



A SYSTEMATIC PHARMACOGNOSTICAL AND PHYTOPHARMACOLOGICAL OVERVIEW OF *VITEX TRIFOLIA* LINN PLANT

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ABSTRACT

Nature is present everywhere in order to fulfil the demands of the human. Along with the food, clothes and shelter nature also provides innumerable plants with medicinal benefits. Only the thing for the man is to search and discover the secrets of nature and use it for the benefit of all the beings. One such medicinal plant is *Vitex trifolia*. *Vitex* is the largest genus in the family Verbenaceae which comprises of 250 species which is distributed all around the world. It is a shrub found in the coastal areas hence also known as sandy shores plants. It is commonly known as chaste tree and is reported to have good medicinal values in traditional system of medicines. It is less than 5 meters in height with the stems covered with soft hairs. *Vitex trifolia* is a herbal plant hence its often preferred over the allopathy drugs. The present study deals with pharmacognostical examination includes the morphological, microscopical characters and physical constants of *vitex trifolia* leaves including determination of loss on drying, ash values and extractive values. The preliminary phytochemical screening of various leaf extracts was also carried out and it is revealed the presence of various phytoconstituents like carbohydrates, flavonoids, protein and amino acids, tannins, phytosterols and saponins. The results of the standardization may throw immense light on the botanical identity of the plant *Vitex trifolia* which may furnish a basis of judging the authenticity of the plant and also to differentiate the drug from its allied species and detect adulterants. *V. trifolia* have a heat producing property, which results in the increase in the bile secretions, development of skin colour. The plant completely eliminates the kapha, vatha, pitta produced disease. *V. trifolia* displays a potent cytotoxicity against a wide range of cancer cell lines via different modes of molecular actions. It is an analgesic, vascular relaxation, antimalarial, trachea- spasmolytic, mouse- repelling properties.

KEYWORDS: Sandy shores, *Vitex trifolia*, Pharmacognostic, Pharmacological, Phytochemistry.

INTRODUCTION

In recent times, interest in plant research has increased all over the world owing to its potential use in traditional systems of medicine for treating a wide variety of diseases. Various medicinal plants have been identified and modern scientific approaches have been used to study their authenticity, safety and efficacy of their therapeutic use. The results highlight the great potential of medicinal plants in the field of pharmacology. The genus *Vitex* includes approximately 270 known species of trees and shrubs in tropical and sub-tropical regions, although a few species are also found in temperate zones.

Vitex trifolia L. is a shrub or shrubby tree that may grow up to 6 meters in height. In the Ayurveda and Unani systems of medicine, the leaves are mostly used as traditional system of medicine.^[1] Plants used in traditional system of medicine have the advantage with little or no side effect. Most of the species of the genus *Vitex* are widely used in traditional Indian systems of

medicine. Around 270 plant species of genus *Vitex* have been reported worldwide.^[2,3] *Vitex trifolia* belongs to the family Lamiaceae/Verbenaceae. *V. trifolia* is an aromatic coastal deciduous shrub or small plant growing from 1–4 m tall.

TAXONOMICAL CLASSIFICATION^[4]

Kingdom: Plantae
Class: Eudicots
Order: Lamiales,
Family: Lamiaceae
Genus: *Vitex*
Species: *Trifolia*.

Vernacular names

Sanskrit: *Jalanirgundi*
Hindi: *Nichinda*
Manipuri: *Urikshibi*
Bengali: *Paniki-shumbala*
Kannada: *Nira-lakki-gida*

Malayalam: Lagondi, Nirnoschi
 Tamil: Nirnochchi, Shirunoch-chi
 Telugu: Niruvavili, Shiruvavili



