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Ethnopharmacology of Indigofera tinctoria (Linn.): From Traditional Use to Evidence-Based Medicine

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ABSTRACT

Indigofera tinctoria (Linn.), commonly known as true indigo, is a medicinal plant traditionally used in various ethnomedical systems, including Ayurveda, Traditional Chinese Medicine (TCM), and African traditional medicine. Beyond its historical role as a natural dye source, the plant has attracted increasing scientific interest due to its diverse pharmacological properties and bioactive constituents. This review aimed to systematically evaluate the ethnopharmacological uses, phytochemical composition, and pharmacological activities of *I. tinctoria*, while highlighting its potential for evidence-based therapeutic applications. A narrative review with a systematic literature search approach was conducted using electronic databases, including Scopus, PubMed, Web of Science, Google Scholar, and ScienceDirect. Relevant studies published between 2020 and 2026 were identified using keywords related to *Indigofera tinctoria*, ethnopharmacology, phytochemistry, and pharmacological activity. Study selection followed PRISMA guidelines, and a total of 45 eligible studies were included in the qualitative synthesis. The findings demonstrated that *I. tinctoria* has been traditionally utilized for the treatment of inflammatory disorders, skin diseases, infections, liver dysfunction, fever, and metabolic abnormalities. Phytochemical analyses revealed the presence of flavonoids, alkaloids, tannins, saponins, terpenoids, phenolic compounds, indigo, and indirubin, which contribute to its biological activities. Experimental studies reported significant anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, antidiabetic, and immunomodulatory effects. Among the identified compounds, indirubin showed notable pharmacological potential due to its anti-inflammatory and antiproliferative properties. However, the current evidence remains predominantly based on in vitro and animal studies, while clinical validation in humans is still limited. In conclusion, *Indigofera tinctoria* possesses promising ethnopharmacological and therapeutic potential supported by growing scientific evidence. Nevertheless, further studies focusing on standardized phytochemical characterization, mechanistic investigations, toxicological assessments, and randomized clinical trials are necessary to facilitate its translation into evidence-based phytotherapeutic applications.

Keywords: *Indigofera tinctoria, ethnopharmacology, phytochemistry, traditional medicine, pharmacological activity*

I. INTRODUCTION

Medicinal plants remain a cornerstone of global healthcare systems, particularly in low- and middle-income countries where access to conventional medical services is often limited

(X. Wang et al., 2025). According to the World Health Organization (WHO), approximately 70–80% of the population in developing regions depends on plant-based traditional medicine for primary healthcare (Dubale et al., 2025; Tuasha et al., 2023). Beyond their cultural and socioeconomic importance, medicinal plants represent a rich and largely untapped reservoir of structurally diverse bioactive compounds that continue to inspire modern drug discovery (Latif & Nawaz, 2025). However, the translation of traditional remedies into evidence-based therapeutics remains a significant scientific challenge (Thamizhoviya, 2025).

Ethnopharmacology has emerged as a critical interdisciplinary approach that systematically investigates traditional medicinal practices to identify pharmacologically active agents (Pirintsos et al., 2022). By integrating ethnobotanical knowledge with phytochemical and pharmacological analyses, this field has contributed to the discovery of numerous clinically important drug (Babalola et al., 2025). Nevertheless, contemporary ethnopharmacological research is often criticized for being overly descriptive, with insufficient emphasis on mechanistic validation, reproducibility, and clinical relevance. This highlights the urgent need for integrative reviews that not only summarize existing knowledge but also critically evaluate the strength of evidence and identify translational gaps (Patwardhan & Aswar, 2025).

Indigofera tinctoria (Linn.), a member of the Fabaceae family, exemplifies a medicinal plant with dual historical significance in both industrial and therapeutic contexts (Shivani, 2025). Traditionally recognized as a primary source of natural indigo dye, the plant has also been extensively utilized in various ethnomedical systems, including Ayurveda, Traditional Chinese Medicine (TCM), and African traditional medicine (Reza & Khouzani, 2022). In these systems, *I. tinctoria* is used to manage a wide range of conditions, such as inflammatory disorders, liver diseases, skin infections, and metabolic abnormalities (Thanuja et al., 2026). Despite this broad spectrum of applications, the pharmacological basis underlying these traditional uses remains only partially understood.

Phytochemical investigations have identified multiple classes of bioactive compounds in *I. tinctoria*, including flavonoids, alkaloids, saponins, tannins, and indole derivatives such as indigo and indirubin (Mishra DN, Gomare KS, 2020). Among these, indirubin has attracted particular attention due to its reported anti-inflammatory and anticancer activities, mediated through modulation of key signaling pathways such as cyclin-dependent kinases and inflammatory mediators (Li et al., 2025; J. Wang, 2024; Xie et al., 2026). While these findings suggest significant therapeutic potential, most evidence is derived from in vitro and preclinical studies, raising concerns regarding their clinical translatability.

A critical limitation in the current body of literature is the fragmentation of knowledge across ethnobotanical, phytochemical, and pharmacological domains. Many studies focus on isolated aspects of the plant without establishing a coherent link between traditional usage and mechanistic evidence (Aierken et al., 2025; Febriyanti et al., 2026). Furthermore, issues such as variability in phytochemical composition, lack of standardized extraction protocols, and insufficient toxicological and clinical data continue to hinder the development of *I. tinctoria*-based therapeutics. This disconnect underscores the necessity for a more integrative and critical synthesis of available evidence.

