

## **Phytochemical and pharmacological activities of *Vitex negundo* L.**

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### **CONTENTS**

- 1. INTRODUCTION**
- 2. BOTANICAL AND TRADITIONAL OVERVIEW**
  - 2.1 Botanical description and morphology**
  - 2.2 Habitat and geographical distribution**
  - 2.3 Uses in Traditional Medicine System**
- 3. PHYTO CHEMICAL CONSTITUENTS**
- 4. PHARMACOLOGICAL PROPERTIES**
  - 4.1 Antioxidant activity**
  - 4.2 Anti-inflammatory Effects**
  - 4.3 Anticancer Activity**
  - 4.4 Antimicrobial Properties**
  - 4.5 Cardioprotective Activity**
  - 4.6 Antipyretic Potential**
  - 4.7 Enzyme Inhibitory Effects**
  - 4.8 Neuroprotective Action**
- 5. BIOLOGICAL ACTIVITIES**
- 6. PHARMACEUTICAL APPLICATIONS**
- 7. CONCLUSIONS**
- 8. REFERENCES**

### **ABSTRACT**

*Vitex negundo* L. family *Verbenaceae*, a medicinal shrub holds immense therapeutic potential owing to its diverse biochemical constituents. Traditionally recognized for its role in Ayurvedic, Unani and Chinese medicine. Every part of the plant viz. leaves, roots, bark, flowers, seeds and fruits-exhibits significant pharmacological activities such as antioxidant, anti-inflammatory, anticancer and antimicrobial properties. Its phytochemical composition includes flavonoids, terpenoids, phenolics, alkaloids and lignans, many of these are under investigation for novel drug development. However, due to overexploitation, habitat loss and lack of sustainable harvesting practices, *V. negundo* faces a decline in its natural populations. This review aimed to analyze the phytochemical profile, biological activities and pharmaceutical applications of *Vitex negundo* and further proposes urgent conservation strategies to safeguard this plant for future biomedical applications.

**Keywords:** Anticancer, Antimicrobial, Antioxidant, Bioactive compounds, Conservation Strategies, Ecological applications, Neuroprotective, Pharmaceutical applications, Pharmacological activities, Traditional Medicine, *Vitex negundo*.

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## 1. INTRODUCTION

The re-emergence of herbal medicine in global health practices has reestablished the importance of medicinal plants such as *Vitex negundo*, (*Verbenaceae* family). Known commonly as the five-leaved chaste tree. Its therapeutic applications range from treating respiratory ailments to hormonal disorders and inflammatory conditions (29). Contemporary phytochemical (Figure 1. Five-leaved Chase Tree *Vitex negundo*). It has been a staple in traditional medicine systems like Ayurveda, Unani and Chinese pharmacopoeia for centuries.



Figure 1. Three-leaved tree *Vitex negundo*

Research reveals a rich profile of bioactive compounds (Vitexin, isovitexin, negundin, ursolic acid and casticin) - each associated with a range of pharmacological actions. Studies have validated its efficacy in antioxidant defense, analgesia, immunomodulation and cytotoxicity against cancer cells. Despite its extensive potential, *V. negundo* is increasingly threatened due to habitat degradation and unsustainable usage. The necessity to integrate conservation strategies with modern pharmaceutical exploration is thus imperative. This review consolidates existing phytopharmacological evidence and emphasizes future biotechnological interventions for its conservation and utilization (30).

## 2. BOTANICAL AND TRADITIONAL OVERVIEW

### 2.1. Botanical Description and Morphology

*V. negundo* L., (*Verbenaceae* family) called Five-Leaved Chaste Tree or "Nirgundi" in Sanskrit (14). It is a woody aromatic shrub that grows 2-5 m and sometimes grows into a small tree. The general identification features of the plant include:

- **Quadrangular branches:** Often covered with fine hairs.
- **Palmately compound leaves:** 5-lanceolate leaflets (hence name *five-leaved*).
- **Bluish-purple to lavender flowers:** Arranged in terminal panicles.
- **Fruits, which are four-seeded drupes:** Turns black upon ripening.

