
- Virtual Screening: This is based on the ability to quickly screen thousands of plant-based compounds against biological targets [7].
- QSAR Modeling: quantifies the correlation between chemical structure and biological activity and is useful in optimization of lead molecules [8].
- Pharmacophore Modeling: Determines major functional groups of activity.

- ADMET Prediction: Compared to other tools, it is a computational evaluation of the absorption, distribution, metabolism, excretion, and toxicity profiles [9].

When such tools are combined, researchers are able to come up with testable hypotheses that save a great deal of time and money in laboratory work. Computational phytochemistry is therefore a digital filter to narrow down on good candidates which can undergo further biological testing.

4. Major Phytochemical Databases

To perform accurate computational analyses, access to high-quality phytochemical data is essential. The following databases have become standard resources in this field:

Table 1: Major phytochemical databases used in computational phytochemistry

Database	**Description**	**Managing Institution**	**Accessibility**
IMPPAT	Indian Medicinal Plants, Phytochemistry and Therapeutics database; provides data on over 1700 Indian plants and 9500 phytochemicals.	CSIR–NCL, India	Free
KNApSAcK	Contains metabolite–species relationships for over 50,000 compounds.	Nara Institute of Science and Technology, Japan	Free
PhytoHub	Database of dietary phytochemicals and metabolites.	INRAE, France	Free
NPASS	Natural Product Activity and Species Source database integrating activity and taxonomy data.	Peking University, China	Free
Dr. Duke’s Phytochemical Database	Classic resource with ethnobotanical and phytochemical profiles of medicinal plants.	USDA, USA	Free

These resources allow retrieval of chemical structures, biological targets, and traditional uses, which form the foundation of computational analyses [10].

5. Key Computational Tools and Software

Table 2: Common computational tools applied in phytochemistry

Tool	Primary Application	Key Features	Availability
AutoDock Vina	Molecular docking	Open-source, widely used for binding affinity prediction	Free
Discovery Studio	Drug design & simulation	Comprehensive suite for docking, QSAR, and visualization	Commercial
SwissADME	ADMET and drug-likeness prediction	Web-based; predicts Lipinski parameters	Free
PASS Online	Biological activity spectrum prediction	Uses training data of >250,000 compounds	Free
PyMOL	Molecular visualization	Used for structure viewing and publication graphics	Free/Commercial

The choice of tool depends on the research objective—whether it is ligand screening, structure refinement, or ADMET prediction [11].

6. Computational Phytochemistry Case Studies

Many studies indicate the effectiveness of computational processes in the case of traditional medicinal plants validation. An example that has been used is molecular docking of the curcumin and its analogs with various inflammatory mediators like COX-2 and it has been established that they do contain anti-inflammatory properties [12]. Similarly, an in-silico binding affinity of alkaloids of Rauwolfia serpentina has been demonstrated to high affinity of dopamine receptors, which explains their antipsychotic use [13].

The second example that can be mentioned is that Azadirachta indica (neem) screening as antiviral agents against SARS-CoV-2 main protease. The results obtained through computation were stable and were experimentally validated [14]. Such evidence clarifies the importance of the computational phytochemistry as the process of ethnobotanical validation.

7. Future Vision and Future Prospects

Artificial intelligence (AI) and machine learning (ML) and big data analytics is the future of the field of computational phytochemistry. Predictive algorithms can be used on large chemical libraries to identify trends in structure-activity that cannot be identified by the human intuition [15]. However, it still has some problems like data inconsistency, unstandardization and inadequate access to 3D phytochemical structures.

Moreover, ethnobotanical data ought to be ethically applied and share the benefits with the indigenous people in order to maintain trust and attain equitable scientific advancement [16]. This interdisciplinary aspect and more efficient nature-inspired therapeutics will be found by further collaboration of chemists, botanists and computational biologists**.**

Conclusion:

Computational phytochemistry is a synthesis of the old wisdom of ethnobotany and the precision of the modern computational science. With the help of the curated databases and powerful in silico tools, researchers will be able to find out the pharmacological potential of the plant-derived molecules within short time, model them and prove them. The combination decreases the onus of experiments as well as maximizing the discovery efficiency. As the field continues to develop, the contribution of computational methods has been one of the most important in transforming the ethnopharmacological knowledge to scientifically tested and clinically applicable medicines.

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A COMPREHENSIVE REVIEW ON *PHLOGACANTHUS THYRSIFORMIS* NEES: BOTANY, DISTRIBUTION, AND MEDICINAL POTENTIALS

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Abstract:

Phlogacanthus thyrsiformis Nees, an evergreen shrub of the family Acanthaceae, is widely distributed across the subtropical regions of the Indian subcontinent and Southeast Asia and has gained attention for its significant medicinal properties. Ethnobotanical reports highlight its use in the treatment of diabetes, respiratory conditions, pain, and infections. Phytochemical investigations have confirmed the presence of bioactive compounds such as flavonoids, alkaloids, diterpene lactones, and glycosides, which contribute to its diverse pharmacological effects including antihyperglycemic, hypolipidemic, antioxidant, analgesic, and hepatoprotective activities. In vitro and in vivo studies support these claims, with recent models showing promising therapeutic outcomes and low toxicity. This chapter presents a comprehensive review of the botany, taxonomy, distribution, phytochemistry, pharmacological activities, and safety profile of *P. thyrsiformis*, emphasizing its potential for development into modern plant-based therapeutics.

Keywords: *Phlogacanthus thyrsiformis*, Acanthaceae, Antihyperglycemic Activity, Ethnomedicine

Introduction:

An evergreen shrub called Phlogacanthus thyrsiformis belongs to the Acanthaceae family and is native to subtropical places such as the Himalayas, Gangetic plains, Bihar, North Bengal, and the northeastern region of India. Locally known by various names such as *titaaphul*, *chuhai*, and *tangkhul*, this plant can grow up to 2.4 meters in height and is characterized by quadrangular branchlets and broad leaves measuring between 13 to 35 cm in length. Among the Indigenous communities, particularly the Jaintia tribe of Meghalaya, *P. thyrsiformis* is significant in traditional medicine. A lot of research has shown that it has antihyperglycemic, hypolipidemic, and hepatoprotective properties. Figure 1 represents the *Phlogacanthus thyrsiformis* plant, a member of the Acanthaceae family, commonly used in traditional medicine for its anti-inflammatory and analgesic properties.

Figure 1: ***Phlogacanthus thyrsiformis***

Phytochemical investigations have revealed that *P. thyrsiformis* is a rich source of secondary metabolites including alkaloids, flavonoids, steroids, triterpenoids, polyphenols, and glycosides, all of which contribute to its diverse pharmacological activities. Recent research has leveraged *Caenorhabditis elegans*, a genetically tractable model organism, to assess the antioxidative and immune-modulatory potential of its bioactive compounds, thereby offering insights into host-pathogen interactions and plant-based therapeutic mechanisms (Suchiang & Kayde, 2017).

The plant is particularly esteemed in Assam for its efficacy in managing diabetes. Its flowers, known for their strong free radical scavenging properties, are widely used in traditional formulations to treat diabetes and other metabolic disorders (Bora *et al.,* 2018). The genus *Phlogacanthus*, comprising approximately 49 species distributed across tropical Asia, is noted for a broad range of biological activities, including anti-inflammatory, antibacterial, and antioxidative effects. Notably, *Phlogacanthus cornutus*, a species endemic to Vietnam, has demonstrated promising antioxidant potential in recent studies exploring its chemical constituents (Ahmed *et al.,* 2016).

Given its longstanding ethnomedicinal use and phytochemical richness, *P. thyrsiformis* presents significant promise for modern pharmacological exploration. Studies have investigated the antidiabetic and antioxidative effects of its flower extracts using alloxan-induced diabetic mice models. These findings contribute to the growing scientific understanding of the plant's therapeutic potential and support its possible integration into contemporary medicinal practices.

Taxonomy

Phlogacanthus thyrsiformis is taxonomically classified under the Kingdom *Plantae* and further categorized based on standard botanical hierarchy. Table no 1, outlines the hierarchical taxonomic classification of *Phlogacanthus thyrsiformis* and further categorizing it into its respective division, class, order, family, genus, and species.(Ningthoujam *et al.,* 2023)

Table 1: Phlogacanthus thyrsiformis taxonomy classification table

Kingdom	**Plantae**
Division	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales
Family	Acanthaceae
Genus	*Phlogacanthus*
Species	*thyrsiformis*
Synonyms:	Phlogacanthus thyrsiformis, thyrsiformis, Justicia thyrsiflora Justicia
Vernacular name	Manipuri: Nongmangkha Assamese: Banheka, Titabahak, titaphul Garo: Ellot Khasi: Baskabomphang, Dieng-soh-ja-buid, dieng-soh-kajut Mikir: Jaogan, Rambha arong Nepali: Chua Lepcha: Rheeom Kumaun: Kaldona, Kawadoni Nepali: Chua Lepcha: Rheeom Kumaun: Kaldona, Kawadoni Bengali: Tamropuspi Basok

Geographical Distribution

These evergreen shrubs, which belong to the Acanthaceae family, are indigenous to Indochina and the Indian subcontinent. They have spread to Bangladesh, Myanmar, Nepal, Thailand, Indonesia, Sri Lanka, and the subtropical Himalayas. The following areas are home to the majority of Phlogacanthus thyrsiformis' distribution:

1. India: North-eastern states such as Assam, Manipur, Mizoram, and Tripura.Bangladesh - Widely found in various parts of the country.
2. Myanmar: Present in different regions.
3. China: Specifically in Yunnan province.
4. Thailand: Various regions, particularly in northern areas.

This plant typically thrives in tropical and subtropical climates and is often found in moist, shady environments such as forest undergrowth and along streams. In the Northeastern region of India, this plant is distributed across several states, as outlined in Table no 2.

