

PHARMACOLOGICAL REVIEW ON VITEX TRIFOLIA LINN.
(VERBANEACEAE)

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Summary

From thousands of years, the traditional medicinal plants were used for the treatment of various diseases based on knowledge and experience. Many of these plants failed to draw attention for their useful medicinal properties and remain to be undiscovered. In recent years, many traditional plants from various genus, species and families were evaluated scientifically for their role in prevention and treatment of many diseased conditions. The genus *Vitex* contains 270 species all over the world with diverse medicinal active constituents and properties. The present review focuses on one of such traditional plant *Vitex trifolia* Linn. from genus *Vitex*, its pharmacological and biological effects, isolated and known potential phytoconstituents of therapeutic importance.

Keywords: Genus *Vitex*, *Vitex trifolia* Linn., Verbanaceae, Phytoconstituents.

Introduction

From ancient years, the traditional medicinal plants were used for the treatment of various diseases based on knowledge and experience. Many of these plants failed to draw attention for their useful medicinal properties and their active pharmacological contents remain to be undiscovered. Even though herbal plants in crude form or 'as it is' are available for the treatment traditionally, their uses and activities has unproven track records. In recent years, many traditional plants from various genus, species and families were evaluated scientifically. Many active phytoconstituents were isolated and evaluated for their role in prevention and treatment of many diseased conditions.

Many studies were reported with underlying the mechanism of action of active principals and various biological and pharmacological activities of traditional plants but the results and data obtained are not still satisfactory and the lack of updating database at regular intervals leads to loss of scientific information on specific plants. Thus more valid scientific data and collective information supports the therapeutic uses of traditional plants and further detailed research.

The genus *Vitex* contains 270 species all over the world with diverse medicinal active constituents and properties. These species are predominantly trees and shrubs, found in tropical and subtropical regions. Other species with fruits, seeds, roots are also important as traditional medicines. In India, some of *Vitex* species viz. *Vitex negundo*, *Vitex glabrata*, *Vitex leucoxylon*, *Vitex penduncularis*, *Vitex pinnata*, and *Vitex trifolia* L. are found. Many of these vitex species and their active principals are studied for Pharmacognostical investigation and phytochemical screening, biological, pharmacological evaluation.

The present review focuses on one of such traditional plant *Vitex trifolia* Linn. from genus Vitex, its pharmacological and biological effects, isolated and known potential phytoconstituents of therapeutic importance.

Plant description:

Botanical name: *Vitex trifolia* Linn. (Verbanaceae)

The plant *Vitex trifolia* Linn (**Verbanaceae**) is well known in Hindi as ‘Pani-ki-Sanbhalu’, ‘Sufed-Sanbhalu’¹. It is stout aromatic shrub or a small tree, found from the foot of Himalayas southwards throughout greater part of India, western ghat and in Andamans.^{1,3} *Vitex trifolia* Linn. (Verbanaceae) is also found in countries such as Sri Lanka, China, Philippines, Indonesia, North Australia, New Caledonia and French Polynesia. It has also been reported from East Africa and islands Central Pacific and Hawaii.¹⁹

Vitex trifolia Linn. is a shrub or small tree growing from 1 to 4 meters in height, sometimes prostrate or ascending in habit. The leaves are simple or 3-foliolate. *V. negundo* closely resembles *V. trifolia* but can be distinguished by its long-petioled median leaflet and 3-5 leaflets. *Agroforestry Database 4.0 (Orwa et al. 2009)*.

In the prostrate form, the leaves are simple, stalkless, oblong to oblong-elliptic, 4 to 7 centimeters long, 1.5 to 4 centimeters wide, pointed at both ends, smooth and shining on the upper surface, and sparsely covered with gray hairs beneath. The flowers are numerous, and borne in terminal, oblong panicles 5 to 10 centimeters in length. The corolla is hairy, and lavender to blue; the tube is about 8 millimeters long; the larger, central lobe of the lower lip has a white blotch at the base; the limb is 12 millimeters in greatest diameter. The fruit is rounded and 4 to 5 millimeters in diameter¹⁻¹⁵.

Active Phytoconstituents:

Casticin, Luteolin, Isoorientin, Alpha-pinene, Linalool, Terpinyl acetate, Beta-caryophylline and Caryophylline oxide, 5-methyl artemitin, Beta-sitosterol, Vitetrolins, dihydrosolidgenone abietatriene, Vitetrolin A, Essential oils of spicy odour⁸ like Limonene, humulene oxide, caryophylline oxide, alpha-humulene, 20 hydroxycyclopentane, ecdysteroids, flavonoids, lignans, triterpenoids, Iridoids, Vitexin, Beta-sitosterols¹⁻¹⁵.