The plant gives off a typical aromatic smell and grows in tropical as well as subtropical climates most preferred in moist humid habitats like riverbanks, edges of a forest and open field (35).

## 2.2. Habitat and Geographical Distribution

*Vitex negundo* is native to India, Sri Lanka, Pakistan and Southeast Asia (China, Malaysia, the Philippines), Eastern Africa and Madagascar. It is found in India, along riverbanks, moist situations, open wastelands and near deciduous forests. It grows in plains and up to altitude of 1,500-2000 m (22). *Vitex negundo's* can grow in a variety of conditions.

## 2.3. Uses in Traditional Medicine Systems

*Vitex negundo*, called five-leaved chaste tree, has been integral to various traditional medicine systems across Asia and Africa (34). As the (Table 1, Traditional Uses of *Vitex negundo* in Various Medical Systems) show that in Ayurveda, it's esteemed for balancing the three doshas-*Vata*, *Pitta* and *Kapha*-and is employed to treat ailments such as rheumatism, respiratory disorders and gynecological issues. Preparations from its leaves, roots and seeds are used for their anti-inflammatory, analgesic and antipyretic properties (5). In the Unani system, *V. negundo* is recognized for its diuretic and anti-inflammatory effects. It's traditionally used to address conditions like arthritis, headaches and digestive disturbances. The plant's various parts are formulated into powders and decoctions for therapeutic use (19). Traditional Chinese Medicine (TCM) also incorporates *V. negundo*, particularly its fruits, known as "Huang Jing Zi." It's prescribed for ailments such as migraines, eye disorders and menstrual irregularities, attributed to its ability to dispel wind and alleviate pain.

Table 1. Traditional Uses of *Vitex negundo* in Various Medical Systems

| System                 | Plant Part           | Form of Use                 | Therapeutic Applications  |
|------------------------|----------------------|-----------------------------|---|
| Ayurveda               | Leaves, Roots, Seeds | Juice, decoction, poultice  | Sinusitis, asthma, fever, rheumatism, wounds, menstrual disorders |
| Unani                  | Seeds, Roots, Leaves | Powder, decoction, tincture | Aphrodisiac, swelling, colic, dyspepsia, respiratory ailments     |
| Chinese Medicine (TCM) | Fruits               | Powder, decoction           | Migraine, blurred vision, arthritis, fatigue, hormonal imbalance  |

## PHYTOCHEMICAL CONSTITUENTS

*Vitex negundo* is pharmacologically significant because of its diverse array of bioactive phytochemicals, which are distributed throughout the plant's anatomical parts - from root to flower. These compounds contribute to its traditional uses and modern therapeutic relevance (20). One of the most studied groups are the flavonoids (vitexin, casticin, luteolin and apigenin). These are primarily found in the leaves and flowers and play crucial roles as antioxidants, anti-inflammatory agents and even anticancer molecules. These flavonoids function by scavenging free radicals and modulating key cellular signaling pathways such as NF- $\kappa$ B and MAPK (8). Triterpenoids such as ursolic acid and oleanolic acid, mainly present in roots and leaves, are known for their anticancer, cardioprotective and hepatoprotective roles. These compounds enhance cellular apoptosis in cancer cells and stabilize lipid membranes in cardiovascular diseases (32). The essential oils extracted from the leaves and seeds are rich in sabinene, 1,8-cineole,  $\alpha$ -pinene and  $\beta$ -caryophyllene. These volatile oils give *V. negundo* its distinctive aroma and contribute to its antimicrobial, insecticidal and anti-inflammatory activities (27). Alkaloids, such as nishindine, are present in roots and seeds, though less explored, exhibit potential antipyretic and pain-relieving properties. The plant also produces iridoid glycosides, like agnuside and negundoside, in the leaves, which are linked to neuroprotective and antioxidant functions (26). Additionally, lignans (e.g., pinoresinol, isoorientin) found in the bark and leaves possess antioxidant and antiviral capabilities. Sterols, such as  $\beta$ -sitosterol and stigmasterol, are dispersed throughout the plant and contribute to its anti-inflammatory and hormone-balancing actions (6). Together, these phytoconstituents reflect the therapeutic synergy of *Vitex negundo*, justifying its widespread use in multiple traditional medicinal systems (7).