Table 2: Distribution of a Species in the North-eastern States of India

State	Specific Areas/Regions
Assam	Various districts, primarily in hilly and forested regions
Manipur	Forested and hilly areas
Meghalaya	Khasi Hills, Jaintia Hills, Garo Hills
Mizoram	Forested and hilly regions
Nagaland	Forested areas, especially in the hilly regions
Arunachal Pradesh	Some parts of the lower hills and forests

Morphology

Botanical Description: The morphology of Phlogacanthus thyrsiformis includes the following characteristics which is mentioned in details in table no 3. (Ningthoujam *et al.,* 2023)

Table 3: Morphology of Phlogacanthus thyrsiformis

Plant Part	Description
Habit	Perennial shrub
Stems	Erect, quadrangular, slightly woody at the base; typically green with a reddish tint
Leaves	Opposite and decussate; lanceolate to ovate-lanceolate; 10–20 cm long, 4–8 cm wide; smooth, glabrous surface; entire or slightly wavy margins
Flowers	Terminal thyrses; tubular, bilabiate flowers; ~4–5 cm long; bright red to reddish-orange; small, leaf-like bracts; five-lobed persistent calyx
Fruit	Capsule; oblong; ~2–3 cm long; contains numerous small, flattened seeds
Root System	Fibrous with adventitious roots
Seeds	Small, flat, and brown, with wing-like structures for dispersal

Phytochemicals and Pharmacological Activities of *Phlogacanthus* Species

In recent years, several species of *Phlogacanthus*, including *Phlogacanthus thyrsiformis*, have been investigated for their phytochemical constituents and pharmacological activities. Ethnomedicinal uses such as the treatment of diabetes, respiratory disorders, and microbial infections have been validated through preliminary phytochemical and pharmacological studies. Notably, different parts of *P. thyrsiformis*—including leaves, flowers, and the whole plant—have shown diverse biological activities such as antihyperglycemic, analgesic, antifungal, and nematocidal effects.

The ability of P. thyrsiformis flower extract to lower blood sugar levels has been confirmed in experimental diabetic models, supporting its traditional use in Assam for managing diabetes. In addition, several bioactive compounds such as diterpene lactones, β-sitosterol, and lupeol have been isolated from various parts of the plant.(Koushik *et al.,* 2020)

These findings are summarized in Table 4**,** which presents the phytochemicals identified and the pharmacological activities reported for different *Phlogacanthus* species.

Table 4: Phytochemicals and Pharmacological Activities of *Phlogacanthus* Species

S. No.	Plant Part Used	Phytochemical(s) Present or Isolated	Pharmacological Activities	References
1	Leaves (P. pubinervius)	Terpenoids include β-sitosterol, stigmasta derivatives, and 3-O-[β-D-glucopyranosyl-(1'→2')-α-L-rhamnopyranosylphlogacanthoside].	Antidiabetic, Cough,Cold, Asthma	A. *et al.,* 2014
2	Roots (P. curviflorus)	Phlogacanthosides A–C (diterpene lactone glucosides), lupeol, betulin, syringaresinol, and phlogacantholides B & C (diterpene lactones)	Tonic, Antipyretic	Teerakitchotikan *et al.,* 2023
3	Leaves (P. thyrsiformis)	Phloganthoside (phlogantholide-A-19-O-β-d-glucopyranoside), betulin, lupeol, and β-sitosterol	Analgesic, Antifungal, Antidiabetic, Nematicidal	*BURUAH InvitropropagationofPhlogacanththyrsiflorus*, n.d.
4	Flowers (P. thyrsiformis)	Phlogantholide-A and other diterpene lactones	Antihyperglycaemic effect	Chakravarty & Kalita, 2012
5	Leaves (P. thyrsiformis)	Not specified (ethanol extract)	Central and peripheral analgesic activity	Mukherjee *et al.,* 2009
7	Leaves (P. thyrsiformis)	Not specified	Antifungal against *Aspergillus* and *Rhizopus* species	Victoria Chanu *et al.,* 2021

Pharmacological Actions of *Phlogacanthus thyrsiformis*

Phlogacanthus thyrsiformis has been studied for a variety of pharmacological activities, supporting its traditional use in ethnomedicine. The flower extract of this plant exhibits significant antihyperglycemic effects. In mice with diabetes induced by streptozotocin, the flower extract significantly decreased blood glucose levels. A dose of 250 mg/kg methanolic flower extract reduced fasting blood glucose by 31.3% at 2 hours, 44.7% at 4 hours, and 50% at 6 hours. Higher doses such as 450 mg/kg and 550 mg/kg led to even greater reductions, reaching up to 79% after 6 hours. These findings suggested that 250 mg/kg was the ideal dosage for further testing.(Ahmed *et al.,* 2016; Chakravarty & Kalita, 2012) (Bora *et al.,* 2018)In diabetic nephropathy models using alloxan-induced diabetic mice, the methanolic flower extract also improved glucose tolerance and restored normal kidney function, suggesting its potential in diabetic kidney complications (Bora *et al.,* 2018). The ethanol extracts from the flowers and leaves produced considerable hypoglycaemic and hypolipidemic effects, further supporting the therapeutic potential of the plant in the management of diabetes and associated metabolic disorders (Koushik *et al.,* 2020). The ethanol leaf extract has demonstrated both central and peripheral analgesic properties in experimental models, including tail flick and acetic acid-induced writhing tests. These findings highlight the plant's ability to reduce pain through multiple mechanisms (Mukherjee *et al.,* 2009).Nematicidal activity is another noteworthy pharmacological property. Leaf extracts effectively controlled *Meloidogyne incognita*, a root-knot nematode, indicating potential for use in botanical pest control and sustainable agriculture (Afzal *et al.,* 2021).GC-MS analysis and phytochemical screening identified a number of bioactive substances that contributed to antioxidant, antibacterial, and anti-inflammatory properties. While *Justicia adhatoda* sometimes exhibited stronger activity, *Phlogacanthus thyrsiformis* still showed significant potential. However, variations in phytochemical content due to geographical and seasonal differences emphasize the need for further standardization and in vivo validation (Ningthoujam *et al.,* 2023).*Phlogacanthus thyrsiformis* possesses diverse pharmacological properties, including antihyperglycemic, hypolipidemic, antifungal, analgesic, nematicidal, antioxidant, and antibacterial effects. These findings provide a scientific basis for its traditional medicinal uses and encourage further research toward novel therapeutic formulations.

Biotechnology

Hassan et al. (2011) developed an efficient protocol for the in vitro shoot proliferation and plant regeneration of *Phlogacanthus thyrsiformis* Nees (Acanthaceae), a rare medicinal shrub of Bangladesh. The study utilised shoot tips and nodal explants for culture initiation. The highest shoot induction response was observed on Murashige and Skoog (MS) medium supplemented with 1.0 mg/L BAP (6-benzylaminopurine) and 0.5 mg/L NAA (α-naphthaleneacetic acid), 84.2% of nodal explants produced the maximum number of shoots per culture (12.4 ± 0.66).(*A.K. M. Sayeed Hassan*, n.d.)

Safety and Toxicological Studies

Phlogacanthus thyrsiformis has been evaluated for safety and toxicity in multiple studies, demonstrating low acute toxicity and potential therapeutic benefits. Here's a synthesis of key findings:

Acute Toxicity

LD_{50} > 2,000 mg/kg: In acute oral toxicity tests, both ethanol leaf extracts and methanolic flower extracts showed no mortality in mice at doses up to 2,000 mg/kg body weight, indicating low acute toxicity(Bora *et al.,* 2018)

No adverse effects were observed in behavioural or physiological parameters at these doses(Bora *et al.,* 2018).

Sub-Acute Toxicity

28-day study (400 mg/kg): Methanolic flower extract administered to mice for 28 days caused no significant changes in body weight, organ weight (liver, kidney, heart), or histopathology (Mukherjee *et al.,* 2009).

Hepatotoxicity and Protective Effects

Diabetic mice model: A 21-day study using flower extract (100–200 mg/kg) in streptozotocin-induced diabetic mice showed **reduced hepatic enzymes** (SGPT, SGOT, ALP), suggesting potential hepatoprotective effects rather than toxicity.

No evidence of liver damage was reported in healthy animals at tested doses(*GOGOI Phytochemistry_and_Pharmacology_of_Phlog (2)*, n.d.)

Analgesic and Anti-Inflammatory Safety

Ethanol leaf extract (500 mg/kg) demonstrated central and peripheral analgesic activity in mice without toxicity, comparable to aspirin and pethidine.

Naloxone reversal experiments indicated partial opioid receptor agonism as a mechanism, with no observed adverse effects(Mukherjee *et al.,* 2009).

Limitations and Gaps

Chronic toxicity, reproductive toxicity, and genotoxicity studies are absent in the available literature.

Most studies used animal models; human safety data are lacking.

P. thyrsiformis exhibits a favorable safety profile in acute and sub-acute studies, with evidence of protective effects in disease models. Further research is needed to confirm long-term safety and validate human applicability.

Conclusion:

Phlogacanthus thyrsiformis Nees, a traditionally revered medicinal shrub of the Acanthaceae family, emerges as a plant of remarkable pharmacological promise. Rooted deeply in the ethnomedical practices of the Northeastern tribes of India—particularly the Jaintia, Khasi, and Assamese communities—this species has long served as a natural remedy for metabolic disorders, pain, inflammation, and microbial infections. The comprehensive phytochemical investigations reveal a rich profile of bioactive compounds, including flavonoids, alkaloids, terpenoids, and glycosides, which scientifically validate many of its folk uses. Recent experimental studies, particularly using diabetic animal models, have demonstrated the plant's potent antihyperglycemic and hypolipidemic effects, with minimal toxicity. Moreover, the flower and leaf extracts have shown additional benefits such as hepatoprotective, analgesic, antifungal, and nematicidal activities. These findings underscore the therapeutic potential of *P. thyrsiformis* and support its development into novel plant-based formulations for the treatment of chronic diseases. Despite these advances, notable gaps remain in long-term safety assessments, human clinical trials, and standardized phytochemical characterizations across different regions and seasons. The promising biotechnological efforts, including successful in vitro propagation protocols, open doors for conservation and sustainable utilization. *Phlogacanthus thyrsiformis* stands at the confluence of traditional knowledge and modern pharmacological science. Its broad-spectrum bioactivity, low toxicity, and ethnobotanical significance warrant deeper exploration and integration into contemporary medicine, potentially contributing to future drug discovery and development from natural sources.

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SUSTAINABLE MUSHROOM PRODUCTION FOR SMALL-SCALE FARMERS

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Introduction:

Growing mushrooms is a great option for small and marginal farmers because it takes little land and yields quick profits. With little funding, it offers a feasible way to make a sizable additional income (*Sarkar & Islam, 2022*). Regular harvests and consistent income flow are made possible by the brief production cycle. Farmers can increase profitability and lower overhead by concentrating on low-cost inputs. Straw, husks, and sawdust are examples of locally accessible agricultural waste that can be recycled into substrate. This encourages environmentally friendly farming methods while simultaneously reducing expenses. Having access to basic technology guarantees production consistency and increases yield. With little financial investment, small-scale manufacturers can create robust microenterprises. These models promote food security and employment in rural areas. All things considered, growing mushrooms provides a scalable route to sustainable agriculture.