Therefore, this study is presented as a narrative review aimed at systematically compiling and critically evaluating the existing literature on the ethnopharmacology of Indigofera tinctoria. Unlike conventional descriptive reviews, this work seeks to bridge traditional knowledge with modern biomedical evidence by examining the relationships among ethnomedicinal uses, phytochemical constituents, and pharmacological activities. Although numerous studies have reported the traditional applications and bioactive compounds of I.

tinctoria, the available evidence remains fragmented. Specifically, there is still limited integration between traditional therapeutic claims, the identification of responsible bioactive metabolites, the underlying molecular and cellular mechanisms of action, and the clinical evidence supporting their efficacy and safety in humans. Most investigations have focused on in vitro and animal-based studies, while clinical validation and translational research remain scarce. Furthermore, the direct correlation between specific phytochemicals and their ethnomedicinal applications has not been comprehensively established. By addressing these gaps, this review aims to provide a more coherent understanding of how traditional uses of I. tinctoria can be scientifically substantiated and translated into evidence-based therapeutic applications. Additionally, this review identifies priority areas for future research, including mechanism-based pharmacological studies, standardization of plant extracts, toxicity assessments, and well-designed clinical trials to facilitate the development of scientifically validated therapeutic strategies.

II. METHODS

2.1 Study Design and Literature Search Strategy

This study was conducted as a narrative review employing a systematic literature search approach to comprehensively evaluate the ethnopharmacological uses, phytochemical constituents, and pharmacological activities of *Indigofera tinctoria* (Linn.). The review was designed to bridge traditional medicinal knowledge with evidence-based biomedical findings through a critical synthesis of the available scientific literature (Calderon Martinez et al., 2025). To ensure comprehensive coverage of relevant studies, a systematic search was performed using several electronic databases, including Scopus, PubMed, Web of Science, Google Scholar, and ScienceDirect. The literature search focused on articles published between 2020 and 2026.

A combination of keywords and Boolean operators was applied to optimize the search process and improve the relevance of retrieved studies. The primary search terms included “Indigofera tinctoria,” “ethnopharmacology,” “traditional medicine,” “phytochemistry,” “pharmacological activity,” “medicinal plant,” “bioactive compounds,” “antioxidant,” and “anti-inflammatory.” The search strategy was formulated using combinations such as: (“Indigofera tinctoria” AND ethnopharmacology) OR (“Indigofera tinctoria” AND pharmacological activity) OR (“Indigofera tinctoria” AND phytochemistry). In addition, the reference lists of selected articles were manually screened to identify potentially relevant studies that were not retrieved during the initial database search.

2.2 Eligibility Criteria and Study Selection

Studies were included in this review if they met the following criteria: (1) published between 2020 and 2026, (2) written in English, (3) specifically focused on *Indigofera tinctoria*, and (4) reported ethnopharmacological, phytochemical, or pharmacological data. Eligible studies included experimental investigations such as in vitro, in vivo, and in silico studies, as well as review articles and ethnobotanical surveys. Conversely, studies were excluded if they were duplicate publications, lacked sufficient methodological details, did not specifically investigate *Indigofera tinctoria*, or were unrelated to medicinal and pharmacological aspects.

Conference abstracts, editorials, and unpublished reports were also excluded from the review process.

All retrieved articles were exported into reference management software to facilitate organization and duplicate removal. Subsequently, titles and abstracts were screened for relevance, followed by full-text evaluation based on the predefined inclusion and exclusion criteria. The study selection procedure was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure transparency and methodological rigor (Figure 1) (Gusenbauer & Gauster, 2025; Page et al., 2021).

The study selection process was conducted according to the PRISMA guidelines (Page et al., 2021). A total of 296 records were identified through database and manual searches. After removing duplicates, 222 articles were screened based on titles and abstracts. Of these, 84 full-text articles were assessed for eligibility, and 45 studies met the inclusion criteria and were included in the qualitative synthesis.

2.3 Data Extraction and Synthesis

Relevant information from the selected studies was systematically extracted and compiled into a structured data extraction table. The extracted information included the authors and publication year, country or region of study, plant parts used, traditional medicinal applications, identified phytochemical constituents, experimental models employed, pharmacological activities observed, and the major findings and conclusions of each study.

The collected studies were analyzed qualitatively using a thematic synthesis approach. The findings were categorized into several major themes, including ethnopharmacological uses, phytochemical constituents, pharmacological activities, toxicological and safety evaluations, as well as research gaps and future perspectives. Furthermore, the evidence was critically interpreted to identify consistencies, limitations, and the translational potential of *Indigofera tinctoria* from traditional medicinal applications toward evidence-based therapeutic development.