MACROSCOPIC EVALUATION

The **green leaves** of *V. trifolia* have resinous glands tomentose beneath and 7–12 pairs of lateral nerves, terminal, and supraaxillary, 5–26 cm long panicles.^[5] Three-foliolate leaflets are elliptic or oblong-obovate, slender, canaliculate, 0.5–3.5 cm long, and grey-appressed petioles. Usually obtuse or acute to cuneate at base, entire along margins, acuminate at apex lengthy leaflet, middle leaflets 2.5–6.5×1–3.5 cm, petiolules 0.5 cm long, lateral leaflets are 2.5–4.5×1–1.5 cm the terminal leaflet sessile, 5–6.3 by 2.5–3.8 cm the lateral smaller, sessile, all glabrous above, very densely white-tomentose beneath, base tapering; common petioles 1.3–1.6 cm long.^[6]

The **stems** are usually procumbent often rooting at the nodes and annulate nodes and 2.5– 5 cm long internodes forming mats several meters in diameter and with soft hair called tomentose, fragrant **flowers** with pedicels of 0.5–1 cm long. Calyx campanulate the flowers are born in panicles with purple to violet two-lipped corollas, The **fruits** 6 mm in diameter bearing four small black seeds.^[7]

MICROSCOPIC EVALUATION

The leaflet has smooth adaxial surface 140 µm thick along the ridged part of the leaflet and 120 µm thick in between the ridges. Thick and prominent adaxial epidermis with rectangular cell and fairly thick walled subepidermal layer with 10 µm thin cuticle. The hypodermal layer is 15 µm thick. The midrib is thick. The epidermal layer is a narrow with small cells. The ground tissue of the midrib consists of circular thin-walled compact **parenchyma** cells.^[6] The vascular system of the midrib has vascular strand consists of several parallel rows of **xylem** which are about six cells angular and thick walled with narrow parenchymatous space in between the xylem rows. The adaxial is circular and collateral the abaxial epidermis is narrow with dense nonglandular **trichomes**. The **mesophyll** consists of four layers cylindrical cells. The height of these palisade and

spongy parenchyma cells reduced gradually toward the lower part.

Physical constant of *Vitex Trifolia* leaves

Table 1: Determination of physical constants.^[6]

Parameter	Value
Total ash	3.64
Water soluble ash	1.54
Acid insoluble Ash	2.46
Sulphated Ash	1.89
Alcohol soluble extractive	8.62
Water soluble Extractive	4.35
Foreign organic matter	0.1
Moisture content	4.0

PHYTOCHEMICAL CONSTITUENTS

The **plant** contains polyphenolic compounds, flavonoids, proteins, tannins, phytosterols, and saponins.^[8]

Fruits consist^[8-10] of essential oil, *Vitex trifolia* A-G, monoterpenes along with diterpenes, dihydrosolidagenone, beta-sitosterol-3-O-glucoside, terpineol, alpha-pinene, 3,6,7-trimethylquercetagenin.^[11]

Stem of *Vitex trifolia* were extracted in hexanic and dichloromethanic solvent.^[12] The methyl-p-hydroxy benzoate was reported.^[13]

Leaves and bark contain Six flavonoids^[14], 15 compound isolated are essential oil, flavones, artemetin and 7-dimethyl artemetin^[15], friedelin, and some non-flavonoids and alkaloids.

Caryophyllene is the major sesquiterpene. Vitricin, a new alkaloid, has been isolated from the plant.^[16] *V. trifolia* contains palmitic acid, ethyl-p-hydroxybenzoate 3,4-dihydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, caffeic acid, hydroxyl ethyl cinnamate, luteolin, quercetin, apigenin, casticin, and 3,6,7-trimethylquercetagenin. Phytochemical investigation of the ethyl acetate fraction afforded stigmaterol^[17], caffeic acid^[18], 7-O-glucopyranoside^[19], 3,6,4'-trimethoxy quercetin 7-O-glucopyranoside^[12] and quercetin 7-O-neohesperidoside.^[20]

Preliminary Phytochemical Screening^[6]

The preliminary phytochemical screening of three different extract of leaves with the various qualitative chemical tests revealed the presence of various phytoconstituents like carbohydrate, phenolic compounds, flavonoids, protein and amino acids, tannins, phytosterols and saponins. The results were shown in Table 2.

