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RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF INDIGOFERA TINCTORIA

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Abstract

Indigofera tinctoria, named true indigo in English, is a leguminous plant displaying a vast traditional and modern medicinal uses. Historical importance, phytochemistry, pharmacological properties, and possible applications of the indigo plant are the major points of discussion in this review. Rich in several bioactive compounds such as flavonoids, alkaloids, saponins, tannins, and phenolics, these together exhibit antimicrobial, antioxidant, anti-inflammatory, and hepatoprotective activities. Antibacterial studies authenticate its potency against several pathogenic strains such as Staphylococcus aureus, Escherichia coli, and Salmonella typhi; antifungal and antiviral potentials are under investigation. It also acts as a sustainable source of natural dye useful for textile industries while offering ecological benefits, such as soil enrichment through nitrogen fixation. Clinical trials and standardized extraction protocols remain limited, even with promising results in vitro and some novel applications in green nanoparticle synthesis. Further research is recommended to fully authenticate the pharmacological applications of Indigofera tinctoria and widen its application in sustainable health and environmental management.

Introduction:-

Importance of Indigofera tinctoria in healthcare

Indigo plant (Avuri), Latin name Indigofera tinctoria, has been practiced as a traditional medicinal plant in India, Africa, and China for the treatments of nervous disorders, epilepsy, bronchitis, and liver diseases, thus being enriched with bioactive ingredients such as flavonoids, saponins, alkaloids, and phenolic compounds that add to its remedial value[1]. Apart from this, research has elaborated the extensive antimicrobial activity of the plant against clinically significant bacteria such as Staphylococcus aureus, Escherichia coli, and Candida albicans. It also has antibiofilm properties, which are very important to prevent chronic infections and, in particular, those associated with catheter-associated urinary tract infections[2].

Antimicrobial effects with anthelmintic activity have been reported to accompany anti-inflammatory and antioxidant functions, such as offsets from oxidative stress and chronic diseases like arthritis and cardiovascular conditions[3]. There appears to be an extensive emphasis on its antimicrobial activity against clinically important bacteria, mostly

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including Staphylococcus aureus, Escherichia coli, and Candida albicans[4]. Furthermore, it has antimicrobial biofilm properties, which help in preventing chronic infections, especially catheter-associated urinary tract infections[5]. Moreover, Indigofera tinctoria has been able to serve in addition to its antimicrobial actions, possible anti-inflammatory and antioxidant functions, reducing oxidative stress in chronic problems such as arthritis and cardiovascular disorders. The extract shows the liver's protective potential and supports liver health[6]. The study suggests providing health benefits from the methanolic extract for non-mutagenic. The plants present natural alternatives for developing new pharmaceuticals. Considering the increase in Bacterial resistance to antibiotics. Ongoing research in cultural and historical significance and medicinal potential, and its traditional and healthcare[7].

Overview of Indigofera tinctoria

The Indigofera tinctoria family, Fabaceae, is of historical and economic importance, particularly in dye production. The lectotype species of the genus Indigofera was first described by Linnaeus in 1753[8].The Ecological and Economic benefits are in warm climates, and predominantly species found in tropical and subtropical regions[9].

TheIndigoferatinctoria plant produces fruits and flowers simultaneouslyfrom perennial shrubs. The leaves are imparipinnate with 5-7 oblong or above leaflets covered in trichomes in flowers, typically pink, and grow in axillary clusters[10]. Indigofera tinctoria is best on leaves, it contains Natural dye extraction in fermentation and is used in manufacturing and including traditional batik production in Java[11]. The dye production the medicinal properties, and applications in soil conservation and fodder production in Indigo plants. The current reports of endangered species indicate thattheirrarity in some regions needs conservation efforts in the highlighted [12].

Traditional uses and significance of Indigofera tinctoria

Indigo is widely used in medicine, culture, and Economics. Tradition is crucial in the treatment of skin and gastrointestinal disorders and inflammation[13]. It enhances therapeutic effectiveness and includes bioactive compounds like alkaloids and flavonoids. The healing attributes in hair health, urinary problems, and Parasitic worm infections are described in ancient Sanskrit texts like Charaka Samhita and Sushruta Samhita[14]. The tradition is crucial in the medicinal treatment of skin, gastrointestinal disorders, and inflammation[15]. Health care in Central and South America has a historical record in medicinal Cairo and is useful for skin treatments[16].

Historically, an important medicine through Indigofera tinctoria, which comes with natural indigo dye from its leaves. Every continent across Asia, Africa, and Europe has associated Indica plants with Indica production, thus accruing an additional dimension to the importance of the plant in trade and world trade over the ages [17]. Crop rotation provides further benefits to agriculture, as it enriches the soil with nutrients and nitrogen-fixing abilities to enhance soil fertility [18]. Indigofera tinctoria also benefits environmental sustainability and erosion control. It has a pivotal role in history and the present age in different and, at times, conflicting ways—for example, in traditional medicine, textile production, and ecological benefits for such cultivation [19].

Objectives of the Review Paper on Indigofera tinctoria

The review paper on Indigofera tinctoria focuses on multiple cultivation and potential applications, and sustainable production. The objectives research gaps to identify the key fertilizer applications to enhance the yield of this quality, particularly in optimizing in cultivation of these techniques[20]. It is mainly about the effect of NPK(16:16:16) fertilization on growth, with sustainable natural dye production at the heart of this research work. The study presented here, in particular, deals with historical and cultural reflections adding to the dyeing methods found in countries such as Indonesia and Thailand from the historical and cultural dimensions of Indigofera tinctoria[21].

The objectives in the global movement of eco-friendly textile production in promoting sustainable agriculture methods it is beneficial for theeconomy while aligning[22]. The review also focuses on the environmental benefits of using natural dyes over synthetic alternatives, focusing on their parts reducing pollution and contributing to a more sustainable textile industry. A comprehensive understanding of research and the mixed methods approach between bibliometric analysis and field experiments can be best illustrated through the explanation of Indigofera tinctoria[23]. Collectively, these objectives seek to advance knowledge on this plant for its sustainable use in agriculture, environmental conservation, and the textile industry[24].

Lately, the human body deposits are that this species represents both comprehensive understanding research and the mixed methods between bibliometric analysis and field experiments. All of the above targets are very well put forth for the advancement of knowledge on the plant for sustainable use in agriculture, environmental conservation, and the textile industry[25].

Morphological and Physiological Characteristics of Indigofera tinctoria

The Indigofera tinctoria, widely known as a true indigo, is a leguminous plant widely recognized for its natural dye properties. The morphological adaptations in plants exhibit notable to varying light intensities. The complete illumination in the reducing light conditions(50% and 25%) stimulates elongation, increasing its height to 117.22 cm, reaching approximately 73.44 and beyond[26]. This is an adaptive response to increase light capture efficiency and is complemented by increasing under lower light intensities, leaf area, and specific leaf area (SLA). While their diameter remains due to auxin-inducing etiolation and shaded environments, their stems tend to elongate. The decreased light intensity contributes to reducing chlorophyll concentration and overall biomass yield, and their functionally increased light exposure improves[27].

The Indigofera tinctoria contains the term of shape, it hasglabrous leaflets and pinnately compound imparipinnate leaves with 5 to 11 obovate leaflets. The closely related species is, in contrast, lacking reddish pigmentation and green stems[28]. The plant features an axillary upright raceme inflorescence measuring 4.56-15.25 cm in length and prominent standards and wings with pink flowers. The pods are cylindrical in shape or cuboid The seeds are dark green and cylindrical, with 3-11 oblong seeds. The stems exhibit a rectangular cross-section with a secondary xylem, phloem, and a prominent pith, and the anatomical leaf structure consists of a well-developed mesophyll layer in the neatly arranged palisade cells. The actinostele arrangement in roots features a dense secondary xylem[29]. The morphological significance of Indigofera tinctoria is that itholds economic and ecologicalvalue, particularly in batik production, and functions as a green manure and fuel crop in various agricultural systems. The major source of serving in natural dye for traditional textile industries[30].

Habitat, Geographical Distribution, and Ecological Significance of Indigofera tinctoria

The Indigofera tinctoria is a true indigo. Theythrive in the tropical and subtropical regions with well-drained soils and moderate slopes. The required temperature range is 7.8 degrees Celsius, annual precipitation is between 800 and 5600mm, and the Isothermality index is between 77 and 88[31]. The species is typically found in areas where precipitation during the driest month remains under 30mm and elevations below 50 meters and in areas. The suitability of the various soil types in diverse environments, including sandy and loamy soils, allows for the growth of disturbed areas[32].

Geographically, Indigofera tinctoria has spread globally due to cultivation, particularly in India, Southeast Asia, and native tropical Asia. It has been recognized and widely introduced to grow for its dye-producing properties in the Americas, Australia, and Africa[33]. The Citarum watershed in West Java, Indonesia, and its species have historical records in the region that show their presence has significantly declined over time. The ecological relevance is further derived for the dye production, as their flora has much for the environment, as it works for nitrogen fixation and improves soil fertility[34]. Besides biodiversity conservation and agroforestry, deep root systems play an additional role in enhancing environmental value, besides checking erosion. The reduction of population densities in selected areas for conservation action is necessary for the sustainability of ecological functions and to address cultural heritage[35].

Cultivation and Harvesting Methods of Indigofera tinctoria

Indigofera tinctoria is highly preferred for this natural indigo dye and requires equilibrated environmental conditions and constant tending to increase yield. The plant grows best under optimum conditions in the tropical and subtropical zones of annual rainfall between 700mm and 4000mm, with an altitude of below 800m[36]. The species needs well-drained soil, which is essential. The importance of this natural dye to the full exposure to sunlight is vital to the growth stages, indicating that 100% sunlight exposure has a great effect on increasing dye yield[37]. Cultivation practices include constant weeding, sowing seeds from March to July, preparation of land, and application of organic manure or fertilizer. The use of intercropping with crops like corn is practiced to maximize land in certain regions. Additionally, including the addition of humans has been shown to improve branching, nutrient management, and overall yield[38].

