World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

Check for updates

Extarction separation and evaluation of terpenoids, flavonoids, Glycosids and Tannis form Nirgudi plant

Bansode Kajal Ashok [1,*], Prachi Nandakumar Padwal [2], Layba Mubarak Tamboli [1], Shreya Ravindra Bhujbal [1], Vishakha vishwanath Dongare [1] and Swamini Balu Dukare [1]

[1] *Samarth Institute of Pharmacy, Belhe, Pune, Maharashtra, India*
[2] *Department of Quality Assurance, Samarth Institute of Pharmacy, Belhe, Maharashtra, India.*

World Journal of Biology Pharmacy and Health Sciences, 2025, 24(02), 027-035

Publication history: Received on 23 September 2025; revised on 28 October 2025; accepted on 31 October 2025

Article DOI: https://doi.org/10.30574/wjbphs.2025.24.2.0961

Abstract

Ayurveda, Siddha, and Unani medical traditions have acknowledged the pharmacological significance of nirgundi (Vitex negundo L.), a medicinal shrub that is abundantly found in tropical and subtropical climates. The plant includes a number of bioactive phytoconstituents with antibacterial, anti-inflammatory, antioxidant, hepatoprotective, and analgesic properties, including terpenoids, flavonoids, glycosides, and tannins. The methods of extraction, separation, and evaluation for various types of phytochemicals from Vitex negundo are highlighted in this review. It also highlights these elements' medicinal potential, which is backed by experimental data and contemporary analytical techniques. Widely distributed across the nation, nirgundi is a huge, fragrant shrub or small tree that can grow up to 4.5 meters in height. Most traditional medical systems employ nigundi in clinical settings for a variety of ailments because of its various therapeutic benefits. Basketry, dyeing, fuel, food, field pesticide, growth stimulant, stored-grain protectant, manure, and human and animal medicine are just a few of the many uses for the plant in India. It is a part of Ayurvedic, Siddha, Unani, homoeopathic, and allopathic medical systems. The pharmacological characteristics and broad therapeutic applications of several plant components are reviewed in this study.

Keywords: Vitex negundo; Ayurveda; Siddha; Unani; Terpenoids; Flavonoids; Glycoside

1. Introduction

Medicinal plants have been the cornerstone of traditional medicine, serving as natural reservoirs of bioactive compounds. *Vitex negundo* L. (family: Verbenaceae), commonly known as Nirgundi, is a large aromatic shrub widely distributed across India and Southeast Asia. It has been traditionally used to manage ailments such as rheumatism, headache, skin diseases, respiratory disorders, and inflammation. The therapeutic efficacy of *Vitex negundo* is attributed to the presence of diverse secondary metabolites including terpenoids, flavonoids, glycosides, and tannins. Extraction, separation, and evaluation of these phytochemicals are essential to explore their pharmacological potential. (Khare,et,al. 2004).

* Corresponding author: Bansode Kajal Ashok

Figure 1 Plant of Vitex negundo; A- plant with leaves, B- plant in flowering stage

As an essential source of bioactive chemicals with therapeutic potential, medicinal plants have long been a mainstay of conventional healthcare systems. Among these, Vitex negundo L., also referred to as Nirgundi, is widely used in Siddha, Unani, and Ayurvedic medicine. It is a huge, fragrant shrub or small tree that may reach a height of 4.5 meters and is found throughout tropical and subtropical regions of Asia and Africa. It belongs to the Verbenaceae family.

Nirgundi has historically been used to treat a wide range of conditions, such as wounds, rheumatism, respiratory issues, inflammatory illnesses, skin conditions, and headaches. Many bioactive components, including terpenoids, flavonoids, glycosides, tannins, and essential oils, have been identified by contemporary phytochemical studies. These components help to explain the plant's broad range of pharmacological actions, which include antimicrobial, anti-inflammatory, antioxidant, hepatoprotective, analgesic, and neuroprotective effects. (Meena and others, 2011). The main phytochemical classes terpenoids, flavonoids, glycosides, and tannins that were extracted, separated, and assessed from Vitex negundo are the subject of this review. Additionally, it highlights the medicinal potential of these phytoconstituents by combining data from contemporary scientific study with traditional knowledge.