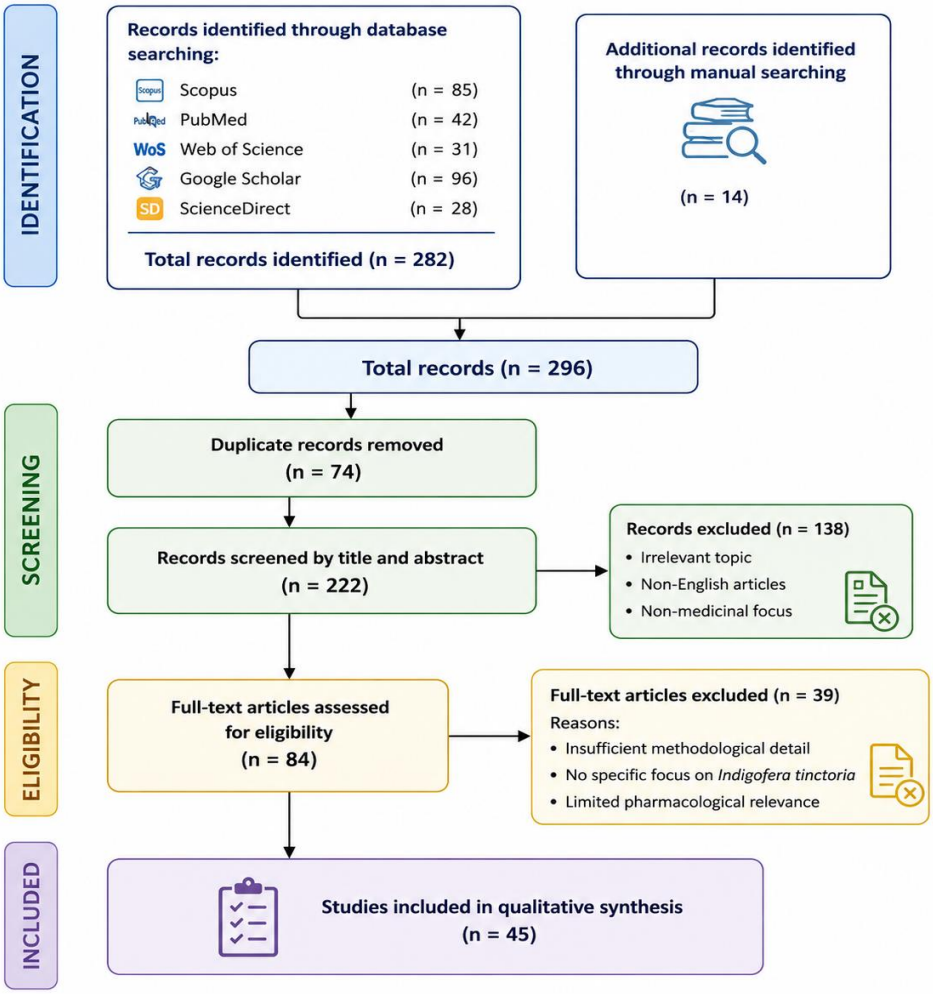

Figure 1. PRISMA flowchart of literature selection process

III. RESULTS AND DISCUSSION

3.1 Study Selection and Characteristics of Included Studies

The literature search process identified a total of 296 records from electronic databases and manual searching. After removing 74 duplicate records, 222 articles were screened based on titles and abstracts. Subsequently, 84 full-text articles were assessed for eligibility, of which 45 studies met the inclusion criteria and were included in the qualitative synthesis (Figure 1). The included studies comprised ethnobotanical surveys, phytochemical investigations, and pharmacological evaluations conducted using in vitro, in vivo, and in silico approaches.

Most of the studies included in this review originated from Asian countries, particularly India and China, which reflects the extensive historical and cultural significance of *Indigofera tinctoria* in traditional medicinal systems such as Ayurveda and Traditional Chinese Medicine (TCM) (Manjusha et al., 2026). *Indigofera tinctoria* has long been utilized for the treatment of skin diseases, inflammatory disorders, liver dysfunction, epilepsy, and metabolic abnormalities due to its perceived detoxifying and anti-inflammatory properties. Similarly, in Traditional Chinese Medicine, indigo-derived preparations, commonly known as Indigo Naturalis or Qing

Dai, have been widely used in the management of psoriasis, ulcerative lesions, fever, and leukemia-associated conditions. The persistent use of this plant across generations indicates a strong ethnomedicinal foundation that has stimulated increasing pharmacological interest in recent decades (Thanuja et al., 2026).

In addition to Asian countries, several studies from African regions were identified, highlighting the role of *I. tinctoria* in indigenous healthcare systems. In African traditional medicine, different parts of the plant are employed for wound healing, pain management, antimicrobial therapy, and the treatment of gastrointestinal and inflammatory disorders. The broad geographical distribution and cross-cultural utilization of *I. tinctoria* suggest that the plant possesses versatile therapeutic properties that have been recognized independently by various traditional communities. This widespread ethnopharmacological relevance further supports the importance of scientifically validating its traditional applications through phytochemical and pharmacological investigations (Elmahaishi et al., 2025).

3.2 Ethnopharmacological Uses of *Indigofera tinctoria*

The reviewed studies demonstrated that *Indigofera tinctoria* has been extensively utilized in traditional medicine systems for the management of a wide range of ailments, reflecting its long-standing ethnopharmacological relevance across different cultures. In Ayurveda, the plant is traditionally recognized for its detoxifying, anti-inflammatory, and rejuvenating properties, and it has been widely used in the treatment of skin disorders, fever, liver dysfunction, epilepsy, and inflammatory conditions. Various plant parts, particularly the leaves and roots, are commonly processed into decoctions, powders, pastes, or herbal formulations for both topical and oral administration. Traditional Ayurvedic preparations frequently employ leaf pastes for treating wounds, ulcers, and skin infections, while root extracts are used for hepatic disorders and inflammatory diseases (Hosain et al., 2024; Muda et al., 2021).