Typically, three cycles are there per season in harvesting, which occurs between June and July. Ensuring the highest dye content in the first harvest takes place in the plants reaching 90-100 days of growth. The stem of regrowth to the preferred technique involves cutting the plant while leaving 30 cm for harvesting, and it is recommended to preserve the leaf quality by harvesting in the early morning (6:00 a.m.) after harvesting is done[39]. Swelling of the leaves occurs in a lime solution to dissolve the dye in post-harvest with a lime concentration for optimum 4% within 24 hours. Based on the biomass, the environmental conditions range from 15 to 40 tons per hectare. The cultivation of Indigofera tinctoria may be optimized to provide a sustainable and efficient means of producing indigo dye by planting structures, fertilizing adequately, and harvesting judiciously[40].

Historical and Cultural Relevance of Indigofera tinctoria in Traditional Medicine

Indigofera tinctoria, otherwise true indigo, has been highly important in the field of traditional medicine as well as the cultural practices of mankind since time immemorial. Indigenously, this plant was used for dyeing purposes and corresponding medicinal benefits in all civilizations, including China, Africa, and, of course, India[41].Traditional healers have long recognized their antimicrobial, analgesic, and anti-inflammatory properties, using wounds, roots, and leaves to treat skin conditions and infections. In some regions, it has also been employed for chronic illnesses like leukemia, eczema, and gastrointestinal problems. The plant's medicinal applications have promoted scientific studies, leading to the identification of over 200 bioactive compounds that support its traditional uses[42].

The cultural importance of Indigofera tinctoria is derived from its use in traditional medicine and also from its role in textile dyeing. Traditionally, dyes made from indigo have been regarded as symbols of honour, skill, and culture; surely, the true king of colours! The study of Ethnobotany stresses the need for documenting such knowledge so that medicinal as well as cultural values may benefit the generations to come[43]. However, with the use of natural indigo declining, the advent of synthetic dyes, through a recent resurgence in interest, driven by concerns over sustainability and health, has led to efforts to revive traditional dyeing and medicinal practices. For example, the dyeing rituals reinforce community bonds, and the indigo-dyed textile is incorporated into their cultural identity of the Landian Yao people of China[44].

Applications of Indigofera tinctoria in Traditional Medicine

Ayurveda is valued for its hepatoprotective properties and is used in blood purification, treatment of poisonous bites, aiding in liver health, anti-inflammatory effects, and skin diseases. Its benefits in the treatment of respiratory ailments, such as coughs and bronchitis, and Unani medicine acknowledge its antimicrobial properties[45]. Being one of the irreplaceable elements of traditional healing systems such as Ayurveda, Unani, Siddha, and folklore, Indigofera tinctoria, indigo in English, and Avuri in common parlance, is suggested for a variety of ailments. Siddha medicines are generally prescribed for skin ailments like eczema and psoriasis, but are also acknowledged for the treatment of disorders of the digestive tract and diseases of the nervous system, like epilepsy[46].

This plant has numerous names: indigo in English, Indigofera in scientific tongues, and Avuri in local Indian dialects, among others. All these names refer to a plant that contributes to human health and wellness. Thousands of years ago, this plant was used as a remedy in different traditional systems of medicine, such as Ayurveda, Unani, Siddha, and folk medicine. Occurrences of what are popularly called diseases have been documented for their use in Siddha medicine: the remedy is for skin disorders like eczema and psoriasis, as well as for digestive health and some neurological disorders such as epilepsy. The plant is traditionally used in folk medicine as a thermogenic and cathartic agent, as well as for hair growth promotion and wound healing[47].

The use of indigo-based treatment due to increasing awareness of its medicinal and environmental benefits and despite the decline in natural dyeing traditions due to synthetic alternatives, there is a growing resurgence. The Indigofera tinctoria has a fundamental role to play in ethnobotanical practices[48]. It has been part of the healing rituals and traditional therapies; very often it is associated with herbal teas and poultices; general healing practices based on that plant become as a bridge between indigenous knowledge and current modern pharmacological research which identifies more than 200 bioactive compounds on scientific studies, and this continues to illustrate the importance of this in natural healing[49].

Use of Indigofera Tinctoria as a Natural Dye and in Cosmetics

This is a natural dye applied in cosmetics. The dye is prized for its stability and eco-friendliness, for not requiring toxic mordants to yield a bright blue shade. Indigo dye is made from the leaves' actual dyeing agents, which can be ordinarily paired with traditional textile fabrics like cotton, linen, and wool. The natural indigo is also regaining its position as a sustainable alternative ink to synthetic dyes, industrial fabrics, and even food colorants, mainly in industries such as textiles, due to the onset of increasing environmental concerns[50].

The Indigofera tinctoria is a sought-after natural colorant used in cosmetic products like soaps, skincare products, and hair dyes. Its low toxicity and antimicrobial and antifungal properties make it preferred in enhancing products safety and effectiveness. Skin benefits, including anti-aging, anti-inflammatory, and moisturizing properties, are believed to suit this plant for sensitive skin formulations, in addition to coloring. Economic and environmental incentives for the reinvigoration of natural indigo in textiles and cosmetics accrue mainly to rural farmers and traditional dyeing industries; likewise, the double gain is the heightened consumer preference for organic and sustainable beauty products[51].

Major bioactive compounds (alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, phenolics) of Indigofera tinctoria
Indigofera tinctoria is a multipurpose medicinal plant based on its rich phytochemistry. The important active ingredients present in this plant are alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, and phenolics, all of which contribute to their therapeutic properties. The alkaloids exhibited analgesic and anti-inflammatory effects, while flavonoids are good antioxidants that reduce oxidative stress and inflammation. Tannins have astringent, wound-healing, and non-infectious healing effects, hence a valuable resource for skin and tissue repair. Saponins perform cholesterol-lowering, immune-boosting, and antimicrobial activities, all increasing the medicinal purposes of the plant[52].

Apart from this, terpenoids also exert anti-inflammatory, antimicrobial, and aromatic properties, while glycosides possess antioxidant and cardioprotective activities. Many phenolic compounds, available in great amounts, exhibit high antioxidant protection from cell damage. The aqueous extract of Indigofera tinctoria is rich in flavonoids (43.94 µg/mg) and phenolics (41.07 µg/mg) according to concentration determination, showing its possible antioxidant potential[53]. From the point of view of quantitative values being very short, tannins, saponins, alkaloids, glycosides, and terpenoids do exist in limited numbers, but their existence in Indigofera species proves that these compounds contribute to the pharmacological as well as traditional medicinal values of the plant. Thus, it can be summarized that the rich bioactivity profiles of Indigofera tinctoria bolster its therapeutic pledge in anti-inflammatory, antimicrobial, and antioxidant applications[54].

Methods used for phytochemical screening (qualitative and quantitative analysis of Indigofera tinctoria
A phytochemical study of Indigofera tinctoria using several qualitative and quantitative means for the testing of bioactive compounds has been undertaken. The qualitative analysis involved air drying fresh leaves, grinding them, and extracting them in second-grade solvents such as petroleum ether, chloroform, ethyl acetate, methanol, and ethanol, either singly or in some combinations of these. Almost all of them, such as alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, phenolics, carbohydrates, steroids, proteins, and amino acids, were confirmed in the preliminary presence, as indicated above by phytochemical screening. Thin-layer and High-Performance thin-layer chromatography compound identification and UV, IR, H1 NMR spectroscopy aided in the characterization of specific compounds[55].

Some additional confirmation of the phytochemical composition was provided by fluorescence analysis in daylight and UV illumination. For the quantitative assay, the extractability of different solvents, besides physicochemical parameters, such as total ash (10.68%), acid-insoluble ash (0.7%), and water-soluble ash (0.93%), was determined with the aim of purifying and determining their quality. Drying indicated that the moisture content (loss on drying) recorded at 3.17% was well within the standards set for proper drying for medicinal purposes. Specific compounds such as alkaloids (2.5%) and other bioactive constituents were quantified by chromatographic and spectrometric techniques, including Gas Chromatography-Mass Spectrometry (GC-MS). All these combined give a comprehensive evaluation of Indigofera tinctoria, which supports the application of this plant medicinally and assures its quality for pharmacological and therapeutic purposes[56].

Extraction Techniques for Indigofera tinctoria
Range of techniques from traditional to advanced innovations for Phyto-extraction and dyeing from Indigofera tinctoria. Water extraction is a simple and natural method of soaking plant material in water to obtain indigo. In a more refined fermentation method, the plant is cut into little pieces and fermented with water, and calcium hydroxide is added to precipitate the indigo dye under controlled pH (~11) and aeration. Maceration is another famous extraction method. Maceration is a process in which the powdered plant materials are soaked in a specific solvent, mostly methanol or ethanol, for 72 hours to extract certain bioactive compounds. Defatting with petroleum

ether (60-80°C) may also be performed before maceration to remove non-polar agents that might interfere with the extraction of polar phytochemicals[57].

More complex extraction: cashing in on Soxhlet extraction ensures the continuous washing of the solvent and thus increases the yield of the compound, while ultrasound-assisted extraction applies sound waves to burst the cell walls and enhance efficiency further. Also, with reflux heating, the plant structures are ruptured to facilitate extraction. Manual stirring or mechanical stirring will render agitation for the penetration of the solvent. The centrifugation (15000 rpm for 5 min) will separate both phases, the indigo and liquid extracts, after the extraction procedure. TLC, HPTLC, and GC-MS are employed to identify and quantify the extracted compounds. Together, these protocols optimally yield pure and efficient extraction of bioactive compounds and dyes from Indigofera tinctoria in the application of traditional medicine and dye manufacture[58].