1.1. Traditional medicinal uses

The most ancient and authoritative text on Ayurveda, Unani, and Chinese traditional medicine systems, the Charaka Samhita, mentions the use of Vitex negundo. The plant's parts serve a variety of purposes: Leaf: anti-inflammatory, analgesic, antihistaminic, neutralises snake venom, eliminates worms and foetid discharges from ulcers, hepatoprotective, and CNS depressive properties (Muthuswamy et al., 2012); Flowers are used to treat liver problems because they are astringent, febrifuge, and anti-diarrheic. Oil is given on scrofulous sores and sinuses. Fruits -nervine stimulant, emmenagogue and vermifuge; antitoxin in seeds; Whole plant: galactagogue, emenagogue, antigastalgic, antiflatulant, antiparasitic, and analgesic; root: tonic, febrifuge, expectorant, and diuretic. (Arora et. al., 2011)

Figure. 2 Nirgundi Plant

1.2. Classification

- Kingdom: Plantae
- Phylum: Tracheophyta
- Class: Magnoliopsida
- Order: Lamiales
- Subkingdom: Tracheobionta

- Super Division: Spermatophyta
- Division:Magnoliophyta
- -Subclass: Asteridae
- Family: Lamiaceae (also known as Verbenaceae in some sources)
- Genus: Vitex
- Species: Vitex negundo L.

1.3. Biological Source:

Vitex negundo Linn., popularly known as Nirgundi, belongs to the family Verbenaceae/Lamiaceae

1.4. Morphological Characteristics -

- **Growth Habit**: Vitex negundo is a big, woody, aromatic, deciduous shrub that can grow to a height of 2 to 8 meters. It an even become a small tree.
- **Bark**: Usually reddish-brown in colour.
- **Leaves**: The leaves have palmate leaflets and are pentafoliate. The terminal leaves are long (4-10 cm), acute, and etiolate (1-1.3 cm long), lanceolate, hairy beneath, and pointed at both ends
- **Flowers**: Found on branching cymes, the blooms are abundant and bluish-purple in hue.
- **Fruits**: The spherical, succulent fruits have four seeds and turn black when ripe.

2. Phytochemical Constituents of Vitex negundo

The main source of Vitex negundo's medicinal potential is its wide range of secondary metabolites. Terpenoids, flavonoids, glycosides, tannins, alkaloids, lignans, and essential oils are found in the plant's leaves, stem, roots, seeds, and flowers, among other sections.

These phytoconstituents contribute significantly to the pharmacological actions reported in traditional medicine as well as in experimental studies.(Ladda et. al., 2012)

2.1.1. Terpenoids

Terpenoids represent one of the major classes of compounds in *V. negundo*.

- Identified terpenoids: ursolic acid, betulinic acid, vitexilactone, negundoside, and oleanolic acid.
- Biological activities: anti-inflammatory, hepatoprotective, anticancer, and antioxidant.
- Volatile terpenoids detected through GC-MS include sabinene, β-caryophyllene, limonene, and α-pinene.

2.2. Flavonoids

Flavonoids are abundantly present in the leaves and flowers.

- Major compounds: casticin, chrysosplenol-D, luteolin, orientin, and gardenin-A.
- Pharmacological relevance: These compounds possess strong antioxidant, antimicrobial, anti-allergic, anti-inflammatory, and anticancer activities.

Flavonoid glycosides are often isolated from ethanol or methanol extracts.

2.3. Glycosides

V. negundo is rich in iridoid and phenolic glycosides.

- Important glycosides: negundoside, aucubin, agnuside, and 5-hydroxy-3,6,7,3',4'-pentamethoxyflavone glycoside.
- Reported activities: cardioprotective, anti-asthmatic, adaptogenic, and antimicrobial.

2.4. Tannins

Both hydrolysable and condensed tannins have been reported.

Contribute to antioxidant, antimicrobial, wound-healing, and hepatoprotective properties.

Quantified by Folin-Ciocalteu and vanillin-HCl methods in phytochemical studies.