Table 2. Major Phytochemicals present in different parts of *Vitex negundo*

| Plant Part | Major Phytochemicals   | Phytochemical Class                | Reported Activities                                |
|------------|--|------------------------------------|--|
| Leaves     | Casticin, Luteolin, Vitexin, Ursolic acid, $\beta$ -sitosterol | Flavonoids, Triterpenoids, Sterols | Antioxidant, anti-inflammatory, hepatoprotective   |
| Roots      | Negundin A and B, Isovitexin, Oleanolic acid                   | Lignans, Flavonoids, Triterpenoids | Cytotoxic, neuroprotective, antipyretic            |
| Seeds      | Artemetin, Vitedoin A and B, $\beta$ -sitosterol               | Flavonoids, Lignans, Steroids      | Analgesic, reproductive health, anticancer         |
| Flowers    | Sabinene, Linalool, Caryophyllene                              | Essential oils, Monoterpenes       | Anti-inflammatory, antimicrobial, aromatherapeutic |
| Fruits     | $\alpha$ -Copaene, Germacrene D, $\beta$ -Selinene             | Sesquiterpenes                     | Anti-inflammatory, insect-repellent, antimicrobial |
| Bark/Stem  | $\beta$ -Amyrin, Hexadecanoic acid, Oleanolic acid             | Triterpenoids, Fatty acids         | Anti-inflammatory, antioxidant, antimicrobial      |

*Vitex negundo* is rich in a variety of phytochemicals (Table 2. Major Phytochemicals Present in Different Parts of *Vitex negundo* Table 3. Key Bioactive Compounds) that contribute to its diverse pharmacological properties (15). The plant contains numerous flavonoids, terpenoids, alkaloids, lignans, phenolic acids and essential oils, each found in varying concentrations across different plant parts. Among the most prominent flavonoids identified in the leaves are casticin, luteolin, vitexin and apigenin, which are known for their potent antioxidant and anti-inflammatory effects (7). Ursolic acid, oleanolic acid and betulinic acid, found primarily in the leaves and roots, are well-recognized triterpenoids with anticancer, hepatoprotective and antimicrobial activities. Roots are a source of distinctive lignans such as negundin A and B, which have shown cytotoxic and neuroprotective potential in preclinical studies. The seeds and bark of *V. negundo* are reported to contain steroidal compounds like  $\beta$ -sitosterol, along with alkaloids and glycosides that support its traditional use in reproductive and hormonal health.

Table 3. Key Bioactive compounds in *Vitex negundo*

| Compound               | Class        | Source              | Activity                            |
|------------------------|--------------|---------------------|-------------------------------------|
| Vitexin and Isovitexin | Flavonoids   | Roots, Seeds        | Antioxidant, anti-proliferative     |
| Casticin               | Flavonoid    | Leaves, Flowers     | Anti-inflammatory, anticancer       |
| Ursolic Acid           | Triterpenoid | Leaves, Roots       | Antitumor, antimicrobial            |
| Negundin A/B           | Lignan       | Roots               | Skin disease treatment, antipyretic |
| $\beta$ -Sitosterol    | Steroid      | Leaves, Seeds, Bark | Immunomodulatory, anticancer        |

Additionally, the essential oils extracted from the flowers and fruits include sabinene, 1,8-cineole and  $\beta$ -caryophyllene, which (Figure 2. Structure of Bioactive Compounds: A. Vitexin, B. Isovitexin, C. Ursolic Acid, D. Casticin, E. Negundin A/B, F.  $\beta$ -Sitosterol) contribute to the plant's antimicrobial and anti-inflammatory effects. These diverse compounds work synergistically, explaining the wide-ranging therapeutic potential of this plant in both traditional and modern medicine (3).