Overview of antimicrobial properties of medicinal plants

Indigofera tinctoria is one of the plants that has an ancient history of folkloric application to treat infection. Recent investigations validate this plant's good repute for having antimicrobial properties. The extracts from the plant using methanol and ethanol were found to be potent antibacterial extracts, especially against many clinically relevant bacteria, such as Staphylococcus aureus (especially its methicillin-resistant strains), Salmonella typhi, Bacillus cereus, and Escherichia coli[59]. The antimicrobial efficacy can be attributed to its rich phytochemical profile that includes such elements as alkaloids, flavonoids, saponins, tannins, and cardiac glycosides. Antimicrobial assays using, among others, the disk-diffusion method and minimum inhibitory concentration (MIC) tests reveal potent antibacterial activities, exhibiting inhibition zones of 6 mm to 25 mm, depending on the type of extract used and the strain used. On the other hand, the plant demonstrated very little antifungal activity as it was ineffective against Aspergillus terreus[60].

These bioactive compounds contained in Indigofera tinctoria are believed to penetrate the bacterial cell wall or interfere with metabolic pathways that lead to pathogen inhibition. Safety assessments further show non-mutagenic behavior. for the extracts up to 5 mg/plate; hence, these extracts are potential alternative agents for medicinal use. Although researches help to validate its traditional applications, more studies should be carried out to assess its complete antimicrobial spectrum, optimize the extraction methods, and develop probable pharmaceutical applications for treating the growing problem of antibiotic resistance[61].

Antibacterial, antifungal, and antiviral activities of Indigofera tinctoria

Indigofera tinctoria has been evaluated for its antibacterial, antifungal, and probable antiviral activities owing to its rich phytochemical composition. Among the bioactive compounds contained by this plant are alkaloids, flavonoids, tannins, saponins, and glycosides, imparting antibacterial activities to the plant. The ethanolic and aqueous extracts are potent bactericidal agents, according to the previous studies, which focused on their activity against Salmonella typhi, Bacillus cereus, Staphylococcus aureus, Escherichia coli, and Streptococcus agalactiae. Among these extracts, the 95 percent ethanol extract provided maximum inhibition activity with a minimum inhibitory concentration (MIC) of 250 mg/mL against S. agalactiae, while the lower concentrations tended to show lesser effects. The antibacterial effect is best believed to be caused by breaking or altering the vulnerability of the bacteria or by inhibiting some metabolic processes[62].

No doubt, infallible information crops up about the antifungal activity of the plants, while certain extract ingredients have been phytochemically characterized to consist of flavonoids and phenols, which are potent in antifungal action. Nevertheless, reports claim that extracts of I. tinctoria do not present any significant inhibition against Aspergillus terreus, thus tending to suggest limited antifungal activity. Concerning their possible antiviral properties, although there are no direct data of activity, flavonoids and glycosides- the known antiviral agents in studies conducted for other plants- denote some potential role in viral inhibition. Further study characterizing antifungal and antiviral potential, as well as prospects for new drug development with Indigofera tinctoria, is warranted[63].

Key studies and findings on its antimicrobial effects

The research regarding Indigofera tinctoria has shown that this plant harbors important antimicrobial activity because of its rich phytochemicals in alkaloids, flavonoids, saponins, tannins, and glycosides. It was seen that the methanol and ethanolic extracts possess strong antibacterial activity against various strains of bacteria such as Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, and Salmonella typhi. Minimum Inhibitory Concentration (MIC) showed a range of 0.125 µg/ml-1000 mg/ml, depending on the extracts and bacteria strains.

The above evidence for the dim-prospecting of this plant as an alternative antibacterial is bolstered when it is stated that this plant inhibits biofilm formation and destroys fully matured biofilms, particularly for the pathogens involved in catheter-associated urinary tract infections[64].

Thus, it is reinforced that I. tinctoria is notably bactericidal, although it is probably incompetent as an antifungal since its extracts do not affect the growth of Aspergillus terreus and Candida albicans. Therefore, the predominant antimicrobial action would be attributed to bacterial control rather than to fungi. Moreover, there is no direct evidence of antiviral properties; however, the presence of bioactive compounds such as flavonoids and glycosides supports the concept of possible antiviral action and requires further study. With a safety profile as evidenced by non-mutagenicity in Ames tests and a history of ethnomedicine use, Indigofera tinctoria would be expected in future pharmacological applications, more against antibiotic-resistant bacteria and biofilm-associated infections[65].

Comparison of Indigofera tinctoria with Standard Antibiotics or Synthetic Drugs

Research findings reveal that Indigofera tinctoria exhibits potent antimicrobial activity, particularly in the extracts in ethanol and the nanoparticles synthesized. The extracts that withstand 95% ethanol have shown an effect against Streptococcus agalactiae by an MIC of 250 mg/mL, and the higher concentration for its bactericidal activity (500 to 1000 mg/mL) suggests its use as an alternative to conventional antibiotics such as ampicillin[66]. Also, gold and silver nanoparticles have shown really promising antibacterial activity; they are most effective against gram-negative bacteria silver nanoparticles, whereas natural phytocompounds such as flavonoids, phenols, and terpenoids obtained from plants show antibacterial activity, paving the way to natural substitutes of the synthetic antibiotics usually targeted to particular bacterial pathways. These nanoparticles have shown excellent antibacterial activity in both gold and silver nanoparticles[67]. It is mainly silver nanoparticles that are resistant to gram-negative bacteria. Such compounds are obtained from plants and comprise flavonoids, phenols, and terpenoids, which produce antibacterial activities and offer natural substitutes to synthetic antibiotics, which usually are targeted at specific bacterial pathways[68].

Unlike conventional antibiotics, I. tinctoria derivatives, such as Indigo natural, contain some bioactive compounds, including tryptanthrin, which are more specifically effective against bacteria by unique mechanisms, such as DNA intercalation, rather than merely by targeting cell wall synthesis. This different mode of action will be beneficial in the fight against resistant strains, including MRSA[69]. Unlike broad-spectrum antibiotics, I. tinctoria selectively acts against pathogenic bacteria while preserving the beneficial gut flora, thereby alleviating associated adverse effects like antibiotic-associated diarrhea. However, it is less effective against fungi and some Gram-negative bacteria, indicating it has a narrower spectrum of activity. Therefore, these properties bring their clinical application into the future; however, further research is warranted to establishthem as a reliable substitute for conventional antibiotics[70].

Possible Mechanisms Behind the Antimicrobial Activity of Indigofera tinctoria

The SNPs synthesized using the leaf extract exhibit antimicrobial activity in Indigofera tinctoria through different mechanisms. The most apparent among these is cell membrane rupture; the SNPs abrade membrane proteins, leading to loss of structural integrity, followed by leakage of intracellular materials into the surrounding medium and consequent cell death of the bacteria. Another process is by enzyme inhibition; here, silver ions react with thiol (-SH) groups in bacterial enzymes, causing a change in structure and a blockade of their functions and impediment of vital processes such as cell division and metabolism. SNPs induce another meaningful mechanism, which is that of reactive oxygen species (ROS) generation; oxidative stress created by SNPs causes damage to the bacterial lipids, proteins, and DNA, leading to subsequent cell impairment for survival[71].

In addition to the primary mechanisms, alterations of genetic material become influential since SNPs interact with phosphorus-bearing molecules, namely DNA and RNA, thereby interfering with replication and transcription processes. Nanoparticles also seem to interfere with cellular signal transduction, disrupting crucial pathways mediating bacterial proliferation or inducing programmed cell death (apoptosis)[72]. Also, the diverse phytochemical composition of I. tinctoria- including alkaloids (Tryptanthrin and indirubin), flavonoids, and polysaccharides- further enhances the antimicrobial action through a multitude of synergistic effects. These compounds may also inhibit the formation of biofilms, further obstructing bacterial resistance. Together, these mechanisms indicate that this plant has a far-reaching antimicrobial capacity that may represent a natural substitute for synthetic antibiotics[73].

Role of Specific Phytochemicals in the Antimicrobial Activity of Indigofera tinctoria

Indeed, the antimicrobial nature of Indigofera tinctoria is well pronounced under bioactive phytochemicals that inhibit bacteria and fungi via different mechanisms. The alkaloids present in all the extracts are responsible for antimicrobial activities, interfering with cellular processes in bacteria and blocking the growth of pathogens, particularly Escherichia coli and Staphylococcus aureus[74]. Cold ethanolic extracts carry flavonoids that inhibit the growth of bacteria and fungi. The flavonoids disintegrate the bacteria's membranes and inhibit enzymes while inducing the antioxidant defence system. The other groups of constituents, like saponins, were identified in hot ethanolic and aqueous extracts to disrupt the microbial membrane, causing the permeability and eventual lysis of the cells. The presence of tannins, found in all extracts and holding similar bioactivities in terms of proteins, limits the activity of bacteria and further directs biofilm development[75].

These phytochemicals act synergistically to enhance the therapeutic efficacy of I. tinctoria, as indicated by the highest undiluted Soxhlet ethanolic extract activity against Salmonella typhi and Bacillus cereus. Extraction methods applied, especially in the case of methanol extraction, seem to have a distinctive effect on antimicrobial potential, with even better yields of phytochemicals. The bulk of bioactive compounds from I. tinctoria have more roles to play in bacterial growth inhibition, destruction of microbial structures, and enhancement of potency from plant-derived antimicrobials[76].

PHP: Phytochemicals work synergistically and thus enhance the therapeutic efficacy of I. tinctoria. This was proved by the highest undiluted Soxhlet ethanolic extract activity against Salmonella typhi and Bacillus cereus. Extraction methods applied, especially in the case of methanol extraction, seem to have a distinctive effect on antimicrobial potential, with even better yields of phytochemicals. The bulk of bioactive compounds from I. tinctoria have more roles to play in bacterial growth inhibition, destruction of microbial structures, and enhancement of potency from plant-derived antimicrobials[77].

Methods of Antimicrobial Testing: Agar Well Diffusion of Indigofera tinctoria

The agar well diffusion method is a common method of evaluating the antimicrobial efficacy of plant extracts, such as Indigofera tinctoria. In this method, agar plates were prepared using different growth media according to the requirement, namely Mueller Hinton Agar (MHA) for bacteria and Potato Dextrose Agar (PDA) for fungi. The plates are then inoculated with standard bacterial and fungal cultures to ensure uniform microbial distribution. Wells of approximately 6-8 mm in diameter were made in the agar using a sterile cork borer. Maceration or infusion techniques were then used to introduce plant extracts into the wells in an aliquot of 50-200 µL. The plates were then incubated at 37°C for the bacterial strains for 24 hours and at 28°C for the fungal strains for about 72 hours, ensuring interaction between the test samples and the microorganisms[78].

