SEARCH FOR ANTIFUNGAL DRUGS
AMONG MEDICINAL PLANTS
Plant volatile oils possess a range of biological activities ranging from stimulation of growth to death of cells. Volatile oils are known for their antimicrobial activity against several organisms of public health significance. Many volatile oils possess antifungal properties and there is a potential application of such oils as fungicidal/fungistatic and antimycotogenic agents (Deans et al., 1989).

It has been established scientifically that about 60% of the essential oils possess antifungal and 35% antibacterial properties. (Chaurasia and Vyas 1977)

It is in this quest for searching new antifungal drugs among the medicinal plants, a study of the literature of various medicinal plants and their biological activities gave the lead to below mentioned plants that were taken for our studies to evaluate their efficacy as antifungal agents against the various strains of Candida albicans.

a) Santolina chamaecyparissus (Family: Compositae)
b) Araucaria bidwillii (Family: Araucariaceae)
c) Allanthus excelsa (Family: Simaroubaceae)
d) Pristimera grahami (Family: Hippocratesaceae)

Preliminary phytochemical and antimycological investigations were carried out on the various extracts of these medicinal plants.
A. LITERATURE SURVEY

a) Santolina chamaecyparissus Linn

Family: Compositae

Common name: Lavender Cotton

A branched, aromatic, evergreen, tufted under-shrub up to 50cm tall; branches densely clustered from the base. Leaves alternate, 1-1.5cm long, subsessile, tomentose, silvery grey, finely divided into 12-16, ovate-oblong, thick, pinnate segments each 2mm, strongly aromatic when crushed; leaf-bases broad without a distinct petiole; leaves 10-15 per flowering branch, of which 2-4 of the uppermost are linear, entire, 8-10 mm long. Heads homogamous, solitary globular, 1-1.5cm across, terminal on green, tomentose peduncles 5-8cm long. Involucre campanulate, 7 x 10mm; involucral bracts imbricated, adpressed, in two whorls; outer ones ovate-acute, 5 x 2mm green outside with white margins; inner ones linear, whitish. Florets numerous, yellow, all bisexual, fertile, each 8mm long, subtended by narrow, chaffy, stiff, white bracts 4mm long with apical pubescence and a strong midrib. Pappus absent. Corolla yellow, 2mm across; lobes 4, spreading. Stamens 4, syngenious; filaments slender, 2mm long; anthers introrose, 2-celled. Ovary inferior 1-celled, with 1 basal ovule; style 3 mm long; stigma 2-lobed; lobes yellow, recurved, exceeding the corolla. Fruit an angled achene 4mm long, without pappus.

Flowering: March to September. Fruiting: May to November.

Field Notes: Young leaves are always green; new shoots appear about January-February and at the time of
flowering are 20-25cm long, with 10-15 leaves.

**Distribution:** Indigenous to the Mediterranean regions; growth in gardens throughout the temperate world.

**Cultivation:** This is a familiar border plant in gardens, often found as an escape, growing gregariously. Plants are easily multiplied from cuttings of tender shoots. Propagation may also be done from seeds, as is the case with the escapes.

The plant is reported to be a vermifuge, emmenagogue, analgesic, stimulant, vulnerary, stomachic and antispasmodic.

The herb owes its strong penetrating odour to the presence of an essential oil, Santolina oil (present to the extent of 1.15%), which is not produced on a commercial scale. Oil obtained by steam distillation of the aerial parts of the plant during the flowering period has the following characteristics: sp.gr. at 15°C 0.9546; [α]d at 6.4°; nD at 1.4908; acid value 9.1; ester value 22.4; and soluble in 0.1 volume of 95 percent alcohol. A recent analysis of a sample of steam volatile oil, obtained from the aerial parts of the plant collected in early winter, shows that it contains a monoterpane, 3,3,6-trimethyl 1-1,5-heptadien-4-one (b.p., 180-82°C), which constitutes 65 percent of the oil and is responsible for the characteristic odour of the plant. Besides the monoterpane, the oil contains three unidentified terpenes.

Extracts of flowers, leaves and roots of the plant are reported to be active against gram-positive bacteria.

Santolina is valuable for its distinct foliage and is used in the south for the specimen planting and in the north
for summer bedding and borders. Cutting for the latter purpose are usually taken in the spring from plants wintered in a frame but may be taken before frost in the fall. They are easily rooted in sand (Bailey, 1919).

