VITEX NEGUNDO L.: PHYTOCHEMICAL CONSTITUENTS, TRADITIONAL USES AND PHARMACOLOGICAL PROPERTIES: COMPREHENSIVE REVIEW

Bansod M.S.*, Harle U.N.

A.I.S.S.M.S. College of Pharmacy, Kennedy Road, Pune-411001, Maharashtra (India).

Summary

Plants used in traditional medicine represent a priceless tank of new bioactive molecules. *Vitex negundo* L. is one of the important plant from traditional system of medicine found all over the world. *Vitex negundo* L is large aromatic shrub distributed throughout India. Plant is easily grown in light well-drained loamy soil in a warm sunny position. It has been reported to possess potent pharmacological properties like anti-inflammatory, anti-rheumatic, antibiotic, hepatoprotective, antioxidant, anticonvulsant, oxidative stress, anti-androgen, snake venome neutralization and anti-allergic activities. The various chemical constituents like flavonoids, flavone glycosides, volatile oil, triterpenes, tannins and lignin many others were identified in this plant. This review gives a bird's eye view mainly on the pharmacognostic characteristics, traditional uses, phytochemistry and pharmacological actions of *Vitex negundo* L.

Keywords: - *Vitex negundo* L., Chastetree, phytochemistry, pharmacological actions.

*Address for correspondence:

Mr. Mangesh S. Bansod
Department of Pharmacology,
AISSMS College of Pharmacy
Kennedy Road, Pune-411001
Phone: 09326406695
E-mail: mee.mangesh@gmail.com

Introduction

There exists a plethora of knowledge and information and benefits of herbal drugs in our ancient literature of Ayurvedic and Unani medicine. One of the earliest treatises of Indian medicine, the Charaka Samhita (1000 B.C.) mentions the use of over 2000 herbs for medicinal purpose. According to the WHO survey 80% of the populations living in the developing countries rely almost exclusively on traditional medicine for their primary health care needs. Exploration of the chemical constituents of the plants and pharmacological screening may provide us the basis for developing the leads for development of novel agents. In addition, herbs have provided us some of the very important life saving drugs used in the armamentarium of modern medicine. However, among the estimated 250,000-400,000 plant species, only 6% have been studied for biological activity, and about 15% have been investigated phytochemically (1,2). This shows a need for planned activity, guided phyto-pharmacological evaluation of herbal drugs.

Vernacular names

It is known as Nigod, Nirgari in Gujrathi, Sambhalu in Hindi, Bilenekki, Lakki, Nirgundi, Nukki in Kannad, Nirgud, Nirgundi in Marathi, Indrani, Nilpushpi, Nirgundi, and Sinduvara in Skanskrit. Sambhalu, fanjankisnt in Unani, Noohi in Siddha, Five – leaved chast tree in English (3).

Taxonomical Hierarchy of *Vitex negundo L (Verbanaceae*) (3)

Kingdom: *Plantae*- Plants
Subkingdom: *Tracheobionta* – Vascular plants
Super division: *Spermatophyte* – Seed plants
Division: *Magnoliophyta* – Flowering plants
Class: *Magnoliopsida* – Dicotyledons
Subclass: *Asteridae*
Order: *Lamiales*
Family: *Verbenaceae* – Verbena family
Genus: *Vitex negundo* L. – Chastetree

Geographical distribution

It is widely distributed throughout India, Sind, Ceylon, Afghanistan, Philippine Islands and Tropical Africa, Madagascar and China. Locally distributed throughout the State Maharashtra (India) along the banks of rivers; very common near the sea-coast in tidal and beach-forests in Konkan; along Deccan rivers. Habitat found to be in Waste lands and moist situations (3, 4).