1. Rationale for Small-Scale Viability

The high yield per unit area and compliance with current agricultural practices are two characteristics that set mushroom production apart. Because of its versatility, it is a great choice for farmers looking to diversify their revenue streams, particularly in areas with weak economies (*Yıldırım & Yıldız, 2025*). Rapid financial returns are made possible by the short production cycle, which usually lasts only a few weeks from spawning to harvest. This is especially advantageous for small-scale farmers that have little money and land. The concept places a strong emphasis on inexpensive inputs, using locally accessible resources like sawdust, straw, and agricultural waste. These resource-efficient methods improve accessibility while lowering overhead. Mushroom cultivation contributes to sustainable living by maximising production and minimising investment. Additionally, it supports the more general objectives of food security and rural development. Long-term viability is ensured by giving affordability and scalability top priority. This makes this cultivation model a useful guide for inclusive agricultural advancement (*Ademola & Oladapo, 2025*).

2. Choosing Sustainable, Low-Cost Substrates

The substrate is the most important cost factor in the mushroom producing process. Using lignocellulosic agricultural waste, which is plentiful and frequently free or extremely affordable, combines sustainability and profitability (*Bhanja et al., 2024*).

Table 1: Type of Substrates

Substrate Type	Source/Relevance	Sustainability Benefit
Paddy/Wheat Straw	Most common, readily available post-harvest waste.	Reduces agricultural burning and pollution.
Rice Husk	Widely available, excellent for blending with other materials.	Cost-effective filler, improves aeration.
Sugarcane Bagasse	Waste product of sugar mills, highly effective substrate.	Turns industrial waste into food.
Coffee Grounds	Waste from processing plants or cafes (if available locally).	High nitrogen content, excellent growth potential.

Input costs are significantly decreased by using locally accessible agricultural leftovers rather than commercial substrates. Straw, husks, and sawdust materials that are frequently thrown away can be used again to grow mushrooms. This method turns agricultural waste into a useful resource rather than a burden on the environment. It reduces reliance on outside inputs and encourages circular farming methods. Improved sustainability and reduced costs are advantageous to farmers. These resource-saving techniques are perfect for inexpensive mushroom models. Additionally, they encourage environmentally responsible rural entrepreneurship. In general, waste-to-resource tactics improve environmental stewardship and profitability (*Ademola & Oladapo, 2025*).

3. Low-Tech Cultivation Techniques

Low-Tech Mushroom Farming Principles must be implemented by a sustainable small-scale business in order to minimise infrastructure costs (*Patil & Singh, 2023*). By using this method, costly and energy-intensive climate control devices are avoided.

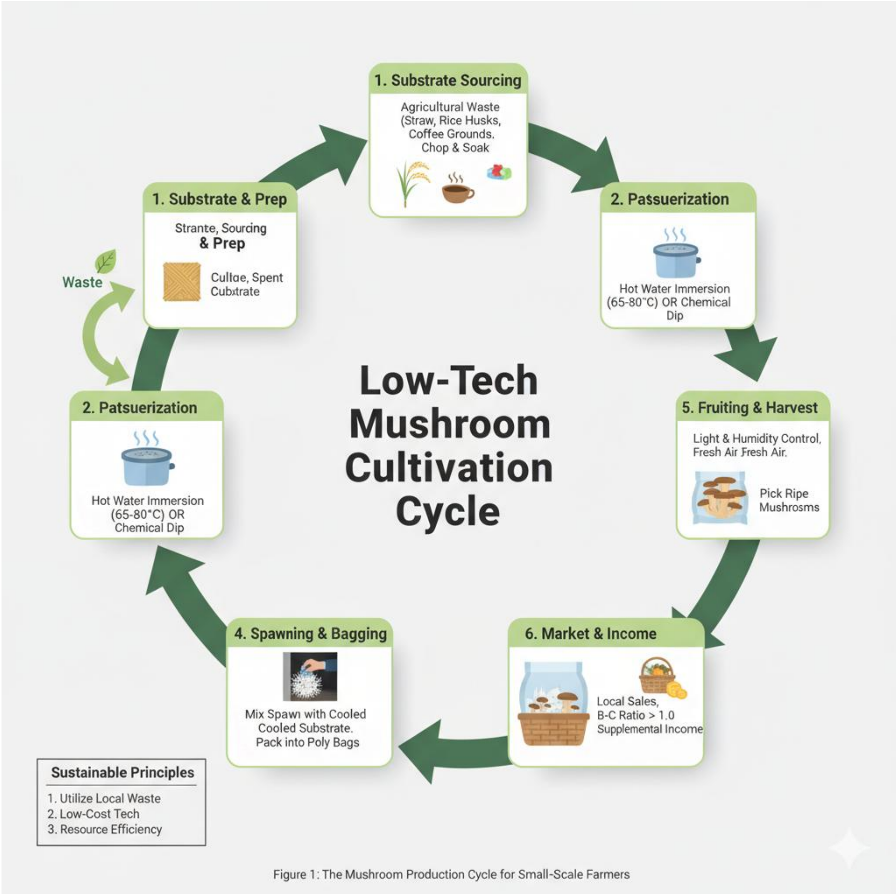


Figure 1: The Mushroom Production Cycle for Small-Scale Farmers

a. Infrastructure

Instead of building high-tech facilities, cultivation can be managed in:

- Clean, well-ventilated shelters or rooms with shade (such as bamboo constructions or mud huts).
- Basic, controlled settings that regulate humidity and temperature with the use of thoughtful airflow and simple coverings (*Ademola & Oladapo, 2025*).

b. Substrate Pasteurization

Sterilization using high-pressure steam is expensive. Low-tech alternatives for pasteurization include:

- **Hot Water Immersion:** Without the use of complex equipment, competitors can be eliminated by soaking the substrate (such as straw) in hot water (65°C to 80°C) for a number of hours (*Kasiraman, 2025; Patil & Singh, 2023*).
- **Chemical Treatment:** using a cold sterilization/pasteurization technique with low-concentration chemical disinfectants (such as formalin or lime) and then letting the food air out completely.

Figure 2: Sustainable Substrate Options for Low-Cost Mushroom Farming

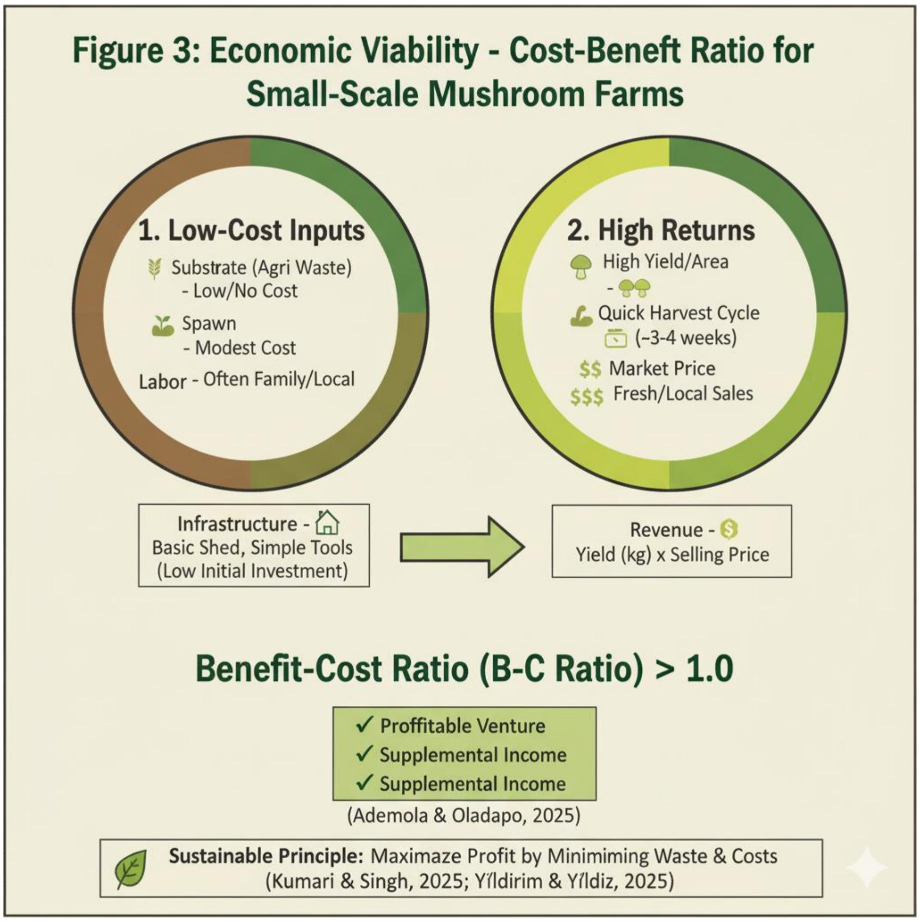
c. Spawning and Incubation

The cultivation process follows standard steps but is scaled down for manual, low-tech operation (*Tewari & Kapoor, 2025*):

i. Preparing the substrate involves chopping and soaking the agricultural waste.
ii. Sterilization/Pasteurization: The substrate is treated.
iii. Spawning: The process of combining the prepared mushroom spawn with the cooled substrate, which is usually placed in columns or polyethylene bags.
iv. Incubation (Spawn Run): Until the mycelium completely colonises the substrate, bags are stored in a clean, dark environment.
v. Fruiting: To enable the mushrooms to fruit, the bags are transported to a well-lit area, the humidity and fresh air levels are regulated, and the bags are cut.

4. Economic Feasibility and Market Strategy

Input management is key to mushroom farming's high profitability. A thorough cost-benefit analysis (CBA) is necessary for a small-scale farmer to succeed in order to determine the return on investment (*Yıldırım & Yıldız, 2025*).



a. Cost-Benefit Analysis

The Benefit-Cost Ratio (B-C Ratio) is a critical measure that needs to be much higher than 1.0 in order for the enterprise to be profitable. Here are six important economic factors to keep an eye on (Kumari & Singh, 2025):

i. Input Costs (Minimised): manpower (which is frequently family labour), plastic bags, and seed.
ii. Infrastructure Costs (Low): The initial outlay for basic tools, storage, and shelters.
iii. Revenue: Based on the selling price per kilogramme and the yield (kg per unit area).

