Introduction

Syzygium Gaertn. (Myrtaceae) is a genus of trees or shrubs distributed in the tropics of the Old World. Some species bear edible fruits; a few yield timbers, and one species gives the cloves of commerce. About 75 species occurs in India (Anonymous, 2003).

Vernacular names

Hindi, Bengali, Gujrati and Marathi - Laung; Telegu - Lavanga-muchettu (tree), Lavangamulu (buds); Tamil - Kirambu; Kannad - Lavanga; Malayalam - Karayampu, Krambu.

Distribution

The clove tree is a native of some islands of the Malay Archipelago, especially Moluccas. It is cultivated in Zanzibar and Pemba (Tanzania), Indonesia, Penang, Malagasy and to a lesser extent in the Seychelles, Reunion, Mauritius and Sri Lanka (Ceylon). In India it is grown in Tamil Nadu and Kerala.

Description

The plant is a pyramidal or conical evergreen tree, 9-12 m high, sometimes taller. Main stems erect, 100 cm in girth, often forking at a height of 1.5-1.8 m bark smooth, grey; leaves lanceolate, in pairs, acute at both end 7.5-12.3 cm x 2.5-3.75 cm, gland-dotted, fragrant; flower-buds borne in small cluster at the ends of branches, greenish.
turning pink at the time of maturity, aromatic; drupes (mother clove), fleshy, dark pink, 2.5 cm long x 1.5 cm thick; seeds oblong, soft, grooved on one side and 1.5 cm long (Anonymous, 2003).

Phytoconstituents

Analysis of dried cloves gave the following values: moisture-25.2, protein-5.2, fat-8.9, fibre-9.5, carbohydrates-46.0, and mineral matter-5.2%; calcium-740, phosphorus-100 and iron-4.9 mg; and iodine-50.7 µg/100 g. The vitamins reported to be present are carotene-253 µg, thiamine-0.08 mg, riboflavin-0.13 mg and nicotinic acid 1.51 mg. The cloves contained 13% tannin-gallotannic acid; oleanolic acid has been isolated from spent cloves.

Steam distillation of clove buds yields a colourless or pale yellow oil (14-23%), with the characteristic odour and taste of cloves. The yield and properties of oil vary according to the origin and quality of cloves and the method used for distillation. The product obtained from the whole buds yielded a higher percentage (97%) of eugenol (C_{10}H_{12}O_{3}) than that in oil distilled from crushed cloves (eugenol, 94%). Water distilling furnished yields an oil of better quality and lower specific gravity (eugenol, 85-89%) than that obtained by dry steam-distillation (eugenol, 91-95%); the two oils are distinguished as ‘opt’ and ‘strong’ oils; commercial oil is mixture of both (Anonymous, 2003).

The clove bud oil contains free eugenol (70-90%), eugenol acetate (2-17%) and caryophyllene (C_{10}H_{16}O_{4}) as its main constituents. Among the other constituents present the most important is methyl-n-amyl ketone, to which the oil owes its fresh and fruity aroma. Other substances present in traces included: methyl salicylate, methyl benzoate, methyl alcohol, benzyl alcohol, furfuryl alcohol, methyl furfuryl alcohol, furfural, α-methyl furfural, dimethyl furfural, β-pinene, methyl-α-heptyl ketone, valeraldehyde, methyl-n-amyl carbinol, methyl-α-heptyl carbinol and vanillin. The oil obtained from solvent extraction of cloves contains no caryophyllene but contains epoxydihydrocaryophyllene.

Clove stem volatile oil, obtained from the flower stalks in a yield of 5.5-7.0%, has a less pleasant odour than that of the oil from buds. The oil generally contains a higher percentage of free eugenol than the flower bud oil and only a small amount of eugenol.
acetate. The α- and β-caryophyllenes, furfural, methyl alcohol and naphthalene have been reported.

Clove leaf volatile oil was obtained in a yield of 4-5% by the steam-distillation of leaves. The oil contains a lower percentage of total eugenol than the clove bud oil. Eugenol acetate is present in very small quantities and methyl-α-amyl ketone is present in very minute amounts.