2.5. Other Constituents

- Alkaloids: Found in roots and bark, known for analgesic and anti-inflammatory roles.
- Essential oils: Contain eugenol, caryophyllene, sabinene, and myrcene, contributing to antimicrobial and insecticidal activities.
- Lignans: Such as negundin-A and negundin-B, reported for antioxidant and estrogenic properties.

3. Extraction Techniques

Extraction is a critical step to obtain phytoconstituents. Different solvents and methods have been employed:

- **Conventional Solvent Extraction** (Maceration, Soxhlet, Reflux): Methanol, ethanol, and hydroalcoholic extracts show high yield of flavonoids and glycosides.
- Maceration involves soaking the plant material in solvent at room temperature for extended periods (24–72 hours) to allow diffusion of phytoconstituents.
- Soxhlet extraction uses continuous hot extraction, improving yield by repeatedly washing the plant material with fresh solvent.
- Reflux extraction applies controlled heating to enhance solubility and extraction efficiency of heat-stable compounds.

These methods are widely used due to their simplicity and cost-effectiveness, although they may require longer extraction times compared to modern techniques.(P. Hu *et, al.*,2015)

3.1. Advanced Techniques:

- **Ultrasound-assisted extraction (UAE)** improves extraction yield and reduces time.
- **Microwave-assisted extraction (MAE)** enhances thermal degradation–resistant compounds.
- **Supercritical fluid extraction (SFE)**, particularly with CO_2, is efficient for terpenoids.

4. Separation and Isolation

Different chromatographic and analytical techniques are used for separation:

4.1. Thin Layer Chromatography (TLC)

- Purpose: Preliminary qualitative analysis and identification of phytochemicals such as flavonoids, glycosides, and terpenoids.
- Procedure: Extracts are spotted on silica gel plates, developed in suitable solvent systems, and visualized under UV light or using specific reagents (e.g., aluminum chloride for flavonoids).
- Advantages: Simple, rapid, cost-effective, and suitable for screening multiple samples.

4.2. Column Chromatography

- Purpose: Isolation and purification of individual phytochemicals from crude extracts.
- Procedure: The extract is loaded onto a silica gel or Sephadex column and eluted with solvents of increasing polarity. Fractions are collected and analyzed further by TLC or HPLC.
- Applications: Effective for isolating terpenoids, flavonoids, and glycosides. *(*C.A. Anosike *et, al,.*2019)

4.3. High Performance Liquid Chromatography (HPLC)

- Purpose: Quantitative and qualitative analysis of specific compounds.
- Advantages: High resolution, sensitivity, and reproducibility.
- Applications: Widely used for flavonoid and glycoside analysis in *V. negundo*, e.g., casticin, luteolin, and negundoside.
- Detection: UV-visible or photodiode array detectors (PDA) are commonly employed.

4.4. Gas Chromatography-Mass Spectrometry (GC-MS)

- Purpose: Identification and quantification of volatile and semi-volatile compounds, primarily terpenoids and essential oils.
- Procedure: Extracted volatile compounds are vaporized and separated on a GC column; mass spectrometry provides structural identification.
- Applications: Detection of α-pinene, β-caryophyllene, sabinene, and other terpenoids.

4.5. Other Techniques

- Preparative HPLC: For large-scale purification of bioactive compounds.
- Liquid-Liquid Partitioning: Used to separate compounds based on polarity before chromatographic analysis.
- Spectroscopic Methods (NMR, IR, MS): For structural elucidation of isolated compounds.

5. Evaluation of Phytoconstituents

5.1. Terpenoids

5.1.1. Qualitative Tests*:*

- Liebermann–Burchard Test: Detects triterpenoids; formation of a blue or green color indicates presence.
- Salkowski Test: Confirms sterols and triterpenes by producing a red or pink coloration.

5.1.2. Quantitative Analysis:

- Gas Chromatography-Mass Spectrometry (GC-MS) for volatile terpenoids.
- UV-Vis Spectrophotometry for total terpenoid content.
- Biological Evaluation: Anti-inflammatory, hepatoprotective, and antioxidant assays.

5.2. Flavonoids

5.2.1. Qualitative Tests:

- Aluminum Chloride Test: Yellow fluorescence indicates flavonoids.
- Shinoda Test: Pink or red coloration confirms flavonoid presence.