Small-scale production can attain a high B-C ratio by sustaining low input and infrastructure costs through local, sustainable methods, which makes it a desirable choice for economic advancement (*Tewari & Kapoor, 2025*).

b. Marketing

Realising the greatest price requires an understanding of the market structure, whether selling directly to customers, regional vendors, or broader markets (*Kumari & Singh, 2025*). For small producers, direct sales at neighbourhood farmers' markets or partnerships with eateries frequently yield the highest returns.

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AN INVESTIGATION ON ETHNOMEDICINAL USES OF NATIVE LEAFY VEGETABLES IN DUNGURIPALI BLOCK OF SUBARNAPUR, ODISHA, INDIA

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Abstract:

Traditional knowledge is crucial for preserving a balance towards biodiversity and sustainability given the current condition of deforestation and the continuously diminishing availability of commercially and medicinally relevant flora. It needs to be addressed and transmitted from generation to generation. In rural places, traditional medicine is often used. It is based on knowledge, experience, and information about the plants that will be used for different purposes. A renewed interest in traditional medicine, particularly herbal medicines, has been spurred by the study of ethnobotany. The ethnomedical qualities of a few native, green leafy vegetables from Dunguripali Block of Subarnapur district are the main subject of this study. In this present study we reported about the ethnomedicinal uses of a total of 30 leafy vegetables by the native of study site. According to ethnobotanical data collected from field surveys it was found that leafy vegetables were used as diuretics, laxatives, digestive aids, antioxidants, tonics, etc. The leafy vegetables are rich sources of iron, proteins, and other vital elements that are utilized in a normal diet. Therefore, in their natural environment, they act as an indirect replacement for easily accessible medicinal supplies. Hence, by analyzing their bioactive components, these plants might be offered as herbal medicine in the future.

Keywords: Leafy Vegetables, Ethnomedicinal, Herbal Medicines, Bioactive Ingredients

Introduction:

India is endowed with an abundance of native and underutilized vegetables, especially green vegetables, which are frequently employed as food sources and sources of income for many of the country's impoverished inhabitants. Since before civilization, man has had a tremendous influence on edible wild plants (Kamble and Jadav, 2013). Due to their high nutritional content, these plants can be employed in a variety of environmental conditions, such as drought. India is a wealthy nation with a diverse population, which contributes to its great biodiversity (Sahu and Ekka, 2021). Leafy vegetables, sometimes referred to as greens or leafy greens, are plant leaves that are occasionally consumed as vegetables along with delicate petioles and shoots. A balanced diet must include leafy vegetables. Because they are more affordable and readily available, they are frequently referred to as "poor man's vegetables." Despite their affordability and accessibility, they are nevertheless underutilized today because people are unaware of their nutritional and therapeutic benefits (Mishra *et al.,* 2008). Furthermore, traditional knowledge about their ethnomedical applications is disappearing daily as a result of urbanization. In addition to their numerous uses in business and agriculture, indigenous leafy vegetables have enormous potential to alleviate the hunger of the nation's expanding population (Sheela *et al.,* 2004). Green leafy vegetables are a significant class of nutrient-dense foods that are thought to be nature's anti-aging gifts (Sahu and Sahu, 2019). Because of their concentrated and abundant supply of minerals and phytochemicals, they are both a cheap food source and a potential herbal remedy (Sharma *et al.,* 2024). Among all vegetable crops, leafy vegetables stand out due to their high nutrient content, exceptional health, and abundance of phytochemicals, antioxidants, vitamins, and minerals, including lutein, zeaxanthin, and beta-carotene, which both prevent cell damage and help treat age-related problems. Tocopherols and polyphenols are essential for general health and provide protection against cancer and coronary heart disease, together with vitamins like A, C, K and minerals like Ca, Fe, Na, and Zn (Negi and Roy, 2000; Lakshmi and Vimala, 2000; Misra and Misra, 2014). Nutrient-dense leafy veggies can help combat malnutrition and strengthen our immune systems. They are the most accessible sources of fibre, vital amino acids, proteins, lipids, carbs, vitamins, and minerals. These are recognized to include antioxidants that are essential for counteracting free radicals, which are recognized chemical risks to humans (Mosha and Gaga, 1999). It is regrettable that some of the beneficial traditional leafy vegetables that local and tribal people have long used for food and medicine are now gradually being neglected. Regretfully, some of them are still only found in their native habitat, are rarely consumed, and are not explored commercially (Noor and Satpathy, 2022, Sahu and Ekka, 2021; Patra *et al.,* 2025). If efforts are not taken to teach younger generations about the significance of wild leafy vegetables, knowledge of them may be lost in the near future. These traditional greens may be used in medicine in the future since they are rich in bioactive chemicals. These underutilized crops can be exploited and used more widely to address social issues including unemployment, poverty, and food security and health (Buragohain *et al.,* 2013). The necessity of the hour is to investigate the hidden potential of native leafy vegetables for future generations by prompt documenting and conservation, as ethnomedical knowledge is disappearing daily. Larger-scale research on native leafy vegetables can yield unexpected variants that could be employed as superfoods and future medications (Noor and Satpathy, 2020).

Several authors were reported the use of leafy vegetables by the native of various regions of Odisha like leafy vegetables of Bargarh (Sahu and Ekka, 2021); Nabarangpur (Sahu and Sahu, 2022); Balangir (Dash and Sahu, 2023); Barpali NAC of Bargarh (Patra *et al.,* 2025), In the state of Odisha, several ethnobotanical studies have been conducted on edible wild plants (Sahu *et al.,* 2010; Sahu *et al.,* 2013; Rana *et al.,* 2020; Sahu and Mishra, 2022; Mishra and Sahu, 2022; Routh and Sahu, 2023; Sahu and Sahu, 2023; Sharma *et al.,* 2024; Sahu *et al.,* 2025); nevertheless, the variety and traditional therapeutic uses of leafy vegetables in this region have not received much attention. Even so, the Subarnapur district of Odisha has a wide range of leafy vegetables. Therefore, the current study's goals are to identify, document, and create a scientific database on the leafy vegetables found in the Subarnapur region's Dunguripali Block as well as to identify the most popular leafy vegetables with medicinal qualities that are effective against a range of illnesses.

Materials and Methods:

Study Area

The administrative district of Subarnapur, commonly known as Sonepur district, is located in the eastern Indian state of Odisha. The district office is in the city of Sonepur. With over a hundred temples, Sonepur is referred to as the Mandiramalini town (city of temples) in Odisha. In 1993, it was formally created as a district. Sambalpur district borders it on the north, Boudh district borders it on the south and southeast, Rairakhol subdivision of Sambalpur district borders it on the east, and Balangir district borders it on the west. The district is 2284 km^2 in size. Sonepur, Ullunda, Dunguripali, Binka, Tarva, and Birmaharajpur are the six administrative blocks that make up the Subarnapur district. The Dunguripali block is well-known for producing paddy crafts. It is further subdivided into many gram panchayats and 132 villages. There were 115,713 people living there as of the 2011 census, and the literacy rate was roughly 73%.

Data Collection

As one author (SP) native village comes under the Dunguripali block of Subarnapur district of Odisha, the seasonal field trips were easier for her. Semi-structured interviews and conversations were used to gather information about the range of native green leafy vegetables that the block's native and tribal residents eat, as well as its ethnomedical applications against different illnesses. For the survey, 120 local informants, including 40% women and 60% men were asked questions. The informants ranged in age from 25 to 80. The botanical name, followed by local name, family, habit, flowering and fruiting season, parts used, diseases treated were arranged in a table.

Plant Identification

Plant specimens collected during field trips were carefully examined taxonomically and identified by consulting the local flora. Digital photos of plants taken in their native environments were used to help with naming and identification by using flora books (Haines 2021-25; Saxena and Brahmam, 1994-96) and available literatures from Odisha (Sahu *et al.,* 2010; Sahu *et al.,* 2013; Sahu *et al.,* 2019; Sahu *et al.,* 2024; Bhoi *et al.,* 2025; Bhoi and Sahu, 2025; Dash *et al.,* 2025; Mallik *et al.,* 2025; Padhan *et al.,* 2025).

Results and Discussion:

A total of 30 indigenous green leafy vegetables are collected from the study areas (Table 1, Figure 1). Out of 30 indigenous leafy vegetables Brassicaceae is the dominant family with five species followed by Amaranthaceae with four species; both the family Convolvulaceae and Cucurbitaceae contribute two species each; rest 17 families (Amaryllidaceae, Apiaceae, Araceae, Basillaceae, Boraginaceae, Caesalpiniaceae, Chenopodiaceae, Fabaceae, Lamiaceae, Lauraceae, Marsileaceae, Meliaceae, Moraginaceae, Nyctaginaceae, Portulaceae, Rutaceae and Tiliaceae) contributes one species each (Figure 2). Habit wise distribution of the leafy vegetables shows 20 numbers (67%) of herbs, six numbers (20 %) of tree, three numbers (10 %) climber, and one (3%) pteridophytes (Figure 3).

The plants like *Allium cepa* L., *Amaranthus* sp., *Azadirachta indica* A. Juss., *Basella alba* L., *Bauhinia* sp., four species of the genus *Brassica, Colocasia esculenta* (L.) Schott, *Ipomoea* Sp., *Mentha spicata* (L.) emend. Nathh., *Momordica charantia* L., *Moringa oleifera* Lam., *Murraya koenigii* (L.) Spreng., and *Raphanus sativus* L. from the kitchen garden of Bargarh district, Odisha, were all commonly used, according to Sahu and Sahu (2019). The indigenous people of Kalahandi district, Odisha, used a variety of plant parts, such as *Allium cepa* L., *Azadirachta indica* A. Juss., and *Moringa oleifera* Lam., for dental care (Sahu *et al.* 2020). The utilisation of *Azadirachta indica* A. Juss. leaves were described by Rana *et al.* (2020), the Gond tribal population in the Amlipali villages of Padampur N.A.C., Bargarh district, Odisha, uses it for skin diseases. According to Sahu *et al.* (2021), the Sahara Tribal Groups of Kangaon Village in Bargarh District, Western Odisha, employ the leaf of *Azadirachta indica* A. Juss. to treat a variety of skin diseases, and twigs were used as toothbrush. According to Sahu and Ekka (2021), the indigenous people of Bargarh district, Western Odisha, India, use a total of 39 plant species from 26 families as leafy vegetables. According to Sahu and Sahu (2022), the tribal peoples of the Jharigaon block of the Nabarangpur district of Odisha recorded 44 plant species from 27 families as leafy vegetables.