Morphology

Figure 1: *Vitex negundo L*

Vitex Negundo L is large and erect aromatic shrubs grow to height 2–5 m or slender tree with quadrangular branchlets distributed throughout India. The leaves have five leaflets in a palmately arrangement, which are lanceolate, 4–10 cm long, hairy beneath and pointed at both ends, quadrangular whites fine tomentum, the leaves are 3-5 foliate, leaflets are lanceolate (5-10 cm), acute terminal leaflet (16-32 mm) with petiolate having 1-1.3 cm long, with a very short petiolate. The bluish purple flowers are numerous. The fruit is succulent, black when ripe, rounded and about 4 mm in diameter (5, 6).

Cultivation Details

Vitex Negundo L is easily grown plant; it prefers a light well-drained loamy soil in a warm sunny position sheltered from cold drying winds (7). The plants require abundant summer sunshine in order to ripen their wood fully; the well-ripened wood is more frost resistant. The leaves and stems are strongly aromatic. The flowers have a most pronounced musk-like perfume (8-10).

Propagation

Germination is usually free and quick. Prick out the seedlings into individual pots when they are large enough to handle and grow them in the greenhouse for their first winter. Plant them out into their permanent positions in early summer of the following year (11, 12).

Phytochemistry

Phytochemical investigation of this plant indicated the presence of monoterpenes agnuside, flavonoids- casticin, chryso-splenol and vitexin, flavonoids (vitexicarpin) also, 5,3'-dihydroxy-3,6,7,4'-tetramethoxyllavone and hydroxy-3,6,7,3',4'-penta methoxy flavone from the leaves (13). Seeds of *Vitex negundo* L agorded a new lignan characterized as 6-hydroxy-4-4-hydroxy-3-methoxyphenyl), 3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde by spectroscopic methods and triterpenoids (betulinic acid and ursolic acid), lignans (negundins, vitedonin), alkaloid (vitrafalal) and diterpene (vitedoin) investigated **Fig 2** (14).

Isolation of the acetoacetate fraction yielded two major lignans: 6-hydroxy-4-(4-hydroxy-3-methoxy-phenyl)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2 napthededyde and andvitedoamine A **Fig 3** (15).

Chromatography of an ethanolic extract of *Vitex negundo* L. resulted in the isolation of another new iridoid glucoside which was characterized as 6'-p-hydroxybenzoyl mussaenosidic acid with derivatives 2-p hydroxyl benzyl mussaenosidic acid (1 b), 6-p-hydroxybenzoylmussaenosidic acid (la), p-hydroxybenzoyl ester of mussaenosidic acid (2), p-hydroxybenzoyl methyl ester of mussaenosidic acid (3) **Fig 4** (16).

Sequiterpenes (17) flavone glycosides, iridoid glycosides, eurotoside aucubin (18, 19), stilbenes (20) have been isolated from roots of *Vitex negundo* L. Chasteberry is thought to contain a progesterone-like compound. The leaves of *Vitex negundo* L are known to possess various antioxidant chemical constituents like flavonoids (20), vitamin C and carotene which may have a modulatory effect on oxidative stress or endogenous antioxidants (21). Tannins were found in the aerial parts of *Vitex negundo* L; Triterpenoids present in *Vitex negundo* L and *Emblica officinalis* may involve in venom inactivation processes (22). Flavonoids are known to inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase (23) and reported to produce significant anti-inflammatory effect (24). Bioassay-guided fractionation of the chloroform-soluble extract of the leaves of *Vitex negundo L* following isolation gives the known flavone vitexicarpin, which exhibited broad cytotoxicity in a human cancer cell line (6).

