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Ethnopharmacological and Biotechnological Significance of *Vitex*

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ABSTRACT

Vitex (Verbenaceae) is a large genus that has a plethora of ethnopharmacological uses. The various species of *Vitex* have been used to treat a range of human ailments, particularly related to insects, fungi, bacteria, snakes and poisonous spiders and diseases associated with menstruation and gynaecological problems. Several secondary metabolites like flavonoids, iridoid glycosides, terpenoids and labdane diterpenes have been reported in different species of *Vitex. Vitex trifolia* and *Vitex negundo* can be propagated vegetatively for cultivation on desecrated lands to produce huge biomass for commercial applications. This review emphasizes the phytochemical and ethnobotanical knowledge on some species of *Vitex* to highlight their traditional and modern usage.

Keywords: antidote, biomass, conservation, genetic diversity, micropropagation, RAPD

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HISTORICAL SIGNIFICANCE

The genus *Vitex* consists of over 270 species, predominantly trees and shrubs, and is restricted to tropical and subtropical regions, although a few species are also found in the temperate zones. Common names of some of the most common *Vitex* species are listed in **Table 1**. Ancient civilizations valued *Vitex* highly for treating many health problems and used its wood for making furniture. According to Pliny, the great writer and compiler on natural history "*the seed extract taste like a wine when a drink is made of them and this was taken to reduce fevers and stimulate perspiretion*". The drink is used in a similar manner today, mainly in European herbalism to promote menstruation and lactation in women. Since ancient times, the plant *Vitex* has been associated with sexual passion, while the seeds were taken to dispel "wind" or flatulence from the bowels, to promote urine and check diarrhoea. It is also immensely beneficial in dropsy and spleen related diseases. The blossom and tender shoots mixed with rose oil is used for headache caused due to intoxication. Finally, an interesting application, though perhaps not so useful today is "It is said that those who keep a twig in their hand or in their girdle do not suffer from chafing between the thighs" (www.tidesoflife.com/*Vitex*.htm). The seeds are also used as an antidote against bites of spiders and snakes (Staden 1939).

Vitex was given as much notice as most other herbs, and it must have been well known in England before 1500 A.D., since Banckes quotes "that it destroys dropsy and cures lethargy". In addition, the herb is said to be good to defy the hardness and stopping of the milt [spleen]. After the early 1700, *Vitex* lost popularity in England where it was not rediscovered until it gathered new interest in the mid 19th century as a herb for female reproductive imbalances. Modern interest in *Vitex* began in Germany, in 1938, when Dr. Madaus, one of the greatest Renaissance herbalists, gathered herbal and folk uses from ancients and professional herba-

Table 1 Common names of selected *Vitex* species.

Different species of *Vitex*	Common names
Vitex agnus-castus	Hemp tree
Vitex rotundifolia	Beach *Vitex*
Vitex negundo	Chinese chaste tree
Vitex diversifolia	--
Vitex cymosa	Taruma Guazu
Vitex megapotamica / Vitex polygama	Taruma
Vitex ovata	Beach *Vitex*, Pohinahina
Vitex glabrata	Blackberry tree
Vitex mollis	Uvalama tree
Vitex doniana	Black plum
Vitex lucens	Puriri
Vitex peduncularis	--
Vitex altissima	Milla
Vitex leucoxylon	Five-leaved chaste tree

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lists and wrote them down in his famous Herbal. His information tends to be fanciful yet pragmatic. On the fanciful side, he considers that *Vitex* will have the same effect whether it is taken in powdered form, tea or the leaves be carried about the body. As is usual throughout this period, Madaus mentions that it is the remedy for those who would live chaste. On the practical side, he also extols it as a cure for "windiness of the stomach", or flatulence. Those who drink an infusion of the fruits in wine can also expect that it can cure the liver and spleen disorders. Madaus also encourages its use as a "female" herb. The seed and leaves are good against pain and inflammations of the uterus, and that the seed drunk with pennyroyal will bring on the menstruation, and as a poultice can cure a headache.

Madaus, who developed a patent medicine from an extract of dried *Vitex* fruits, conducted the first scientific research on the plant's effects on the female hormonal system. This medicine trade marked as "Agnolyt" has subsequently been used in almost all scientific studies on *Vitex*. The fruits were found to have 1.3-1.6% yield of essential oil, 22% sabinene, 20% 1.8-cineole, and 6% α-pinene. The essential oil has an antibacterial activity (Kastrak *et al.* 1992). They also contain flavonoids and iridoid glycosides (Kuruuzum-Uz *et al*. 2003). The iridoid glycosides have recently been quantified and consist of 0.3% acubin, 0.6% agnoside and 0.07% eurostoside (Azarnia *et al.* 2007). No individual constituent of *Vitex* has been shown to have an intrinsic hormonal activity and the chemical composition responsible for its action has not yet been elucidated.