Table 2: Results of Phytochemical screening.

Constituents	Tests	Pet. Ether	Ethanol	Aqueous
Alkaloid	a) Mayer's reagent	-	-	-
	b) Dragendorff's reagent	-	-	-
	c) Hager's reagent	-	-	-
	d) Wagner's reagent	-	-	-
Sugars and Carbohydrates	a) Molish's reagent	-	+	+
	b) Barfoed's test	-	+	+
	c) Fehling's solution	-	+	+
	d) Benedict's test	-	+	+
Phenolic compound	a) Ferric chloride solution	-	+	+
	b) Lead acetate solution	-	+	+
	c) Pot. dichromate test	-	+	+
Flavanoids	a) Shinoda test	-	+	+
	b) Conc. H ₂ SO ₄	-	+	+
	c) NaOH test	-	+	+
Protein and amino acids	a) Biuret test	-	-	+
	b) Ninhydrin test	-	-	+
	c) Million's test	-	-	+
Tannins	a) Ferric chloride solution	+	+	+
	b) Potassium dichromate	-	+	+
Phytosterols	Salkowski reaction	-	+	-
Saponins	Foam test	-	+	-
Fixed oil and Fats	a) Spot test	-	-	-
	b) Saponification test	-	-	-

TRADITIONAL USES

It is used as anti-inflammatory in Chinese folk medicine in the treatment of headaches, colds, migraine, eye pain^[21], rheumatic pains and cancer.^[22] The leaves possess insecticidal, antibacterial activity^[23], eczema, ringworm, liver disorders, spleen enlargement, rheumatic pain, gout, abscess, antiseptic, beriberi, diuretic, febrifuge, treating sprains, contusions and swollen testicles^[24,25] and fruits an acid alkaloid and coloring matter are used in the treatment of amenorrhea, common cold, headache, watery eyes and mastitis, to control population, nervine, cephalic, and emmenagogue which stimulate blood flow in the pelvic area and uterus. *V. trifolia* is known as sambhalu in Unani medicine in the treatment of decreasing libido. The inner bark is used for dysentery, expectorant, lowering blood pressure and treating common cold, prosopalgia, chronic tracheitis, sinusitis, periodontitis and rheumatism and used in the treatment of pulmonary tuberculosis. The roots are used as anthelmintic, insecticidal, and diuretic.

PHARMACOLOGICAL STUDIES

Antiasthmatic

The extracts of the leaves of *V. trifolia* were subjected to chromatography lead to isolation of three compounds, namely Viteosin-A, Vitexicarpin, and Vitetrifolin-E. Vitexicarpin was effective in controlling asthma through stabilization of mast cells membrane and non-competitive antagonism to histamine.^[26]

Insect growth regulatory activity

Essential oil of *Vitex trifolia* castus were evaluated against fifth instar larvae of *Spilozoma obliqua* when topically on the

dorsal side of metathoracic region, for insect growth and regulatory activity caused extended period of larval and pupal, increasing in the larval mortality and adult deformity and decrease in adult emergence, fecundity of female and egg fertility of test insect.^[27]

Mosquito Larvicidal Activity of Methyl-P-Hydroxybenzoate Isolated From the Leaves

The vector-borne diseases caused by mosquitoes are one of the major health problems in many countries especially in tropical and sub-tropical countries. The resistance of mosquitoes to synthetic chemicals and environmental toxicity created by the chemicals raised the demand for finding of alternate natural molecules that control mosquito. In the present study, a crystalline compound methyl-p-hydroxybenzoate was isolated from the methanol extract of leaves and it was identified by (1)H and (13)C NMR and single crystal X-ray diffractometer. The larvicidal potential of the isolated compound was evaluated against early 4th instar larvae of *Culex quinquefasciatus* and *Aedes aegypti*. The compound exhibited 100% larval mortality of both the mosquitoes at 20 ppm with LC(50) values of 5.77 and 4.74 ppm against *C. quinquefasciatus* and *A. aegypti*, respectively.^[12]