In Traditional Chinese Medicine (TCM), indigo-containing preparations derived from *I. tinctoria*, commonly referred to as Indigo Naturalis or Qing Dai, have historically been used to manage psoriasis, ulcerative lesions, inflammatory conditions, and leukemia-related disorders. Bioactive compounds such as indirubin and indigo are considered the principal therapeutic agents responsible for these medicinal effects. Recent pharmacological investigations have further demonstrated that indirubin possesses anti-inflammatory and antiproliferative activities through modulation of inflammatory mediators and cell-signaling pathways, supporting several of its traditional applications in TCM (Muda et al., 2021; Zourob & Lio, 2026).

Similarly, African traditional medicine employs different parts of *I. tinctoria* for wound healing, pain relief, diarrhea, microbial infections, and fever management. Ethnobotanical surveys conducted in several African regions reported the use of leaf and root preparations as antimicrobial and anti-inflammatory remedies, particularly in rural communities where medicinal plants remain an essential component of primary healthcare systems. The plant is also traditionally applied to treat gastrointestinal disorders and skin-related infections due to its perceived antiseptic and healing properties (Y. Wang et al., 2021).

The widespread ethnomedicinal use of *I. tinctoria* across geographically and culturally distinct regions suggests the presence of diverse bioactive compounds with broad therapeutic potential. This cross-cultural consistency in medicinal application provides an important ethnopharmacological basis for scientific investigation. Indeed, phytochemical studies have

identified multiple secondary metabolites, including flavonoids, alkaloids, tannins, saponins, indigo, and indirubin, which are associated with antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective activities (L et al., 2025; Thanuja et al., 2026). However, despite the growing body of experimental evidence, many traditional claims remain insufficiently validated through rigorous clinical studies. Most available studies are limited to in vitro assays or animal models, with relatively few well-designed human trials available to confirm efficacy and safety. Furthermore, inconsistencies in extraction methods, phytochemical standardization, and dosage formulations continue to limit the reproducibility and translational applicability of current findings. These limitations highlight the urgent need for integrative and translational research approaches that combine ethnopharmacological knowledge with modern biomedical methodologies to support the development of evidence-based therapeutic applications derived from *I. tinctoria* (Matsuno et al., 2022).

3.3 Phytochemical Constituents

Phytochemical analyses revealed that *Indigofera tinctoria* contains a wide diversity of secondary metabolites that contribute to its pharmacological potential. Various studies have identified the presence of flavonoids, alkaloids, tannins, saponins, terpenoids, glycosides, phenolic compounds, and indole derivatives such as indigo and indirubin. These bioactive constituents are believed to play important roles in the therapeutic properties traditionally attributed to the plant. Among these compounds, indirubin has gained considerable scientific interest due to its notable anti-inflammatory, antiproliferative, and anticancer activities. Previous investigations demonstrated that indirubin can regulate cell-cycle progression and inhibit inflammatory signaling pathways, including cyclin-dependent kinases (CDKs) and nuclear factor-kappa B (NF-κB), thereby supporting its therapeutic relevance in inflammatory and neoplastic disorders (Sun et al., 2021).

Flavonoids identified in *I. tinctoria* are strongly associated with antioxidant activity through their ability to scavenge free radicals and reduce oxidative stress. These compounds function by donating hydrogen atoms or electrons to neutralize reactive oxygen species (ROS), thereby protecting cellular components from oxidative damage. Antioxidant activity is particularly important because oxidative stress has been implicated in the pathogenesis of numerous chronic diseases, including diabetes, inflammation, cardiovascular disorders, and cancer (Roy et al., 2022; Zahra et al., 2024). In addition to flavonoids, tannins and saponins present in the plant contribute significantly to antimicrobial and anti-inflammatory activities by disrupting microbial cell membranes and modulating inflammatory mediators. Terpenoids and phenolic compounds have also been reported to exhibit antimicrobial and hepatoprotective properties, further broadening the pharmacological spectrum of the plant (Asaduzzaman et al., 2026; Parham ve ark., 2020; Sarkar et al., 2025).

The occurrence of indigoid compounds such as indigo and indirubin further enhances the medicinal significance of I. tinctoria beyond its traditional industrial application as a natural dye source. Historically, indigo was primarily valued for textile production; however, modern pharmacological studies have revealed that these compounds possess important biological activities, including immunomodulatory, antioxidant, and anticancer effects. This transition from industrial utility to biomedical relevance demonstrates the growing scientific interest in the therapeutic potential of indigo-producing plants (Qi-yue et al., 2020; Rao et al., 2025; Sarkar et al., 2025).

Despite the promising phytochemical profile of *I. tinctoria*, several studies have reported considerable variability in its chemical composition depending on geographical origin, climatic conditions, soil characteristics, harvesting period, and extraction techniques (Hosain et al., 2024). Environmental stressors and cultivation practices may significantly influence the biosynthesis of secondary metabolites, leading to differences in the concentration and activity of bioactive compounds among plant samples. Furthermore, variations in solvent polarity and extraction procedures can affect the qualitative and quantitative composition of phytochemical extracts, thereby complicating direct comparisons between studies (Kimta et al., 2024; Qi-yue et al., 2020).

This phytochemical variability represents a major challenge for the standardization of herbal formulations and may compromise the reproducibility and reliability of pharmacological investigations. The lack of standardized extraction protocols and quality control measures remains one of the primary obstacles in translating *I. tinctoria* from traditional medicine into evidence-based therapeutic products. Therefore, future studies should prioritize phytochemical standardization, advanced analytical characterization, and quality assurance to ensure consistency, safety, and efficacy in medicinal applications derived from *I. tinctoria* (Bhosale et al., 2024).