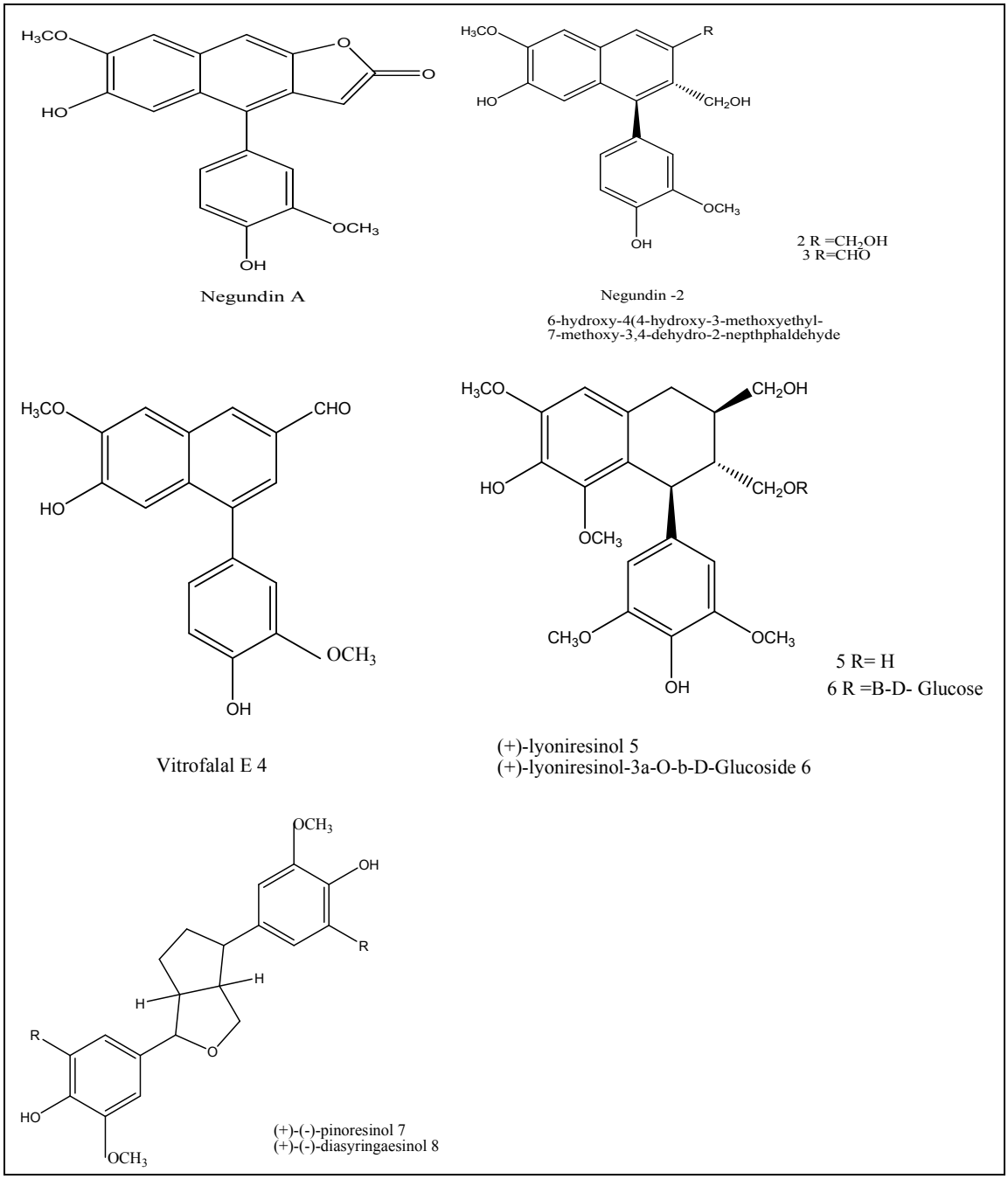

Fig 2: Structures of the lignans (Negundin A, Negundin 2, vitrofalal, lyoniresinol and pinoresinol) isolated from the roots of *Vitex negundo* L.