According to Dash and Sahu (2023), the indigenous people of Balangir area, Odisha, use 49 green vegetables from 28 families. According to Rout and Sahu (2023), the indigenous people of the Bhawanipatna region in the Kalahandi district use *Azadirachta indica* A. Juss. as an antibacterial and to treat skin conditions and diabetes. Sahu and Sahu (2023) reported that *Allium* bulb and *Marsilea quardifolia* L. leaves were utilized for the treatment of asthma by Gonds tribal peoples in Jharigaon area of Nabarangur district. They used 50 gm fresh decoction of *Allium* bulb is given every morning and evening in empty stomach to lessen the effect of asthmatic pain. Further regular use of *M. quardifolia* L. leaves as food provides relief from breathlessness due to asthma. Sahu *et al.* (2024) reported that the seeds of *Brassica campestris* L. were used for are excellent for digestion, improves cardiovascular health, helps to control diabetes, prevent asthma by the natives of Bargarh municipality in the state of Odisha, India. Leaves of *Coriandrum sativum* L. prevent anaemia, improve skin health when consumed regularly, good for digestion, reduce blood sugar levels, and have anti-inflammatory properties by the natives of Bargarh municipality in the state of Odisha, India. According to Sahu and Sahu (2024) plants like *Azadirachta indica* Juss., *Coriandrum sativum* L., and *Mentha spicata* (L.) emend. Nathh. were used as natural dye yielding ethnomedicinal plants by the native of Jharigaon Block of Nabarangpur district. Sahu *et al.* (2024) reported that both the *Azadirachta indica* A. Juss., *Capsicum* sp. were used for ethnomedicinal purposes by the natives of Barpali N.A.C. of Bargarh district of western Odisha, India. Sahu *et al.* (2024) reported that twigs of *Azadirachta indica* A. Juss. used as a toothbrush by the tribal peoples of Jharigaon block of Nabarangpur District, Odisha, India. The phytochemical constituents of *Marsilea minuta* leaves, which are responsible for several known pharmacological traits, were examined by Sharma *et al.* (2024). Padhan *et al.* (2025) reported that *Moringa* leaves contained tannins, saponins, flavonoid, steroids, terpenoids, cardiac glycosides, anthraquinones and alkaloids as secondary metabolites. Sahu *et al.* (2025) reported that the ethanol extract of *Allium sativum* (bulb) showed positive results for alkaloids, flavonoids, phenolic compounds, tannins, glycosides, saponins, steroids and carbohydrates. The ethanol extract of *Coriander sativum* (seeds and leaves) showed positive results for alkaloids, flavonoids, phenolic compounds, terpenoids & triterpenoids, saponins, steroids and carbohydrates. Bhoi and Sahu (2025) reported 40 leafy vegetables from 19 families used by the residents of Hemgir block in Sundargarh district, Odisha, use as green vegetables. With nine species, the Amaranthaceae family is the most abundant, followed by the Brassicaceae and Fabaceae families with six each, the Cucurbitaceae family with three, and two species each from the family Amarylidaceae, Basellaceae and Solanaceae. Each of the remaining

12 families produced one species. Of the 40 species, there are 26 (65%) herbaceous plants, two (5%) shrubs, seven (18%) climbers, and five (12%) trees.

Figure 1: Photographs of representative leafy vegetables namely *Allium cepa* L. (a), *Amaranthus tricolor* L. (b), *Bauhinia purpura* L. (c), *Boerhavia diffusa* L. (d), *Brassica oleracea* var. *capitata* L. (e), *Cinnamomum tamala* Nees. (f), *Cucurbita maxima* Duchesne. (g), *Mentha spicata* (L.) emend. Nathh. (h) and *Moringa oleifera* Lam. (i).

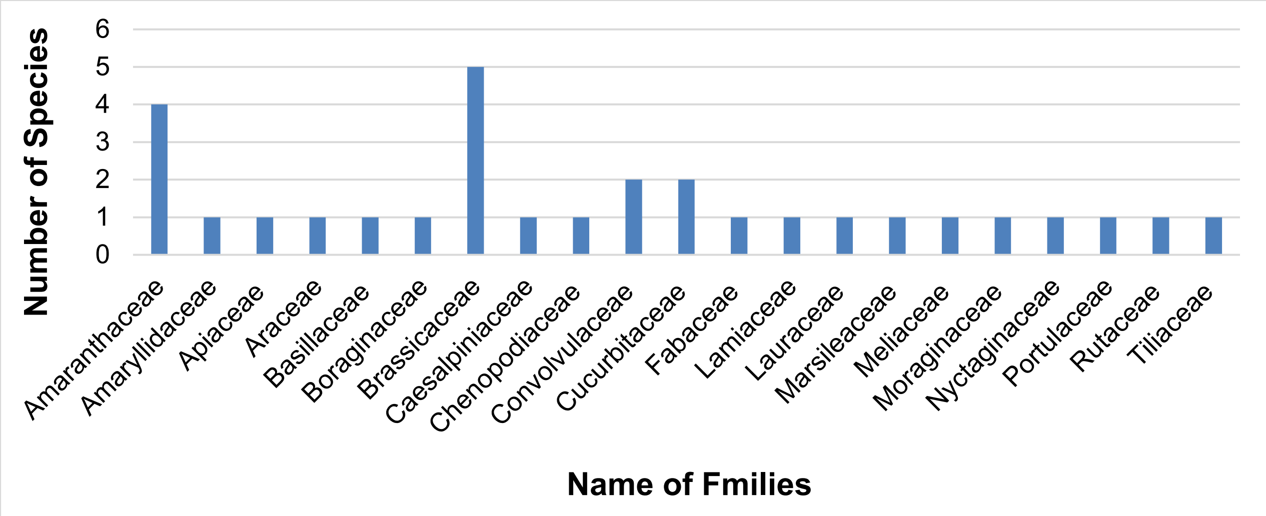

Figure 2: Family-wise distribution of leafy vegetable species used by the native of Dunguripali block of Subarnapur district of Odisha.

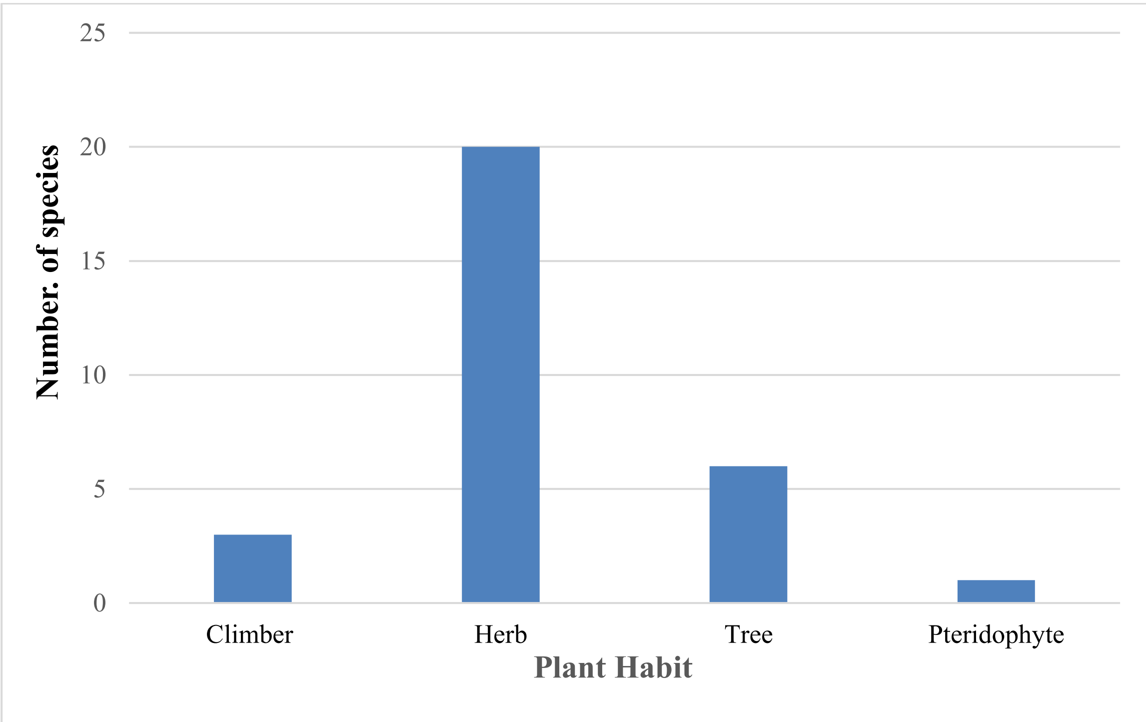

Figure 3: Diversity of plant species by habit.