The microbial activity will be determined using the zones of inhibition measured in millimeters after incubation around the wells. A larger inhibition zone defines better antimicrobial activity. Chloramphenicol (bacteria) and streptomycin (fungi) were included as standards for positive comparisons for antimalarial activity. Studies suggest that I. tinctoria has considerable antimicrobial activity against Escherichia coli, Staphylococcus aureus, Bacillus cereus, while also showing some effect against Salmonella typhi and a few fungal isolates like Aspergillus Niger. The silver nanoparticles (AgNPs) generated from I. tinctoria show enhanced antimicrobial efficiency, especially and specifically against Gram-negative bacteria. Thus, the agar well diffusion method is a simple and effective method to determine the antimicrobial activity of such plant extracts against microbial infections[79].

Methods of Antimicrobial Testing for Indigofera tinctoria

Different microbiological tests have also been used for estimating minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), to assess the antimicrobial efficacy of Indigofera tinctoria against Streptococcus agalactiae. Inoculum preparation was by even spreading of S. agalactiae on Trypticase Soy Agar (TSA) plate and subsequent addition of 30 µl of the Indigofera tinctoria extract to 6 mm filter paper discs[80]. For the control group, 100 µg/mL of ampicillin was used. Incubation was done at 37ºC for 24 hours, after which the inhibition zones were measured with a vernier caliper. MIC and MBC were done by broth dilution method where the extract is serially diluted and a suspension of the organism introduced into Trypticase Soy Broth (TSB), with the MIC being the lowest concentration without visible growth and the MBC being determined by inoculating samples from MIC wells onto TSA plates to confirm death of the organisms[81].

The research procedure for MIC and MBC determination followed strict specifications; the microdilution technique with two-fold serial dilutions of the extract was followed by adding a suspension of bacteria in each well of a 96-

well plate, and so forth until turbidity was evidenced for MIC observation. Subcultures from wells without visible growth were done on Mueller-Hinton blood agar plates, incubated, and the lowest concentration without any growth on the agar plate was recorded as MBC. Positive controls, such as vancomycin and solvent controls such as DMSO, were used for quality assessment. Both methods show the antibacterial potential of Indigofera tinctoria and hence may provide some insights for therapeutic and chemical applications as a natural antibacterial agent[82].

In Vivo Studies and Synergistic Effects of Indigofera tinctoria

Despite limited in vivo studies on its antimicrobial activity, Indigofera tinctoria is well-documented against some pathogens. During an assessment of its potential as an anthelmintic agent, sheep infected with Haemonchus contortus were treated with varying doses of Indigofera tinctoria aqueous extract (IAE). Fecal egg count reduction was highest in the 62 mg/mL group, which was linked with weight gain and various hematological parameters. The likely impact was because of phytochemicals such as tannins and flavonoids known for having anthelmintic properties. Nevertheless, the antimicrobial synergism of the plant with other agents was not covered, allowing for future in-depth studies[83].

On the synergism front, Indigofera tinctoria has, however, shown antimicrobial activity against some pathogens, including Staphylococcus aureus, Escherichia coli, Salmonella typhi, and Candida albicans. In this study, interaction with other antimicrobial agents was not specifically investigated, but the presence of bioactive compounds such as phenols and flavonoids suggests a potential for combination therapy[84]. In herbal medicine, extracts from plants exhibiting enhancement of the efficacy of synthetic drugs, thereby lessening the likelihood of development of resistance to the given drug, are not uncommon. Therefore, in the future, studies should be focused on in vivo research evaluating synergistic effects of Indigofera tinctoria with standard antimicrobials in terms of treating bacterial and fungal infections[85].

Other pharmacological properties (antioxidant, anti-inflammatory, anticancer, etc.)

Indigofera tinctoria is a very versatile medicinal plant with numerous pharmaceutical potentials. Strong antioxidants have a lot to do with flavonoids and tannins in the plant and are very effective in neutralizing free radicals, thus reducing oxidative stress. Its leaf extracts are fermented and have proven to be significantly more effective in inducing anti-inflammatory effects. The other major pharmacological activity possessed by Indigofera tinctoria is that the plant has demonstrated antimicrobial activity against both gram-positive and gram-negative bacteria, particularly Staphylococcus aureus and Klebsiella pneumoniae. These features indicate that it might be a premise for the development of a new generation of antimicrobial agents from natural sources[86].

Antimicrobial activities have been demonstrated, as well as antidiabetic, hepatoprotective, and anti-dyslipidemic activities. The findings imply that the methanolic extract of Indigofera tinctoria root has antidiabetic and antioxidant properties, leading to a management measure for diabetes. Furthermore, protection of the liver from damage would also be conferred through the hepatoprotective effects, and lipid-lowering properties would also contribute to cardiovascular health[87]. The anticancer potentials of this plant can also be evidenced by its cytotoxic and antiproliferative characteristics: in vitro studies have also revealed antimalarial activity against Plasmodium falciparum. The wide range of pharmacological properties described must make this plant worth researching in-depth to allow the clinical validation of the ethnotherapeutics of Indigofera tinctoria[88].

Toxicity and safety profile based on animal and human studies

Indigofera tinctoria studies indicate that safety was generally favorable, particularly with animal models. When tested in Wistar rats for acute oral toxicity, no significant adverse effects were noted when administered orally in doses of up to 1000 mg/kg body weight, with observations revealing no disturbances in metabolism and tissue toxicity. Furthermore, the lowest amounts of liver enzymes indicated potential liver-disturbing activity at lower doses[89]. With these findings, it was evident that cattle were fed with indigo waste-enriched diets, for which there were no adverse reactions regarding hematological parameters, immune response, and growth performance, indicating its safety as a source of protein in animal feed. However, there are scant human studies, and further studies are warranted to substantiate safety for human consumption[90].

Ecotoxicity studies, however, raise concerns about the effects an indigo dye has on aquatic life. Studies involving Oreochromis niloticus (Nile tilapia) have shown the indigo dye to be toxic at concentrations above 1.3 mg/L, causing death, behavioral alterations, and damage to internal organs. These results point towards the conclusion that while Indigofera tinctoria is largely safe for animal consumption, it may pose a danger to the environment if used as a dye. Some may seem to suffer from allergic reactions with exposure. Its traditional use in herbal medicine and cosmetics highlights that the long-term safety needs to be assessed through thorough toxicological studies in humans[91].

Dosage and potential side effects

Research has been carried out on the methanolic extraction of Indigofera tinctoria to study its dosage and efficacy in seizure models, the extract with a test dose of 200 mg/kg and 400 mg/kg body weight showed a dose-dependent reduction in duration of seizures in both Maximal Electroshock (MES) and Pentyl Tetrazole (PTZ) models. The higher doses were effective in delaying the onset of convulsions, thus indicating potential as an antiepileptic agent. Acute toxicity studies also indicated that the extract was non-toxic to experimental animals in doses up to 2000 mg/kg body weight according to OECD guidelines, hence reasonably safe within the tested doses[92].

No stringent effects were noted; there are scant data on specific side effects of the herb. Although the study did not report any adverse effects, there may be instances where herbal extracts cause allergic reactions, gastrointestinal disturbances, or sedation in some sensitive individuals. As a precautionary measure, it is better to consult a healthcare professional before using this herb, especially those having some prior conditions or on medications. Long-term safety and side effects require extensive research for proper assessment[93].

Potential pharmaceutical applications

As a treatment and dye-making plant, Indigofera tinctoria is very useful in pharmaceutical applications as it possesses various phytochemicals. This plant bears bioactive compounds like flavonoids, tannins, saponins, and indole alkaloids that usher in antioxidant, anti-inflammatory, and antimicrobial effects, functioning towards its possible treatment of inflammatory conditions such as rheumatoid arthritis and inflammatory bowel disease, as well as infections caused by antibiotic-resistant pathogenic bacteria[94]. Other therapeutic indications are efficacy in psoriasis and ulcerative colitis, with a vast range of ongoing studies into its cancer therapy potentials, particularly because of the presence of indirubin, which has anti-leukemic activity. Further, it can be used for the provision of medicinal benefits whilst also providing to supply nutritional dividends to animals thatenhance the quality of the diet, reducing dependence on synthetic antibiotics[95].

Furthermore, the crude fiber in the plant acts in regulating blood sugar and digestion, thus showing promise for use in diabetes management. This plant can be sustainably cultivated, thereby ensuring a constant supply of it for future pharmaceutical and nutritional applications. Nevertheless, and notwithstanding the great potential, there is still much work to be done in terms of standardizing quality control methods, pharmacokinetic studies with multiple components, and unearthing hitherto unexplored chemical compounds. Induction of Indigofera tinctoria into modern medicine through scientific validation of its traditional uses may yield inventive therapeutic applications and environmental sustainability[96].

Use in formulations for antimicrobial therapy

Indigofera tinctoria has been evaluated as an effective antimicrobial agent in formulations, especially for green synthesizing silver nanoparticles (AgNPs) and its extracts from nature. The plant AgNPs were effective as broad-spectrum antimicrobial agents against Gram-positive and Gram-negative bacteria and fungi; therefore, they are important for clinical and pharmaceutical applications. In addition, they can be applied as efficient drug carriers to control drug release and increase the efficacy of antimicrobial treatments. In addition, the synthesis of AgNPs from I. tinctoria is an environmentally friendly process, which is low in price and has less impact on the environment[97].

Apart from its nanoparticle applications, the extracts from Indigofera tinctoria have shown great antimicrobial properties, especially against pathogenic strains such as Streptococcus agalactiae, thus being applicable in areas of natural antimicrobial therapy, including aquaculture, veterinary medicine, and human healthcare. The extracts can be applied in formulations such as dietary supplements, topical applications, and textiles with antimicrobial characteristics[98].While this plant may have a potential use in high-tech textile applications where microencapsulation or nanotechnology could enhance its durability and controlled release of antimicrobial compounds, in vivo, this would require extensive research to standardize and optimize bioavailability and to conduct regulatory and safety assessments before any mass clinical and commercial applications[99].

