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A Comprehensive Comparative Study on the Chemical Constituents and Pharmacological Activities of Regional Vitex negundo Linn

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ABSTRACT

Vitex negundo Linn. is a deciduous shrub with significant clinical and economic value across global tropical and subtropical regions. This comprehensive review addresses the active chemical architecture of V. negundo , analyzing over 120 bioactive compounds categorized mainly into flavonoids, iridoids, lignans, and terpenoids. Recent advanced phytochemical analyzes reveal distinct regional heterogeneities in chemotypes among samples from India, China, the Philippines, Nigeria, and Nepal, heavily influenced by environmental factors, extraction techniques, and soil composition. For instance, Indian studies primarily focus on iridoids (eg, negundoside) for anti-inflammatory standardization, whereas Chinese research targets V. negundo var. cannabifolia for respiratory conditions. The Philippines has successfully translated these ethnopharmacological insights into commercialized respiratory treatments and is currently pioneering anti-SARS-CoV-2 investigations. The multi-target mechanisms of V. negundo manifest through the modulation of pathways such as NF-κB, AMPK/PI3K/Akt, and TGF-β, conferring potent anti-inflammatory, antioxidant, antimicrobial, and antitumor effects. The study concludes that standardizing regional chemical variations using multi-marker quality control systems, alongside clinical pharmacokinetic studies, is vital for the future development of V. negundo as a standardized global botanical drug.

Keywords

Chemotypes, Flavonoids, Iridoids, Pharmacognosy, *Vitex negundo.*

Introduction

A Global Overview of Vitex negundo in Modern Pharmacology and Traditional Medicine Vitex negundo (*L. negundo* Linn.), botanically classified as belonging to the Lamiaceae family (formerly Verbenaceae), is a deciduous shrub or small tree of extremely high economic and medicinal value in tropical and subtropical regions worldwide [1,2]. Its height typically ranges from 2 to 8 meters, with reddish-brown, coarse bark [2,3]. The leaves of Vitex negundo are characteristically palmate five-lobed compound leaves with serrated edges and densely covered with white downy hairs on the lower surface [3-5]. Throughout thousands of years, Vitex negundo has been a core element of the medical systems of many countries in Asia, Africa, and South America. In Ayurveda, it is given the name "Nirgundi", meaning "protection of the body from disease"; in Traditional Chinese

Medicine (TCM), it is widely used in the treatment of rheumatic pain, respiratory infections, and gastrointestinal diseases [1,6-8].

With the rapid development of modern phytochemical detection technology, the global scientific research community's focus on Vitex negundo has shifted from simple ethnopharmacological descriptions to molecular-level chemical characterization analysis and cross-regional comparative studies. Currently, over 120 bioactive compounds have been isolated and identified from different parts of this plant, endowing Vitex negundo with a wide range of pharmacological functions, including anti-inflammatory, antioxidant, analgesic, antipyretic, antibacterial, antitumor, and antiviral effects [9]. However, due to differences in growing environment, soil composition, climate fluctuations, and extraction techniques, Vitex negundo from China, India, the Philippines, Malaysia, and Africa exhibits significant heterogeneity in chemotypes and the yield of active ingredients [10-12]. This study aims to comprehensively analyze nearly one hundred authoritative academic reports, systematically outline the bioactive chemical framework of Vitex negundo, and deeply explore the similarities and differences in research directions, chemical marker screening, and clinical applications among various countries, providing a scientific basis for the future development of standardized herbal medicines.

A comprehensive overview of the chemical taxonomy and active ingredients of Vitex negundo

The pharmacological basis of Vitex negundo stems from its complex matrix of secondary metabolites. Using analytical techniques such as high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR), researchers have classified its active ingredients into four main categories: flavonoids, iridoids, lignans, and terpenoids [9,13].