**Phytochemical and Pharmacological studies reported:**

Baig et al., (1989) had reported that the callus culture established from explants of apical meristem of Santolina chamaecyparissus produced beta-phellandrene and myrcene in yields (total Ca 0.02% wt/wet wt) similar to that from foliage of the parent plant. In addition, significant (0.01%) levels of 3, 3-dimethyl allyl alcohol and traces of trans-chrysanthemyl alcohol were detected. Thus the biosynthetic pathway to the immediate precursors of artemesia ketone, the main terpenoid of the plant, was demonstrated. Suspended callus or fine cell-suspensions were not similarly biosynthetically active.

Guella et al., (1984) had reported that Santoline-none [=(+)-(4R)-(7)-p-menthen-2-one was obtained from the volatile oil of Santolina chamaecyparissus. Its synthesis has been achieved in 30% yield from (+)-(4R)-p-menthene.

Olner and others (1986a,b) have reported the pharmacological study of Santolina chamaecyparissus. Acute toxicity, antiinflammatory and antiulcer activity are also discussed.

In another report by Villar et al., (1986) the chemical composition of the essential oil of Santolina chamaecyparissus SSP squarrosa has been given in detail. The identified compounds and their peak area percentages are listed in Table below according to their order of elution.
The main compounds (in%) of essential oil from *Santolina chamaecyparissus* asp. *squarrosa*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Peak area(%)</th>
<th>Technique</th>
</tr>
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<tbody>
<tr>
<td>Pinene</td>
<td>0.26</td>
<td>gc, gc/ms</td>
</tr>
<tr>
<td>Camphene</td>
<td>2.96</td>
<td>gc, gc/ms</td>
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<tr>
<td>Sabinene</td>
<td>0.23</td>
<td>gc, gc/ms</td>
</tr>
<tr>
<td>b-Pinene</td>
<td>0.99</td>
<td>gc, gc/ms</td>
</tr>
<tr>
<td>Myrcene</td>
<td>t a</td>
<td>gc</td>
</tr>
<tr>
<td>a-Phellandrene</td>
<td>0.14</td>
<td>gc, gc/ms</td>
</tr>
<tr>
<td>Carene</td>
<td>0.14</td>
<td>gc, gc/ms</td>
</tr>
<tr>
<td>a-Terpinene</td>
<td>0.83</td>
<td>gc, gc/ms</td>
</tr>
<tr>
<td>Limonene</td>
<td>1.24</td>
<td>gc</td>
</tr>
<tr>
<td>b-Phellandrene</td>
<td>t</td>
<td>gc, gc/ms</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>9.99</td>
<td>gc, gc/ms</td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>9.99</td>
<td>gc, gc/ms</td>
</tr>
<tr>
<td>Terpinene</td>
<td>1.55</td>
<td>gc, gc/ms</td>
</tr>
<tr>
<td>Artemisia ketone</td>
<td>0.64</td>
<td>gc, gc/ms</td>
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<tr>
<td>Terpinolene</td>
<td>0.15</td>
<td>gc, gc/ms</td>
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<tr>
<td>p-Cymenene</td>
<td>0.56</td>
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<tr>
<td>Fenchone</td>
<td>0.37</td>
<td>gc</td>
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<tr>
<td>Thujone</td>
<td>0.20</td>
<td>gc</td>
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<tr>
<td>cis-Sabinene hydrate</td>
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<tr>
<td>Camphor</td>
<td>25.19</td>
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<tr>
<td>Borneol</td>
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<tr>
<td>Terpinen-4-ol</td>
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<td>gc</td>
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<tr>
<td>Myrtenal</td>
<td>1.31</td>
<td>gc, gc/ms</td>
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<tr>
<td>Bornyl acetate</td>
<td>6.61</td>
<td>gc, gc/ms</td>
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<tr>
<td>Compound</td>
<td>%</td>
<td>Method</td>
</tr>
<tr>
<td>-------------------------------</td>
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</tr>
<tr>
<td>Isobornyl acetate</td>
<td>6.61</td>
<td>gc,gc/ms</td>
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<tr>
<td>a-Cubebene</td>
<td>1.47</td>
<td>gc,gc/ms</td>
</tr>
<tr>
<td>a-Copaene</td>
<td>1.47</td>
<td>gc,gc/ms</td>
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<tr>
<td>4-Isopropyl benzaldehyde</td>
<td>0.14</td>
<td>gc,gc/ms</td>
</tr>
<tr>
<td>a-Ylangene</td>
<td>0.14</td>
<td>gc,gc/ms</td>
</tr>
<tr>
<td>b-Gurjunene</td>
<td>0.19</td>
<td>gc,gc/ms</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>0.19</td>
<td>gc,gc/ms</td>
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<tr>
<td>a-Gurjunene</td>
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<td>gc,gc/ms</td>
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<tr>
<td>Isocaryophyllene</td>
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<tr>
<td>allo-Aromadendrene</td>
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<td>gc,gc/ms</td>
</tr>
<tr>
<td>a-Muurolene</td>
<td>7.28</td>
<td>gc,gc/ms</td>
</tr>
<tr>
<td>Germacrene-b</td>
<td>0.45</td>
<td>gc,gc/ms</td>
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<tr>
<td>b-Cadinene</td>
<td>1.29</td>
<td>gc,gc/ms</td>
</tr>
<tr>
<td>Sesquiterpene alcohol</td>
<td>0.12</td>
<td>gc,gc/ms</td>
</tr>
<tr>
<td>Sesquiterpene alcohol</td>
<td>0.75</td>
<td>gc,gc/ms</td>
</tr>
<tr>
<td>Cadinol</td>
<td>0.83</td>
<td>gc,gc/ms</td>
</tr>
</tbody>
</table>