Need for further research (clinical trials, molecular studies)

Research on Indigofera tinctoria continues in different fields owing to its immense potential in medicine, pharmaceuticals, and industries. Clinical trials need to be conducted to prove their therapeutic efficacy, especially in nanoparticle synthesis, antimicrobial formulations, and flavonoid-based treatments. The study of molecular mechanisms underlying its action, including biochemical pathways involved in nanoparticle formation and flavonoid biosynthesis, can help in controlling synthesis conditions, which further contributes to particular biological activity. Pharmacological investigations should be focused on establishing its mode of action and possible health benefits, along with its impact on other disease conditions, thus opening pathways for its use in modern medicine [100].

Additionally, research needs to be undertaken to develop sustainable cultivation and environmentally friendly extraction techniques to improve the productivity of the plant with minimum impact on the environment. Reports on advanced processing techniques can improve the quality of natural dyes and antimicrobials from the plant so that they can be used as viable alternatives to synthetic ones[101]. This will further enhance and augment the commercial value of Indigofera tinctoria by widening its applications into agriculture, food safety, textiles, and environmental remediation. Evaluation of regulatory provisions, safety assessments, and awareness studies will become necessary to secure the acceptance of the plant in the global market. Indigofera tinctoria can, thus, become an important natural resource to support research and development towards sustainable therapeutic and industrial applications if the focused research is executed in these directions[102].

Summary of Indigofera tinctoria

Indigofera tinctoria is worth its application in natural dyeing, medicinal, and extraction. The studies for application of its dyeing properties indicate preparing dyestuff through cold soaking leaves for a maximum absorbance for 48 hours, with lime (CaO) playing a major role in acidifying the solution to subsequently promote oxidation and improve color intensity[103]. Such fermentation conditions as optimum temperature and pH greatly affect dye extraction. Further, the studies on extraction methods have established that the most suitable ethylene glycol solvent is dimethyl sulfoxide (DMSO) for the extraction of indigo from wool fiber in optimal conditions defined by specified temperature and time balances to prevent degradation. The study concluded that indirubin is more stable than indigotin, whereas prolonged heating at elevated temperature results in the formation of degradation products, isatin being one among them[104].

Apart from dyeing functions, Indigofera tinctoria holds considerable medicinal value. Traditionally, the leaves and roots have been used to cure many diseases like epilepsy, bronchitis, and skin ulcers. Investigations in pharmacognosy point towards important morphological characteristics like the presence of sclerenchyma, stomata, and vascular bundles in the microscopic analysis[105]. Preliminary phytochemical screening has established its existence in flavonoids, glycosides, tannins, terpenoids, and saponins. The treated phytoconstituents reveal the pharmacological potential of the plant. Standards such as physicochemical and microscopic analyses should be maintained for authentication and quality control. Thus, due to its medicinal and economic importance, Indigofera tinctoria shows great potential for the sustainable production of natural dyes and pharmaceuticals and deserves further research regarding its optimum areas of use and environmental sustainability[106].

Limitations of existing studies

Limited investigations on Indigofera tinctoria exist within the domains of agriculture, dye extraction, and medicinal use. The biggest setback remains geo-oriented research, as far as the studies have only been done in some specific regions, namely Uzbekistan and Kerala. This makes data less authentic to other regions having entirely different climates and soils. The situation is further worsened by the lack of well-rounded investigations concerning the cultivation, biological attributes, and long-term sustenance of the plant, as regards standardizing agriculture and extraction practices for the plant. Inhibiting this further are the differences in stimulant effects and seed yield, as well as a limited number of adaptability trials for the plant species[107].

From a pharmacognostic and phytochemical viewpoint, there seems to be an acute shortage of studies with adequate standardization and quality assurance paradigms, thus obstructing any meaningful attempts to introduce Indigofera tinctoria into contemporary medicine. The list of bioactive compounds is still very limited, and documentation of medicinal properties and pharmacological activity is poor. Furthermore, it is poorly understood how environmental variability affects phytochemical composition; modern methods for quality control are minimally employed. Closing such gaps would thus involve extensive and long-term studies utilizing state-of-the-art methodologies aimed at the full agricultural, industrial, and medicinal exploitation of Indigofera tinctoria[108].

Future research directions

Indigofera tinctoria holds great promise for more agricultural, medicinal, and industrial applications, provided that research continues in the future. Agriculture requires that a constant evaluation of how soil type, soil composition, fertilizer application, and environmental factors affect plant growth and dye yield takes place. Also, studies regarding the genetics may formulate varieties resistant to diseases and maximize yield, while studies that compare Indigofera tinctoria with other sources of indigo could standardize and optimize the agronomic practices. Furthermore, sustainable agricultural practice, incorporating traditional knowledge, should be evaluated for greenness[109].The different pharmacological investigations on the medicinal side revolved around the usual anti-inflammatory, antimicrobial, and anticancer screening, carried out normally in vitro and in vivo. Quality and chemical composition analysis are mandatory to standardize herbal medicine, thus ensuring safety and efficacy in action. Clinical trials thus appear to be a viable line of action in the inquiry of therapeutic benefits of Indigofera tinctoria against dermatologic disorders like psoriasis or ulcerative colitis[110]. There would also have to be scrutiny of the traditional medicinal values to be tapped for the present applications.Studies ought to investigate whether Indigofera tinctoria wastes could be an alternative feed supplement to augment industrial and livestock applications. Such investigations should also envisage those promising avenues as regards nutrients, digestibility, and physiological efficacy of the supplement[111].

Conclusion:

Indigofera Tinctoria, once considered sacred, has many modern applications, ranging from medicine to agriculture and sustainable industries. Its wealth of phytochemical constituents, namely alkaloids, flavonoids, saponins, tannins, glycosides, and many other constituents, justify its numerous antimicrobial, antioxidant, anti-inflammatory, and hepatoprotective activities. Experimental findings, including many using ethanolic and methanolic extracts, prove their efficacy on many pathogenic bacteria such as Staphylococcus aureus and Escherichia coli. Its potential in finding another application in green synthesis of silver nanoparticles also shows grounds for advancing eco-friendly antimicrobial approaches. Beyond medicines, Indigofera tinctoria renders services as a part of sustainable dyeing processes in textiles and helps the environment in nitrogen fixation and reducing soil erosion. Though many promises have been made, limitations like inadequate clinical studies, non-standardization, and environmental toxicity merit further investigation. Research in the future should focus more on clinical validation, molecular exploration, sustainable cultivation, and wider test application for extracting the full therapeutic and industrial promise of this plant.

References:

[1] O. O. Ogbole, O. D. Akin-Ajani, T. O. Ajala, Q. A. Ogunniyi, J. Fettke, and O. A. Odeku, "Nutritional and pharmacological potentials of orphan legumes: Subfamily faboideae," Heliyon, vol. 9, no. 4, Apr. 2023, doi: 10.1016/j.heliyon.2023.e15493.

[2] F. Zafar et al., "Antibiofilm and Quorum Sensing Inhibition (QSI) Potential of Lagerstroemia speciosa Leaves Extract," Dose-Response, vol. 20, no. 4, Oct. 2022, doi: 10.1177/15593258221132080.

[3] H. Hasan, H. Ismail, Y. El-Orfali, and G. Khawaja, "Therapeutic benefits of Indole-3-Carbinol in adjuvant-induced arthritis and its protective effect against methotrexate induced-hepatic toxicity," BMC Complement Altern Med, vol. 18, no. 1, Dec. 2018, doi: 10.1186/S12906-018-2408-1.

[4] J. E. McLain, E. Cytryn, L. M. Durso, and S. Young, "Culture-based Methods for Detection of Antibiotic Resistance in Agroecosystems: Advantages, Challenges, and Gaps in Knowledge," J Environ Qual, vol. 45, no. 2, pp. 432–440, Mar. 2016, doi: 10.2134/JEQ2015.06.0317.

[5] J. Wong, D. Manoil, P. Näsman, G. N. Belibasakis, and P. Neelakantan, "Microbiological Aspects of Root Canal Infections and Disinfection Strategies: An Update Review on the Current Knowledge and Challenges," Frontiers in Oral Health, vol. 2, 2021, doi: 10.3389/FROH.2021.672887/FULL.

[6] W. He et al., "Efficacy and safety of preventing catheter-associated urinary tract infection by inhibiting catheter bacterial biofilm formation: a multicenter randomized controlled trial," Antimicrob Resist Infect Control, vol. 13, no. 1, Dec. 2024, doi: 10.1186/S13756-024-01450-0.

[7] S. R. Panda, A. Meher, G. Prusty, S. Behera, and B. R. Prasad, "Antibacterial properties and therapeutic potential of few medicinal plants: current insights and challenges," Discover Plants, vol. 2, no. 1, p. 21, Jan. 2025, doi: 10.1007/S44372-025-00097-4.

[8] M. J. Datiles and P. Acevedo-Rodríguez, "Indigofera tinctoria (true indigo)," CABI Compendium, Mar. 2014, doi: 10.1079/CABICOMPENDIUM.28613.

[9] E. Kebede, "Contribution, Utilization, and Improvement of Legumes-Driven Biological Nitrogen Fixation in Agricultural Systems," Front Sustain Food Syst, vol. 5, Nov. 2021, doi: 10.3389/FSUFS.2021.767998/FULL.

[10] I. L. Mouafon et al., "Chemical constituents of the medicinal plant Indigofera spicata Forsk (Fabaceae) and their chemophenetic significance," Biochem Syst Ecol, vol. 95, Apr. 2021, doi: 10.1016/j.bse.2021.104230.

[11] K. Hu et al., "All-Season Production of Plant Indigo Based on Insights into Heat Stress for Strobilanthes cusia Leaves," ACS Sustain Chem Eng, vol. 11, no. 1, pp. 426–435, Jan. 2023, doi: 10.1021/ACSSUSCHEMENG.2C06375.