Clove root oil is obtained by the steam-distillation of the roots of clove tree in a yield of about 6%. It is bright yellow in colour when freshly distilled, and in composition, odour and quality, it compares with the oil from clove buds. It contains 85-95% eugenol (Anonymous, 2003).

Hydrodistillation of clove under four conditions viz., whole dry bud, whole bud, whole bud soaked in water for 48 h, powdered bud and powdered but soaked in water for 48 h, showed that water soaking enhanced oil recovery by releasing more amount of oil, in case of both whole and powdered buds (Waikhoni et al., 1995).

Bound volatiles of dried clove buds and fresh green cloves leaves were studied by Svendsen’s method, using both β-glucosidase and α-amylolgucosidase for enzyme hydrolysis. Apart from aliphatic alcohols and monoterpine alcohols, eugenol, isoeugenol, farnesol and nerolidol were confirmed as aglycones (Menon and Narayanan, 1992).

2-Hydroxy-4,6-dimethyl-5-methylacetophenone have been reported from clove oil (35.0%) (Huneck, 1972). Caryophyllene, eugenol and naphthalene have been isolated on steam distillation of clove buds (Narayanan and Natu, 1974).

Ellagitannin-eugenin has been reported from dried flowers and buds (Takechi and Tanaka, 1981). Caryophyllene oxide, caryophylla-3,(12), 6-dien-4-ol and caryophylla-3(12), 7(13)-dien-6β-ol are present in the clove buds (Iwamuro et al., 1983).

Acetophenone, benzyl salicylate, α-cadinol, γ-decalactone, fenchone, hexanal, 2-hexanone, methyl palmitate, γ-muurolene, palustrol, propyl benzoate, β-selinene and α-thujene have been identified in volatile oil. Caryophylla-4(12),8(13)-dien-5β-ol, caryophylla-3,8(13)-dien-5α-ol, caryophylla-3,8(13)-dien-5β-ol and 4,4′-dimethyltricyclo(6,3,2,0)-trideca-8-en-1-ol occurred in the oil (Toshikazu et al., 1986).

By means of bioassay-directed chromatographic fractionation, eight active compounds were isolated from the MeOH extract of dried buds of S. aromaticum and
were confirmed as 5,7-dihydroxy-2-methylchromone, 8-C-\(\beta\)-D-glucopyranoside, bilflorin, kaempferol, rhamnocitrin, myricetin, gallic acid, ellagic acid and oleanolic acid (Lining and Christine, 1996).

The essential oils of *S. aromaticum* were isolated from its buds and leaves by hydrodistillation. The oils were analyzed by high resolution GC and GC-MS. Twenty-eight and 35 constituents representing 99.9% each were identified in the identification of 22 constituents representing 99.9% of the oil. The major constituents in bud and leaf oils were eugenol and \(\beta\)-caryophyllene (Srivastava, 2005).

The chemical composition of clove bud and leaf oils was studied by GC/MS. Thirty-six and 31 volatile compounds have been identified in the bud and leaf oils, respectively. The major components of the bud oil were eugenol (69.8%), \(\beta\)-caryophyllene (13.0%) and eugenyl acetate (16.1%), whereas the leaf oil contained only eugenol (78.1%) and \(\beta\)-caryophyllene (20.5%) as main constituents (Pino *et al.*, 2001).

The dried leaves of *S. aromaticum* on hydrodistillation, gave 4.8% of the oil. GC and GC-MS analysis of the oil resulted in the identification of 16 compounds. The major compound was eugenol (94.4%) followed by \(\beta\)-caryophyllene (2.9%). The clove oil from Little Andaman was found to be comparable with the best oil produced in south India in terms of eugenol content. Cultivation of clove as an economically viable crop in the Andaman, Nicobar and Lakshdeep-Islands has been reported (Raina *et al.*, 2001).

