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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 ethnomedecological 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 perspiration”. 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 twigs of the plant are said to stop the flow of urine. It is also used as a contraceptive

- In ancient times, the plant Vitex was 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 twigs of the plant are said to stop the flow of urine. It is also used as a contraceptive.

Table 1 Common names of selected Vitex species.

<table>
<thead>
<tr>
<th>Different species of Vitex</th>
<th>Common names</th>
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<tbody>
<tr>
<td>Vitex agnus-castus</td>
<td>Hemp tree</td>
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<tr>
<td>Vitex rotundifolia</td>
<td>Beach Vitex</td>
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<tr>
<td>Vitex negundo</td>
<td>Chinese chaste tree</td>
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<td>Vitex diversifolia</td>
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<tr>
<td>Vitex cymosa</td>
<td>Taruma Guazu</td>
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<td>Vitex megapotamica / Vitex polygama</td>
<td>Taruna</td>
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<td>Vitex ovata</td>
<td>Beach Vitex, Pohinahina</td>
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<td>Vitex glabrata</td>
<td>Blackberry tree</td>
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<tr>
<td>Vitex mollis</td>
<td>Uvalama tree</td>
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<tr>
<td>Vitex doniana</td>
<td>Black plumb</td>
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<tr>
<td>Vitex lucens</td>
<td>Puriri</td>
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<tr>
<td>Vitex peduncularis</td>
<td></td>
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<tr>
<td>Vitex altissima</td>
<td>Milla</td>
</tr>
<tr>
<td>Vitex leucoxylon</td>
<td>Five-leaved chaste tree</td>
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</tbody>
</table>

Review
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 extols it as a cure for "windiness of the stomach", or flatulence. Those who dried the infused fruits in the sun, he says, can cure the liver and spleen disorders. Madaus also encourages its use as a "feminine" 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 menstrual, 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 (Kuruzum-Uz et al. 2003). The iridoid glycosides have recently been quantified and consist of 0.3% acubin, 0.6% agnoside and 0.07% eurystoside (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 pharmacopoeias, including the influential first Pharmacopoeia Londinensis (Urdang 1618) but quickly was dropped from official status. Including the influential first Pharmacopoeia Londinensis (Urdang 1618) but quickly was dropped from official status. Including the influential first Pharmacopoeia Londinensis (Urdang 1618) but quickly was dropped from official status.

ETHNOPHARMACOLOGICAL 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 Pharmacopoeia 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 Göttingen in 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 luteinizing 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 indicate 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 amenorrhea, infertility and hyperprolactinemia (Daniele et al. 2005). Vitex leucocyma 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 as expectorant, astringent and febrifuge (Chopra et al. 1956; Ambastha 2000). Various phytochemical studies on this plant have revealed the presence of flavonoids, iridoids, and sterols (V. leucocyma et al. 1992). The leaves and flowers of 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 Ganapathy et al. (2005). The methanolic extract of V. leucocyma tested positive for Lieberman Bauchard's and Shinoda's tests confirming the presence of sterols, triterpenes and flavonoids. Antibacterial and anti-fungal 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. leucocyma leaf exhibited good anti-bacterial activity against Gram-positive (+ve) and -negative (-ve) bacteria. The methanolic extracts of V. subhirtella, V. negundo, V. agnus castus, and V. negundo 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 (Ganapathy et al. 2005).

The ethanol extract of V. negundo leaves resulted in the isolation of a new flavone glycoside along with five known compounds which were evaluated for their anti-microbial activities. The new flavones were found to have significant anti-fungal 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 (Tripathi and Tripathi 2007). The plant extract was also used as a botanical insecticide against the rice leaf folder (Cnaphalocrocis medialis) 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, anti-histaminic, 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 (Usmanahewsara et al. 2007). The methanolic root extract of V. negundo was active against herpes simplex virus, the first time for antisnake venom. The plant extracts significantly antagonized the Vipera russelli and Naja kaouthia venom-injected lethal activity both in in vitro and in vivo studies. V. russelli venom-injected 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.
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 (Brown 1999; Halaska et al. 2003). The alcoholic extracts and hexane extracts of Vitex lucens 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 (Chanpatty 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. leucoxylon.

**VITEX: SAFETY, RISKS AND SIDE EFFECTS**

Good quality chast 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 endocrin 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 selegiline, amantadine, carbidopa, levodopa, 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 prolactin-inhibiting actions (Williamson 2006). Several