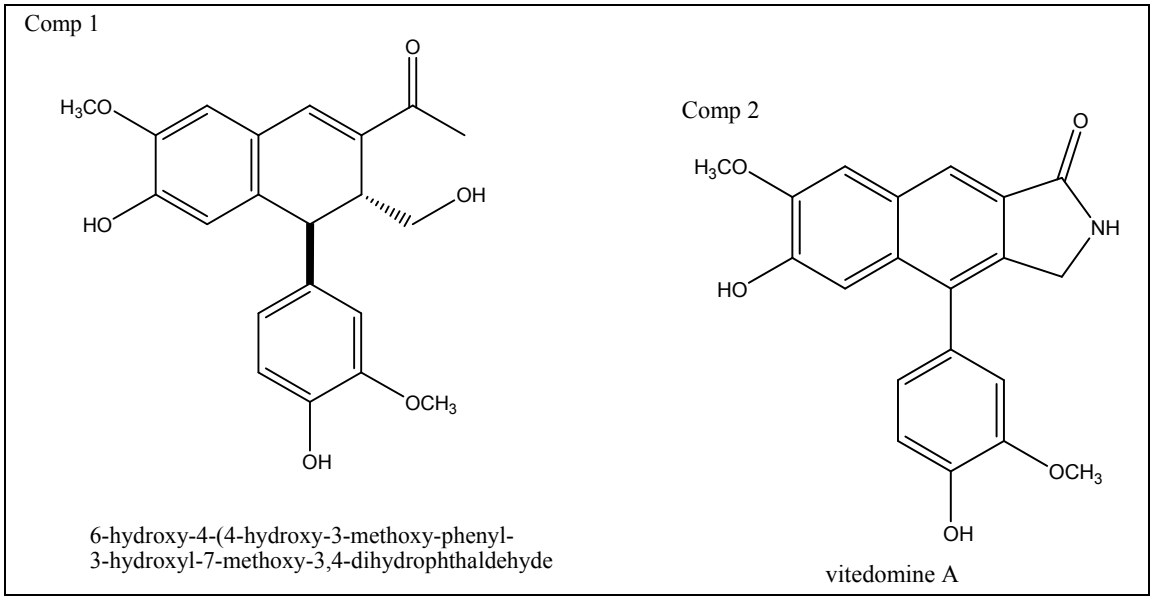

Fig 3: Chemical structure of two Isoflavonol phenylnaphthalene-type lignans isolated from *Vitex negundo* L. seeds (*6-hydroxy-4-(4-hydroxy-3-methoxy-phenyl)-3-hydroxymethyl-7-methoxy-3,4-dihydro-phthaldehyde(1) and vitedoamineA (2).*

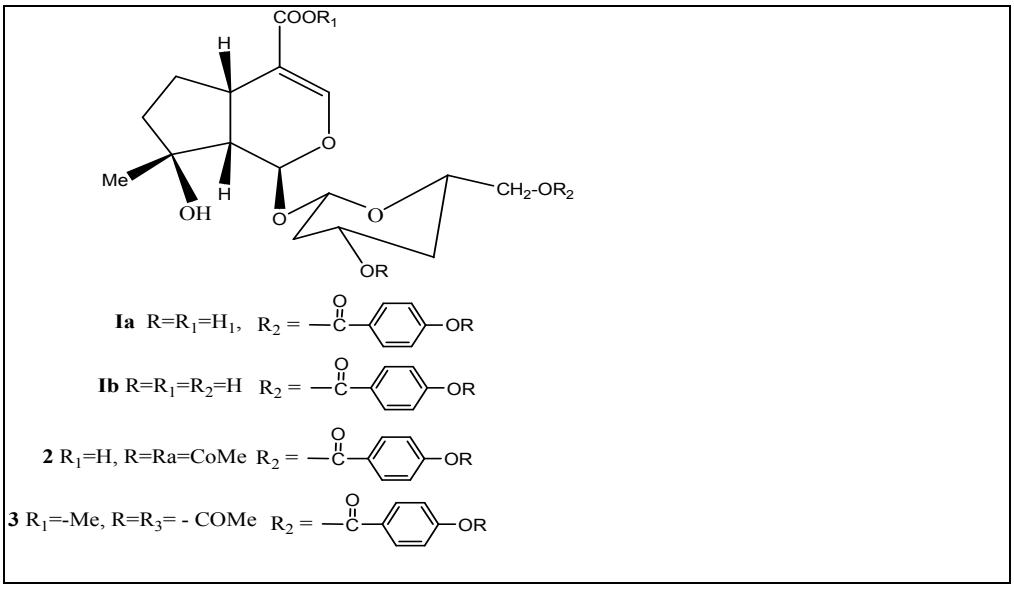

Fig 4: Structure of 6'-p-hydroxybenzoylmussaenosidic acid-an iridoid glucoside isolated from *Vitex negundo* L (2,-phydroxybenzolymussaenosidic acid (1 b), 6'-p-hydroxybenzoylmussaenosidic acid (la), p-hydroxybenzoyl ester of mussaenosidic acid (2), p-hydroxybenzoyl methyl ester of mussaenosidic acid(3)).