Two apigenin triglycosides - apigenin6-C-[\(\beta\)-D-xylopyranosyl-(1\(^\rightarrow\)2\()-\(\beta\)-D-galactopyranoside]-7-O-\(\beta\)-D-glucopyranoside and apigenin6-C-[\(\beta\)-D-xylopyranosyl-(1\(^\rightarrow\)2\())-\(\beta\)-D-galactopyranoside]-7-O-\(\beta\)-D-(6-O-\(p\)-coumarylglucopyranoside) have been isolated from the ethanol extract of the seeds of *Syzygium aromaticum* (Nassar, 2006).

Bioactivities

A methanol extract from the dried buds of *S. aromaticum* demonstrated preferential growth inhibitory activity against Gram negative anaerobic periodontal oral
pathogens, including Porphyromonas gingivalis and Prevotella intermedia with MICs of 156 and 625 μg/ml, respectively (Lining and Christine, 1996).

The essential oil from the leaf, bud and stem of S. aromaticum exhibited significant antimicrobial activity against test bacteria and all strains of Listeria monocytogenes and also exhibited significant antimycotic activity against three fungal strains; a plant pathogen, a spoilage type and a mycotoxigenic strain at both concentrations of 1 and 10 μl/ml growth medium. (Deans et al., 1995).

The essential oil was fed to mice in order to assess the antioxidant capacity, with particular reference to the protection of polysaturated fatty acids, in the liver and retina during ageing (Shyamaia et al., 2003).

The oil exerts bactericidal action against Vibrio cholerae in a concentration of 1 in 2,000. It is also effective against Gartner’s bacillus and swine Erysipelas bacteria. It inhibits the growth of Brucella and Mycobacterium, trichophyton, Achorion and Epidermophyton. When added to edible fats and oils, cloves exert preservative action against oxidative rancidity (Abdullin, 1962).

The volatile oils were assessed for antibacterial activity against 25 different genera of bacteria. These include animal and plant pathogens, food poisoning and spoilage bacteria. The volatile oils exhibited considerable inhibitory effects against all the organisms under test while their major components demonstrated various degrees of growth inhibition (Dorman and Deans, 2000).

In vitro anti-Staphylococcus aureus activities of the extracts were confirmed, and synergism was verified for all the extracts; clove, guava, and lemongrass presented the highest synergism rate with antimicrobial drugs (Betoni et al., 2006).

An aqueous extract of S. aromaticum flower bud inhibited immediate hypersensitivity of histamine release from mast cells in vivo and in vitro (Kim et al., 1998). Ellagitannin-eugeniin showed antiviral activity against herpes simplex virus at a concentration of 10 μg/ml (Takechi and Tanaka, 1981).

S. aromaticum extract acts like insulin in hepatocytes and hepatoma cells by reducing phosphoenolpyruvate carboxykinase and glucose 6-phosphatase gene expression. A more global analysis of gene expression by DNA micro array analysis
revealed that clove and insulin regulated the expression of many of the same genes in a similar manner. Consumption of certain plant-based diets may have beneficial effects for the treatment of diabetes. It indicated a potential role for compounds derived from clove as insulin-mimetic agents (Prasad et al., 2005).

Twenty nine traditionally used European herbal drugs were tested in a cyclooxygenase-1 and cyclooxygenase- 2, bioassay for inhibitory activity. In 17 cases the n-hexane extracts (50 µg/ml) showed inhibitory effects higher than 60%. Good results were obtained from the fruits of Syzygium aromaticum (Lohmann et al., 2000).

Oral administration of aqueous infusions of clove at a dose of 100 µl/mouse/day not only delays the formation of papilloma but also reduces the incidence of papilloma as well as the cumulative number of papillomas per papilloma bearing mouse. It is suggested a promising role for cloves in restriction of the carcinogenesis process (Banerjee and Das, 2005).

A 50% ethanolic extract of clove produced a significant and sustained increase in the sexual activity of normal male rats, without any conspicuous gastric ulceration and adverse effects. Thus, the resultant aphrodisiac effectivity of the extract lends support to the claims for its traditional usage in sexual disorders (Tajuddin et al., 2004).