3.4 Pharmacological Activities

3.4.1 Anti-inflammatory Activity

Anti-inflammatory activity represents one of the most extensively investigated pharmacological properties of *Indigofera tinctoria*, largely due to its long-standing traditional use in the treatment of inflammatory disorders and skin-related diseases. Numerous experimental studies have demonstrated that extracts of *I. tinctoria* possess significant anti-inflammatory effects through the modulation of key inflammatory mediators and signaling pathways. Specifically, plant extracts have been reported to inhibit the production and expression of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which are critically involved in the initiation and progression of inflammatory responses (Sun et al., 2021). In addition, several studies showed that *I. tinctoria* extracts suppress cyclooxygenase (COX) activity and downregulate the nuclear factor-kappa B (NF-κB) signaling pathway, a central regulator of inflammation and immune responses (Lotts et al., 2020).

The inhibition of NF-κB activation is particularly significant because this transcription factor regulates the expression of multiple inflammatory genes, including cytokines, chemokines, and enzymes associated with chronic inflammatory diseases. By suppressing these pathways, *I. tinctoria* may reduce inflammatory tissue damage and oxidative stress, thereby supporting its traditional application in managing inflammatory skin conditions, wounds, ulcers, and rheumatic disorders. Experimental evidence from in vitro and in vivo studies further demonstrated reductions in edema formation, inflammatory cell infiltration, and oxidative biomarkers following administration of I. tinctoria extracts (Hosain et al., 2024).

Among the bioactive constituents identified in *I. tinctoria*, indirubin has received considerable scientific attention as one of the principal compounds responsible for its anti-inflammatory activity. Indirubin has been shown to modulate several intracellular signaling pathways involved in inflammation and immune regulation, including inhibition of cyclin-dependent kinases (CDKs), glycogen synthase kinase-3β (GSK-3β), and NF-κB-mediated transcriptional activity (Qi-yue et al., 2020). These mechanisms contribute to the suppression

of excessive immune responses and inflammatory mediator production. Furthermore, indirubin derivatives have demonstrated immunomodulatory effects that may be beneficial in autoimmune and chronic inflammatory disorders, including psoriasis and ulcerative conditions traditionally treated with indigo-containing preparations in Traditional Chinese Medicine (Sun et al., 2021).

The anti-inflammatory properties of *I. tinctoria* are also closely associated with its antioxidant capacity. Oxidative stress is recognized as a major contributor to chronic inflammation through the generation of reactive oxygen species (ROS), which activate inflammatory signaling cascades. Flavonoids and phenolic compounds present in I. tinctoria may exert synergistic effects by scavenging free radicals and reducing oxidative damage, thereby indirectly attenuating inflammatory responses (Duraisamy et al., 2023). This dual antioxidant and anti-inflammatory mechanism may explain the broad traditional application of the plant in inflammatory and dermatological disorders.

Despite the promising pharmacological evidence, most current findings are derived from preclinical investigations involving cell cultures and animal models. Clinical validation of the anti-inflammatory efficacy and safety of *I. tinctoria* in humans remains limited. Moreover, differences in extraction methods, phytochemical composition, and experimental protocols among studies present challenges for reproducibility and standardization. Therefore, further research involving standardized extracts, mechanistic studies, and well-designed clinical trials is necessary to establish the therapeutic potential of *I. tinctoria* as an evidence-based anti-inflammatory agent..

3.4.2 Antioxidant Activity

Numerous in vitro studies have demonstrated that *Indigofera tinctoria* possesses significant antioxidant activity, primarily through its ability to scavenge free radicals and reduce oxidative stress. Antioxidant evaluations using commonly employed assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′ -azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) revealed strong radical scavenging capacities of various *I. tinctoria* extracts (Duraisamy et al., 2023). These findings indicate that the plant contains bioactive constituents capable of donating electrons or hydrogen atoms to stabilize reactive free radicals, thereby preventing oxidative cellular damage. In several studies, methanolic and ethanolic extracts exhibited higher antioxidant activities compared to aqueous extracts, suggesting that solvent polarity significantly influences the extraction efficiency of antioxidant compounds (Hosain et al., 2024)

The antioxidant potential of *I. tinctoria* is primarily attributed to the presence of flavonoids, phenolic compounds, and other secondary metabolites that act as natural antioxidants. Flavonoids are known to neutralize reactive oxygen species (ROS) by inhibiting free radical formation, chelating metal ions, and enhancing endogenous antioxidant defense systems. Phenolic compounds also contribute substantially to antioxidant activity due to their redox properties, which enable them to function as reducing agents and hydrogen donors. These mechanisms are particularly important because excessive ROS production is closely associated with oxidative stress, a major pathological factor involved in chronic inflammation, aging, cardiovascular diseases, diabetes mellitus, neurodegenerative disorders, and hepatic injury (Hassanpour & Doroudi, 2023).

The antioxidant effects of *I. tinctoria* may therefore contribute significantly to its therapeutic potential in preventing and managing oxidative stress-related disorders.

Experimental studies have suggested that antioxidant activity may partially explain the hepatoprotective, anti-inflammatory, and antidiabetic effects traditionally associated with the plant. By reducing oxidative damage to lipids, proteins, and DNA, the plant extracts may help preserve cellular integrity and regulate inflammatory pathways (Sun et al., 2021). In liver injury models, antioxidant compounds present in *I. tinctoria* were reported to decrease lipid peroxidation and improve antioxidant enzyme activity, thereby protecting hepatic tissues from toxic insults (Hosain et al., 2024).