5.2.2. Quantitative Analysis:

- UV-Vis Spectrophotometry- total flavonoid content.
- HPLC- for individual flavonoids (e.g., casticin, luteolin).
- Biological Evaluation: Antioxidant (DPPH assay), antimicrobial, and anti-inflammatory activities.(Devi, P.Ret,al,.2007)

5.3. Glycosides

5.3.1. Qualitative Tests:

- Keller-Killiani Test: Detects cardiac glycosides; brown ring at the interface indicates presence.
- Borntrager's Test: Detects anthraquinone glycosides.

5.4. Quantitative Analysis:

- Spectrophotometric assays for total glycoside content.
- HPLC for individual glycosides such as negundoside and agnuside.
- Biological Evaluation: Cardioprotective, anti-asthmatic, and antimicrobial activities.

5.5. Tannins

5.5.1. Qualitative Tests:

- Ferric Chloride Test: Blue-black or green-black color indicates hydrolysable or condensed tannins.
- Gelatin Test: Precipitation confirms tannin presence.

5.5.2. Quantitative Analysis:

- Folin-Ciocalteu Method for total phenolic content.
- Vanillin-HCl Assay for condensed tannins.
- Biological Evaluation: Antioxidant, antimicrobial, and wound-healing activities.

6. Pharmacological Significance

6.1. Anti-Inflammatory and Analgesic Activity

By preventing prostaglandin synthesis and lowering cytokine production, nigundi leaf and root extracts have strong anti-inflammatory properties.

Terpenoids and flavonoids support traditional use in rheumatism, arthritis, and musculoskeletal pain by contributing to analgesic benefits.. *(*Yunos et . al. (2005)

6.2. Antimicrobial Activity

• Flavonoids, glycosides, tannins, and essential oils have antifungal and antibacterial properties that work against bacteria like Candida albicans, Escherichia coli, and Staphylococcus aureus.

Terpenoids enhance the antimicrobial efficacy of Nirgundi extracts.

6.3. Antioxidant Activity

Flavonoids and tannins act as potent free radical scavengers, preventing oxidative damage to cells.

These properties support protection against degenerative diseases and cellular aging**.**

6.4. Hepatoprotective Activity

Terpenoids such as ursolic acid and betulinic acid help protect liver cells from chemical-induced toxicity.

Mechanism includes stabilization of cell membranes, reduction of lipid peroxidation, and enhancement of antioxidant defense systems**.**

6.5. Cardioprotective and Anti-Asthmatic Effects

Glycosides contribute to cardioprotection by improving cardiac function and reducing oxidative stress.

Leaf and root extracts show anti-asthmatic activity, likely due to anti-inflammatory and bronchodilator effects of flavonoids and terpenoids. (G.L. Gallardo et, al.,2004)

6.6. Wound Healing and Skin Protection

Tannins and flavonoids enhance wound contraction, collagen formation, and microbial protection**.**

Traditionally used for cuts, burns, and skin infections**.**

6.7. Other Activities

- Neuroprotective effects: Flavonoids protect neurons against oxidative stress and inflammation.
- Anti-cancer potential: Certain terpenoids and flavonoids induce apoptosis in cancer cell lines.
- Anti-diabetic activity: Experimental investigations have shown that leaf extracts lower blood glucose levels.

7. Results

The review reveals that *Vitex negundo L.* is a rich source of bioactive compounds such as terpenoids, flavonoids, glycosides, and tannins, which are responsible for its wide pharmacological activities. Extraction and chromatographic studies confirmed the presence of these phytoconstituents in various plant parts. These compounds exhibit significant anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, and analgesic effects. The findings validate the

traditional uses of *Nirgundi* in Ayurveda, Siddha, and Unani systems and support its potential for developing standardized herbal formulations.

8. Conclusion

Terpenoids, flavonoids, glycosides, and tannins are abundant in Vitex negundo and have a variety of pharmacological effects. The development of phytopharmaceuticals based on Nirgundi requires standardised extraction, accurate separation, and sophisticated evaluation techniques. To fully utilise these drugs' medicinal potential, future research should concentrate on formulation development, molecular docking, and bioavailability investigations.