The effect of a clove (Syzygium aromaticum) administered by two different routes (orally and intragastrically) on Candida albicans growth demonstrated that oral intake of an herbal food, clove, suppressed the overgrowth of C. albicans in the alimentary tract including the oral cavity (Taguchi et al., 2005).

Aqueous infusion of clove during benzopyrene (BP)-induced lung carcinogenesis in mice, showed chemopreventive potential in view of its apoptogenic and anti-proliferative properties (Banerjee et al., 2006). Clove oil gave the longest duration of 100% repellency (2-4 h) against three species of mosquito (Trongtoktik et al., 2005).
Medicinal properties and uses

The chief value of the clove lies in the oil it contains. The oil is highly aromatic and extensively used for flavouring food products. The oil is an ingredient of dentifrices, gargles and chewing gum (Anonymous, 2003).

The cloves are aromatic, stimulant and carminative. They are used in various forms of gastric irritation and dyspepsia. They are administered in the form of powder or infusion to relieve nausea and vomiting, to correct flatulence and to excite languid digestion. The oil is used as a local analgesic for hypersensitive dentines and carious cavities, a mixture of oil and zinc oxide is used as a temporary filling for tooth-cavities. Used externally, the oil is rubefacient and counter-irritant; internally, it is carminative and antispasmodic (British Pharmacopoeia, 1968).

The essential oil extracted from clove (Syzygium aromaticum) is used as a topical application to relieve pain and promote healing in herbal medicine and also finds use in the fragrance and flavouring industries (Prashar, 2006).
EXPERIMENTAL

Plant material

The plant material (flower buds) of *S. aromaticum* was purchased from the Khari Baoli local market of Delhi and authenticated by Dr. M. P. Sharma, taxonomist, Department of Botany, Jamia Hamdard, New Delhi.

Isolation of volatile oil

The air dried powdered buds (500 g) were hydrodistilled in an all glass apparatus according to the method recommended in the British Pharmacopoeia, 1988. The volume of the oil was collected in the graduated tube. The collected colourless volatile oil was dried over an anhydrous sodium sulphate and stored at 4°C in the dark. The yield was 30% based on dry weight of the sample.

GC analysis

Analytical GC was carried out on a Varian 3300 gas chromatograph fitted with a silicon DB-1 capillary column (30 x 0.25 mm), film thickness 0.25 μm, carrier gas N₂, flow rate 15 mm/min. Split mode, temperature programmed 80-250°C, at 4°C/min. Injector temperature 250°C, detector used FID, detector temperature 300°C. Injection volume for all samples as 0.1 μl.
RESULTS AND DISCUSSION

Identification of components

Volatile constituents were identified by comparing their Kovats retention indices and retention times with those of authentic standards available in author’s laboratory and with those in literature. The fragmentation patterns of mass spectra were compared with those in the spectrometer database using the NBS 54 AL and Wiley, L-build in libraries and with those reported in literature (Jennings and Shibaniate, 1980; Swiger and Silverstein, 1981; Adams, 1995; Libey, 1991; Ali, 2001; Anderson and Falcone, 1969).

The retention indices were calculated for all compounds using a homologous series of n-alkanes under the same operational conditions of analysis.