Pharmacological Action

Analgesic and Anti-Inflammatory Activities

The isolation of the acetoacetate fraction yielded two major lignans: 6-hydroxy-4-(4-hydroxy-3-methoxy-phenyl)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2 phthaldehyde (1) and vitedoamine A (2) (**fig. 3**). Compound 1(in fig 3) which was more productive, showed significant inhibitions on chemical nociception induced by intraperitoneal acetic acid and subplantar formalin injections and exhibited notable anti-inflammatory activities in dimethyl benzene-induced ear edema test in a dose-dependent manner. Since co-administration of naloxone fails to antagonize the analgesic activity of compound 1(in fig 3) in the formalin test, that suggest compound 1 (in fig 3) possesses potent analgesic effects which are most likely to be mediated by its anti-inflammatory activity rather than through opioid receptor system and therefore could partially explain the anti-nociceptive effect of *Vitex negundo* L. seeds in mice (15). Water extract of mature fresh leaves (MFL) of *Vitex negundo* L. (Verbenaceae) showed anti inflammatory, analgesic and antihistamine properties against carrageenan-induced rat paw oedema, formaldehyde-induced rat paw edema , tail flick test , in vivo antihistamine and in vitro prostaglandin (PG) synthesis inhibition, membrane stabilising and antioxidant activities in rats (25). It is also reported that the hydroalcoholic extract of *Vitex negundo* L. leaves, in the doses of 500 and 1000 mg/kg possess anti-inflammatory and analgesic attributed to its flavonoid contents, which are known to act through inhibition of prostaglandin biosynthesis (26-28).

Hepatoprotective activity

The ethanolic extract of *Vitex negundo* L. at 250 and 500 mg/kg doses significantly decrease Serum Bilirubin (TB), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphates (ALP) and Total Protein (TP) levels against hepatotoxicity (HT) produced by administering a combination of three anti-tubercular drugs isoniazid (INH)-7.5 mg/kg, rifampin (RMP)-10 mg/kg and pyrazinamide (PZA)-35 mg/kg (29). Also alcoholic extract of the seeds of *Vitex negundo* L. showed the hepatoprotective action against carbon tetrachloride-induced liver damage. The extract was found to be effective in preventing liver damage which was evident by morphological, biochemical and functional parameters (30).

Antioxidant Activity

The antioxidant potency of *Vitex negundo* L was investigated by employing various established *in vitro* systems, such as 2,2′-azino-bis 3-ethyl benzothiazoline-6-sulfuric acid (ABTS*+)/Lipid Peroxides (LPO)/Superoxide/Hydroxyl radical scavenging and iron ion chelation. All the fractions of *Vitex negundo* L exhibited a potent scavenging activity for (2, 2′-azino-bis 3-ethyl benzothiazoline-6-sulfuric acid) ABTS radical cations in a concentration dependent manner, showing a direct role in trapping free

radicals. This property may be attributed to the presence of polyphenolics and flavones in the *Vitex negundo* L. The inhibition of lipid peroxidation may be either due to chelation of Fe or by trapping of the Free Radicals. The polar fractions of *Vitex negundo* L. possess potent antioxidant properties, which may be mediated through direct trapping of the free radicals and also through metal chelation (31). Tondon and Gupta (2005) have also reported similar antioxidant properties of *Vitex negundo* L. in rats, by using ethanol induced oxidative stress model (32).

