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Review Article

***Vitex trifolia* linn. (Verbanaceae): A Review on pharmacological and biological effects, isolated and known potential phytoconstituents of therapeutic importance**

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ABSTRACT

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 more than 270 species all over the world with diverse medicinal active constituents and properties. In India, some of *Vitex* species viz.

Vitex negundo, *Vitex glabrata*, *Vitex leucoxydon*, *Vitex penduncularis*, *Vitex pinnata*, and *Vitex trifolia* L. are found. Many of these vitex species and their active principals were studied for Pharmacognostical investigation and phytochemical screening and 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' (CSIR 1995). 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 (CSIR 1995, Agrawal 1997). *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 (Neosom 2011, Fact sheet 1999).

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

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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 diameter (Meena 2012, Padmalatha 2009).

Active Phytoconstituents

Casticin, Luteolin, Isoorientin, Alpha-pinene, Linalool, Terpinyl acetate, Beta-caryophylline and Caryophylline oxide, 5-methyl artemitin and 7-desmethyl artemitin (Nair 1975) Beta-sitosterol, Vitetrifolins, dihydrosolidgenone abietatriene, Vitetrifolin A, Essentials oils of spicy odour (Pan 1989) like Limonene, humulene oxide, caryophylline oxide, alpha-humulene, 20 hydroxyecdysone, ecdysteroids, flavonoids, lignans, triterpenoids, Iridoids, Vitexin, Beta-sitosterols, Flavone glycosides (Ramesh 1986).



Figure 1: *Vitex trifolia* linn. (Verbanaceae)

Traditional Uses

Leaves are used medicinally, for rheumatic pain, inflammation (Chatterjee 2003, Nair 1997), analgesic, anticonvulsant and sedative, hypnotic (Saxena 1992) etc. Leaves also possess insecticidal, cytotoxic, fungicidal properties (Shastry 2002). Leaves showed inhibitory action against *Mycobacterium tuberculosis* (Kirtikar 1999). The roots are antiemetic, expectorant, tonic and beneficial in thirst (Nair 1997). 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 evalua-

tion 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 phytoconstituents (Thenmozhi 2011).

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 safe.

Free radical scavenging and *In-vitro* antioxidant activity

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. The same author studied hepatoprotective activity (Sreedhar et al.).

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 compounds (Anandam 2009).

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 activities

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 anti-feeding 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, 2µl 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 (Hernandez 1999).

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 (Manjunatha 2007).

Anticancer activity

Six flavonoids, persicogenin, artemetin, luteolin, pendentin, vitexicarpin and chryso-splenol-D and five labdane-type diterpenes, vitexilactone, rotundifuran, vitetrifolin D, and vitetrifolin 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 (Li 2005, Wen 2005).

Mosquito Larvicidal activity

The vector borne disease was targeted with *Vitex trifolia*. Methyl-p-hydroxybenzoate, a crystalline compound, was isolated from the leaves methanolic extract which exhibited 100% Mosquito larval mortality against *C. quinquefasciatus* and *A. aegypti*. (Kannathasan K 2011)

Antimicrobial and Antibacterial activity:

Geetha evaluated the plant for the antimicrobial potential on against pathogenic bacterial strains (Geetha 2004) whereas Hossain carried out investigation on pet ether extract and ethanolic extracts for antibacterial activity against both gram-positive and gram-negative bacteria (Hossain MM).

Tracheospasmolytic Activity

Two compounds, viteosin-A and vitexicarpin, from n-hexane extract were isolated, identified and studied for tracheospasmolytic activity. Vitexicarpin, at minimum concentration of 1.3 x 10⁻⁵ M exhibited sensitization of guinea pig trachea and thus block effects of hista-

mine possibly by stabilizing the mast cells membrane function (Alam 2002).

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 activity (Kiuchi 2004).

Alfi Khatib 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 (Khatib 2010). 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 (Woradulayapinij 2005). Vitexicarpin isolated flavonoid significantly inhibited the proliferation of human cancer cells. The cells treated with vitexicarpin showed characteristic morphology typical for apoptosis (Wang 2005).

Simultaneous quantification method for the detection of major bioactive metabolites in *Vitex trifolia* and their antioxidant potential was studied by Tiwari (Tiwari 2012). GuQ isolated a new benzofuran-type lignan, vitrifol A, as a dimer of dihydrobenzofuran-type lignan from the fruits of *Vitex trifolia*, along with three known compounds (GuQ 2008) whereas Four new halimane-type (rearranged labdane-type) diterpenes, vitetrifolins D-G were isolated by Ono from the fruit (Ono 2001). Chen YS carried out Chromatography and spectroscopic analysis and identified five triterpenoids (ursolic acid (I), 2 α ,3 α -dihydroxyurs-12-en-28-oic acid (II), betulinic acid (11), taraxerol (IV), 2 α ,3 β , 19-trihydroxyurs-12-en-28-oic acid (V).) present in the plant for the first time (Chen 2010). Matsui M studied aqueous extract of *Vitex trifolia* leaves and concluded that the plant has significant dose- and time-dependent inhibitory activity on interleukin and iNOS mRNA synthesis, with slight effect on tumor necrosis factor (TNF)- α , and induction of LPS-dependent IL-10 anti-inflammatory cytokine, confirmed by ELISA and PCR techniques and showed that the plant has a potential therapeutic value against inflammatory diseases (Matsui 2009). Zeng isolated and identified rho-hydroxybenzoic acid, beta-sitosterol, beta-sitosterol-3-O-glucoside, casticin and, 3,6,7-trimethylquercetagenin from the fruits of *Vitex trifolia*. Except beta-sitosterol all of the compounds were obtained from the fruits of the plant for the first time (Zeng 1996).

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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- Zullies ikawati, Nunung yuniarti. Faculty of pharmacy, Gadjah Mada University, Yogyakarta, Indonesia. Abstract Acute toxicity study of extract combination of *Vitex trifolia* leaves and *Curcuma xanthorrhiza* rhizome on wistar rats.