Table 1: List of indigenous leafy vegetables found in the study areas

Botanical name	Local Name	Family	Habit	Flowering and Fruiting Season	Traditional Medicinal Uses
Allium cepa L.	Uel	Amaryllidaceae	Herb	Feb–Apr	Insect Bites, Ulcers, Sunstroke, Blood Purity, Arthritis; Bulb juice used to cure toothache, bleeding gums.
Alternanthera sessilis L.R. Br.	Madaranga	Amaranthaceae	Herb	Jul–Jan	treating eye diseases like night blindness, skin conditions, and digestive issues like diarrhea, dysentery
Amaranthus blitum L.	Kosila	Amaranthaceae	Herb	Jan–Dec	treating mouth and throat ulcers with a fluid extract, using the juice for headaches, and applying poultices to inflammations and boils.
Amaranthus tricolor L.	Lal Bhajee	Amaranthaceae	Herb	Jan–Dec	Laxative, abdominal pain, stomach ache, chronic inflammation of skin, laxative blood purifier
Amaranthus viridis L.	Leutia saga	Amaranthaceae	Herb	Jan–Dec	Root paste is used for Boils for suppuration, leafy parts are used as blood purifier
Azadirachta indica A. Juss.	Lim	Meliaceae	Tree	Feb–Jul	Skin diseases, Diabetics/ Contraceptive, antimalarial/ Tooth ache, antimalarial/ Tooth ache, Ulcers, Piles, Antipox
Basella alba L.	Poi	Basillaceae	Climber	Dec–Feb	Constipation; Skin diseases and as laxative in children and pregnant women
Bauhinia purpura L.	Kuelar	Caesalpiniaceae	Tree	Sep–Mar	Root bark is used for the treatment of TB,

Boerhavia diffusa L.	Gadhapurni	Nyctaginaceae	Herb	Jan–Dec	Whole plants are used for Bronchitis, Leucorrhoea, asthma, Anemia
Brassica napus L. var. *glauca* (Roxb.) Schulz	Surso	Brassicaceae	Herb	Sep–Feb	Rheumatism, Bronchitis, Massages
Brassica oleracea var. *botrytis* L.	Phula-kobi	Brassicaceae	Herb	Nov–Mar	antioxidant, anti-inflammatory, antimicrobial, and gastrointestinal benefits
Brassica oleracea var. *capitata* L.	Bandha Kobi	Brassicaceae	Herb	Dec–Feb	gastrointestinal issues, inflammation, and headaches.
Brassica oleracea L. var. *gongylodes* L.	Goint kobi	Brassicaceae	Herb	Dec–Feb	used to aid digestion and for skin issues
Cinnamomum tamala Nees.	Tejpatra	Lauraceae	Tree		treating digestive issues like diarrhea and flatulence, alleviating cough and bronchitis, and reducing inflammation
Colocasia esculenta (L.) Schott	Saru	Araceae	Herb	Jun–Nov	used to treat various ailments like asthma, arthritis, diarrhea, and anti-diabetic.
Corchorus capsularis L.	Nalita	Tiliaceae	Herb	May–Nov	Root is used to cure Dysentery, blood purifier
Cordia dichotoma G.Forst.	Bahal	Boraginaceae	Tree	Mar–Sep	anti-inflammatory, analgesic, and wound-healing properties, and is also used to treatment of diarrhea, fever, and coughs
Coriandrum sativum L.	Dhania	Apiaceae	Herb	Nov–Mar	Diarrhoea, dyspepsia, boost immunity, aid digestion
Cucurbita maxima Duchesne.	Makhan	Cucurbitaceae	Climber	Mar–Aug	intestinal parasites, urinary disorders, wound healing, benefits for blood pressure regulation, blood sugar control

Ipomoea aquatica Forssk.	Kalamasaga	Convolvulaceae	Herb	Nov–Mar	jaundice, constipation, liver disease, blood sugar control
Ipomoea batatas (L.) Lam.	Kanda	Convolvulaceae	Herb	Dec–Jan	used for the treatment of diabetes, inflammation, and wound healing
Marsilea minuta L.	Sunsunia	Marsileaceae	Pteridophyte	Nov–Mar	used to treat nervous system disorders, respiratory issues, and skin ailments
Mentha spicata (L.) emend. Nathh.	Podina	Lamiaceae	Herb	Jul–Sep	Toothache, Increase in milk flow, Jaundice; mouth freshener
Momordica charantia L.	Karla	Cucurbitaceae	Climber	Jun–Feb	Fruits are used for antipox, diabetes, Sex debility
Moringa oleifera Lam.	Munga	Moraginaceae	Tree	Jan–Jun	Leaf Juice and Seed are used for Blood Pressure, Weakness, Diabetes
Murraya koenigii L. Spreng.	Lesinga	Rutaceae	Tree	Feb–Sep	Tooth brush of stem is found to be effective to cure toothache.
Portulaca oleracea L.	Chanti sag	Portulaceae	Herb	Jan–Dec	used as antiseptic, vermifuge, burns, headaches, coughs, and gastrointestinal problems
Raphanus sativus L.	Mula	Brassicaceae	Herb	Jan–Feb	Seeds are used for Sexual Debility
Spinacia oleracea L.	Palanga	Chenopodiaceae	Herb	Nov–Feb	managing blood sugar for diabetes, supporting eye health, improve digestive health
Trigonella foenum-graecum L.	Methi	Fabaceae	Herb	Dec–Mar	improve blood sugar levels, aid digestion, stimulate milk production in breastfeeding mothers, alleviate menstrual cramps and menopausal symptoms

Conclusion:

The current study shows that a variety of native green vegetables greatly contribute to rural households' nutritional needs in the Subarnapur area of Odisha, India. According to the study, traditional knowledge and use of native leafy vegetables continue to play a significant role in village life and culture. Numerous anthropogenic and natural factors, including land use, habitat damage, migration, scientific harvesting, overgrazing, and alien species invasion, pose a threat to these valuable resources. In addition to improving the people's economic situation, domestication of the mentioned species will contribute to the preservation of biodiversity, ethnomedical and traditional knowledge of the species, and food security. Additionally, the ongoing pandemic has increased demand for nutrient-dense foods like salad greens and other green leafy vegetables since it has taught us the value of adopting a healthy diet that helps boost immunity. To uncover their potential as future medications, further extensive investigation is needed. Therefore, in order to properly preserve this popular traditional knowledge and to improve the relationship between human society and nature, a larger survey, contacts with the inhabitants, and data gathering are crucial.

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PRODUCTION AND FORMULATIONS OF ENTOMOPATHOGENIC NEMATODES

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Abstract:

Entomopathogenic nematodes (EPNs), primarily from the genera *Steinernema* and *Heterorhabditis*, are obligate insect parasites that have emerged as promising biological control agents against a wide spectrum of agricultural pests. Their ability to actively search for hosts, kill them rapidly through symbiotic bacteria, and persist in soil ecosystems makes them integral to integrated pest management (IPM) programs. This chapter presents a comprehensive overview of EPN production and formulation technologies, emphasizing the scientific principles, technological innovations, and industrial applications underpinning their large-scale deployment. Production strategies are broadly categorized into in vivo methods, where nematodes are cultured within live insect hosts, and in vitro techniques, which use artificial media in either solid or liquid fermentation systems. The latter allows for mass production at industrial scale but requires careful optimization of nutrient composition, aeration, and nematode-bacteria interactions. Formulation technologies are discussed with a focus on extending nematode shelf life, preserving infectivity, and facilitating field application. Formulations vary from simple aqueous suspensions to advanced water-dispersible gels, alginate beads, and partially anhydrobiotic products. Comparative analyses of production methods, formulation strategies, and their suitability for specific pest targets are presented, supported by recent case studies and commercial examples. Challenges, including production costs, storage stability, and environmental constraints, are critically examined. The chapter concludes with future research directions, including genetic improvement, nanotechnology-based formulations, and precision delivery systems for sustainable pest management.

Keywords: Entomopathogenic Nematodes, *Steinernema*, *Heterorhabditis*, In Vivo Production, In Vitro Production, Biological Control, Formulation Technology, Integrated Pest Management, Symbiotic Bacteria, Sustainable Agriculture

1. Introduction:

Entomopathogenic nematodes (EPNs) are obligate insect parasites predominantly belonging to the families *Steinernematidae* and *Heterorhabditidae*, which have gained significant attention as biocontrol agents in sustainable agriculture (Shapiro-Ilan *et al.,* 2017). These nematodes establish a mutualistic relationship with specific bacteria - *Xenorhabdus* spp. in *Steinernema* and *Photorhabdus* spp. in *Heterorhabditis* which play a critical role in host insect mortality by producing toxins and causing septicemia (Gulcu *et al.,* 2017; Adams *et al.,* 2019). The infective juvenile (IJ) stage of EPNs is the only free-living stage capable of surviving in soil, locating, and infecting susceptible insect hosts (Gupta *et al.,* 2003; Grewal *et al.,* 2017).

The use of EPNs in biological control has expanded due to their rapid kill rates, broad host range, environmental safety, and ability to persist in soil ecosystems (Kaya & Gaugler, 1993; Lacey & Georgis, 2012). Over 100 species of *Steinernema* and approximately 20 species of *Heterorhabditis* have been described worldwide, highlighting their global distribution and ecological importance (Bhat *et al.,* 2020). Initial mass production methods relied on in vivo propagation in insect hosts, which, while effective, presented challenges in scale-up and cost (Shapiro-Ilan & Gaugler, 2016). Subsequently, in vitro liquid fermentation techniques were developed to produce EPNs more economically at industrial scales (Koppenhöfer, 2000).

Formulation technologies have similarly evolved to address the challenges of storage, transport, and field application. EPNs are highly sensitive to desiccation, UV radiation, and temperature extremes, necessitating innovative formulations such as gels, powders, and granules to improve shelf life and ease of application (Chen & Glazer, 2005; Shapiro-Ilan *et al.,* 2014). Despite these advances, factors such as production costs, environmental stressors, and strain specificity still limit widespread adoption (Miller & Kaya, 2017).

This chapter provides a detailed review of EPN production systems and formulation approaches, with emphasis on technological advances and commercial applications. Challenges and future prospects for improving the efficacy and scalability of EPN-based biocontrol products are also discussed.

2. Biology and Life Cycle of Entomopathogenic Nematodes

EPNs are soil-dwelling nematodes that parasitize a wide range of insect hosts, often killing them within 24–72 hours through a symbiotic relationship with bacteria (Shapiro-Ilan *et al.,* 2017). The families *Steinernematidae* and *Heterorhabditidae* differ in morphology, bacterial symbionts, and reproductive biology, but share a similar overall life cycle centered around the infective juvenile (IJ) stage (Gaugler & Kaya, 1993).

Table 1: Biological comparison of *Steinernema* and *Heterorhabditis* species

Characteristic	***Steinernema* spp.**	***Heterorhabditis* spp.**
Symbiotic bacteria	*Xenorhabdus* spp.	*Photorhabdus* spp.
Morphology	Lacks stylet; specialized anterior vesicle	Has tooth-like stylet for penetration
Foraging strategy	Ambush and intermediate	Cruiser and intermediate
Infection mode	Natural openings, rarely cuticle penetration	Natural openings and direct cuticle penetration
Reproduction mode	Amphimictic	Hermaphroditic in first generation, amphimictic thereafter
Host range	Broad, including Lepidoptera and Coleoptera	Broad, similar to *Steinernema*

2.1 Morphology and Symbiotic Association

Adult EPNs are morphologically distinct, with *Steinernema* spp. typically lacking a stylet, whereas *Heterorhabditis* spp. possess a specialized tooth-like structure used during host penetration (Liu *et al.,* 2019). Their mutualistic bacteria reside within the IJ gut: *Xenorhabdus* spp. in *Steinernema* and *Photorhabdus* spp. in *Heterorhabditis* (Goodrich-Blair & Clarke, 2007; Saravanapriya, 2005). These bacteria are essential for killing the host and creating a suitable environment for nematode reproduction.