Vitex was official in some European pharmacopeias, including the influential first *Pharmacopoeia Londinensis* (Urdang 1618) but quickly was dropped from official status and by 1733, Alleyne, in his *New English Dispensatory*, reporting on official drugs, could only say, "not now in esteem, or scarce ever made or used in the shops".

ETHNOPHARMOCOLOGICAL IMPORTANCE

The belief of the ancients that it was efficacious to quell excess sexual passions was often quoted, but it was not much used in medical practice by "the moderns". James, in his *Pharmacopeia Universalis* (1747), asserts that the common belief of the current practitioners was that the herb was only repressive to the passions in people. It is interesting that many of the energetic properties of *Vitex* and other herbs were ascribed to them, in some cases quite close to ones given in current Traditional Chinese Medicine.

One of the most cited studies of the pharmacological effects of *Vitex* was carried out by Haller at the University of Gottingen in the early 1960s. Female Guinea pigs were given *Vitex* tincture orally at normal to high dose for 90 days (Mediherb Pvt Ltd. 1989). At the end of this time the animals were examined for any changes in organ structure or weight. It was concluded that at normal doses *Vitex* clearly demonstrated a decrease of oestrogen effects and an increase of progesterone levels. This effect was mediated by the pituitary gland. Follicle stimulating hormone secretion was decreased and simultaneous increase in the lutenizing hormone and prolactin hormones was observed. Consistent with this hypothesis, corpus luteal development and glandular proliferation in breast tissue were enhanced whereas follicular development and uterine weight were slightly decreased. *Vitex* has been traditionally used to treat a number of ailments, but with particular emphasis on menstrual disorders and related hormonal problems. These are all situations that indicated corpus luteum insufficiency and suboptimal ovarian function. This is usually due to the abnormal low progesterone levels three weeks after the onset of menstruation (serum progesterone below 10-12 ng/ml). This state is normal during puberty and at menopause, but it is considered abnormal when occurring in women between ages 20 to 40 years. This includes pre-menstrual syndrome (Loch *et al*. 2000), polymenorrhea, an ovulatory cycle, secondary amenorrhoea, infertility and hyperprolactinemia (Daniele *et al.* 2005). *Vitex leucoxylon* L. (Verbenaceae) is a deciduous (large and lofty) tree rarely found in tropical forests. It possesses anti-inflammatory and antibacterial pro-perties (Kapoor and Kapoor 1980). All parts of the plant are useful. The hot water extract of the fruits is used as a vermifuge while the dried roots are used asexpectorant, astringent and febrifuge (Chopra *et al.* 1956; Ambastha 2000). Various phytochemical studies on this plant have revealed the presence of flavonoids, iridoids, and sterols (Sarma *et al.* 1990; Krishnarao *et al.* 1997). Another species, *Vitex altissima*, possesses white flowers, tinged blue or violet. The leaf extract is used against fungal infections and in inflammatory conditions. Flavonoids have been reported from the leaves of this plant. The antimicrobial properties have been reported by Ganapaty *et al.* (2005). The methanolic extract of *V. leucoxylon* tested positive for Liebermann Bauchard's and Shinoda's tests confirming the presence of sterols, triterpenes and flavonoids. Antibacterial and antifungal properties of leaf and bark were observed by the agar cup plate method, and compared with reference standards *viz.*, chloramphenicol and nystatin apart from measuring the diameter of zone of inhibition. These activities were attributed due to the presence of sterols and iridoids. The methanolic extracts of *V. leucoxylon* leaf exhibited good antibacterial activity against Gram-positive (+ve) and -negative (-ve) bacteria, but for *Bacillus subtilis*, it was low compared to other bacteria. The response was found to be dose-dependent whereas in the case of stem bark it was reported to be active against all bacteria tested (Ganapaty *et al.* 2005).