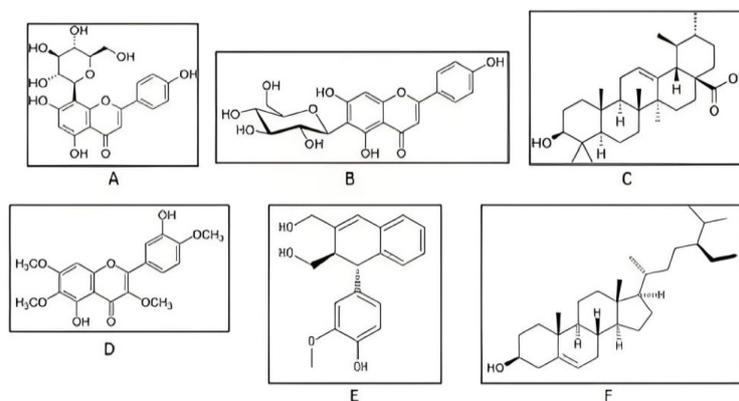


Figure 2. Structure of Bioactive Compounds: A. Vitexin, B. Isovitexin, C. Ursolic Acid, D. Casticin, E. Negundin A/B, F.  $\beta$ -Sitosterol in *Vitex negundo*

#### 4. PHARMACOLOGICAL PROPERTIES

*Vitex negundo* has gained significant attention for its wide spectrum of pharmacological properties, supported by both traditional use and modern research (23). One of its most extensively studied properties is its antioxidant potential, which is attributed to flavonoids and phenolic acids like vitexin and luteolin that scavenge free radicals and protect cellular structures. Its anti-inflammatory action is evident from both *in-vitro* and *in-vivo* studies, showing suppression of pro-inflammatory cytokines and enzymes like COX and LOX. These anti-inflammatory effects explain its traditional use in managing arthritis, wounds and muscular pain. The plant has also demonstrated anticancer properties, particularly due to compounds such as casticin and ursolic acid, which induce apoptosis and inhibit cancer cell proliferation. Extracts of *V. negundo* have shown strong antimicrobial activity against a range of bacterial and fungal pathogens, attributed to its essential oils and flavonoid content (2). Furthermore, the plant exhibits cardioprotective effects, helping in the regulation of blood pressure, lipid profile, and myocardial oxidative stress (13). Its antipyretic activity, validated by traditional use and scientific studies, is linked to the modulation of prostaglandins and thermoregulatory pathways. Some studies also suggest that *V. negundo* possesses enzyme-inhibitory properties, particularly against acetylcholinesterase and  $\alpha$ -glucosidase, making it a candidate for neurodegenerative and diabetic conditions (18). Lastly, its neuroprotective potential is emerging, with evidence of its ability to reduce oxidative stress in brain tissues and enhance cognitive function, likely due to its lignan and flavonoid-rich profile. This pharmacological diversity supports the continued interest in *V. negundo* as a multi-target herbal therapeutic (10) as shown in (Figure 3).