Effect of *Vitex negundo* on Oxidative Stress

The leaves of *Vitex negundo* L. are known to possess various antioxidant chemical constituents like flavonoids, vitamin C and carotene, which might possibly be responsible for the reduction of lipid peroxidation production. As there are several measurement of malondialdehyde (MDA) by the Thiobarbituric acid (TBA) method, Glutathione (GSH), Glutathione S – transference (GST), Glutathione perixodise (GPx), Glutathione reductase (GR) and catalase along with (MDA) and superoxide dismutase(SOD) are evaluated effect of Vitex negundo on oxidative stress (32).

Anticonvulsant Activity

The antiepileptic activity of hydrophilic extract of *Vitex agnus castus* fruit was evaluated by the kindling model of epilepsy. Rats were stereotaxically implanted with a tripolar and two monopolar electrodes in amygdala and dura, respectively. The after discharge (AD) threshold was determined in each animal and stimulated daily until fully kindled. *Vitex agnus* can reduce or prevent epileptic activity as demonstrated by reduction of ADD and S5D (length of convulsion) in a dose dependent manner (33). Also it is reported that *Vitex negundo* L. leaf extracts showed anticonvulsant activity against maximal electroshock seizures (MES) in albino rats and pentylenetetarazole (PTZ) induced seizures in albino mice. Showed 50% protection in clonic seizures and 24-hour mortality against PTZ induced seizures. It also decreased number and duration of convulsions significantly. *Vitex negundo* L. potentiated anticonvulsant activity of valporic acid (34).

Antibacterial studies

Essential oils and successive ethyl acetate and ethanol extracts of *Vitex negundo* L. showed antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* bacterial strains. Main constituents identified in leaves oil were d-guaiene, carryophyllene epoxide and ethyl-hexadecenoate; in flowers oil - a-selinene, germacren-4-ol, carryophyllene epoxide and (E)-nerolidol while fruit oil showed β -selinene, a-cedrene, germacrene D and hexadecanoic acid as the main constituents which help for antibacterial activity (35).

Antiandrogenic effects

The ability of the flavonoid-rich fraction (5, 7, 3'-trihydroxy, 6, 8, 4'-trimethoxy flavones) of *Vitex negundo* L. seeds antagonized the androgen action of exogenous testosterone propionate on the male reproductive system in castrated prepubertal and intact adult dog. Treatment with F (5, 7, 3'-trihydroxy, 6, 8, 4'-trimethoxy flavones) caused disruption of the latter stages of spermatogenesis. The epididymides were devoid of spermatozoa. Protein, sialic acid and RNA contents of the testes and epididymides were reduced significantly. Reduced androgen production was reflected in low levels of sialic acid in testes and epididymides. Castration alone brought about reduction in size and weight of the epididymides whereas castration followed by F (5, 7, 3'-trihydroxy, 6, 8, 4'-trimethoxy flavones) treatment caused a reduction in the cellular heights of epididymides. Simultaneous administration of testosterone propionate to F-treated intact adult and castrated prepubertal dogs maintained the viability of spermatozoa, kept epididymal physiology relatively normal and significantly increased the cellular heights of epididymides (36).

Antiallergic Activity

Ethanolic extract of *Vitex negundo* L. showed antiallergic activity against immunologically induced degranulation of mast cells better than that with compound 40/80. It also inhibited edema during active paw anaphylaxis in mice, effects on mediator release and smooth muscle contractions of sensitized and non-sensitized guinea pig trachea using antigen and compound 48/ 80 respectively. The extract significantly inhibited both the initial and later sustained phases of tracheal contractions. The initial phase was primarily due to histamine release which was blocked by the extract (confirmed in guinea pig ileal studies). The latter phase was due to release of lipid mediators from arachidonic acid. Inhibition of the latter phase may be secondary to inhibition of arachidonic acid by the ethanolic extract (37, 38).

Snake venom neutralization activity

The methanolic root extracts of *Vitex negundo* L. and *Emblica officinalis* Gaertn. Showed antisnake venom activity. The plant (*Vitex negundo* L. negundo and *Emblica officinalis*) extracts significantly antagonized the Vipera russellii and Naja kaouthia venom induced lethal activity both in in vitro and in vivo studies. Vipera russellii venom-induced haemorrhage, coagulant, defibrinogenating and inflammatory activity were significantly neutralized by both plant extracts. No precipitating bands were observed between the plant extract and snake venom (39).