The ethanolic extract of *V. negundo* leaves resulted in the isolation of a new flavone glycoside along with five known compounds which were evaluated for their antimicrobial activities. The new flavones were found to have significant antifungal activity against *Trichophyton mentagrophytes* and *Cryptococcus neoformans* (Sathiamoorthy *et al.* 2007). The antioxidant potency was investigated by employing various established *in vitro* systems, such as 2,2-azino-bis 3-ethyl benzothiazoline-6-sulfuric acid, lipid peroxides (LPO), superoxide, or hydroxyl radical scavenging and iron ion chelation. Therefore, its reported anti-inflammatory properties could be due to the down regulation of the free radical-mediated pathway of inflammation (Tiwari and Tripathi 2007). The plant extract was also used as a botanical insecticide against the rice leaf folder (*Cnaphalocrocis medinalis*) by indirectly acting as a strong enzyme inhibitor (Nathan *et al.* 2006). When water and 80% ethanol extracts of *Vitex* sp. were used to treat AIDS and for their HIV type 1 reverse transcriptase inhibitory activity, the water extracts of *V. glabrata* (branch), *V. trifolia* (aerial part) and *V. negundo* (aerial part) showed HIV-1 RT inhibition ratio higher than 90% at 200 g/ml (Woradulayapinij *et al.* 2005).

Anti-inflammatory, analgesic and antihistamine properties of mature fresh leaves of *V. negundo* were claimed in Ayurveda medicine by orally treating water extract of the leaves to rats. Flowering of the tree did not abolish the analgesic and anti-inflammatory activities of the leaves. These observations revealed that the fresh leaves of *V. negundo* have anti-inflammatory, pain suppressing, antihistamine, membrane-stabilizing and antioxidant activities. The antihistamine activity can produce the anti-itching effect claimed in Ayurveda medicine (Dharmasiri *et al.* 2003). Xanthine oxidase inhibitory activity was assayed for the methanolic and water extracts which showed an *in vivo* hypouricaemic activity against potassium oxonate-induced hyperuricaemia in mice (Umamaheswari *et al.* 2007). The methanolic root extract of *V. negundo* was explored for the first time for antisnake venom. The plant extracts significantly antagonized the *Vipera russellii* and *Naja kaouthia* venom-induced lethal activity both in *in vitro* and *in vivo* studies. *V. russellii* venom-induced haemorrhage, coagulant, defibrinogenating and inflammatory activity was significantly neutralized by using the plant extract. No precipitating bands were observed between the plant extract and snake venom. The above observations confirmed that the plant extracts possess potent snake venom neutralizing

Table 2 Ethno-botanical/pharmacological importance of the genus *Vitex* in general and its ethno-botanical usage.

Name of the disease	Symptoms	References
Premenstrual syndrome (PMS)	Cyclic mood swings after menopause, sore breasts, bloating, fatigue and psychological changes such as increased appetite, sweet cravings, nervousness/ restlessness, anxiety, depression, lack of concentration, headaches, sweet cravings, palpitations and dizziness.	Brown 1994; Lauritzen *et al.* 1997; Berger *et al.* 2000; Huddleston and Jackson 2001; Atmaca *et al.* 2002; Wuttke *et al.* 2003; Daniele *et al.* 2005; Prilepskaya *et al.* 2006; Yuan *et al.* 2007; Rapkin *et al.* 2008
Abnormal menstrual cycle	Secondary amenorrhoea, menstrual irregularity, cystic hyperplasia, dysfunctional bleeding.	Probst and Roth 1954; Westphal *et al.* 2004; Daniele *et al.* 2005; Chamandoosti, 2007; Chowdhury *et al.* 2008
Breast feeding	Increased milk flow and ease of milk release.	Brown 1994; Miller *et al.* 1998; Schellenberg 2001; Artz 2006; Chamandoosti 2007
Infertility	Normal prolactinaemia but showed pathologically low serum levels at day 20 of the menstrual cycle. Shortening of the luteal phase and a positive change in the LHRH test dynamic, decreased corpus luteal function.	Mediherb News Letter 1994; Das *et al.* 2004; Westphal *et al.* 2004; Artz 2006
Hyperprolactinemia	Reduction of Prolactin release, shortened luteal phases and deficits in progesterone production.	Brown 1994; Halaska *et al.* 1999; Loch *et al.* 2000; Wuttke *et al.* 2003; Daniele *et al.* 2005; Hu *et al.* 2007; Tamagno *et al.* 2007
Menopause	Hot flushes and menstrual irregularities such as flooding, clotting and irregular cycle, mood savings depression.	Taylor 2001; Carmichael 2007; Hu *et al.* 2007
Acne	Menstrual cycle abnormalities.	Probst and Roth 1954; Mediherb Newsletter 1994; Berger *et al.* 2000; Daniele *et al.* 2005

capacity and need further investigation (Alam and Gomes 2003). The petroleum ether and ethanol extracts of *V. trifolia* leaves exhibited moderate inhibiting activity against both $Gram^+$ and $Gram^-$ bacteria (Hossain *et al.* 2001).