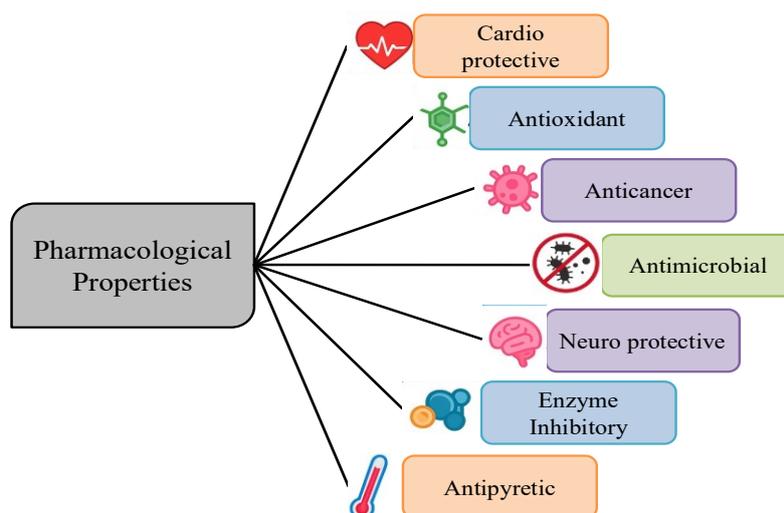


Figure 3. Pharmacological properties of *Vitex negundo*

#### 4.1. Antioxidant Activity

*Vitex negundo* is recognized for its strong antioxidant potential, largely attributed to its flavonoids and polyphenolic constituents like vitexin, luteolin and apigenin (28). These compounds neutralize reactive oxygen species (ROS), thus protecting cellular structures from oxidative stress. Several *in-vitro* studies using DPPH and ABTS assays have confirmed the free radical scavenging activity of leaf and root extracts (17). This antioxidant defense system plays a critical role in reducing the risk of chronic diseases such as cancer, cardiovascular conditions and neurodegenerative disorders.

#### 4.2. Anti-inflammatory Effects

The anti-inflammatory potential of *V. negundo* has been validated by both traditional applications and scientific research. Flavonoids such as casticin and triterpenoids like ursolic acid to downregulate pro-inflammatory mediators including TNF- $\alpha$ , IL-6 and prostaglandins. Animal studies have demonstrated reduced paw edema and improved joint inflammation, supporting its use in arthritis and musculoskeletal pain relief. These effects are thought to be mediated through inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) pathways (11).

#### 4.3. Anticancer Activity

Several bioactive compounds from *V. negundo* exhibit cytotoxic properties against cancer cell lines. Casticin and betulinic acid induce apoptosis by activating caspase pathways and disrupting mitochondrial membrane potential. Extracts have shown antiproliferative effects on breast, colon and liver cancer cells. These findings suggest that *V. negundo* may serve as a source of natural compounds for developing anticancer therapies with fewer side effects compared to synthetic drugs (21).

#### 4.4. Antimicrobial Properties

Essential oils and extracts from *Vitex negundo* demonstrate broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as fungi. Active constituents like 1,8-cineole, sabinene and  $\beta$ -caryophyllene damage microbial cell walls and interfere with protein synthesis. The antimicrobial efficacy supports its traditional use for treating wounds, respiratory infections and skin disorders (24).

#### 4.5. Cardioprotective Activity

Preclinical studies indicate that *V. negundo* can protect the heart against ischemic damage and oxidative stress. Its flavonoid-rich extracts reduces lipid peroxidation, improves lipid profiles and modulates nitric oxide levels, helps to maintain vascular integrity and lowers blood pressure. These properties make it a promising candidate for managing hypertension and other cardiovascular disorders (16).

#### 4.6. Antipyretic Potential

Traditional use of *V. negundo* as a febrifuge is supported by studies showing that its root and leaf extracts lower the fever in animal models. The mechanism is believed to involve inhibition of prostaglandin synthesis, similar to the action of NSAIDs. This makes it useful in managing fever associated with infections and inflammatory conditions (4).

#### 4.7. Enzyme-Inhibitory Effects

*V. negundo* contains compounds that inhibit enzymes like acetylcholinesterase, relevant in Alzheimer's disease and  $\alpha$ -glucosidase, important for managing blood sugar in diabetic patients. This enzyme-inhibitory activity expands the therapeutic horizon of the plant into metabolic and neurodegenerative diseases.

#### 4.8. Neuroprotective Action

Emerging studies indicate that *Vitex negundo* may protect neural tissues from oxidative and inflammatory damage. Lignans and flavonoids contribute to improved cognitive function and reduced neuroinflammation. These effects may offer potential for managing conditions such as dementia and epilepsy (33).

### 5. BIOLOGICAL ACTIVITIES

In addition to its pharmacological benefits, *Vitex negundo* demonstrates notable biological and ecological activities shown in (Table 4).