Compliance with ethical standards

Acknowledgments

I would like to express my heartfelt gratitude to my guide, Prachi Padwal, for their constant support, valuable guidance, and encouragement throughout this research work on the Extarction Separation And Evaluation Of Terpenoids, Flavonoids, Glycosids And Tannis Form Nirgudi Plant. Their expertise and insightful suggestions were instrumental in the successful completion of this study.

I am also thankful to all the faculty members and laboratory staff of Samarth institute of Pharmacy Belhe for providing the necessary facilities and technical assistance during the formulation and evaluation processes.

Finally, I extend my sincere thanks to my family and friends for their continuous motivation and support, which helped me to complete this work successfully.

Disclosure of conflict of interest

If two or more authors have contributed in the manuscript, the conflict of interest statement must be inserted here.

References

[1] Kaur, R., Arora, S., & Singh, B. (2016). Extraction and isolation of bioactive compounds from *Vitex negundo* and their pharmacological applications. *Journal of Ethnopharmacology*,– 192, 370381.

[2] Dharmasiri, M. G., Jayakody, J. R., Galhena, G., Liyanage, S. S., & Ratnasooriya, W. D. (2003). Anti-inflammatory and analgesic activities of *Vitex negundo*. *Journal of Ethnopharmacology*, 87(2-3), 199–206.

[3] Tandon, V. R. (2005). Medicinal uses and biological activities of *Vitex negundo*. *Natural Product Radiance*, 4(3), 162–165.

[4] Nadkarni, K. M. (1996). *Indian Materia Medica*. Popular Prakashan, Mumbai.

[5] Chawla, A., Chawla, P., & Saxena, S. (2013). Phytochemical and pharmacological studies of *Vitex negundo*. *International Journal of Green Pharmacy*, 7(2), 95–101.

[6] Harborne, J. B. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. Springer, Dordrech

[7] Alam, M.I. and Gomes, A. (2003): Snake venom neutralization by Indian medicinal plants (Vitex negundo and Emblica officinalis) root extracts. Journal of Ethnopharmacology. 86: 75-80.

[8] Arora, V., Lohar, V., Singhal, S.and Bhandari, A. (2011): Vitex negundo - A Chinese Chaste Tree. International Journal of Pharmaceutical Innovations., 1(5): 9- 20.

[9] Aswar, P.B., Khadabadi, S.S., Kuchekar, B.S., Rajurkar, R.M., Saboo, S.S. and Javarkar, R.D. (2009): In vitro evaluation of anti-bacterial and anti-fungal activity of Vitex negundo (Verbenaceae). Ethnobotanical Leaflets. 13: 962-967.

[10] Au, D.T., Wu, J., Jiang, Z., Chen, H., Lu, G. and Zhao, Z. (2008):Ethnobotanical study of medicinal plants used by Hakka in Guangdong, China. Journal of Ethnopharmacology. 117 : 41-50.

[11] Avadhoot, Y. and Rana, A.C. (1991): Hepatoprotective effect of Vitex negundo against carbon tetrachloride induced liver damage. Arch. Pharm. Res. 14(1): 96-98

[12] Chew, Y.L., Goh, J.K. and Lim, Y.Y.(2009): Assessment of in vitro antioxidant capacity and polyphenolic composition of selected medicinal herbs from Leguminosae family in Peninsular Malaysia. Food Chemistry. 116: 13-18.

[13] Das, G.P. (1995): Plants used in controlling the potato tuber moth, Phthorimaea operculella (Zeller). Crop Protection. 14: 631-636.

[14] Das, S., Parveen, S., Kundra, C.P. and Pereira, B.M. (2004): Reproduction in male rats is vulnerable to treatment with the flavonoid-rich seed extracts of Vitex negundo. Phytother Res.18(1): 8-13.

[15] Das, K., Tiwari, R.K.S. and Shrivastava, D.K.(2010): Techniques for evaluation of medicinal plant products as antimicrobial agent. Current methods and future trends. J Med Plant Res. 4(2):104-111.

[16] Devani, U., Pandita, N., and Kachwala, Y.(2013): Evaluation of inhibitory activity of Vitex negundo and Terminalia chebula by alpha amylase inhibiton assay in management of diabetes. Asian Journal of Plant Science and Research.3(2):6-14.