Biological assays of *V. trifolia* organic extracts have shown relevant activities. Hexanic and dichloromethanic extracts have proved to be very toxic against several cancer cell lines and antifeeding activity against the insect pest *Spodoptera frugiperda* (Lepidoptera: Noctuidae). The hexanic extract of leaves, completely inhibited the growth of the fungal plant pathogen *Fusarium* sp. (Hernández *et al.* (1999). The alcoholic extracts and hexane extracts of *V. trifolia* selected on the basis of medicinal folklore for asthma treatment in Indonesia were studied for their activity in inhibiting histamine release from RBL-2H3 cells (rat basophilic leukemia cell line), a tumor analog of mast cells. The inhibitory effects were found to be more than 80% for extract concentrations of 0.5 mg/ml. The extracts contain active compounds that inhibit mast-cell degranulation and provide insight into the development of new drugs for treating asthma and/or allergic disease (Ikawati *et al.* 2001). *V. rotundifolia* and *V. trifolia* were reported to be the strongest emitters of methyl chloride. In *V. altissima*, the leaf extract showed moderate activity against both $Gram^+$ and $Gram^-$ bacteria, but at 50 mg/ml, it showed no antifungal activity against *Aspergillus niger*. Overall it shows considerable anti-microbial activities (Ganapaty *et al.* 2005).

In medical applications, *Vitex* has been used in different features summarized in **Table 2**.

DIFFERENT SPECIES OF *VITEX*

There are many species of *Vitex*, which have medicinal and phytochemical importance, out of them, 15 are mostly explored in various studies, hence they are described in **Table 3**: *Vitex agnus-castus*, *V. rotundifolia*, *V. negundo*, *V. diversifolia*, *V. cymosa*, *V. glabrata*, *V. megapotamica*, *V. mollis*, *V. limonifolia*, *V. doniana*, *V. lucens*, *V. polygama*, *V. peduncularis*, *V. altissima* and *V. leuxoxylon*.

VITEX: SAFETY, RISKS AND SIDE EFFECTS

Good quality chaste berries have a strong, warming taste somewhat akin to black pepper. However, a number of *Vitex* species used in Ayurvedic and Chinese medicine do not possess this characteristic and, therefore, may not be effective for the purposes mentioned here. For ensuring quality, taste is really the best test. Even a small taste from your tablet, tincture, or capsule will tell you very quickly if the herbal product possesses the characteristic pungency. *Vitex agnus-castus* (chastetree) is exceptionally safe. In one study, chastetree berries was given up to nine years with very few side effects. It is, however, not advisable for pregnant women. Studies aimed at studying interactions with hormone replacement therapy, animal studies and human data have reported that chastetree berry constituents affect endocrinal activity that may alter the effects of medications and possibly the dose needed for treatment (www.ovarian-cysts-pcos.com/*Vitex*.html). In a recently conducted systematic review of adverse events of chastetree used as single treatment, it was found that side effects potentially caused by *V. agnus-castus* were mild and reversible (Daniele *et al.* 2005). The most frequently cited adverse events include: nausea, mild gastrointestinal complaints, fatigue, menstrual disorders, dry mouth, acne, pruritus and erythematous rash, mild digestive upset or skin rash, rapid heartbeat, hair loss, headache, itching and bleeding between periods. *Vitex* may decrease the effectiveness of oral contraceptives or female hormone replacement. It could also theoretically increase the risk of side effects. There was one report of a case of nocturnal seizures in a patient taking a combination of herbs that included chastetree, however, it is unlikely that *Vitex* was the causative agent. People with hormone dependent conditions such as endometriosis, uterine fibroids, and cancers of the breast, ovaries, uterus or prostate should not take *Vitex*. *Vitex* is not recommended during pregnancy (Lucks *et al.* 2002). Small amounts of *Vitex* could increase the production of breast milk in post-partum women. High doses may have the opposite effect and decrease the production of breast milk. *Vitex* may affect levels of the neurotransmitter dopamine (Roemheld-Hamm 2005). People with Parkinson's disease, schizophrenia, or any other condition in which dopamine levels are affected should avoid *Vitex* unless under the supervision of a qualified health professional. Some of the drug-herb interactions are listed below.