Table 4. Biological activities of *Vitex negundo*

| Activity              | Target Organism  | Plant Part Used              | Active Constituents                         | Applications  |
|-----------------------|--|------------------------------|---|---|
| Antifungal            | <i>Aspergillus</i> ,<br><i>Candida</i> , <i>Fusarium</i><br>spp. | Leaves,<br>Seeds             | Flavonoids,<br>Essential oils               | Plant disease<br>control, topical<br>fungal creams  |
| Antibacterial         | <i>E. coli</i> , <i>S. aureus</i> ,<br><i>Pseudomonas</i> spp.   | Leaves, Oils                 | Sabinene, 1,8-<br>Cineole,<br>Caryophyllene | Antiseptics, natural<br>disinfectants               |
| Anti-filarial         | <i>Brugiamalayi</i> ,<br><i>Wuchereriabancrofti</i>              | Leaves,<br>Roots             | Alkaloids,<br>Flavonoids                    | Herbal deworming<br>agents, filariasis<br>treatment |
| Anti-larval           | <i>Aedesegypti</i> ,<br><i>Anopheles stephensi</i><br>larvae     | Leaf<br>extracts             | Essential oils                              | Larvicides in vector<br>control programs            |
| Mosquito<br>repellent | Adult mosquitoes<br>(various species)                            | Essential oil<br>from leaves | Terpenoids,<br>Monoterpenes                 | Natural repellents,<br>fumigation products          |

Biological and Ecological Activities in *Vitex negundo*), making it useful in managing infectious diseases and controlling environmental health risks (25). One of its well-documented properties is its antifungal activity, especially against phytopathogenic fungi such as *Aspergillus niger*, *Candida albicans* and *Fusarium* species. This activity is primarily attributed to flavonoids and essential oils, which inhibit spore germination and disrupt fungal membrane integrity. The antibacterial properties of *V. negundo* have been validated through

various studies involving both Gram-positive and Gram-negative bacteria. Compounds like 1,8-cineole, sabinene and  $\beta$ -caryophyllene from its essential oil interferes with bacterial cell wall synthesis, thereby reducing bacterial proliferation. This makes it a valuable candidate for natural antiseptic formulations (12). Another important application is its anti-filarial activity, particularly against *Wuchereria bancrofti* and *Brugia malayi*, the parasites responsible for lymphatic filariasis. Extracts from *V. negundo* leaves have shown significant larvicidal and adulticidal effects, supporting its use in traditional remedies and vector-control strategies. *Vitex negundo* also exhibits anti-larval properties, especially against mosquito species such as *Aedesaegypti* and *Anopheles stephensi*. The plant's leaf extract, when used as a larvicidal agent, has shown significant reduction in larval populations under lab and field conditions. This is further supported by its role as a mosquito repellent, where volatile oil compounds effectively repel adult mosquitoes when applied topically or used in fumigation.

## 6. PHARMACEUTICAL APPLICATIONS

The pharmacological versatility of *Vitex negundo* has opened up a wide range of pharmaceutical and therapeutic applications, many of which bridge traditional practices and modern science as shown in (Table 5. Key Pharmaceutical and Industrial Applications of *Vitex negundo*). With its abundance of bioactive constituents (flavonoids, terpenoids, phenolics, alkaloids and essential oils)-the plant is being utilized in diverse forms, from topical preparations to advanced drug delivery systems. One of the most commercially successful applications is in the formulation of herbal soaps and skin-care products, particularly those targeting acne, skin inflammation and fungal infections. Its antimicrobial and anti-inflammatory properties, especially from compounds like casticin and cineole, make it effective in cleansing and protecting the skin. Herbal companies have adopted *V. negundo* as base ingredient for natural antiseptic creams, face washes and gels (1).