Molecular Characteristics and Distribution of Flavonoids

Flavonoids are the most representative polyphenolic components in Vitex negundo, mainly found in leaves and seeds. Their chemical structures are mostly based on benzopyran rings, exhibiting diverse biological activities through phenolic group substitutions at different positions [3,14]. Detailed distribution and pharmacological functions are shown at the end of the article (Table 1). The content of flavonoid components directly determines the antioxidant efficacy of Vitex negundo extracts. For example, a comparative study showed that the total flavonoid content of Vitex negundo (63.11 mg QE/g) was significantly higher than that of its close relative, *Vitex trifolia* , which was highly correlated with its lower IC50 value (70.20 μg/mL) in the DPPH free radical scavenging experiment [15,16].

Iridoids

Key Quality Control Markers Iridoids are important indicators of Vitex negundo in chemical taxonomy, mainly monoterpenoid derivatives with cyclopentane ring structures [3]. In the pharmaceutical industry, negundoside and agnuside have been established as core chemical markers for evaluating product quality [11,22]. Negundoside has significant anti-inflammatory and anticonvulsant effects, while agnuside shows unique efficacy in regulating the reproductive endocrine system (e.g., reducing serum prolactin levels in hyperprolactinemia) [3,13,19]. Indian researchers used qNMR technology to quantitatively analyze Vitex negundo collected from different geographical regions and found that the yield of these compounds was drastically affected by the seasonal harvesting time, highlighting the necessity of harvesting standardization [11].

Volatile oils is the most prominent phenomenon in studies from various countries. For example, in samples found in Nigeria, the content of β-pinene can be as high as 42.04% in some areas, while it may be only trace amounts in other areas. This chemotype inconsistency is mainly attributed to the total nitrogen content in the soil and the ambient temperature [10] (Table 2).

A comparative analysis of regional studies on Vitex negundo in various countries reveals that research on Vitex negundo globally exhibits clear regional characteristics, which is not only related to the distribution of local plant resources but also deeply driven by its traditional medical background.

Table 1: Molecular characteristics and distribution of flavonoid compounds in *Vitex negundo*.

Ingredient name	Main locations	Pharmacological functions	References
Vitexin	Leaves, roots, bark	Inhibits leukocyte migration, anti-inflammatory, and cardioprotective effects.	[3,17]
Luteolin	blade	Powerful antioxidant, induces apoptosis, and has anti-cancer properties.	[3,14,18]
Vitexin caffeate	stem bark	Anti-inflammatory and antifungal	[9,19]
Isoorientin	Leaves, seeds	Free radical scavenging and anti-diabetic	[15,20,21]
Quercetin	seed	Antibacterial, inhibits gyrase B.	(twenty one)
Casticin (Vitex trifolia flavonoids)	Leaves, seeds	Regulates menstruation, reduces inflammation, and inhibits prostaglandins.	[2,15,19]

Table 2: Key volatile and terpenoid constituents and their pharmacological characteristics.

Volatile components	Main proportions	Pharmacological properties	References
α-pinene	High (especially in Nigerian/Indian samples)	Antibacterial, bronchiectasis	[10,23]
Sabinene	Significant fluctuations (11%-12%)	Anti-inflammatory and antioxidant	[2,10,24]
β-caryophyllene	Significant fluctuation (around 5%)	Analgesia, antimicrobial	[2,19,24]
Globulol (a type of terpineol)	High (up to 30%)	Antibacterial and antiseptic	[2,19,23]
Linalool	medium	Sedative and anti-anxiety	[10,24,25]