INTERACTIONS WITH ORAL CONTRACEPTIVES AND DOPAMINE AGONISTS

Experimental data on animals and human clinical studies have reported that the phytocomponents of *Vitex* exhibit hormonal activity and may alter the pharmacological effects of hormonal medications like norethindrone, ethynodiol diacetate, norgestrel, norgestimate, ethinyl estradiol, drospirenone, desogestrel, levonorgestrel, and possibly necessitate dose adjustments to derive clinical benefit. An *in vitro* study reported that chasteberry constituents possess dopaminergic activity that is able to modify the effects of medications like selegeline, amantadine, carbidopa, levadopa, pramipexole, ropinirole, bromocriptine, pergolide. Patients who are taking any of these medications should consult their physician before taking *Vitex*. The emerging consensus is that dopaminergic effects of *Vitex* may be partly responsible for its prolaction-inhibiting actions (Williamson 2006). Several

Table 3 Distribution, phytochemical constituents, and economic importance of different species of *Vitex*.

***Vitex* sp.**	**Distribution**	**Phytochemical(s)**	**Medicinal/economic importance**	**References**
V. rotundifolia	Sea coast in Asia	Phenylnapthalene	Antibacterial	Kawazoe *et al.* 2001
		Phenylnapthalene; Polymethoxyflavonoids	Leukamia, anticarcinogenic and antimutagenic	Ko *et al.* 2000
		Diterpenoids	Mosquito repellent	Ono *et al.* 1997
		Veterinary crude drugs	Pest control	Sudarsanam *et al.* 1995
		Rotundiferan		Rahman and Bhattacharya 1982; Epila and Ruyooka 1988; Watanabe *et al.* 1995
		Iridoid and phenolic glucoside		Kouno *et al.* 1988
		Prerotundifuranne and rotundifuranne		Asaka *et al.* 1973; Kondo *et al.* 1986
		Perrotundiferan; aucubin; 2^1,3^1,5-trymethoxyflavone (Vx-1); vitexicarpin (Vx-5); artemetin (Vx-6); dialdehyde rotundial; mussaenosidic acid		Hänsal *et al.* 1965
		Lignans and agnuside		Hu *et al.* 2007
V. agnus-castus	Asia and Mediterranean	2',7'-dichlorofluorescin diacetate		Wuttke *et al.* 2003; Liu *et al.* 2004; Ohyama *et al.* 2005
		1,8-cineole, sabinene		Galletti *et al.* 1998
		α-pinene; β-farnenes; β-caryophyllene, α-terpinenyl acetate	Secondary metabolite	Sorensen and Katsiotis 2000; Baser 2002
		6 β, 7 β-diacetoxy-13-hydroxy-labda-8,14-dienerotundifuran	Prolactin secretion	Artz 2006
			Postpartum	Stevinson and Ernst 2001
		Vitexilactone	Anti-oxidant	Kondo *et al.* 1986
		Linoleic acid	Estrogen receptors	Liu *et al.* 2004
		Clerodadienols	Premenustrual	Wuttke *et al.* 2003
		Luteolin (6-C-(4^{11}-methyl-6^{11}-*O-trans*-caffeoylglucoside); luteolin 6-C-(6^{11}-*O-trans*-caffeoylglucoside; luteolin 6-C-(2^{11}–*O-trans*-caffeoylglucoside); luteolin-7-O-(6^{11}-*P*-benzoylglucoside); 5,4^1-dihydroxy-3,6,7,3^1, tetramethoxyflavone artemetin and isorhamnetin, vitexlactan A; 6 β-acetoxy-9 α-hydroxy-13 (14)-labden-16,15-amide; iridoid glycosides	Tumor inhibition or apoptosis	Hirobe *et al.* 1997
		Artemetin; luteolin-7-*O*-β-glucuronide	Insecticide	Hirobe *et al.* 1997
			Haemorrhages controls hormonal imbalances	Alam and Gomes 2003
			Stimulates lactation	Halaska *et al.* 1999
			Mastalgia, antioxidant	Dennehy *et al.* 2006
V. negundo	Eastern Africa and South East Asia	Lignans	Anti-inflammatory activity	Ono *et al.* 1997
		Negundin A; negundin B; vitrofolal E; lyoniresinol, (+) olyoniresinol-3-α-*O*-β-D-glucoside; (+)-(-)-pinoresinol; (+)-diaasyringaaresinol	Hyperpigmentation	Azhar-ul-Haq *et al.* 2006
		6-hydroxy-4-(4-hydroxy-3-methyoxy)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2-napthaledehyde	Anti-inflammatory activity	Yamasaki *et al.* 2008
			Larvicidal	Das *et al.* 2004
			Anti-inflammatary	Ravishankar *et al.* 1985; Pushpalatha and Muthukrishnan 1995; Telang *et al.* 1999
			Necroisis, analgesic	Alam and Gomes 2003
			Nephrotoxicity, hypersensitive	Hebbalkar *et al.* 1992
			Antidote to scorpion-sting and snake poison	Chopra *et al.* 1956; Sutherland 1977; Stahel *et al.* 1985; Corrigan *et al.* 1987; Sudarsanam *et al.* 1995
			Antihistamine activity	Dharmasiri *et al.* 2003
V. trifolia	India and Mexico		Anti microbial, anti feeding and cytotoxic activity	Hosozawa *et al.* 1974
		Casticin; 3,6,7-trimethylquercetagetin	Wound healing potency	Zeng *et al*. 1996
		Luteolin-3-*O*-β-D-glucuronide and isoorientin	Medicinal and insecticidal properties	Ramesh *et al.* 1986
		Alpha-pinene, linalool, terpinyl acetate, beta-caryophyllene and caryophyllene oxide		Pan *et al.* 1989
		5-methyl artemetin		Nair *et al.* 1975
		7-desmethyl artemetin; luteolin; B-sitosterol-β-D-glucoside		
			Sprained joints, vomiting, rheumatism, intermittent fever	Hernández *et al.* 1999