Acute Toxicological Studies

The acute toxicity study was carried out by administering *Vitex negundo* L. leaf extract. LD_{50} of the extract was determined by histomorphological changes in vital organs of rats. The rats were observed continuously for two hours, then occasionally

for a further four hours for any mortality onset and for the severity of any toxic sign. The stomach showed no histomorphological changes in any of the doses of the extract. *Vitex negundo* L. which is known to act by prostaglandin inhibition may be expected to cause gastric damage but on the contrary it produced no histomorphological changes in the stomach even in toxic doses (40).

Uses in western herbal medicine

Modern medical world with *Vitex* began with the introduction of concentrate extracts of vitex fruits in the 1950. From 1943 to 1997, approximately -32 clinical trial were conducted on a propriety *Vitex agnus* berry product (Angolut, Madus, Gernany) for evaluating its effectiveness in treating PMS, mastitis and fibrocystic diseases, monopuasal symptoms ,poor lactation, hyperprolactinomia, uterine bleeding disorder, and menstrual irregularities . German commission E approved the use of *Vitex agnus* costus fruits for irregularities of the menstrual completes and mastodynia. T. Michal murrey and Joseph Pizzorno have quoted one study which shows that *Vitex agnus* castus extracts lowers prolactin level in men at a dose of 400 mg/day (male with hyperprolactenemia frequently face impotence) (41). In homoeopathic medicine, vitex agnus is used for various sexual debilities- marked depression of vital power, premature old age with apathy, self contempt for the sexual abuse nervous debility in unmarried person feeble errection without sexual desire, emission of prolactic fluid when straining at stool, cold, hard, swollen, painful testicle (42). In general practice ,the drug is prescribes to female for leukemia staining yellow suppressed menses, slangy or suppressed breast milk , inflammation of uterus(4). The flowers are astringents and used in fever, diarrhea and liver complaints. The fruits are prescribed in headache catarrh and watery eyes when dried .They is consider vermifuge. They are much valued medininally in china. An aquqes exstract of the fruits was found to be good analgesic action (4). In Philipins – the seed are reported to eaten after boiling. The young shoots are used in the basket making .The ash of the plants is source of patasium , corbonate or peer ash and is reported to be used as an alkaqli in drying (4).

Medicinal Uses

Leaves: The leaves *Vitex negundo* L are Antibacterial; Antitumor, Astringent, Febrifuge, Sedative, Tonic and Vermifuge (54). They are useful in dispersing swellings of the joints from acute rheumatism, and of the testes from suppressed gonorrhea (54). The juice of the leaves is used for removing foetid discharges and worms from ulcers, whilst oil prepared with the leaf juice is applied to sinuses and scrofulous sores (54). They are harvested in early summer and used fresh or dried. Extracts of the leaves have shown bactericidal and antitumor activity. Leaves-anti-parasitical, alterative, aromatic, vermifuge, pain reliever. Leaves are insect repellents. It is also useful for rheumatic swellings. Extracts of the leaves have insecticidal activity (55). The fresh leaves are burnt with grass as a fumigant against mosquitoes (58).

Table 1: some common, ethnomedicinally important species of genus Vitex

Name	Uses	Distribution
Vitex agnus-castus	Premenstrual syndrome (PMS) treat pain, swelling, inflammation, headaches, rheumatism, and sexual dysfunction	Mediterranean and western Asia. It is now cultivated all over the world, including the southern part of the United States (43).
Vitex trifolia L	Fungicidal, bactericidal, cytotoxic and insecticidal	India and Mexico (44).
Vitex rehmannii Guerke	Treat hysteric fits	Zulu Africa (45).
Vitex wilmsii Guerk	Bark infusions are taken as purifying emetics by adults when a kraal member is dying. treat hysteric fits	Zulu (45).
Vitex negundo var. cannabifolia (Sieb. et Zucc.) Hand.-Mazz.	Diarrhea, stomachache, asthma, detoxification	Guangdong southeast coast of China, Hong Kong (46).
Vitex rehmannii	Enema for stomachache	Zulu, Africa (47).
Vitex rotundifolia	Upper respiratory infection	Korea (48).