- **India: The Intersection of Ayurvedic Tradition and Modern Bioanalysis.** As a leader in Vitex negundo research, India's work mainly revolves around verifying the mechanism of "Nirgundi" in anti-inflammatory and analgesic effects. Indian scholars Basu and Singh conducted the earliest chemical investigation in 1947, pioneering global research [9]. The depth of Indian research is reflected in the interpretation of the mechanisms of specific diseases. For example, for sciatica (Gridrasi), clinical studies have confirmed that the Ayurvedic prescription "Nirgundi khanavati" can effectively increase the pain threshold [6]. In addition, India has the most complete system for validating HPLC and HPTLC methods for vitexin and vitexin, aiming to provide accurate quantitative standards for the global supply chain [22,26].
- **Chemical and Mechanistic Studies of Vitex *negundo* var. *cannabifolia*** Chinese research has primarily focused on a specific variety of Vitex negundo – Vitex negundo. In traditional Chinese medicine, Vitex negundo oil is widely used to treat chronic bronchitis. Chinese research teams have conducted detailed comparisons of its different medicinal parts (roots, stems, leaves, and fruits) using UPLC-Q-TOF/MS [27]. Analysis showed that the stems and leaves of Vitex negundo were more effective than the roots in inhibiting xylene-induced ear swelling in mice, providing a scientific basis for the traditional Chinese medicine practice of "using leaves instead of roots" [27]. Chinese scholars have also successfully screened 39 antioxidant components from the leaves using advanced two-dimensional liquid chromatography combined with electrochemical detection (2D-LC-ECD) and confirmed the strong binding affinity of isovitexin, chlorogenic acid, and baicalin to antioxidant target proteins through molecular docking technology [21].
- **Philippines: A Pioneer in Successful Commercialization and Antiviral Research.** Philippine research is highly practically oriented, and its experience in transforming *Lagundi* (locally known as *Vitex negundo*) into a nationally recognized cough and asthma medication is considered a benchmark for developing countries [2,15,28]. Specific clinical results are shown in Table 3. Philippine research extends beyond traditional cough remedies, recently focusing on antiviral applications. Studies have demonstrated that the dichloromethane extract of *Vitex negundo* significantly inhibits the growth of the original SARS-CoV-2 strain and its variants, and exhibits a synergistic effect with remdesivir [29].
- **Nigeria and Nepal: Environmental Stress and Chemotypic Diversity**. Nigeria's research primarily focuses on chemotoxotaxonomy. By comparing samples from different states, researchers found that the concentrations of nitrogen, potassium, and sodium in the soil directly affect terpene biosynthetic pathways [10]. Nepalese research, on the other hand, focused on samples from low-altitude areas of the Himalayas, identifying specific flavonoid glycosides that inhibited local multidrug-resistant bacteria such as Staphylococcus aureus, with a minimum inhibitory concentration (MIC) of up to 6.25 mg/mL [30].

A deep analysis of the pharmacological mechanism reveals that the bioactivity of Vitex negundo is not determined by a single component, but rather by the combined action of multiple secondary metabolites on multiple cell signaling pathways.

- **Anti-inflammatory and Immunomodulatory Mechanisms:** The anti-inflammatory pathway of Vitex negundo exhibits multi-level characteristics. First, it exerts an analgesic effect by inhibiting prostaglandin synthesis, which has been confirmed in multiple animal models [19,27,31]. Second, Vitex negundo extract can significantly downregulate the NF-κB signaling pathway and reduce the expression of pro-inflammatory factors such as TNF-α, IL-1β, and IL-6 [17,32,33]. Recent immunomodulatory studies indicate that Vitex negundo aqueous extract can induce a conversion of macrophages from the pro-inflammatory M1 type to the anti-inflammatory M2 type in a simulated hypertension model [33]. This phenotypic conversion has profound clinical significance for controlling atherosclerosis and the course of chronic inflammation.
- **Respiratory protection and anti-asthmatic effects:** In a mouse model of allergic asthma, Vitex negundo leaf extract reduced pulmonary fibrosis, congestion, and alveolar collapse by modulating the AMPK/PI3K/Akt/p38 pathway [17,34]. Its antitussive mechanism is partly attributed to papaverine-like phosphodiesterase (PDE) inhibition and diltiazem-like calcium channel blocking, which together promote bronchial smooth muscle relaxation [35].
- **Antimicrobial and antitumor activities** : Vitex negundo exhibits an antibacterial spectrum covering both Gram-positive and Gram-negative bacteria [20,24,36,37]. The mechanism involves the disruption of cell membranes by monoterpenoid components, leading to intracellular potassium leakage [24]. In antitumor studies, lignans from Vitex negundo seeds and polyphenols from its leaves have shown cytotoxicity against various human cancer cell lines [9,17,38]. Their apoptosis-inducing process involves caspase activation, PARP protein cleavage, and DNA breakage [17,25].