Table 3 (Cont.)

Vitex sp.	Distribution	Phytochemical(s)	Medicinal/economic importance	References
V. trifolia	India and Mexico	Vitetrifolins D-G; vitetrifolins B and C; dihydrosolidagenone abietatriene; 3b-ol diterpenes; Vitetrifolin A	Anti-oxidant activity	Ono *et al.* 2000, 2001
			Leucoderma	Rajendran *et al.* 2003; Tiwari and Tripathi 2007
			Amenorrhoea	Daniele *et al.* 2005; Manjunatha and Vidya 2008
		Essential oils like limonene; humulene oxide; caryophyllene oxide; α-humulene	Tooth and skin diseases; intestinal bilharzias; headache; amoebiasis	Nebie *et al.* 2005
		-	Alleviate dysentery; analgesic; anti-inflammatory; scorpion stings; stomach ache	Argueta *et al.* 1994
		20-hydroxyecdysone; ecdysteroids	Glycemia	Zannata *et al.* 2007
		Flavonoids, triterpenoids, lignans, iridoids	Anti-inflammatory	Sridhar *et al.* 2004
			Antioxidant	Sridhar *et al.* 2005
			Stomatitis; cardiac diseases; anorexia; blindness; leprosy; worm infestation; digestive; carminative rheumatic swellings; chest pains	Parrotta 2001
		Vitexin; β-sitosterol	Promote cardiovascular health by improving blood and nutrient flow to the heart muscle	Lindsay and Cambie 1958; Seikel *et al.* 1959
V. diversifolia	Africa	20-hydroxyecdsone; 20-dihydroxyecdysone (turkesterone); pterosterone	Astringents; anthelmintic; gastrointestinal disorders	Suksamran *et al.* 1998, 1999
V. mollis	-	(-)-Limonidilactone; limonidilactone; andrographolide	-	Aphaijitt *et al*. 2006
V. megapotamica	Brazil, Argentina, Paraguay, Uruguay	20-hydroxyecdysone; ecdysteroids	Glycemia	Zannata *et al*. 2007
V. altissima	South East Asia	Flavonoids, triterpenoids, lignans, iridoids	Anti-inflammatory	Sridhar *et al.* 2004
			Antioxidant	Sridhar *et al.* 2005
			Stomatitis; cardiac diseases; anorexia; blindness; leprosy; worm infestation; digestive; carminative rheumatic swellings; chest pains	Parrotta 2001
V. lucens	New Zealand	Vitexin; β-sitosterol	Promote cardiovascular health by improving blood and nutrient flow to the heart muscle	Lindsay and Cambie 1958; Seikel *et al.* 1959
V. glabrata	-	20-hydroxyecdsone; 20-dihydroxyecdysone (turkesterone); pterosterone	Astringents; anthelmintic; gastrointestinal disorders	Suksamran *et al.* 1998, 1999
V. limonifolia	-	(-)-Limonidilactone; limonidilactone; andrographolide	-	Aphaijitt *et al.* 2006
V. doniana	Nigeria	Vitamin C	Nutritive sweetener	Egbekun *et al.* 1996
			Diarrhoea	Ladeji *et al*. 2004
V. polygama	-	Flavonoids	Antiviral activity	Gonçalves *et al.* 2001
V. peduncularis	-	Peduncullariside; iridoid agnuside	Inhibition of COX-2; anti-inflammatory	Suksamran *et al.* 2002
		Vitexin	Promote cardiovascular health by improving blood and nutrient flow to the heart muscle	Bheemasankarrao and Venkateswarlu 1956
		Triterpenoids and flavonoids	Anti-inflammatory	Sahu *et al.* 1984
V. cymosa	Central and Brazil	Iridoid; viteoid 11; agnuside		Tereza *et al.* 2001
V. piramidata	-	-	Gastrointestinal disorders	Hernández *et al.* 1999; Argueta *et al.* 1994; Ahmad and Holdsworth 1995
V. gaumeri				
V. pubescens				Bajpai *et al.* 1995