<table>
<thead>
<tr>
<th>Name of the disease</th>
<th>Symptoms</th>
<th>References</th>
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<tr>
<td>Premenstrual syndrome (PMS)</td>
<td>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.</td>
<td>Brown 1994; Lauritzen et al. 1997; Berger et al. 2000; Huddleston and Jackson 2001; Atmaca et al. 2002; Wuttke et al. 2003; Daniel et al. 2005; Prilepskaya et al. 2006; Yuan et al. 2007; Rapkin et al. 2008</td>
</tr>
<tr>
<td>Breast feeding</td>
<td>Increased milk flow and ease of milk release.</td>
<td>Brown 1994; Miller et al. 1998; Schellenberg 2001; Artz 2006; Chamandoost 2007</td>
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<tr>
<td>Infertility</td>
<td>Normal prolatinicaemia but showed pathologically low serum levels at day 20 of the menstrual cycle. Shortening of the luteal phase and a positive change in the LHRR test dynamic, decreased corpus luteal function.</td>
<td>Mediherb News Letter 1994; Das et al. 2004; Westphal et al. 2004; Artz 2006</td>
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<td>Menopause</td>
<td>Hot flushes and menstrual irregularities such as flooding, clotting and irregular cycle, mood savings depression.</td>
<td>Taylor 2001; Carmichael 2007; Hu et al. 2007</td>
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<tr>
<td>Acne</td>
<td>Menstral cycle abnormalities.</td>
<td>Probst and Roth 1954; Mediherb Newsletter 1994; Berger et al. 2000; Daniel et al. 2005</td>
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<tr>
<td>Vitex sp.</td>
<td>Distribution</td>
<td>Phytochemical(s)</td>
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<tr>
<td><strong>V. rotundifolia</strong>&lt;br&gt;Sea coast in Asia</td>
<td>Phenylisophtalene; Phenylisophtalene; Polyphenolxanthinoids; Diterpenoids; Veteranary crude drugs Rotundiferan&lt;br&gt;Iridoid and phenolic glucoside</td>
<td>Antibacterial; Leukamia, anticarcinogenic and antimutageneic; Mosquito repellent; Pest control</td>
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<td></td>
<td>2',7'-dichlorofluorescin diacetate</td>
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<td>1,8-cineole, sabinene α-pinene; β-farnesene; β-caryophyllene, α-terpinenyl acetate&lt;br&gt;6 β, 7 β-diacetoxy-13-hydroxy-labda-8,14-dien rotundifuran&lt;br&gt;Vitexilactone</td>
<td>Secondary metabolite&lt;br&gt;Prolactin secretion</td>
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<td>Linoleic acid&lt;br&gt;Clerodadienol&lt;br&gt;Lutetin (6-C-(4'-methyl-6'-O-trans-caffeoylglucoside); luteolin 6-C-(6'-O-trans-caffeylglucoside; luteolin 6-C-(2''-O-trans-caffeoylglucoside); luteolin-7-O(6''-P-benzoylglucoside); 5,4'-dihydroxy-3,6,7,3' metathymoeflavone&lt;br&gt;arretin and isofarmenam, vitexlactan A; 6 β-acetoxy-9 α-hydroxy-13 (14)-labden-16,15-amide; tridio glycosides</td>
<td>Postpartum&lt;br&gt;Anti-oxidant&lt;br&gt;Estrogen receptors&lt;br&gt;Progestin&lt;br&gt;Tumor inhibition or apoptosis</td>
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<td>Artemetin; luteolin-7-O-β-glucuronide</td>
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<td></td>
<td>6-hydroxy-4-(4-hydroxy-3-methoxy)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaledehyde</td>
<td></td>
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<td></td>
<td>Nafunin A; negundin B; vitrofolal E; lymonresinol, (+) olyonresinol3-α-O-β-glucoside; (+)-(-)-pineoresinol; (+)-diasyringaressinol&lt;br&gt;Lignans</td>
<td>Anti-inflammaratory activity&lt;br&gt;Insecticide&lt;br&gt;Haemorrhages controls&lt;br&gt;Stimulates lactation&lt;br&gt;Mastalgia, antioscident</td>
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<td>Casticin; 3,6,7-trimethylquercetagetin&lt;br&gt;Luteolin-3-O-β-d-glucuronide and isoorientin&lt;br&gt;Alpha-pinene, linalool, terpinyl acetate, beta-caryophyllene and caryophyllene oxide</td>
<td>Anti-inflammatory activity&lt;br&gt;Antiamoebic, anti feeding and cytotoxic activity</td>
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<td>5-methyl artemetin&lt;br&gt;7-desmethyl artemetin; luteolin; B-sitosterol-β-D-glucoside&lt;br&gt;Casticin; 3,6,7-trimethylquercetagetin</td>
<td>Wound healing potency&lt;br&gt;Medicinal and insecticidal properties</td>
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<tr>
<td></td>
<td></td>
<td>Sprained joints, vomiting, rheumatism, intermittent fever</td>
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experimental studies using rodent striatum, calf striatum, and human recombinant dopamine D₂ 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 bio-fence 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), Stk 1-naphthaleneacetic acid (NAA with sodium as active ingredient), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and gibberellic acid (GA₃) (10-3000 ppm) on the growth and productivity of the plant in vegetative propagation was studied using stem cuttings of V. negundo. Stk-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. GA₃ 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 – N₂-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 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 Jayabal (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 (5 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, GA₃, 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 = 0.22±0; GD = 0.086). A relatively high genetic differentiation (Gₑ = 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 bioexploitation. Efforts should be made to conserve these plants (in situ and ex situ) due to overexploitation and awareness of these plants among tribes 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.

ACKNOWLEDGEMENTS

Financial support from the Department of Biotechnology, Govt. of India, New Delhi (Ref: BT/PR2273/PBD/17/117/2000 dt.7-9-01) during 1-10-2001 to 31-3-2005 and part of the ex-situ conservation received financial assistance from the Ministry of Environment and Forest, GOI ref No.10/03/2003-CS/ BG dt. 8.2.2005 (Botanic Garden scheme) are gratefully acknowledged.

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