Stem: A decoction of the stems of *Vitex negundo* L is used in the treatment of burns and scalds (55).

Fruit: The dried fruit of *Vitex negundo* L is vermifuge (54). The fruit is also used in the treatment of angina, colds, coughs, rheumatic difficulties etc (55). The fresh berries are pounded to a pulp and used in the form of a tincture for the relief of paralysis, pains in the limbs, weakness etc (56). Fruit-nervine, cephalic, emengogue, dried fruit-vermifuge employing an aqueous extract from the fruit, a 1979 study reported good results on premenstrual water retention. Women were able to sustain a good level of milk production for breast feeding while taking this herb. While it took some time for the drug to take effect, the women were able to continue the use of the drug for months without harmful side effects.

Root: The root of *Vitex negundo* L is expectorant, febrifuge and tonic (54). It is used in the treatment of colds and rheumatic ailments (55). It is harvested in late summer and autumn, and dried for later use. Roots are tonic, febrifuge, expectorant, diuretic. The leaves are used to repel insects in grain stores (57). Root juice is said to increases the growth of hair. Decoction of leaves may improve eyesight.

Edible Uses

Seed of *Vitex negundo* L occasionally used as a condiment, it has pepper substitute. When washed to remove the bitterness it can be ground into a powder and used as a flour, though it is very much a famine food used only when all else fails .A tea is made from the roots and leaves (59).

Therapeutic uses mentioned in Ayurvedic Pharmacopoeia

Sul, Sopha, Vatavyadhi, Amavata, Kustha, Kandu, Kasa, Pradara, Adhmana, Piniroga, Gulma,Aruci,Karma,Vrana Nadi Vrana, Karnasula, Sutika, Jwara (60).

Discussion

Medicinal plants have provided copious leads to combat diseases, from the dawn of civilization. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs (61, 62). The extensive survey of literature revealed that *Vitex negundo* L. is important medicinal plant with diverse pharmacological spectrum. The plant shows presence of many chemical constituents, which are responsible of the various activities of the plant such as anticonvulsant effect , CNS-depressant activity ,antiarthritic effect , antiallergic activity were reported in literature. *Vitex negundo* L. embibing a tremendous potential deserves a special attention of the scientific fraternity to emerge as a milestone for medical science of this millennium due to its various medicinal uses. Further evaluation needs to be carried out on *Vitex negundo* L. in order to explore the concealed areas and their practical clinical applications, which can be used for the welfare of the mankind.

Table 2: Region-wise ethnomedical uses of *Vitex Negundo* L.

Part	Ethno medical uses	Region	Reference
Leaves (Samalu)	Leaves (50 g) with equal amount of *Azadirachta indica* leaves are boiled in water and legs are warmed in that water to cure encephalitis.	Melghat, Amaravati (Maharastra)	(49).
Leaves	leaves of *Vitex negundo* are burned to repel mosquitoes from houses.	Southern India	(50).
Fresh leaves	Juice of fresh leaves was applied externally to treat joint pain.	Satpuda hill in India	(51).
Leaves	Leaf juice useful in eye pain, Leaves boiled in urine of cow and applied on sprains and swellings.	Jaunsar-Bawar Utter Pradesh,	(52).
Leaves and roots	Skin aliments Urticaria- The crushed form is applied on the skin and extracted juice is orally taken. Cellulitis- The paste (crushed form) is layered on the infected place. Abscess Carbuncle - The paste is layered on the boils Abscess. The juice extracted from the crushed part is mixed with the oil extracted from Sesamum indicum and applied on the skin. Eczema - The paste is mixed with Sesamum indicum and then layered on the infection.	Assamese people	(53).

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