Despite these promising findings, direct comparisons among antioxidant studies remain challenging due to methodological inconsistencies. Variations in extraction solvents, plant parts used, extraction techniques, concentrations, and assay protocols may substantially influence the measured antioxidant activity. For instance, differences in solvent polarity can alter the phytochemical profile of extracts, resulting in variability in flavonoid and phenolic content. Furthermore, antioxidant assays such as DPPH, ABTS, FRAP, and reducing power assays evaluate different mechanisms of antioxidant action, making standardization difficult. These inconsistencies limit the reproducibility and comparability of results across studies and highlight the need for standardized methodologies in future antioxidant research involving *I. tinctoria* (Chodok et al., 2025; Shahidi & Samarasinghe, 2025).

Overall, the available evidence supports the view that *I. tinctoria* is a promising natural source of antioxidant compounds with potential applications in the prevention of oxidative stress-mediated diseases. However, further investigations involving standardized extraction methods, advanced phytochemical characterization, and clinical validation are necessary to establish its efficacy and therapeutic relevance in evidence-based medicine.

3.4.3 Antimicrobial Activity

The antimicrobial activity of *I. tinctoria* is believed to be associated with the synergistic action of multiple phytochemical constituents, particularly alkaloids, tannins, flavonoids, saponins, and phenolic compounds. Alkaloids may interfere with microbial DNA replication and protein synthesis, while tannins are known to exert antimicrobial effects by precipitating microbial proteins and disrupting cell membrane integrity. Phenolic compounds and flavonoids further contribute to antimicrobial activity through oxidative damage induction, inhibition of microbial enzymes, and disruption of membrane permeability. In addition, saponins may enhance antimicrobial efficacy by increasing membrane permeability and facilitating the penetration of other bioactive compounds into microbial cells (Hosain et al., 2024)

Several studies also suggested that methanolic and ethanolic extracts of *I. tinctoria* demonstrate stronger antimicrobial activity compared to aqueous extracts, likely due to the higher solubility of phenolic and flavonoid compounds in organic solvents. This observation indicates that extraction methods play an important role in determining the antimicrobial efficacy of the plant. Furthermore, some investigations reported dose-dependent inhibitory effects, where increasing extract concentrations resulted in larger zones of microbial inhibition and reduced microbial growth. (Speranza et al., 2020)

The antifungal properties of *I. tinctoria* are particularly relevant in the context of increasing resistance to conventional antifungal agents. Bioactive compounds present in the plant may inhibit fungal growth by altering membrane structure, suppressing ergosterol biosynthesis, and interfering with fungal metabolic pathways (Qi-yue et al., 2020). These mechanisms suggest the potential application of *I. tinctoria* as a natural antimicrobial agent for pharmaceutical and therapeutic purposes.

Despite these promising findings, several limitations remain within the current body of antimicrobial research involving *I. tinctoria*. Many studies utilized crude extracts with limited phytochemical characterization, making it difficult to determine which specific compounds are primarily responsible for the observed antimicrobial effects. In addition, variations in extraction procedures, microbial strains, assay methods, and concentrations complicate direct comparisons between studies and reduce reproducibility. Most available investigations are also limited to in vitro assays, while in vivo and clinical studies remain scarce (De Rossi et al., 2025).

Therefore, the identification and isolation of specific active compounds, along with detailed investigations into their molecular mechanisms of action, remain important priorities for future research. Advanced analytical techniques such as high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC-MS), and metabolomic profiling may help clarify the bioactive constituents responsible for antimicrobial activity. Moreover, further studies focusing on synergistic interactions, toxicity evaluations, and clinical efficacy are necessary to support the development of *I. tinctoria*-based antimicrobial therapies within evidence-based medicine (De Rossi et al., 2025).

3.5 Bridging Traditional Knowledge and Modern Biomedical Evidence

One of the major findings of this review is the increasing convergence between traditional medicinal knowledge and modern pharmacological evidence regarding the therapeutic potential of *Indigofera tinctoria* (Table 1). Across various traditional medical systems, including Ayurveda, Traditional Chinese Medicine (TCM), and African ethnomedicine, *I. tinctoria* has historically been used to manage inflammatory disorders, skin diseases, wounds, infections, fever, and metabolic abnormalities. Interestingly, many of these ethnomedicinal claims are now being supported by contemporary pharmacological studies demonstrating anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, and immunomodulatory activities of the plant and its bioactive constituents (Sun et al., 2021). This consistency between traditional usage and experimental findings strengthens the ethnopharmacological relevance of *I. tinctoria* and highlights its potential as a promising source of therapeutic agents.

Particularly, preclinical investigations have demonstrated that extracts and isolated compounds from *I. tinctoria* can modulate inflammatory mediators such as TNF-α, IL-6, COX, and NF-κB pathways, supporting its traditional application in inflammatory and dermatological conditions. Likewise, antioxidant and antimicrobial activities observed in laboratory studies provide scientific support for its use in wound healing and infection management (Hosain et al., 2024). The bioactive compound indirubin, widely recognized in TCM-based preparations, has also shown significant antiproliferative and immunoregulatory properties, further validating traditional medicinal practices associated with indigo-producing plants (Qi-yue et al., 2020).