2.2 Infective Juvenile Stage and Host-Seeking Behavior

The IJ is developmentally arrested, non-feeding, and adapted to survive harsh soil conditions (Bongers, 2018). It actively searches for insect hosts using chemical cues such as CO_2, host volatiles, and temperature gradients (Campbell & Gaugler, 1993). Foraging strategies vary among species: ambush foragers like *S. carpocapsae* wait near the soil surface for passing hosts, while cruisers such as *H. bacteriophora* actively move through the soil profile (Poinar, 1990).

2.3 Infection and Pathogenesis

IJs enter the insect host through natural openings or by penetrating the cuticle directly (Adams & Nguyen, 2002). Upon entry, they release their symbiotic bacteria into the hemocoel, where bacterial proliferation causes septicemia and host death within 48 hours (Ciche & Ensign, 2003; Ciche *et al.,* 2006). The bacteria also produce antimicrobials that inhibit competing microbes, ensuring the cadaver remains a nutrient-rich environment for nematode development (Cowles & Goodrich-Blair, 2008).

2.4 Reproduction and Emergence

Within the cadaver, EPNs develop through four juvenile stages into reproductive adults, completing one or more generations depending on host size and environmental conditions (Kaya & Gaugler, 1993). After resource depletion, newly formed IJs emerge into the soil to seek new hosts, perpetuating the cycle. The duration of the life cycle ranges from 7 to 21 days, depending on temperature and species (Lacey & Georgis, 2012).

3. Production of Entomopathogenic Nematodes

The commercial utilization of entomopathogenic nematodes (EPNs) depends heavily on efficient production methods that yield large quantities of high-quality infective juveniles (IJs) at a reasonable cost. Production systems for EPNs are broadly categorized into **in vivo** and **in vitro** methods, each with advantages and limitations (Koppenhöfer, 2000; Shapiro-Ilan & Gaugler, 2016).

3.1 In Vivo Production

In vivo production involves the multiplication of EPNs within live insect hosts, typically larvae of susceptible species such as *Galleria mellonella* (greater wax moth), *Helicoverpa armigera*, and *Corcyra cephalonica* (rice moth) (Kaya & Gaugler, 1993). This method is favored for producing highly virulent nematodes due to the natural host environment promoting optimal development and bacterial symbiosis (Ehlers, 2001).

3.1.1 Host Rearing

Mass rearing of host insects requires maintaining healthy insect colonies on artificial diets or natural food sources under controlled temperature and humidity. For instance, *G. mellonella* larvae are cultured on a diet consisting of beeswax, honey, and bran at 27°C and 70% relative humidity to ensure a consistent supply for nematode inoculation (Shapiro-Ilan *et al.,* 2010).

3.1.2 Nematode Inoculation and Harvest

Insects are inoculated with a suspension of infective juveniles by immersion or topical application. Post-inoculation, insects are incubated in Petri dishes lined with moist filter paper at 25°C. Within 2–5 days, infected larvae succumb to infection, and nematodes reproduce inside the cadaver (Shapiro-Ilan & Gaugler, 2006).

Harvesting IJs typically employs the White trap method (White, 1927), where infected cadavers are placed on a water-filled platform allowing emerging nematodes to migrate into the water for collection. The harvested nematodes are then concentrated by sedimentation or centrifugation and stored at 8–12°C for further use (Koppenhöfer, 2000).

3.1.3 Advantages and Limitations

In vivo production is advantageous due to simplicity, low infrastructure cost, and high nematode virulence (Kaya & Gaugler, 1993). However, it has drawbacks including labor intensity, scalability issues, batch variability, and the requirement for continuous host insect rearing, which increases production costs and complicates quality control (Shapiro-Ilan *et al.,* 2006).

3.2 In Vitro Production

In vitro production involves culturing EPNs in artificial media, either on solid substrates or in liquid fermentation systems. It allows large-scale, standardized production and reduces dependency on live insect hosts (Ehlers, 2001).

3.2.1 Solid Culture Systems

Solid-state fermentation utilizes substrates such as cooked grains, wheat bran, or foam cubes impregnated with nutrient media. Nematodes are inoculated onto the substrate, where they complete their life cycle. Foam-based substrates, for example, provide high aeration and moisture retention, facilitating nematode propagation (Bhat *et al.,* 2020).

3.2.2 Liquid Fermentation

Liquid fermentation is the most widely adopted industrial method, involving the co-cultivation of EPNs with their symbiotic bacteria in bioreactors under controlled conditions (Kaya & Gaugler, 1993). This method typically proceeds in two stages:

- **Bacterial culture phase**: Symbiotic bacteria are grown to high density in nutrient-rich media (e.g., yeast extract, soy flour, oils).
- **Nematode growth phase**: IJs or juveniles are inoculated into bacterial broth, where they feed on bacteria and multiply.

Optimizing parameters such as pH, temperature (usually 25–28°C), oxygen levels, and nutrient concentrations is critical for maximizing yields (Grewal *et al.,* 2017).

3.2.3 Harvesting and Processing

After fermentation, IJs are separated from the culture medium by filtration or centrifugation, rinsed to remove residual bacteria and media, and concentrated for formulation (Koppenhöfer, 2000). Maintaining IJ viability and infectivity during downstream processing is a key challenge.

3.2.4 In vitro mass production

In vitro mass production utilizes artificial media to culture EPNs either in liquid or solid culture systems, providing greater scalability and cost efficiency for commercial use. Several media formulations have been developed and optimized over the years.

Table 2: Artificial media and their composition to culture EPNs

Medium Name	Ingredients (per 100 ml or g)
Wout's Medium	Nutrient broth (0.88 g), Yeast extract (0.32 g), Soybean flour (14.4 g), Groundnut oil (10.4 g), Distilled water (60 ml)
Modified Wout's Medium	Nutrient broth (0.5 g), Yeast extract (0.2 g), Soybean flour (16 g), Groundnut oil (12 g), Distilled water (30 ml)
Wheat Flour Medium	Wheat flour (15 g), Kabuli gram flour (5 g), Beef extract (5 g), Agar agar (1 g), Coconut oil (6 g), Distilled water (60 ml)
Modified Wheat Flour Medium	Wheat flour (15 g), Kabuli gram flour (5 g), Beef extract (5 g), Agar agar (1 g), Groundnut oil (10 g), Distilled water (60 ml)
Egg Yolk Medium I	SDEY (10 g), Yeast extract (2 g), NaCl (0.8 g), Oil (15 g), Distilled water (60 ml)
Egg Yolk Medium II	SDEY (10 g), Yeast extract (5 g), NaCl (0.8 g), Oil (12 g), Distilled water (60 ml)
Modified Egg Yolk Medium	SDEY (7 g), Soybean flour (20 g), Yeast extract (2 g), NaCl (0.8 g), Oil (15 g), Distilled water (60 ml)
Dog Biscuit Medium	Dog biscuit (15 g), Yeast extract (1 g), Peptone (3 g), Agar agar (2 g), Oil (10 g), Distilled water (60 ml)
Modified Dog Biscuit Medium	Dog biscuit (20 g), Yeast extract (1 g), Peptone (0.5 g), Beef extract (5 g), Oil (7 g), Distilled water (100 ml)

These ingredients are mixed and coated onto polyether polyurethane foam cubes (approximately 1.5 g foam chips soaked with 8-9 g medium). The foam-media is autoclaved, cooled, and inoculated aseptically with freshly harvested infective juveniles (1,000 IJs per flask). The flasks are incubated at 28 °C for approximately 25 days, after which nematode progeny emerge and are harvested (Reddy, 2009).

Harvested IJs are purified by washing and sieving, sterilized briefly with 0.1% Hyamine solution, and stored until use (Reddy, 2009). In vitro production allows for large-scale nematode cultivation but requires precise control of conditions and quality assurance to maintain nematode virulence (Shapiro-Ilan & Gaugler, 2016).

3.2.5 Advantages and Limitations

In vitro fermentation offers consistent large-scale production, reduced labor, and cost-effectiveness once optimized. However, initial setup requires significant capital investment and technical expertise. Additionally, IJs produced in vitro may have lower virulence if bacterial symbiont density is suboptimal, necessitating rigorous quality control (Shapiro-Ilan & Gaugler, 2016).

Table 3: Comparison of In Vivo and In Vitro EPN Production Methods

Feature	**In Vivo Production**	**In Vitro Production**
Host dependency	Requires live insect hosts	No live hosts; culture in media
Production scale	Limited scalability	High scalability possible
Cost	Lower initial cost; higher per unit cost	Higher initial cost; lower per unit cost
Labor intensity	High	Lower
Virulence of IJs	Generally high	May vary; depends on bacterial symbiosis
Quality control	Challenging due to batch variability	Easier with controlled conditions
Infrastructure needs	Simple	Requires bioreactors and sterile facilities

4. Formulation of Entomopathogenic Nematodes

Formulation is a critical step in transforming freshly produced entomopathogenic nematodes (EPNs) into stable, user-friendly products that maintain nematode viability and efficacy during storage, transport, and field application (Shapiro-Ilan *et al.,* 2017). Due to the delicate nature of infective juveniles (IJs), formulation strategies must protect them from environmental stressors such as desiccation, UV radiation, temperature fluctuations, and oxygen deprivation (Chen & Glazer, 2005).

4.1 Objectives of Formulation

The main goals of EPN formulation include:

- Maintaining nematode viability and infectivity for extended periods.
- Facilitating ease of handling, storage, and transport.
- Enhancing field persistence and efficacy.
- Allowing compatibility with existing application equipment.

To achieve these objectives, formulations may contain a combination of active (nematodes) and inert ingredients such as carriers, surfactants, binders, humectants, antioxidants, and UV protectants (Greenslade *et al.,* 2020).

4.2 Types of EPN Formulations

Formulations are broadly classified into two categories based on nematode mobility:

4.2.1 Formulations with Actively Moving Nematodes

These formulations maintain EPNs in an active, motile state, which is essential for immediate infectivity upon application.