Comparison of Extraction Processes and Biotechnology

The choice of extraction technology directly affects the integrity and purity of active ingredients. Different countries around the world have adopted different paths in pursuing high yield and environmental protection and energy conservation, as detailed in [Table 4]. Studies show that although the traditional methanol reflux method is the most efficient for extracting luteolin, for large-scale industrial production, ultrasonic-assisted extraction is increasingly valued for its protective effect on heat-sensitive components at low temperatures [5,12,41]. To address the problem of limited wild resources, the intervention of biotechnology has become a new trend. Studies have found that by adding growth regulators to MS medium, the active ingredients induced in Vitex negundo callus tissue differ significantly from those in wild plants [13]. For example, the concentration of octadecadienoic acid in the callus tissue can reach 47.79%, far higher than the 21.93% in natural leaves, indicating the possibility of large-scale production of specific active ingredients using bioreactors [13,32].

Table 3: Regional research focus and clinical achievements in the Philippines.

Field of study	Key Results	Clinical/Technical Background
Respiratory medications	Lagundi Syrup/Tablets (Ascof)	The top-ranked herb among the ten medicinal herbs approved by the Ministry of Health [2,15,39]
COVID-19 clinical trials	Relieves mild symptoms (especially loss of smell)	A multicenter randomized controlled trial with a 10-day treatment period [40]
Molecular docking (In silico)	Inhibit HRV-3C protease	Potential inhibitory effect of iridoids on rhinovirus [39]
Antiviral screening	Inhibition of SARS-CoV-2 RdRp activity	The dichloromethane extract showed a 5-log reduction in virus [29].

Table 4: Effect of extraction methods on target constituent yield and efficiency.

Extraction method	Main application target components	Yield and efficiency observation	References
Methanol reflux extraction	Luteolin, flavonoids	It is considered the most ideal process for extracting flavonoids.	[41,5]
Ethanol impregnation	Total phenols, total flavonoids	High yield (approximately 15.73%), but long processing time.	[15,42]
Supercritical carbon dioxide	Volatile oils, nonpolar terpenes	The yield was low (0.88%), but there was no solvent residue.	[24,42]
20% ethanol-water system	Vitexin, Vitexin	The yield is superior to that of a single solvent (up to 42.19% extraction rate).	[43]
Water extraction	Saponins, polar polyphenols	The most commonly used traditional dosage forms in the Philippines and India	[5,12,36]

Conclusions and Global Development Outlook

Based on an in-depth analysis of global research data, Vitex *negundo* has transformed from a traditional ethnic herbal medicine into a globally recognized herbal medicine with a solid scientific foundation. The diversity of its active ingredients and its multi-target mechanism of action make it play an irreplaceable role in the management of chronic inflammation, the treatment of respiratory diseases, and the prevention and control of emerging viral infections.

Cross-national comparative studies have revealed two parallel development paths: one, represented by India and China, focuses on analyzing the ancient wisdom of traditional medicine at the molecular level and establishing stringent quality standards through advanced chemometric methods [11,27,44]; the other, represented by the Philippines, has successfully established a transformation path from ethnic pharmacology research to the modern pharmaceutical industry chain, providing an economical and effective solution for global public health [2,40]. Future R&D should focus on addressing the following challenges: first, standardizing the regional differences in chemical components, requiring the establishment of a quality control system based on multiple biomarkers [32,11]; second, strengthening clinical drug interaction research [31,32,35]. Furthermore, as the impact of climate change on the metabolic pathways of medicinal plants intensifies, exploring the deep connection between environmental factors and chemotype evolution will be crucial for maintaining the stability of global medicinal material supply [10,45].

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