Traditional Uses¹⁻¹⁵:

Leaves are used medicinally, for rheumatic pain, inflammation^{2, 4}, analgesic, anticonvulsant and sedative, hypnotic⁶ etc. Leaves also possess insecticidal, cytotoxic, fungicidal properties⁷. Leaves showed inhibitory action against *Mycobacterium tuberculosis*⁵. The roots are antiemetic, expectorant, tonic and beneficial in thirst². Fruits are nervine, cephalic, and emmenagogue.

Pharmacological and Biological Properties:**Pharmacognostical investigation and phytochemical screening**

S. Thenmozhi et al. authenticated and standardized *Vitex trifolia* Linn leaves for pharmacognostical evaluation viz. the morphological, microscopical characters and determination of physical constants including loss on drying, ash values, extractive values, moisture contents and foreign organic matter. The preliminary phytochemical screening with the various qualitative chemical tests of various leaf extracts revealed the presence of carbohydrates, flavonoids, protein and amino acids, tannins, phytosterols and saponins phytoconstituents¹⁶.

Toxicity Studies

Zullies Ikawati from Gadjah Mada University, Indonesia studied the acute toxicity of the extract combination of *V.trifolia* leaves and *C. xanthorrhiza* rhizome in rats. The acute administration of the extract at single dose showed that there were no remarkable changes and histopathological findings in all doses revealed no gross abnormalities. The LD50 value showed that the highest dose can be administered without lethal effect, indicating that the extract has become safe¹⁷.

Free radical scavenging and In-vitro antioxidant activity¹⁸

The methanolic & chloroform Extracts of *Vitex trifoliata* roots tested for its free radical scavenging and antioxidant property by using different in vitro models. The methanolic and chloroform root extracts were found to scavenge the superoxides generated by photoreduction of riboflavin in dose dependent manner. The extracts were investigated in comparison with the known antioxidant ascorbic acid. The chloroform extract showed better hydroxyl radicals, lipid peroxidation, DPPH radical scavenging activity as compared to methanolic extract. (Sreedhar et al.). The same author studied hepatoprotective activity

Hepatoprotective activity

The hepatoprotective activity was studied in CCL4 induced hepatic damage model. The ethanolic extract of flowers of *Vitex trifolia* Linn. significantly decreased the CCL4 induced elevated biochemical markers like SGPT, SGOT, ALP, and bilirubin, which indicated the enhancement of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the extract. Decrease in the bilirubin level indicated the effectiveness of the extract in the normal functioning of the liver. The size of liver and enzyme level were almost restored to normal. The histopathological findings such as formation of normal hepatic cords, absence of necrosis and vacuoles suggested the protective effect of flower extract against the CCL4 induced hepatic damage due to the active principles of the plant namely, flavonoids, tannins and other polyphenolic compounds¹⁹.

In another study, Chloroform extracts of *Vitex trifoliata* roots at 200, 400 and 800 mg/kg showed a significant hepatoprotective effect against paracetamol induced hepatitis in rats.

Biological activities²⁰

The organic crude extracts of *vitex trifolia* Linn. leaves and stems were studied for various biological activities. The cytotoxic effect of various extracts was evaluated in different cell line cultures ranging from more sensitive to less sensitive (SQC-1 UIISO, OVCAR-5, HCT-15 COLADCAR, and KB). Hexanic and dichloromethanic (DCM) stems and foliage extracts exhibited significant cytotoxic effect against several cancer cell lines in culture. Although DCM extract exhibited more cytotoxic effect than other extracts against all the cancer cell line cultures but became highly active (ED50 <1µg/ml) against the most sensitive cell line HCT-15 COLADCAR. The leaves and stems methanolic extracts did not exhibit any significant cytotoxic activity. An important antifeeding activity against the insect pest *Spodoptera frugiperda* (Lepidoptera: Noctuidae) was observed and reported in DCM leaves extract. The antifungal activity of crude extracts with five microorganisms viz. *Penicillium species*, *Aspergillus flavus*, *A.parasiticus*, *Trichoderma species* and *fusarium species* was studied with the evaluation criteria the percentage of mycelium growth inhibition. The leaves hexanic extract completely inhibited the fungal pathogen *Fusarium sp.* growth within the first 2 days of growth, but dropped later significantly at day 6 (15% inhibition). DCM extract showed initial growth inhibition (54%) but after 6 days the percentage of growth inhibition dropped significantly. All the extracts exerted no significant growth inhibition when tested with the other fungal species. The potential of crude extracts (10mg/ml, 5 mg/ml, 2.5 mg/ml