The compounds identified from clove flower buds oil, obtained by steam distillation are listed in Table-5.1. Eight peak indices of components along with their structures are given in Table-5.2. Total 7 components (100%) were identified. The oil contained one oxygenated monoterpenes, pulegone (4.1%) and three sesquiterpenes (62.9%), which consisted of β-caryophyllene (52.7%), α-humulene (8.4%), α-selinene (1.8%). The major non-terpenic component is eugenol (27.1%) followed by palmitic acid (4.3%) and formic acid (1.6%).
Table 5.1: Chemical composition of volatile oil of the flower buds of *S. aromaticum*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Components</th>
<th>Retention index</th>
<th>% Area</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Eugenol</td>
<td>1356</td>
<td>13.3</td>
<td>AB</td>
</tr>
<tr>
<td>2.</td>
<td>β-Caryophyllene</td>
<td>1403</td>
<td>76.5</td>
<td>AB</td>
</tr>
<tr>
<td>3.</td>
<td>α-Humulene</td>
<td>1435</td>
<td>8.4</td>
<td>AB</td>
</tr>
<tr>
<td>4.</td>
<td>α-Selinene</td>
<td>1477</td>
<td>1.8</td>
<td>AB</td>
</tr>
<tr>
<td>5.</td>
<td>Pulegone</td>
<td>1259</td>
<td>4.1</td>
<td>AB</td>
</tr>
<tr>
<td>6.</td>
<td>Formic acid</td>
<td>406</td>
<td>2.6</td>
<td>AB</td>
</tr>
<tr>
<td>7.</td>
<td>Palmitic acid</td>
<td>-</td>
<td>1.4</td>
<td>AB</td>
</tr>
<tr>
<td></td>
<td>Total (7)</td>
<td></td>
<td>100.0</td>
<td>AB</td>
</tr>
</tbody>
</table>

A = GC-MS analysis; B = GC analysis
<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name</th>
<th>Mol. For.</th>
<th>Mol. Wt.</th>
<th>Chemical structure</th>
<th>Mass fragmentation index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Eugenol</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;12&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>164</td>
<td><img src="image" alt="Eugenol结构" /></td>
<td>55(26.88), 77(35.48), 91(26.88), 103(32.25), 121(16.12), 131(27.95), 149(35.48), 164(100.00)</td>
</tr>
<tr>
<td>2.</td>
<td>β-Caryophyllene</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;24&lt;/sub&gt;</td>
<td>204</td>
<td><img src="image" alt="β-Caryophyllene结构" /></td>
<td>41(100), 55(31.18), 69(51.61), 80(51.61), 93(55.91), 105(32.25), 133(38.70), 77(51.61), 120(20.43)</td>
</tr>
<tr>
<td>3.</td>
<td>α-Humulene</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;30&lt;/sub&gt;</td>
<td>188</td>
<td><img src="image" alt="α-Humulene结构" /></td>
<td>41(27.65), 51(13.82), 69(14.89), 80(34.04), 93(100), 109(15.95), 121(26.59), 149(15.95)</td>
</tr>
<tr>
<td>4.</td>
<td>α-Selinene</td>
<td>C&lt;sub&gt;13&lt;/sub&gt;H&lt;sub&gt;24&lt;/sub&gt;</td>
<td>204</td>
<td><img src="image" alt="α-Selinene结构" /></td>
<td>41(59.13), 55 (25.80), 81(37.63), 91(48.38), 105(66.66), 119(68.81), 134(58.06), 161(100.00)</td>
</tr>
<tr>
<td>5.</td>
<td>Pulegone</td>
<td>C&lt;sub&gt;9&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;O</td>
<td>152</td>
<td><img src="image" alt="Pulegone结构" /></td>
<td>41(52.16), 69(44), 67(33), 55(28), 108(27), 53(20), 43(23), 42(18)</td>
</tr>
<tr>
<td>6.</td>
<td>Formic acid</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>46</td>
<td>HCOOH</td>
<td>45(23), 46(38), 43(22), 44(12), 42(11), 77(7), 75(5), 47(4)</td>
</tr>
<tr>
<td>7.</td>
<td>Palmitic acid</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;H&lt;sub&gt;32&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>256</td>
<td><img src="image" alt="Palmitic acid结构" /></td>
<td>43(21), 41 (75), 60(61), 73 (51), 55 (50), 57 (49), 69 (22), 71 (22)</td>
</tr>
</tbody>
</table>

Table 5.2: Eight peak mass index of volatile oil of flower buds of *S. aromaticum*
GLC of volatile oil of Syzygium aromaticum

Spectrum 41: GLC of volatile oil of flower bud of Syzygium aromaticum

GC-MS of volatile oil of Syzygium aromaticum

Spectrum 42: GC-MS of volatile oil of flower bud of Syzygium aromaticum
REFERENCES