[12] K. su Kim et al., "Assessment of Indigo (Polygonum tinctorium Ait.) water extracts' bioactive compounds, and their antioxidant and antiproliferative activities," LWT, vol. 46, no. 2, pp. 500–510, 2012, doi: 10.1016/j.lwt.2011.11.017.

[13] F. Nouban and M. Abazid, "Plastic degrading fungi Trichoderma viride and Aspergillus nomius isolated fromNouban, F. and Abazid, M. (2017) 'Plastic degrading fungi Trichoderma viride and Aspergillus nomius isolated

from local landfill soil in Medan', Iopscience.Iop.Org, 8(February ," Iopscience.Iop.Org, vol. 8, no. February 2018, pp. 68–74, 2017, doi: 10.1088/1755-1315/824/1/012070/PDF.

[14] M. Riaz et al., "Phytobioactive compounds as therapeutic agents for human diseases: A review," Food Sci Nutr, vol. 11, no. 6, pp. 2500–2529, Jun. 2023, doi: 10.1002/FSN3.3308.

[15] R. S. Chaughule and R. S. Barve, "Role of herbal medicines in the treatment of infectious diseases," Vegetos, vol. 37, no. 1, pp. 41–51, Feb. 2024, doi: 10.1007/S42535-022-00549-2.

[16] S. Y. Pan et al., "Historical perspective of traditional indigenous medical practices: The current renaissance and conservation of herbal resources," Evidence-based Complementary and Alternative Medicine, vol. 2014, 2014, doi: 10.1155/2014/525340.

[17] M. J. Datiles and P. Acevedo-Rodríguez, "Indigofera tinctoria (true indigo)," CABI Compendium, Mar. 2014, doi: 10.1079/CABICOMPENDIUM.28613.

[18] S. D. Logsdon and P. L. O'Brien, "Runoff and nutrient losses in extended and conventional crop rotations," Agrosystems, Geosciences and Environment, vol. 5, no. 4, 2022, doi: 10.1002/AGG2.20318.

[19] M. J. Datiles and P. Acevedo-Rodríguez, "Indigofera tinctoria (true indigo)," CABI Compendium, Mar. 2014, doi: 10.1079/CABICOMPENDIUM.28613.

[20] A. M. Martins and J. M. Marto, "A sustainable life cycle for cosmetics: From design and development to post-use phase," Sustain Chem Pharm, vol. 35, Oct. 2023, doi: 10.1016/j.scp.2023.101178.

[21] S. Li, A. B. Cunningham, R. Fan, and Y. Wang, "Identity blues: The ethnobotany of the indigo dyeing by Landian Yao (Iu Mien) in Yunnan, Southwest China," J Ethnobiol Ethnomed, vol. 15, no. 1, Feb. 2019, doi: 10.1186/S13002-019-0289-0.

[22] A. M. Thakker and D. Sun, "Sustainable Development Goals for Textiles and Fashion," Environmental Science and Pollution Research, vol. 30, no. 46, pp. 101989–102009, Oct. 2023, doi: 10.1007/S11356-023-29453-1.

[23] K. S. Randhawa, "Synthesis, Properties, and Environmental Impact of Hybrid Pigments," The Scientific World Journal, vol. 2024, no. 1, Jan. 2024, doi: 10.1155/TSWJ/2773950.

[24] R. Rame, P. Purwanto, and S. Sudarno, "Industry 5.0 and sustainability: An overview of emerging trends and challenges for a green future," Innovation and Green Development, vol. 3, no. 4, Dec. 2024, doi: 10.1016/j.igd.2024.100173.

[25] J. Mensah, "Sustainable development: Meaning, history, principles, pillars, and implications for human action: Literature review," Cogent Soc Sci, vol. 5, no. 1, Jan. 2019, doi: 10.1080/23311886.2019.1653531.

[26] A. Hansen, A. Gladala-Kostarz, R. Hindhaugh, J. H. Doonan, and M. Bosch, "Mechanical stimulation in plants: molecular insights, morphological adaptations, and agricultural applications in monocots," BMC Biol, vol. 23, no. 1, Dec. 2025, doi: 10.1186/S12915-025-02157-3.

[27] J. Long, T. Tan, Y. Zhu, X. An, X. Zhang, and D. Wang, "Response of blueberry photosynthetic physiology to light intensity during different stages of fruit development," PLoS One, vol. 19, no. 9, Sep. 2024, doi: 10.1371/JOURNAL.PONE.0310252.

[28] Q. Li, M. Deng, Y. Xiong, A. Coombes, and W. Zhao, "Morphological and photosynthetic response to high and low irradiance of aeschynanthus longicaulis," Scientific World Journal, vol. 2014, 2014, doi: 10.1155/2014/347461.

[29] D. Gudavalli, K. Pandey, V. G. EDE, D. Sable, A. S. Ghagare, and A. S. Kate, "Phytochemistry and pharmacological activities of five species of Bauhinia genus: A review," Fitoterapia, vol. 174, Apr. 2024, doi: 10.1016/j.fitote.2024.105830.

[30] S. A. Bhat et al., "In-vitro evaluation of Indigofera heterantha extracts for antibacterial, antifungal and anthelmintic activities," J Pharm Health Care Sci, vol. 10, no. 1, Dec. 2024, doi: 10.1186/S40780-024-00328-Y.

[31] L. Ma and G. Li, "Auxin-dependent cell elongation during the shade avoidance response," Front Plant Sci, vol. 10, Jul. 2019, doi: 10.3389/FPLS.2019.00914/FULL.

[32] W. Bin Wang et al., "High resource capture and use efficiency and prolonged growth season contribute to invasiveness of Eupatorium adenophorum," Plant Ecol, vol. 214, no. 6, pp. 857–868, Jun. 2013, doi: 10.1007/S11258-013-0214-X.

[33] R. Surjit, H. Shanruthi, V. Sruthi, and K. S. Tarunvalavan, "Natural Indigo Dyes: A Potential Dye for Sustainability," pp. 193–210, 2023, doi: 10.1007/978-3-031-47471-2_9.

[34] P. Dey et al., "Sustainable and eco-friendly natural dyes: a holistic review on sources, extraction, and application prospects," Textile Research Journal , 2025, doi: 10.1177/00405175251321139.

[35] A. K. Kabish, M. T. Abate, Z. A. Alemar, and S. Girmay, "The Importance of Natural Indigo Dye and Its Revitalization and Ethiopian Potential for Indigo Growing," Advances in Materials Science and Engineering, vol. 2023, 2023, doi: 10.1155/2023/2135014.

[36] M. J. Datiles and P. Acevedo-Rodríguez, "Indigofera tinctoria (true indigo)," CABI Compendium, Mar. 2014, doi: 10.1079/CABICOMPENDIUM.28613.

[37] E. Lee, X. Yang, J. Ha, M. Y. Kim, K. Y. Park, and S. H. Lee, "Identification of a Locus Controlling Compound Raceme Inflorescence in Mungbean [Vigna radiata (L.) R. Wilczek]," Front Genet, vol. 12, Mar. 2021, doi: 10.3389/FGENE.2021.642518/FULL.
[38] M. J. Datiles and P. Acevedo-Rodríguez, "Indigofera tinctoria (true indigo)," CABI Compendium, Mar. 2014, doi: 10.1079/CABICOMPENDIUM.28613.
[39] S. Nichols and G. Cottam, "HARVESTING AS A CONTROL FOR AQUATIC PLANTS," JAWRA Journal of the American Water Resources Association, vol. 8, no. 6, pp. 1205–1210, 1972, doi: 10.1111/J.1752-1688.1972.TB05263.X.
[40] R. Surjit, H. Shanruthi, V. Sruthi, and K. S. Tarunvalavan, "Natural Indigo Dyes: A Potential Dye for Sustainability," pp. 193–210, 2023, doi: 10.1007/978-3-031-47471-2_9.
[41] J. Song, H. Imanaka, K. Imamura, K. Kajitani, and K. Nakanishi, "Development of a highly efficient indigo dyeing method using indican with an immobilized β-glucosidase from Aspergillus niger," J Biosci Bioeng, vol. 110, no. 3, pp. 281–287, Sep. 2010, doi: 10.1016/j.jbiosc.2010.03.010.
[42] M. J. Datiles and P. Acevedo-Rodríguez, "Indigofera tinctoria (true indigo)," CABI Compendium, Mar. 2014, doi: 10.1079/CABICOMPENDIUM.28613.
[43] Y. Shi et al., "Quality blues: traditional knowledge used for natural indigo identification in southern China," J Ethnobiol Ethnomed, vol. 17, no. 1, Dec. 2021, doi: 10.1186/S13002-021-00454-Z.
[44] A. Kamboj, M. Tamta, P. Kundal, and B. Soun, "Eco-friendly Dyeing Approach: Natural Dyeing—A Need of the Hour," pp. 91–107, 2024, doi: 10.1007/978-981-99-9856-2_7.
[45] M. Emerald, "Medicinal Plants: Therapeutic Potential, Safety, and Toxicity," Drug Discovery and Evaluation: Safety and Pharmacokinetic Assays, pp. 1–71, 2024, doi: 10.1007/978-3-030-73317-9_90-1.
[46] T. P. Ijinu, V. George, and P. Pushpangadan, "History of Research on Medicinal Plants in India," pp. 35–61, 2022, doi: 10.1007/978-3-030-98701-5_2.
[47] K. Kovendan, S. Arivoli, R. Maheshwaran, K. Baskar, and S. Vincent, "Larvicidal efficacy of sphaeranthus indicus, cleistanthus collinus and murraya koenigii leaf extracts against filarial vector, culex quinquefasciatus say (Diptera: Culicidae)," Parasitol Res, vol. 111, no. 3, pp. 1025–1035, Sep. 2012, doi: 10.1007/S00436-012-2927-5.
[48] A. K. Kabish, M. T. Abate, Z. A. Alemar, and S. Girmay, "The Importance of Natural Indigo Dye and Its Revitalization and Ethiopian Potential for Indigo Growing," Advances in Materials Science and Engineering, vol. 2023, 2023, doi: 10.1155/2023/2135014.
[49] S. Y. Pan et al., "Historical perspective of traditional indigenous medical practices: The current renaissance and conservation of herbal resources," Evidence-based Complementary and Alternative Medicine, vol. 2014, 2014, doi: 10.1155/2014/525340.
[50] J. A. Mora-Jiménez, V. A. Alvarez-Rodriguez, S. Cisneros-Hernández, C. Ramírez-Martínez, and A. Ordaz, "Natural pigment indigoidine production: process design, simulation, and techno-economic assessment," Chemical Product and Process Modeling, vol. 19, no. 4, pp. 551–572, Aug. 2024, doi: 10.1515/CPPM-2023-0098/HTML.
[51] L. Saikhao, N. Kaew-unruan, K. Thanomsith, R. Dulyasucharit, and W. Munmuangsan, "A study of the influence of the addition of lime to natural indigo production," Appl Surf Sci, vol. 689, Apr. 2025, doi: 10.1016/j.apsusc.2025.162474.
[52] M. R. Arif et al., "Traditional uses, phytochemistry, and pharmacology of Flemingia macrophylla, an important traditional medicinal plant," Discover Pharmaceutical Sciences, vol. 1, no. 1, p. 2, Feb. 2025, doi: 10.1007/S44395-025-00003-7.
[53] Y. Zang, R. nan Sun, R. qi Feng, H. hui Zhu, and X. wen Li, "Recent Advances of Terpenoids with Intriguing Chemical Skeletons and Biological Activities," Chin J Chem, Feb. 2024, doi: 10.1002/CJOC.202400697.
[54] E. Gerometta, I. Grondin, J. Smadja, M. Frederich, and A. Gauvin-Bialecki, "A review of traditional uses, phytochemistry and pharmacology of the genus Indigofera," J Ethnopharmacol, vol. 253, May 2020, doi: 10.1016/j.jep.2020.112608.
[55] S. Devanesan, M. Jayamala, M. S. AlSalhi, S. Umamaheshwari, and A. J. A. Ranjitsingh, "Antimicrobial and anticancer properties of Carica papaya leaves derived di-methyl flubendazole mediated silver nanoparticles," J Infect Public Health, vol. 14, no. 5, pp. 577–587, May 2021, doi: 10.1016/j.jiph.2021.02.004.
[56] S. Dhakal, D. Pandey, M. E. van der Heide, J. V. Nørgaard, U. Vrhovsek, and P. Khanal, "Effect of different drying methods on the nutritional composition and phenolic compounds of the brown macroalga, Fucus vesiculosus (Fucales, Phaeophyceae)," J Appl Phycol, vol. 36, no. 6, pp. 3649–3663, Dec. 2024, doi: 10.1007/S10811-024-03343-6.
[57] J. Petrović et al., "Different extraction methodologies and their influence on the bioactivity of the wild edible mushroom Laetiporus sulphureus (Bull.) Murrill," Food Funct, vol. 5, no. 11, pp. 2948–2960, Oct. 2014, doi: 10.1039/C4FO00727A.