Despite these encouraging findings, the translation of ethnopharmacological knowledge into evidence-based therapeutic applications remains incomplete and faces several scientific and methodological challenges. Current research on *I. tinctoria* is still heavily dominated by in vitro assays and animal experiments, whereas well-designed clinical investigations in humans remain extremely limited. Although preclinical studies provide important mechanistic insights, their findings cannot always be directly extrapolated to clinical efficacy and safety in humans due to differences in metabolism, dosage, and pharmacokinetics.

Consequently, the absence of robust clinical evidence remains a major obstacle to the integration of *I. tinctoria*-based therapies into modern healthcare systems (N et al., 2020).

Another important limitation concerns the lack of standardized extraction procedures and quality control protocols. Significant variability in phytochemical composition has been reported depending on geographical origin, environmental conditions, harvesting periods, and extraction solvents. Such inconsistencies may substantially affect pharmacological outcomes and reproducibility across studies. In many cases, crude extracts are used without adequate phytochemical standardization, making it difficult to determine the specific compounds responsible for therapeutic activity. This issue not only limits scientific reproducibility but also complicates the development of safe, effective, and commercially viable herbal formulations.

To address these limitations, future research should adopt more integrative and multidisciplinary approaches that combine ethnobotanical knowledge with advanced biomedical technologies. The integration of ethnopharmacological data with modern omics approaches, including metabolomics, proteomics, transcriptomics, and genomics, may provide deeper insights into the molecular mechanisms underlying the therapeutic effects of *I. tinctoria* (Sun et al., 2021). Furthermore, molecular pharmacology and systems biology approaches could help elucidate complex interactions between phytochemicals and biological pathways, thereby improving the understanding of synergistic effects among plant-derived compounds.

Such integrative strategies may also facilitate the discovery of novel lead compounds for drug development and enhance the scientific validation of traditional medicine. Advanced analytical techniques such as liquid chromatography–mass spectrometry (LC-MS), high-performance liquid chromatography (HPLC) (N et al., 2020), and network pharmacology could support the identification of bioactive compounds and their molecular targets. Ultimately, bridging traditional medicinal knowledge with modern evidence-based research may contribute to the development of standardized phytopharmaceutical products derived from *Indigofera tinctoria*, while also preserving the cultural and medicinal value of traditional healing practices.

Table 1. Correlation Between Traditional Uses, Bioactive Constituents, Pharmacological Activities, and Levels of Evidence of *Indigofera tinctoria*

Traditional Use	Plant Part	Major Bioactive Compounds	Pharmacological Activity	Experimental Model	Level of Evidence	Key References
Skin diseases, psoriasis, wound healing	Leaves, Indigo Naturalis	Indirubin, Indigo, Flavonoids	Anti-inflammatory, wound healing, immunomodulatory	In vitro, animal models, limited clinical observations	Moderate	Muda et al. (2021); Sun et al. (2021); Matsuno et al. (2022)
Inflammatory disorders and rheumatism	Leaves, Roots	Indirubin, Flavonoids, Phenolics	NF-κB, TNF-α, IL-6 inhibition; anti-inflammatory activity	In vitro and in vivo studies	Moderate	Sun et al. (2021); Hosain et al. (2024); Lotts et al. (2020)
Fever and infectious diseases	Leaves	Alkaloids, Tannins, Saponins, Phenolics	Antimicrobial and anti-inflammatory effects	Primarily in vitro studies	Low–Moderate	Y. Wang et al. (2021); Hosain et al. (2024);

						Speranza et al. (2020)
Gastrointestinal disorders and diarrhea	Leaves, Roots	Tannins, Flavonoids, Saponins	Antimicrobial and anti-inflammatory activities	In vitro and animal studies	Low	Y. Wang et al. (2021); Hosain et al. (2024)
Liver disorders	Roots, Leaves	Flavonoids, Phenolic compounds	Hepatoprotective and antioxidant activities	Animal studies	Low–Moderate	Hosain et al. (2024); Hassanpour & Doroudi (2023)
Metabolic abnormalities	Whole plant extracts	Flavonoids, Terpenoids	Antioxidant and metabolic regulation	Experimental animal models	Low	Roy et al. (2022); Hassanpour & Doroudi (2023)
Leukemia-related conditions (TCM)	Indigo-containing preparations	Indirubin	Antiproliferative activity, cell-cycle regulation, CDK inhibition	In vitro, animal studies, limited clinical observations	Moderate	Muda et al. (2021); Sun et al. (2021); Qi-yue et al. (2020)
General detoxification and health maintenance	Various plant parts	Flavonoids, Phenolics, Alkaloids	Antioxidant activity	In vitro antioxidant assays (DPPH, ABTS, FRAP)	Low	Duraisamy et al. (2023); Roy et al. (2022); Zahra et al. (2024)

3.6 Research Gaps and Future Perspectives

This review identified several critical research gaps in the current literature concerning the ethnopharmacological and biomedical potential of *Indigofera tinctoria*. Although numerous studies have reported promising pharmacological activities, the majority of available evidence remains preliminary and fragmented. One of the most significant limitations is the lack of well-designed clinical trials evaluating the efficacy, safety, and therapeutic applicability of *I. tinctoria* in humans. Most current findings are derived from in vitro experiments and animal models, which, although valuable for mechanistic exploration, cannot fully predict clinical outcomes in human populations. Consequently, the clinical relevance of many reported biological activities, including anti-inflammatory, antioxidant, antimicrobial, and hepatoprotective effects, remains insufficiently validated.