experimental studies using rodent striatum, calf striatum, and human recombinant dopamine D_2 receptors with two different ligand probes, sulpiride and spiroperidol, suggest that a variable degree of binding occurs between crude extracts and diterpene fractions of *Vitex* (Daniele *et al*. 2005).

PROPAGATION AND LARGE-SCALE CULTIVATION ON WASTE LANDS

In view of its medicinal and ethnobotanical importance, agrocultivation techniques for cultivating *V. negundo* and *V. trifolia* on farms are essential. The plants can be propagated

Fig. 1 Five-year-old *Vitex trifolia* and *V. negundo* plants used as a biofence at the University of Hyderabad Campus.

vegetatively using stem cuttings and large-scale cultivation on waste lands has been achieved in India (**Fig. 1**). The effects of different plant growth regulators (PGRs), Stik l-naphthaleneacetic acid (NAA with sodium as active ingredient), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and gibberellic acid (GA_3) (10-3000 ppm) on the growth and productivity of the plant in vegetative propagation was studied using stem cuttings of *V. negundo*. Stik-treated cuttings showed maximum effect on rooting (100%), length of the root (30.5 cm) and node sprouting (91%) at 500-1000, 500 and 1500 ppm, respectively. They also showed maximum effect on average number of leaves (69/ cutting) and average number of lateral branches (10.2/ cutting) at 1500 ppm. GA_3 showed inhibitory effect on rooting (Badola and Badoni 1990; Tewary *et al.* 2004). *In vitro* shoot induction and plant regeneration was achieved from mature nodal explants of *V. trifolia* on MS (Murashige and Skoog 1962) medium fortified with benzylaminopurine (BAP), kinetin (KN), thidiazuron (TDZ), adenine (ADE), and 2-isopentenyladenine (2-iP) at 0.25–10.0 µM (Hiregoudar *et al*. 2006). Similarly *in vitro* culture of *V. negundo* by nodal segments from mature plants was developed using cytokinins – N^6-benzyladenine (BA), KN, and TDZ on to MS medium. BAP at an optimal concentration of 2.0 mg/l was most effective in inducing bud break, although callus-free multiple-shoot formation was a function of cytokinin activity. The frequency of shoot proliferation was markedly influenced by the explanting season. The percentage of shoot multiplication (98–100%) as well as the number of shoots per node (6-8) was highest during the first three culture passages, after which there was a gradual decline in shoot development. Rooting was best induced (94%) in shoots excised from proliferated shoot cultures on half-strength MS medium augmented with an optimal combination of IAA and IBA each at 1.0 mg/l. High-frequency survival (93%) rate was observed in the field (Sahoo and Chand 1998). *In vitro* flowering and an efficient micropropagation protocol was developed for *V. negundo* by using nodal segments of mature plants in MS medium supplemented with 4.4 µM BAP and 0.53 µM NAA. MS medium supplemented with 4.4 µM BAP and 0.53 µM NAA induced an average of five shoots per node and was the best for axillary bud proliferation. Full strength MS solid medium with 3.69 µM indole-3-butyric acid (IBA) exhibited the best *in vitro* rooting. 90% of the rooted shoots survived when transferred to green house and subsequently to the field (Vadawale *et al.* 2006). Thiruvengadam and Jayabalan (2000), Jeong *et al.* (2004) also reported *in vitro* mass propagation of *V. negundo* and *V. rotundifolia*. Chandramu (2003) and Usha *et al.* (2007) reported the micropropagation of *V. negundo* using nodal explants on MS medium with sodium sulphate and shoot tip culture respectively and large-scale propagation of *V. negundo* by *in vitro* culture of nodal segments was also reported using a mature plant explants. The efficiency of two nutrient media, viz., MS and Woody Plant Medium (WPM) supplemented with varied concentrations of BAP, KN and IAA was compared in producing multiple shoots and roots. A maximum of 11 shoots with a frequency of 80% regeneration was found in combinations of BA (1 mg/l) and IAA (0.05 mg/l). The explants responded better in MS medium containing higher concentrations of BA (>1 mg/l). The *in vitro* generated shoots were best rooted in liquid MS medium containing IBA (3 mg/l), which were successfully established in soil (Handique 2007). An efficient protocol was established for rapid and large scale propagation of woody aromatic medicinal plant *V. negundo* by *in vitro* shoot multiplication from shoot tips and nodal segments of mature plant. Of the four different PGRs, BA, KN, GA_3, NAA with coconut water, MS fortified with BA 1.0 mg/l was found to be the most effective for inducing multiple shoots from nodal explants. The percentage (96%) of shoot multiplication per node (21.83) was highest up to second subculture passages, after which there was a gradual decline in shoot development. Best rooting was induced (93%) in excised shoots on half-strength MS medium supplemented with an optimal combination of NAA (0.3 mg/l). Soil, compost and sand (1:1:1) mixture was the most suitable planting substrate for hardening. The survival rate was 80% and the regenerated plants were successfully transferred to the soil (Afroz *et al.* 2008). In addition to the above reports, efficient and improved shoot regeneration technique for the micropropagation of *V. negundo* was developed using *in vitro* culture of nodal segments with axillary buds with TDZ at 1.0 mM. Initiation of multiple shoot proliferation at the rate of 25 microshoots per nodal explant after 4 weeks of culture was observed. Optimum shoot multiplication and elongation was achieved when TDZ exposed explants were subcultured on MS media containing a combination of 1.0 mM BA and 0.5 mM NAA. Efficient rooting was achieved directly in Soilrite when basal portion of the shoots were treated with 500 mM IBA for 10 min which was the most effective in inducing roots, as 97% of the micro shoots produced roots. Plantlets went through a hardening phase in a controlled plant growth chamber, prior to *ex-vitro* transfer. Micropropagated plants grew well, attained maturity and flowered. No phenotypical differences for morphogenesis were observed among the regenerants (Ahmad and Anis 2007).