Hepatoprotective activity

Aqueous and ethanol extracts of leaf of *Vitex trifolia* was investigated for hepatoprotective activity against carbon tetrachloride induced liver damage. To assess the hepatoprotective activity of the extracts, various biochemical parameters viz., total bilirubin, total protein, alanine transaminase, aspartate transaminase and alkaline

phosphatase activities were determined. Results of the serum biochemical estimations revealed significant reduction in total bilirubin and serum marker enzymes and increase in total protein in the animals treated with ethanol and aqueous extracts. However significant rise in these serum enzymes and decrease in total protein level was noticed in CCl₄ treated group indicating the hepatic damage. The hepatoprotective activity is also supported by histological studies of liver tissue. Histology of the liver tissue treated with ethanol and aqueous extracts showed normal hepatic architecture with few fatty lobules. Hence study revealed that *Vitex trifolia* could afford significant protection against CCl₄ induced hepatocellular injury.^[28]

Wound healing activity

The wound healing potency of ethanol leaf extracts of *V. trifolia* L. was evaluated in excision, incision and dead space wound models. It was found to possess significant wound healing activity which was evidenced by a decrease in the period of epithelialization, an increase in the rate of wound contraction, skin breaking strength, granulation tissue dry weight, hydroxyproline content and breaking strength of granulation tissue. Histopathological study of the granulation tissue also showed an increased collagenation when compared with the control group of animals.^[29]

Anti- bacterial activity

The petroleum ether and ethanol extracts of *Vitex trifolia* leaves exhibited moderate inhibiting activity against both gram-positive and gram-negative bacteria. For the evaluation of antibacterial activity, The test was done by the disc diffusion technique in which gram-positive organism like *-B. crenus*, *B. subtilis*, *Sarcina hutae*, and gram-negative microbes like *E. coli*, *Salmonella typhi*, *S. boydii*, *V mimicus* microbes were taken.^[30]

Anticancer activity

Evaluate cytotoxicity of n-Hexane fractions of *Vitex trifolia* in two cancer cell lines viz., Hep G2 and HeLa. Different concentrations of extract were tested for cytotoxicity using MTT Assay. MTT Assays showed that hexanic extract of *Vitex trifolia* were highly effective against both Hep G2 and HeLa cancer cell lines at a concentration of 80 µg/ml. This conclude that *Vitex trifolia* can be potent drug for cancer treatment and an alternative medicine.^[31] Biological assays of *Vitex trifolia* L. organic extracts have shown relevant activities. Hexanic and dichloromethanic (DCM) extracts, when prepared from **stems and foliage**, have proved to be very toxic against several cancer cell lines in culture (SQC-1 UISO, OVCAR-5, HCT-15 COLADCAR, and KB). Also, an important anti-feeding activity against the insect pest *Spodoptera frugiperda* (Lepidoptera: Noctuidae) was recorded. The hexanic extract from **leaves** completely inhibited the growth of the fungal plant pathogen *Fusarium* sp. within the first 2 days of the experiment, but dropped significantly at day 6 (15% inhibition).^[11] To evaluate the effect of Ethanol

Extract of *Vitex trifolia* against trypan blue dye exclusion assay against *Daltons ascites* lymphoma cell lines. DAL cells were injected intraperitoneally to the mice. The Ethanolic Extract of *Vitex trifolia* were administered orally for 14 consecutive days to the tumor bearing group of animals. Derived parameters, hematological parameters, packed cell volume and WBC, RBC count were measured and compared to the control group. 5-Flurouracil was used as a standard drug. The dose of Ethanolic Extract of *Vitex trifolia* decreased average increase in body weight, reduced the packed cell volume (PCV) viable tumor cell count and increased the life span of DAL treated mice and brought back the hematological parameters near to normal values. These observations are suggestive of the protective effect of extracts in Dalton's Ascitic Lymphoma (DAL).^[5]