[58] C. Wen et al., “Advances in ultrasound assisted extraction of bioactive compounds from cash crops – A review,” Ultrason Sonochem, vol. 48, pp. 538–549, Nov. 2018, doi: 10.1016/j.ultsonch.2018.07.018.

[59] A. Sharma, N. Kumar, A. Dhanda, A. Yadav, and N. K. Aggarwal, “In vivo assessment of antimicrobial, antioxidant, antigenotoxic and antimutagenic effects of different extracts from Camellia sinensis,” Systems Microbiology and Biomanufacturing, vol. 4, no. 1, pp. 282–293, Jan. 2024, doi: 10.1007/S43393-023-00196-X.

[60] C. M. Wangui, E. S. Madivoli, W. Waudo, and J. Gichuki, “Evaluation of in vitro antimicrobial and antioxidant properties of Ziziphus robertsoniana Beentje aqueous and methanol extracts,” Discover Applied Sciences, vol. 7, no. 1, Jan. 2025, doi: 10.1007/S42452-024-06338-7.

[61] I. I. Ozyigit et al., “Production of secondary metabolites using tissue culture-based biotechnological applications,” Front Plant Sci, vol. 14, 2023, doi: 10.3389/FPLS.2023.1132555/FULL.

[62] Y. H. Gonfa et al., “Anti-inflammatory activity of phytochemicals from medicinal plants and their nanoparticles: A review,” Curr Res Biotechnol, vol. 6, Jan. 2023, doi: 10.1016/j.crbiot.2023.100152.

[63] A. H. Hashem, A. M. Shehabeldine, A. M. Abdelaziz, B. H. Amin, and M. H. Sharaf, “Antifungal Activity of Endophytic Aspergillus terreus Extract Against Some Fungi Causing Mucormycosis: Ultrastructural Study,” Appl Biochem Biotechnol, vol. 194, no. 8, pp. 3468–3482, Aug. 2022, doi: 10.1007/S12010-022-03876-X.

[64] G. F. Swandiny et al., “Potent antibacterial and cytotoxic bioactive compounds from endophytic fungi Diaporthe sp. associated with Salacia intermedia,” Arch Microbiol, vol. 207, no. 2, p. 40, Feb. 2025, doi: 10.1007/S00203-025-04236-Z.

[65] A. Y. Moussa, “The limitless endophytes: their role as antifungal agents against top priority pathogens,” Microb Cell Fact, vol. 23, no. 1, Dec. 2024, doi: 10.1186/S12934-024-02411-3.

[66] A. M. Shehabeldine, A. M. Abdelaziz, M. A. Abdel-Maksoud, M. A. El-Tayeb, B. H. Kiani, and A. S. Hussein, “Antimicrobial characteristics of endophytic Aspergillus terreus and acute oral toxicity analysis,” Electronic Journal of Biotechnology, vol. 72, pp. 1–11, Nov. 2024, doi: 10.1016/j.ejbt.2024.07.003.

[67] M. T. Shaaban, B. S. Mohamed, M. Zayed, and S. M. El-Sabbagh, “Antibacterial, antibiofilm, and anticancer activity of silver-nanoparticles synthesized from the cell-filtrate of Streptomyces enissocaesilis,” BMC Biotechnol, vol. 24, no. 1, Dec. 2024, doi: 10.1186/S12896-024-00833-W.

[68] Hemlata, P. R. Meena, A. P. Singh, and K. K. Tejavath, “Biosynthesis of Silver Nanoparticles Using Cucumis prophetarum Aqueous Leaf Extract and Their Antibacterial and Antiproliferative Activity against Cancer Cell Lines,” ACS Omega, vol. 5, no. 10, pp. 5520–5528, Mar. 2020, doi: 10.1021/ACSOMEGA.0C00155.

[69] H. H. Gong, K. Baathulaa, J. S. Lv, G. X. Cai, and C. H. Zhou, “Synthesis and biological evaluation of Schiff base-linked imidazolyl naphthalimides as novel potential anti-MRSA agents,” Medchemcomm, vol. 7, no. 5, pp. 924–931, 2016, doi: 10.1039/C5MD00574D.

[70] J. Zhu, F. Xia, S. Wang, Y. Guan, F. Hu, and F. Yu, “Recent advances in nanomaterials and their mechanisms for infected wounds management,” Mater Today Bio, vol. 31, Apr. 2025, doi: 10.1016/j.mtbio.2025.101553.

[71] D. Ravi, B. Gunasekar, V. Kaliyaperumal, and S. Babu, “A recent advances in antimicrobial activity of green synthesized selenium nanoparticle,” OpenNano, vol. 20, Nov. 2024, doi: 10.1016/j.onano.2024.100219.

[72] J. M. Sasso et al., “The Progress and Promise of RNA Medicine-An Arsenal of Targeted Treatments,” J Med Chem, vol. 65, no. 10, pp. 6975–7015, May 2022, doi: 10.1021/ACS.JMEDCHEM.2C00024.

[73] H. Cao et al., “Phytochemicals from fern species: potential for medicine applications,” Phytochemistry Reviews, vol. 16, no. 3, pp. 379–440, Jun. 2017, doi: 10.1007/S11101-016-9488-7.

[74] U. Anand et al., “Safer plant-based nanoparticles for combating antibiotic resistance in bacteria: A comprehensive review on its potential applications, recent advances, and future perspective,” Science of the Total Environment, vol. 821, May 2022, doi: 10.1016/j.scitotenv.2022.153472.

[75] T. Suwandi, M. Sundjojo, C. C. Panjaitan, E. R. Hepziba, and T. O. Setiabudi, “Antibacterial effects of rosella petal extract (Hibiscus sabdariffa L.) in mouthwash formulation against Streptococcus sanguinis and Porphyromonas gingivalis Biofilms: An in vitro study,” Saudi Dental Journal, vol. 37, no. 1, Mar. 2025, doi: 10.1007/S44445-025-00007-0.

[76] S. P. Mahire and S. N. Patel, “Extraction of phytochemicals and study of its antimicrobial and antioxidant activity of Helicteres isora L.,” Clinical Phytoscience, vol. 6, no. 1, Dec. 2020, doi: 10.1186/S40816-020-00156-1.

[77] A. Phuyal, P. K. Ojha, B. Guragain, and N. K. Chaudhary, “Phytochemical screening, metal concentration determination, antioxidant activity, and antibacterial evaluation of Drymaria diandra plant,” Beni Suef Univ J Basic Appl Sci, vol. 8, no. 1, Dec. 2019, doi: 10.1186/S43088-019-0020-1.

[78] M. Ocal et al., “A broad-spectrum biological activities of Heracleum humile extracts: A first report of the antiviral, anti-cancer and chemical properties,” Food Biosci, vol. 62, Dec. 2024, doi: 10.1016/j.fbio.2024.105195.