In addition to the micropropagation diversity analysis was also reported in few of the *Vitex* species. *V. rotundifolia* efficient use and conservation, genetic diversity and clonal variation in China were investigated using inter-simple sequence repeat (ISSR) markers. The overall genetic diversity (*GD*) of *V. rotundifolia* populations in China was moderate (*GD* = 0.190), with about 40% within-population variation. Across all populations surveyed, the average within-population diversity was moderate (*P* = 22.6%; *GD* = 0.086). A relatively high genetic differentiation (G_{st} = 0.587) among populations was detected based on the analysis of molecular variance data. Such characteristics of *V. rotundifolia* are likely attributed to its sexual/asexual reproduction and limited gene flow. The genotypic diversity (*D* = 0.992) was greater than the average values of a clonal plant, indicating its significant reproduction through seedlings. Spatial autocorrelation analysis showed a clear within-population structure with gene clusters of approximately 20 m. Genetic diversity patterns of *V. rotundifolia* in China provide a useful guide for its efficient use and conservation by selecting particular populations displaying greater variation that may contain required medicinal compounds, and by sampling individuals in a population at >20 m spatial intervals to avoid collecting individuals with identical or similar genotypes (Hu *et al.* 2008).

CONCLUSION AND FUTURE PROSPECTS

To summarize, most of the plant species of the genus *Vitex* were revered for many ailments, mostly related to female reproductive imbalances apart from colic, gas and other digestive problems. Initially in England, the plant was considered to be useful for the above conditions based on the writings of the Greeks and Romans, which were conceded for centuries. The fruits are reported to be bitter and aromatic, a promoter of good digestion, diuretic, carminative and are useful in removing visceral obstructions. All the parts of the plant are medicinally important, but mostly the seeds are highly potent for the medicinal value. Indian *Vitex* sp. such as *V. negundo* and *V. trifolia* definitely are promising for bioprospection. Efforts should be made to conserve these plants (*in situ* and *ex situ*) due to overexploitation and habitat alteration. The pharmaceutical companies especially need to take the initiative to cultivate the plant for commercial exploitation in view of the clinical potential, primarily in problems related to women health care. Attempts must be made to conserve different germplasm accessions and protocols must be developed for rapid multiplication and storage of the rare and elite accessions. In future lot of measures should be implemented like inculcating awareness of these plants among tribals and similarly to urban people about their medicinal and commercial importance, to develop efficient methods for propagation of these species in order to restrict them moving into the threatened category and detailed studies on the chemistry and mode of action of these medicines should be taken up as one of the most challenging issues in the medicinal plant research.

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