- **Sponge formulations:** Polyether polyurethane sponges impregnated with aqueous suspensions of nematodes (500–1000 IJs/cm^2) provide a moist environment that supports IJ survival during storage and shipping (Bedding& Akhurst, 1975). Before application, nematodes are extracted by soaking and squeezing the sponge in water. Sponge formulations are ideal for small-scale use but have limited shelf life and require refrigeration (Shapiro-Ilan *et al.,* 2016).
- **Vermiculite-based formulations:** Vermiculite, a hydrated laminar mineral, serves as an inert carrier mixed homogeneously with nematode suspensions. It improves storage life, concentration, and ease of handling compared to sponges. The vermiculite-nematode mixture is typically packaged in polyethylene bags and can be diluted in water for spraying or soil drenching (Akbulut & Starnes, 2021).
- **Soil formulations:** For short-term storage, moist soil or sterilized substrates like peat can be mixed with nematodes and stored under controlled conditions. Earthen pots containing nematode-infused soil can be buried to buffer against environmental extremes. However, oxygen depletion in such closed systems reduces nematode viability over time (Chen & Glazer, 2005).

4.2.2 Formulations with Reduced Nematode Mobility

These formulations render nematodes partially anhydrobiotic (dormant), improving shelf life and tolerance to desiccation and temperature stress.

- **Gel formulations:** Nematodes are mixed in hydrogels such as polyacrylamide, creating a hydrated matrix that sustains IJ survival. While gels offer moisture retention and protect nematodes from desiccation, difficulties in dissolving gels and potential clogging of spray equipment limit their practical use (Shapiro-Ilan *et al.,* 2016).
- **Powder formulations:** EPNs are adsorbed onto inert powders like clay or talc, often arranged in ‘sandwich’ layers to reduce moisture and improve dispersibility in water (Bedding & Akhurst, 1975). Such formulations enable storage at ambient temperatures for up to 3 months with minimal viability loss and are suitable for broad-scale application (Akbulut & Starnes, 2021).
- **Granular formulations:** Nematodes are encapsulated in granules composed of natural materials such as lucerne, alginate, or wheat gluten. Granules protect nematodes from

environmental stress and allow controlled release in the soil (Miller & Kaya, 2017). However, granules tend to dry out rapidly during storage, which can reduce nematode survival (Shapiro-Ilan *et al.,* 2017).

Table 4: Advantages and Limitations of Common EPN Formulations

Formulation Type	Advantages	Limitations
Sponge	Simple preparation, moist environment for IJs	Short shelf life, refrigeration required
Vermiculite	Improved storage, easy application	Requires moisture management
Soil	Natural substrate, buffers environmental extremes	Oxygen depletion, limited shelf life
Gel	High moisture retention	Difficult to dissolve, spray equipment clogging
Powder	Good storage stability, easy dispersibility	Possible desiccation over long-term storage
Granules	Protection against stress, controlled release	Rapid drying during storage, reduced survival

4.3 Additives and Enhancers in EPN Formulations

Additives improve formulation performance by enhancing nematode survival and efficacy.

- **Humectants** (e.g., glycerol, polyethylene glycol) reduce desiccation by retaining moisture (Akbulut & Starnes, 2021).
- **UV protectants** such as optical brighteners or lignin derivatives protect IJs from solar radiation damage during foliar applications (Chen & Glazer, 2005).
- **Surfactants** enhance nematode distribution and penetration in soil or on plant surfaces (De Waal *et al.,* 2013).
- **Antimicrobials and antioxidants** prevent contamination and oxidative damage during storage (Greenslade *et al.,* 2020).

4.4 Commercial Formulations and Products

Various commercial products based on EPNs are available worldwide with different formulations adapted to target pests and cropping systems (Grewal *et al.,* 2017).

Table 5: Commercial Formulations and Products

EPN species	Product formulation	Country
Steinernema carpocapsae	ORTHO Biosafe, X-GNAT, Vector TL	USA
	Green commonda, Bouncer	India
	CAPSANEM	Netherland
	Mioplant	Austria
	Sanoplant	Switzerland
	Boden Nutzlinge	Germany
	Helix	Canada
	Nemastar	Italy
S. feltiae	Magnet, Entonem	USA
	Nemasys, Stealth	UK
S. kushidai	SDS Biotech	Japan
S. riobrave	Vector MG, Bio Vector	USA
S. scapterisci	Proactant SC	USA
Heterorhabditis bacteriophora	Otinem	USA
	E-Nema Gmbh	Germany
	Nemopak HB	Italy
H. megidis	Nemasys	UK
	Larvanem	Netherland
	NovoNem	Germany
Phasmarhabditis hermaphrodita	Nemaslug	UK
Heterorhabditis indica strain NBAII HI1 and *Heterorhabditis bacteriophora* strain NBAII HB5	Solder, Armour, Caltterm, Grubcure, Nema power	ICAR–NBAIR, Karnataka, India

5. Application Technologies and Field Use

Entomopathogenic nematodes (EPNs) are increasingly recognized as effective biological control agents for a broad range of insect pests in diverse cropping systems. However, their successful field application requires careful consideration of delivery methods, environmental factors, and pest biology to maximize efficacy (Shapiro-Ilan *et al.,* 2017).

5.1 Application Methods

EPNs can be applied through various conventional and specialized delivery systems adapted to target soil-dwelling or foliar insect pests.

5.1.1 Soil Application

Soil application is the most common method for EPN deployment, targeting subterranean insect pests such as white grubs, root weevils, and fungus gnats.

- **Sprayers and Drenchers:** Nematode suspensions are applied using ground sprayers, drip irrigation systems, or soil drenches to ensure even distribution within the soil profile (Kaya & Gaugler, 1993).
- **Granule Application:** Granular formulations are broadcast or banded into the soil, allowing gradual nematode release and enhanced persistence (Miller & Kaya, 2017).
- **Injection:** For high-value crops or confined root zones, nematodes may be injected into the soil or irrigation lines for precision delivery (Shapiro-Ilan *et al.,* 2010).

5.1.2 Foliar Application

Although EPNs are primarily soil organisms, they can be applied to foliar pests under conditions that reduce UV and desiccation stress.

- **Spray Application:** Electrostatic sprayers or hydraulic nozzles can be used to apply nematode suspensions onto foliage, targeting pests such as diamondback moth larvae or aphids (Schroer & Ehlers, 2005).
- **Gel and Polymer Formulations:** Encapsulation in gels or polymers improves adhesion and survival on leaf surfaces, enhancing efficacy in above-ground applications (Shapiro-Ilan *et al.,* 2010).

5.2 Timing and Environmental Considerations

To optimize EPN survival and infection rates, applications are ideally timed and conducted under favorable environmental conditions.

- **Time of Day:** EPNs are sensitive to ultraviolet (UV) radiation and desiccation; thus, applications are recommended in early morning, late afternoon, or cloudy days to minimize exposure (Kaya & Gaugler, 1993).
- **Soil Moisture:** Adequate soil moisture is crucial for nematode movement and host-seeking behavior. Irrigation prior to or after application can improve nematode persistence and control efficacy (Shapiro-Ilan *et al.,* 2006; Orozco *et al.,* 2014).
- **Temperature:** EPNs perform optimally within moderate temperature ranges (20–30°C), and extreme heat or cold reduces infectivity and survival (Grewal *et al.,* 2017).

- **pH and Soil Type:** Most EPN species tolerate a range of soil pH values and textures, but highly acidic or alkaline soils and heavy clays may limit effectiveness due to reduced nematode mobility or survival (Koppenhöfer, 2000).

5.3 Compatibility with Other Pest Management Practices

Integrating EPNs with other pest control strategies enhances overall management efficacy and sustainability.

- **Chemical Pesticides:** Many synthetic pesticides negatively affect EPN survival; thus, selecting compatible chemicals or reducing pesticide use is critical when integrating EPNs (Shapiro-Ilan *et al.,* 2017).
- **Biological Agents:** EPNs can be combined with other biocontrol agents such as entomopathogenic fungi or predatory insects for synergistic effects (Dowds & Peters, 2002; Grewal *et al.,* 2017).
- **Cultural Practices:** Soil tillage, irrigation scheduling, and crop rotation can influence EPN persistence and distribution, and should be optimized for nematode performance (Koppenhöfer, 2000).

6. Future Perspectives

Entomopathogenic nematodes (EPNs) hold great promise as sustainable biological control agents, but realizing their full potential requires addressing current limitations and leveraging emerging technologies.

6.1 Advances in Strain Improvement and Selection

Modern molecular and genomic tools enable identification and development of EPN strains with enhanced traits such as increased virulence, environmental tolerance, and host specificity (Campos-Herrera *et al.,* 2015). Genetic engineering and selective breeding could further improve nematode efficacy and adaptability under diverse field conditions (Shapiro-Ilan & Han, 2014).

6.2 Novel Formulations and Delivery Systems

Research continues to optimize formulations that improve nematode survival under harsh field conditions. Innovations include microencapsulation, nanotechnology-based carriers, and polymer gels with UV protectants and humidity regulators (Greenslade *et al.,* 2020). Additionally, development of smart delivery systems that release nematodes in response to pest cues could increase precision and effectiveness (Jansson *et al.,* 2021).

6.3 Integration into IPM and Sustainable Agriculture

Greater adoption of EPNs depends on their integration with other pest management approaches, crop management practices, and organic farming systems (Grewal *et al.,* 2017). Collaborative efforts among researchers, extension services, and farmers to promote awareness, training, and field demonstrations will facilitate wider use.

6.4 Cost Reduction and Mass Production

Innovations in cost-effective, large-scale in vitro production methods and low-cost substrates remain crucial to reduce product prices and improve accessibility, particularly for smallholder farmers (Shapiro-Ilan & Gaugler, 2016). Automation and bioreactor technologies may enhance production efficiency.

Conclusion:

Entomopathogenic nematodes are a potent, eco-friendly alternative to chemical insecticides for controlling a broad spectrum of insect pests. Their mutualistic relationship with bacteria enables efficient insect killing, while their soil-dwelling infective juveniles make them suitable for targeting subterranean pests. Advances in in vivo and in vitro production, formulation technologies, and application methods have expanded their practical use.

However, challenges related to environmental sensitivity, production costs, and delivery systems persist. Addressing these through multidisciplinary research and development will enhance EPN efficacy and facilitate their integration into sustainable integrated pest management programs worldwide.

With continued innovation, education, and regulatory support, entomopathogenic nematodes will become increasingly important tools in achieving sustainable agriculture and reducing reliance on harmful pesticides.

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