[79] X. Zhang, X. Hou, L. Ma, Y. Shi, D. Zhang, and K. Qu, “Analytical methods for assessing antimicrobial activity of nanomaterials in complex media: advances, challenges, and perspectives,” J Nanobiotechnology, vol. 21, no. 1, Dec. 2023, doi: 10.1186/S12951-023-01851-0.

[80] K. M. Craft, H. C. Thomas, and S. D. Townsend, “Sialylated variants of lacto-: N -tetraose exhibit antimicrobial activity against Group B Streptococcus,” Org Biomol Chem, vol. 17, no. 7, pp. 1893–1900, 2019, doi: 10.1039/C8OB02080A.

[81] P. Zajkoska et al., “Immobilised whole-cell recombinant monoamine oxidase biocatalysis,” Appl Microbiol Biotechnol, vol. 99, no. 3, pp. 1229–1236, Feb. 2015, doi: 10.1007/S00253-014-5983-1.

[82] A. Singh, L. B. Prasad, K. Shiv, R. Kumar, and S. Garai, “Synthesis, characterization, and in vitro antibacterial and cytotoxic study of Co(II), Ni(II), Cu(II), and Zn(II) complexes of N-(4-methoxybenzyl) N-(phenylethyl) dithiocarbamate ligand,” J Mol Struct, vol. 1288, Sep. 2023, doi: 10.1016/j.molstruc.2023.135835.

[83] S. A. Bhat et al., “In-vitro evaluation of Indigofera heterantha extracts for antibacterial, antifungal and anthelmintic activities,” J Pharm Health Care Sci, vol. 10, no. 1, Dec. 2024, doi: 10.1186/S40780-024-00328-Y.

[84] A. W. Alshameri and M. Owais, “Antibacterial and cytotoxic potency of the plant-mediated synthesis of metallic nanoparticles Ag NPs and ZnO NPs: A review,” OpenNano, vol. 8, Nov. 2022, doi: 10.1016/j.onano.2022.100077.

[85] P. Gupta, S. Kalvatala, A. Joseph, A. Panghal, and S. Santra, “Outline of Therapeutic Potential of Different Plants Reported Against Psoriasis via In Vitro, Pre-Clinical or Clinical Studies,” Phytotherapy Research, Feb. 2025, doi: 10.1002/PTR.8405.

[86] J. S. Nissi, S. Vyaishnavi, R. Sivaranjanee, M. P. Sekar, D. Sundaramurthi, and V. Vadivel, “Development and characterization of Morinda tinctoria incorporated electrospun PHBV fiber mat for wound healing application,” Macromol Res, vol. 31, no. 4, pp. 393–405, Apr. 2023, doi: 10.1007/S13233-023-00149-2.

[87] A. Arshad et al., “In vitro enzyme inhibition, antibacterial, UHPLC-MS chemical profiling and in silico studies of Indigofera argentea Burm. f. for potential biopharmaceutical application,” South African Journal of Botany, vol. 143, pp. 322–329, Dec. 2021, doi: 10.1016/j.sajb.2020.12.001.

[88] A. Rauf et al., “Anticancer therapeutic potential of genus Diospyros: From phytochemistry to clinical applications—A review,” Food Sci Nutr, Oct. 2024, doi: 10.1002/FSN3.4375.

[89] M. A. Costa et al., “Acute and chronic toxicity of an aqueous fraction of the stem bark of stryphnodendron adstringens (Barbatimão) in rodents,” Evidence-based Complementary and Alternative Medicine, vol. 2013, 2013, doi: 10.1155/2013/841580.

[90] C. Novais et al., “Natural Food Colorants and Preservatives: A Review, a Demand, and a Challenge,” J Agric Food Chem, vol. 70, no. 9, pp. 2789–2805, Mar. 2022, doi: 10.1021/ACS.JAFC.1C07533.

[91] O. V. Ayebidun and A. O. Ajibare, “Sub-lethal toxicity of indigo dye (Indigofera tinctoria) on Oreochromis niloticus juveniles,” Bull Natl Res Cent, vol. 47, no. 1, Jun. 2023, doi: 10.1186/S42269-023-01060-7.

[92] E. Gerometta, I. Grondin, J. Smadja, M. Frederich, and A. Gauvin-Bialecki, “A review of traditional uses, phytochemistry and pharmacology of the genus Indigofera,” J Ethnopharmacol, vol. 253, May 2020, doi: 10.1016/j.jep.2020.112608.

[93] S. J. L. Yoon and C. H. Home, “Herbal products and conventional medicines used by community-residing older women,” J Adv Nurs, vol. 33, no. 1, pp. 51–59, Jan. 2001, doi: 10.1046/J.1365-2648.2001.01637.X.

[94] A. T. B. dos Santos et al., “Organic extracts from Indigofera suffruticosa leaves have antimicrobial and synergic actions with erythromycin against Staphylococcus aureus,” Front Microbiol, vol. 6, no. FEB, 2015, doi: 10.3389/FMICB.2015.00013/FULL.

[95] I. Bassukas, G. Gaitanis, K. Katsanos, D. Christodoulou, E. Tsianos, and C. Vlachos, “Psoriasis and inflammatory bowel disease: links and risks,” Psoriasis: Targets and Therapy, vol. Volume 6, pp. 73–92, Jul. 2016, doi: 10.2147/PTT.S85194.

[96] D. A. Timm and J. L. Slavin, “Dietary Fiber and the Relationship to Chronic Diseases,” Am J Lifestyle Med, vol. 2, no. 3, pp. 233–240, 2008, doi: 10.1177/1559827608314149.

[97] G. S. Reddy, K. V. Saritha, Y. M. Reddy, and N. V. Reddy, “Eco-friendly synthesis and evaluation of biological activity of silver nanoparticles from leaf extract of Indigofera barberi Gamble: an endemic plant of Seshachalam Biosphere Reserve,” SN Appl Sci, vol. 1, no. 9, Sep. 2019, doi: 10.1007/S42452-019-1008-0.

[98] M. M. Hossain et al., “Advancements of eco-friendly natural antimicrobial agents and their transformative role in sustainable textiles,” SPE Polymers, vol. 5, no. 3, pp. 241–276, Jul. 2024, doi: 10.1002/PLS2.10135.

[99] N. T. T. Nguyen, L. M. Nguyen, T. T. T. Nguyen, T. T. Nguyen, D. T. C. Nguyen, and T. Van Tran, “Formation, antimicrobial activity, and biomedical performance of plant-based nanoparticles: a review,” Environ Chem Lett, vol. 20, no. 4, pp. 2531–2571, Aug. 2022, doi: 10.1007/S10311-022-01425-W.

[100] M. I. Attia, W. M. Eldehna, S. A. Afifi, A. B. Keeton, G. A. Piazza, and H. A. Abdel-Aziz, “New hydrazonoindolin-2-ones: Synthesis, exploration of the possible anti-proliferative mechanism of action and encapsulation into PLGA microspheres,” PLoS One, vol. 12, no. 7, Jul. 2017, doi: 10.1371/JOURNAL.PONE.0181241.

[101] U. P. Albuquerque et al., “Natural products from ethnodirected studies: Revisiting the ethnobiology of the zombie poison,” Evidence-based Complementary and Alternative Medicine, vol. 2012, 2012, doi: 10.1155/2012/202508.

[102] A. M. Martins and J. M. Marto, “A sustainable life cycle for cosmetics: From design and development to post-use phase,” Sustain Chem Pharm, vol. 35, Oct. 2023, doi: 10.1016/j.scp.2023.101178.

[103] H. Purnama, N. Hidayati, D. S. Safitri, and S. Rahmawati, “Effect of initial treatment in the preparation of natural indigo dye from Indigofera tinctoria,” AIP Conf Proc, vol. 1855, Jun. 2017, doi: 10.1063/1.4985467/13746337/020022_1_ONLINE.PDF.

[104] O. V. Kharissova, B. I. Kharisov, C. M. O. González, Y. P. Méndez, and I. López, “Greener synthesis of chemical compounds and materials,” R Soc Open Sci, vol. 6, no. 11, Nov. 2019, doi: 10.1098/RSOS.191378.

[105] E. Gerometta, I. Grondin, J. Smadja, M. Frederich, and A. Gauvin-Bialecki, “A review of traditional uses, phytochemistry and pharmacology of the genus Indigofera,” J Ethnopharmacol, vol. 253, May 2020, doi: 10.1016/j.jep.2020.112608.

[106] T. Ahmadu and K. Ahmad, “An Introduction to Bioactive Natural Products and General Applications,” Advanced Structured Materials, vol. 140, pp. 41–91, 2021, doi: 10.1007/978-3-030-54027-2_2.

[107] P. B. Tayade and R. V. Adivarekar, “Extraction of Indigo dye from Couroupita guianensisand its application on cotton fabric,” Fashion and Textiles, vol. 1, no. 1, Dec. 2014, doi: 10.1186/S40691-014-0016-3.

[108] M. El Haouari et al., “An Insight into Phytochemical, Pharmacological, and Nutritional Properties of Arbutus unedo L. From Morocco,” Evidence-based Complementary and Alternative Medicine, vol. 2021, 2021, doi: 10.1155/2021/1794621.

[109] S. Babu et al., “Nanofertilizers for agricultural and environmental sustainability,” Chemosphere, vol. 292, Apr. 2022, doi: 10.1016/j.chemosphere.2021.133451.

[110] S. M. Yu, S. J. Kim, Y. C. Yoon, and J. H. Kim, “Development and application of a chemical profiling method for the assessment of the quality and consistency of the Pelargonium sidoides extract,” J Anal Sci Technol, vol. 12, no. 1, Dec. 2021, doi: 10.1186/S40543-021-00297-Z.

[111] V. Rajani, S. Umadevi, and C. Naga Raju, “ A Review on Exploring the Phytochemical and Pharmacological Significance of Indigofera astragalina ,” Pharmacogn Mag, vol. 20, no. 2, pp. 363–371, Jun. 2024, doi: 10.1177/09731296231215911/ASSET/31D87972-0EAD-4C4D-9B66-A3CB06995C71/ASSETS/IMAGES/LARGE/10.1177_09731296231215911-FIG2.JPG.