Anti- inflammatory activity

Haze anti-inflammatory potential of an aqueous extract of *Vitex trifolia* leaves was evaluated by monitoring its effects on the modulation of cytokines, the mediators of inflammation, as well as on the expression profiles of inducible nitric oxide synthase (iNOS) which produces the free radical nitric oxide (NO). Aqueous extract of leaves showed significant dose- and time-dependent inhibitory activity on interleukin (IL)-1 beta, IL-6 and iNOS mRNA synthesis, but slight effect on tumor necrosis factor (TNF)-alpha, all of which are involved in the inflammatory response. Moreover, the plant extract seemed to induce the LPS-dependent IL-10 anti-inflammatory cytokine. These results were further confirmed by ELISA using specific antibodies to mouse IL-6, IL-10 and TNF-α.^[32, 33]

GOLD NANOPARTICLE FROM VITEX TRIFOLIA AS ANTIMICROBIAL PROPERTIES

To evaluate the antibacterial activities of these aqueous extracts were determined by disc diffusion method. From these five extract (petroleum ether, acetone, ethyl acetate, chloroform and distilled water) ethyl acetate shows strong antimicrobial effect on *E.coli*, *Acenatobactor*, *Proteus*, *Staphylococcus*, *Klebsiella*. None of leaf extract no more activity in *Pseudomonas*. Shade dried **leaves** of *Vitex Trifolia* was used for the synthesis of gold nanoparticles.^[34]

AYURVEDIC USES OF VITEX TRIFOLIA

Ring worm Infection^[35,36]

Collected leaves completely crushed and mixed with ghee. That mixture is applied on infected area then it will be cured.

For Leprosy^[37]

Vitex leaves and roots are collected and crushed immediately and that crushed material immediately filter it and collect the juice. The juice mixed with equal portion honey then boiled on burner up to the loss of water content only collect oil portion and filter it, then take orally morning and evening with full spoon. Along

with that portion is applied infected area, vatha pains, skin diseases vanished.

Blood Pitha^[38]

Vitex leaves and jasmine leaves are collected and prepared the curry with cow ghee. That curry eat two times daily morning and evening along with controls blood vomiting, blood diarrhoea, blood running nose, urinal blood and other blood related diseases eliminated.

For Tuberculosis^[30, 39]

Vitex trifolia plant was washed with water and crushed to collect the juice. To this juice mixed with honey equal amount. That mixture is poured in to the dish and boiled up to the loss of juice only collect oil content of medicament and filter it, prepared medicine is taken orally with tea spoon full daily morning and evening it will completely eradicate the TB disease.

Kapha fever^[40]

Vitex trifolia leaves are crushed to collect the juice mixed with pepper powder and that mixture was taken orally up 2-3 days immediately relieve the kapha fever.

For Vatha Diseases^[41]

Vitex trifolia leaves are crushed to collect the juice mixed with castor oil and that mixture was taken orally up 2-3 days immediately relieve the vatha diseases.

For Joint pains^[42]

Vitex trifolia leaves are taken in glass of water and boiled up to remaining cup of water and filter it and the daily one spoon honey mixed with boiled cup water consumed it immediately relieve the joint pains and sciatica pains.

Skin rashes^[43]

Vitex trifolia leaves are crushed to collect the juice and applied on infected surface of skin immediately vanished that skin rashes.

CONCLUSION

Medicinal plants are the local heritage with the global importance. World is endowed with a rich wealth of medicinal plants. Medicinal plants also play an important role in the lives of rural people, particularly in remote parts of developing countries with few health facilities. The present review reveals that *Vitex trifolia*. Overview of the plant *Vitex trifolia* Linn consist of its taxonomy, morphology, microscopy, Phytochemical studies, traditional uses, pharmacological studies and Ayurvedic uses. The findings provide scientific evidence for the traditional use of *V. trifolia* leaves (a sustainable resource) and highlight the importance of conservation of medicinal plants as resources for drug discovery. Our results together with others suggest further investigation on *V. trifolia* and constituents to develop novel treatment

strategies in immune-mediated inflammatory conditions is warranted.

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