Another major challenge involves the inadequate standardization of extraction procedures and phytochemical profiling. Variability in geographical origin, cultivation conditions, harvesting periods, and extraction solvents can substantially influence the phytochemical composition of *I. tinctoria* extracts, leading to inconsistencies in pharmacological results across studies. In many investigations, crude extracts are used without detailed characterization of their active constituents, thereby limiting reproducibility and

scientific comparability. The absence of standardized quality control measures also presents significant obstacles for the development of reliable phytopharmaceutical products and regulatory approval processes.

Furthermore, mechanistic investigations at the molecular level remain limited for many of the pharmacological activities attributed to *I. tinctoria*. While some studies have identified the involvement of signaling pathways such as NF-κB, COX, and oxidative stress-related mechanisms, comprehensive molecular evidence explaining the interactions between bioactive compounds and cellular targets is still lacking. This gap restricts the understanding of the precise therapeutic mechanisms of the plant and hinders the identification of specific lead compounds with clinical potential.

To overcome these limitations, future research should prioritize several important areas. First, standardized phytochemical characterization using advanced analytical techniques such as high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC-MS), and liquid chromatography–mass spectrometry (LC-MS) is essential to ensure consistency, quality, and reproducibility of herbal preparations. Such approaches would facilitate the identification and quantification of bioactive compounds responsible for pharmacological effects.

Second, mechanistic studies employing molecular and systems biology approaches are needed to elucidate the biochemical pathways and molecular targets associated with the therapeutic properties of *I. tinctoria*. The application of metabolomics, transcriptomics, proteomics, and network pharmacology could provide deeper insights into synergistic interactions among phytochemicals and their pharmacodynamic actions.

Third, clinical validation through randomized controlled trials (RCTs) is urgently required to establish the efficacy and safety of *I. tinctoria* in humans. Well-designed clinical studies involving standardized extracts, appropriate dosing regimens, and long-term follow-up would significantly strengthen the evidence base supporting its medicinal applications. Such investigations are particularly important for conditions in which traditional claims and preclinical evidence demonstrate strong consistency, such as inflammatory disorders and skin diseases.

In addition, comprehensive toxicological and pharmacokinetic evaluations remain essential before therapeutic applications can be recommended. Current toxicological evidence is limited primarily to acute toxicity studies, while long-term toxicity, genotoxicity, reproductive toxicity, and herb–drug interactions remain poorly understood. Pharmacokinetic investigations focusing on absorption, distribution, metabolism, and excretion (ADME) profiles of bioactive compounds such as indirubin are also necessary to support dosage optimization and safety assessments.

Finally, future efforts should focus on the development of evidence-based herbal formulations derived from *I. tinctoria*. Integrating traditional medicinal knowledge with modern pharmaceutical technologies may facilitate the formulation of standardized, safe, and clinically effective herbal products. Such translational approaches could improve the acceptance of traditional medicinal plants within modern healthcare systems while preserving their ethnopharmacological value.

Overall, addressing these research gaps will be crucial for translating the traditional use of *Indigofera tinctoria* into scientifically validated therapeutic applications. A multidisciplinary research framework integrating ethnopharmacology, phytochemistry, molecular pharmacology, toxicology, and clinical science will be essential for unlocking the

full medicinal potential of this plant and supporting its future development as an evidence-based phytotherapeutic agent.

IV. CONCLUSION

Indigofera tinctoria (Linn.) represents a medicinal plant with substantial ethnopharmacological significance and considerable therapeutic potential. This review demonstrated that the plant has been extensively utilized in traditional medicinal systems, including Ayurveda, Traditional Chinese Medicine (TCM), and African ethnomedicine, for the treatment of inflammatory disorders, skin diseases, infections, liver dysfunction, and metabolic abnormalities. The widespread traditional use of *I. tinctoria* across diverse cultural regions highlights its long-standing medicinal relevance and supports its importance as a valuable source of natural therapeutic agents.

Phytochemical investigations revealed that *I. tinctoria* contains diverse bioactive compounds, including flavonoids, alkaloids, tannins, saponins, terpenoids, phenolic compounds, indigo, and indirubin, which collectively contribute to its pharmacological activities. Experimental studies have demonstrated significant anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, antidiabetic, and immunomodulatory effects, thereby providing scientific support for several traditional medicinal claims. Among these compounds, indirubin has emerged as a particularly promising bioactive constituent due to its anti-inflammatory and antiproliferative properties.

Despite these encouraging findings, the current body of evidence remains largely dependent on in vitro and animal studies, while robust clinical validation in humans is still limited. In addition, variability in phytochemical composition, lack of standardized extraction methods, and insufficient toxicological and pharmacokinetic evaluations continue to hinder the development of reproducible and evidence-based herbal formulations derived from *I. tinctoria*. These limitations underscore the need for more rigorous and integrative research approaches.

Future investigations should prioritize standardized phytochemical characterization, molecular mechanistic studies, comprehensive toxicological assessments, and well-designed randomized clinical trials to establish the safety and efficacy of *I. tinctoria* for therapeutic applications. Furthermore, integrating ethnopharmacological knowledge with modern omics technologies, systems biology, and pharmaceutical sciences may facilitate the discovery of novel lead compounds and improve the translational potential of this medicinal plant.

Overall, this review highlights the importance of bridging traditional medicinal knowledge with modern scientific evidence to support the development of *Indigofera tinctoria* as a scientifically validated phytotherapeutic agent. Continued multidisciplinary research will be essential for unlocking its full medicinal potential and promoting its integration into evidence-based healthcare systems..

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