and 1.25 mg/ml dissolved in mixture of 20% DMSO and Tween 20) of leaves for bacterial growth inhibition was assessed by conventional disk assay method. All the extracts produced bacterial growth inhibition against two gram positive (*S. aureus* and *S. faecalis*), four gram negative (*E. coli*, *P. mirabilis*, *S. sonnei*, *S. typhi*) and one yeast (*C. albicans*) species. The broth culture of strength 10⁸ colony forming unit (CFU) per ml was prepared for preparing microorganism inoculums. For assay purpose, 2 µl calibrated micropipette was used to place a tiny droplet (5-8 mm in diameter, 10⁴ CFU/ml). Gentamicin (Pharmacia) was used as a standard drug (Positive control) for treatment. The plates after inoculation were incubated for 24 hr at 37°C.

The growth of Gram positive organisms were completely inhibited by all the extracts except lower dose of methanolic extract. In case of Gram negative organisms, the complete growth inhibition was observed except for *S. typhi* species. Over 50% growth inhibition was observed in *S. typhi* species when treated with dichloromethane extract (5 mg/ml) while no bacterial growth was observed in other species. When compared with all extracts at dose 2.5 mg/ml, DCM extract partially inhibited *S. sonnei* and *S. typhi* species growth. Methanolic extract showed greater than 50% growth inhibition in *E. coli* and *P. mirabilis* species. It was found that only DCM extract at two different doses (5 mg/ml and 10 mg/ml) inhibit the fungal species growth whereas no other extract showed significant growth inhibition.

Wound healing activity

The comparative wound healing activity of ethanolic extracts of leaves of *Vitex trifolia* L. and *Vitex altissima* L. was performed in various wound healing models viz. incision, excision and dead space models. Both the plant extracts showed significant wound healing activity which was supported by a decreased in the epithelialization period, an increase in the wound contraction rate, breaking strength of skin, dry weight of granulated tissue, hydroxyproline content and granulation tissue breaking strength. Between the two extracts, ethanolic extract of leaves of *Vitex trifolia* L. exhibited more wound healing activity than *Vitex altissima* L. An increased collagenation in drug treated group was major findings in Histopathological study of the granulation tissue as compared to the control.²¹

Anticancer activity^{23,24}

Six flavonoids, persicogenin, artemetin, luteolin, penduletin, vitexicarpin and chrysofenolone and five labdane-type diterpenes, vitexilactone, rotundifuran, vitetrolin D, and vitetrolin E were isolated, identified by spectroscopic methods and evaluated for the antiproliferative effect on mammalian cancer cells and their effects on cell cycle and apoptosis by flow cytometry. IC₅₀ values and Flow cytometric investigations demonstrated that both the constituents inhibited the proliferation of cancer cells by inducing apoptosis and inhibiting the cell cycle.

Miscellaneous activities

The two new norditerpene aldehydes and five known diterpenes viz. vitexifolin E, vitexifolin F, vitexilactone, 6-acetoxy-9-hydroxy-13(14)-labden-16,15-olide, and previtexilactone were isolated from acetone fruit extract of *Vitex trifolia* Linn. and were responsible for exhibiting trypanocidal activity²⁵.

Alfi Khatib²⁶ developed new approach two dimensional TLC pattern comparison for the estimation of active constituents in the leaves of *Vitex trifolia* Linn. and concluded that the plant exhibit anti-tracheospasmodic activity due to semipolar compounds present in leaves. The aqueous extract of *Vitex trifolia* Linn. (aerial part) showed HIV-1 reverse transcriptase inhibitory activity with RT inhibition ratio (% IR) higher than 90% at a 200 µg/ml concentration.²⁷ Vitexicarpin isolated flavonoid significantly inhibited the proliferation of

human cancer cells. The cells treated with vitexicarpin showed characteristic morphology typical for apoptosis.²⁸

Conclusion

The present review covers many useful medicinal properties with further mechanism of actions to be established. But still more research studies on various plant extracts will be needed to explore the plant for more traditional medicinal properties as well as mechanism of actions and toxicity studies to be further investigated. Research Studies on the plant extract could be targeted to develop novel anticancer agent, potential anti-HIV drug, analgesic and anti-inflammatory agent from the active constituents.

The active constituents from the plant could be isolated and carried over clinical evaluations to develop potential therapeutic agent with adequate safety, efficacy and tolerability. Apart from this, the novel approach could be developed for the herbal product preparation and/or with combination with other plants. Other pharmacological properties such as anti-atherosclerotic, antihypertensive, effect on CNS, Endocrine systems, neurological disorders immunomodulatory effect, antiviral activity could be evaluated depending upon the plant properties similar to other species from the same family/genus. The comparative evaluation with other plants could also be considered.

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