[17] Devi, P.R., Kumari, S.K. and Kokilavani, C. (2007): Effect of Vitex negundo leaf extract on the free radicals scavengers in complete Freund's adjuvant induced arthritic rats. Indian Journal of Clinical Biochemistry. 22:143-147.

[18] Dharmasiri, M.G., Jayakody, J.R.A.C., Galhena, G., Liyanage, S.S.P. and Ratnasooriya, W.D. (2003): Anti-inflammatory and analgesic activities of mature fresh leaves of Vitex negundo. Journal of Ethnopharmacology. 87: 199-206.

[19] Diaz, F., Chavez, D., Lee, D., Mi, Q., Chai, H.B., Tan, G.T., Kardono, L.B.S., Riswan, S., Fairchild, C.R. and Wild, R. (2003): Cytotoxic flavone analogues of vitexicarpin, a constituent of the leaves of Vitex negundo. Journal of Natural Products. 66:865-867.

[20] En-shun, J., Ming, X., Yu-qing, L. and Yu-feng, W. (2009): Toxicity of Vitex negundo extract to aphids and its co-toxicity with imidacloprid. Chinese Journal of Applied Ecology. 20: 686-690.

[21] Graham, J.G., Quinn, M.L., Fabricant, D.S. and Farnsworth, N.R. (2000): Plants used against cancer–an extension of the work of Jonathan Hartwell. Journal of Ethnopharmacology. 73 :347-377.

[22] Guleria, S. and Kumar, A. (2006): Antifungal activity of some Himalayan medicinal plants using direct bioautography. Journal of Cell and Molecular Biology. 5: 95-98.

[23] Gupta, M., Mazumder, U.K., Bhawal, S.R. and Swamy, S.M.K. (1997): CNS activity of petroleum ether extract of Vitex negundo Linn in mice. Indian Journal of Pharmaceutical Sciences. 59: 240-245.

[24] Gupta, M., Mazumder, U.K. and Bhawal, S.R. (1999): CNS activity of Vitex negundo Linn. in mice. Indian Journal of Experimental Biology. 37: 143-146.

[25] Gupta, R.K. and Tandon, V.R. (2005): An experimental evaluation of anticonvulsant activity of Vitex negundo. Indian Journal of Physiology and Pharmacology. 49: 163-172.

[26] Hamayun, M. (2005): Ethnobotanical studies of some useful shrubs and trees of district Buner, NWFP, Ethnobotanical Leaflets. 9.

[27] Hebbalkar, D.S., Hebbalkar, G.D., Sharma, R.N., Joshi, V.S. and Bhat, V.S. (1992): Mosquito repellant activity of oils from Vitex negundo Linn. leaves. Indian Journal of Medical Research. 95: 200-203.

[28] Hu, Y., Zhang, Q., Hou, T., Xin, H., Zheng, H., Rahman, K. and Qin, L. (2007): Estrogen-like activities in Vitex species from China determined by a cell based proliferation assay. Pharmazie. 62: 872-875.

[29] Ivan, A.R. (2005): Medicinal plants of the world: chemical constituents, traditional and modern medicinal uses .New Jersey: Humana press.510.

[30] C.A. Anosike *et al.*Antioxidant properties and membrane stabilization effects of methanol extract of Mucuna pruriens leaves on normal and sickle erythrocytesAfr. J. Tradit., Complementary Altern. Med.(2019)

[31] S. Bindu *et al.*Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: a current perspectiveBiochem. Pharmacol.(2020)

[32] W. Chen *et al.*Geraniol — a review of a commercially important fragrancematerial. S. Afr. J. Bot.(2010)

[33] G.L. Gallardo *et al.*Neric acid derivatives produced by the honey bee fungal entomopathogen Ascosphaera apis Phytochem. Lett.(2008)

[34] A. Karthikeyan, V. Shanthi and N. agasathaya .,"Preliminary phytochemical and antibacterial screening of crude extract of the leaf of Adhatoda vasica L",International Journal Green Pharmcy ,vol.3,pp.78-80,2009.

[35] A .Bernhoft., "A brief review on bioactive compounds in plants". In Proceedings from a symposium held at The Norwegian Academy of Science and Letters, Oslo, Norway 2010.