Table 5. Key Pharmaceutical and industrial applications of *Vitex negundo*

| Application                   | Form/Product                    | Functional Role                                    | Plant Part Used             |
|-------------------------------|---------------------------------|--|-----------------------------|
| Herbal soap                   | Medicinal soap bar              | Antiseptic, anti-inflammatory for skin             | Leaf extract, essential oil |
| Natural dye                   | Textile and cosmetic dyes       | Eco-friendly pigment with antioxidant protection   | Leaves, bark                |
| Silver nanoparticle synthesis | Nanoparticles for drug delivery | Antibacterial, anticancer, biosafe synthesis agent | Leaf extract                |
| Defluoridation                | Water treatment powder (ash)    | Fluoride adsorption for safe drinking water        | Leaf ash                    |
| Topical pain relief           | Herbal balms, sprays            | Muscle relaxant, anti-arthritis, anti-inflammatory | Leaf oil and extracts       |
| Respiratory health            | Syrups, decoctions, inhalers    | Bronchodilator, anti-asthmatic                     | Leaf and root extract       |
| Gynecological formulations    | Capsules, herbal tonics         | Menstrual regulation, uterine tonic                | Seeds, leaves               |
| Wound healing dressings       | Herbal-infused bandages         | Antimicrobial, promotes skin regeneration          | Leaves                      |
| Aromatherapy                  | Essential oil diffusers         | Stress relief, anti-migraine                       | Essential oil               |
| Oral care products            | Herbal mouthwash, oral gel      | Antibacterial, anti-ulcer                          | Leaf and bark extract       |
| Antifungal creams             | Topical ointments               | Treatment for skin infections                      | Leaf oil, flavonoids        |

In the field of nanomedicine, *Vitex negundo* has proven to be a bio-reducing agent for the synthesis of silver nanoparticles (AgNPs). These nanoparticles exhibit superior antibacterial, antifungal and anticancer activity compared to conventional forms. In modern herbal pharmacology, *V. negundo* is used in polyherbal pain-relief sprays and balms, often combined with eucalyptus, menthol and camphor. These formulations are widely used for muscle pain, arthritis and sprains. Similarly, its role in respiratory care is highlighted through its inclusion in herbal cough syrups, expectorants and steam inhalation formulations, owing to its bronchodilator, decongestant and anti-asthmatic actions. The plant's effects on women's reproductive health have led to its use in gynecological formulations aimed at regulating menstruation, relieving menstrual cramps, and improving uterine tone. Traditionally, decoctions from its seeds and roots were used for this purpose, and modern formulations are now following suit with standardized extracts (9). Further pharmaceutical exploration includes its use in oral health care-where mouthwashes and dental gels made from leaf and bark extracts show promising anti-plaque and anti-ulcer activity. Wound-healing dressings and antiseptic sprays infused with *V. negundo* have also shown potential, particularly in reducing microbial contamination and promoting tissue regeneration.

Finally, the essential oil of *V. negundo*, known for its aromatic and therapeutic properties, is being developed for use in aromatherapy and stress-relief applications. It is incorporated in diffusers, massage oils and inhalers for managing anxiety, migraines and fatigue. With increasing evidence of its neuroprotective potential, researchers are also exploring nano-emulsion and transdermal gel formulations for targeted delivery in neurodegenerative conditions (3). These pharmaceutical advancements, both traditional and modern, highlight the adaptability of *Vitex negundo* as a multi-functional bioresource. Ongoing innovations are expected to bring even more sophisticated formulations, particularly in the areas of nanomedicine, cosmeceuticals and herbal environmental therapeutics.

## 7. CONCLUSIONS

*Vitex negundo* is a multifaceted medicinal plant with a long-standing history of use in traditional systems like Ayurveda, Unani and Chinese medicine. Rich in a variety of bioactive compounds including flavonoids, terpenoids, lignans and essential oils, the plant exhibits a wide range of pharmacological properties such as antioxidant, anti-inflammatory, anticancer, antimicrobial and neuroprotective effects. These activities underline its potential in developing natural therapeutics for various chronic and infectious diseases. However, the increasing demand for its extracts and the lack of sustainable harvesting practices are leading to the depletion of wild populations in several regions. This underscores the urgent need for integrated conservation strategies, combining *in-situ* and *ex-situ* approaches with biotechnological tools such as tissue culture and genetic conservation. In conclusion, *Vitex negundo* holds significant promise not only as a medicinal plant but also as a sustainable resource for future pharmaceutical and ecological innovations. Future research should focus on the formulation and standardization of *V. negundo*-based therapeutics, including *in-vivo* pharmacokinetic and toxicological studies. This will help validate its safety and efficacy for clinical applications.

## AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception, literature collection, drafting and final approval of the manuscript.

## DECLARATION

We declare that the manuscript has not been published previously and is not under consideration elsewhere.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ETHICAL APPROVAL

This study does not involve any human or animal experiments requiring ethical approval.

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