\[ t^a = \text{traces (}< 0.10\%\) }\]

Identification is based on comparison of the mass spectral data obtained with literature data (2-3) and also by comparing the retention data with those of pure compounds. A total of five components were identified only by glc. These were: myrcene, limonene, fenchone, thujone and a-terpineol.

Rios, Giner and Villar. (1989) had reported the isolation and identification of an antiinflammatory principle from Santolina chamaecyparissus. The antiinflammatory activity of the dried aerial parts of Santolina chamaecyparissus showed antiinflammatory activity in mice against carrageenan induced paw edema. In an another report Giner et al., (1988) had
described the CNS depressant effects, antiinflammatory activity, analgesic activity and anticholinergic activity of the various extracts of the dried aerial parts of Santolina chamaecyparissus. In a yet another study with rats, Ginére et al., (1986.a,b) had reported antigastric ulcer activity with various extracts of the aerial parts of Santolina chamaecyparissus.

b) Araucaria bidwillii

Family: Araucariaceae

Araucaria (chilean name). Including columbea and Eutacta. Pinaceae. Large South American and Pacific Australian evergreen trees (about a dozen species), grown in their juvenile state in greenhouses and windows and often used in summer for lawn decoration; they are very decorative pot-plants.

Tall strict or widely branching conical trees: lvs. small, scale-like and stiff, clothing all the branches uniformly and usually closely imbricated; mostly deciduous, the staminate terminal and solitary or disposed in fascicles; anthers 6-8 celled; pistillate fls. in ovoid or globose heads that become large woody cones with only 1 seed underneath each scale. The South American species (columbea) have scarcely winged cone-scales, the cotyledons 2, and germination hypogeal (cotyledons remaining below ground); the Australian and Pacific species (Eutassa) have winged scales, cotyledons 4, and germination epigeal.

Araucaria are probably the most prized pot evergreens in cultivation. They are much used in house decoration,
particularly at Christmas time, as they are not only attractive but will stand much hard usage. A. excelsa is the one commonly seen in residences. Propagation is by seeds and cuttings, as for A. excelsa. Symmetrical plants are secured from the leading shoots. Side shoots are likely to make misshapen specimens.

Rather narrow in growth, especially with age, the branches simple: lvs. in two rows, lanceolate and very sharp pointed, thick, firm and shining. Australia, where it attains a height of 150ft., and is known as bunya-bunya. R.H. 1897, p.500, desc. G.C. III, 15:465, showing the pineapple-like cone. One of the best handsomest species for pots.

There are several species of Araucaria of which some of them are:

1. Araucaria bidwilli Hook. rather narrow in growth, especially with age the branches are simple. Leaves in two rows, lanceolate and very sharp pointed thick, firm and shining. Australia, where it attains a height of 150ft and is known as bunya-bunya

2. Araucaria balansae (Balansa's) male cones cylindrical conical, 2 inch, female cones elliptic, globose 4 (in) scales obovate, cuneate, arcuately-uncinate, ovate triangular, imbricated round the distichous, simple branchelets height 130 ft to 160 ft.

3. Araucaria brasiliensis (Brazilian)L Oblong-lacceolate much attenuated at the point, loosely imbricated. Deep green, lower part of the trunk usually free from branches, terminating in a rounded head height 7ft to
100 ft. Araucaria brasiliensis gracilis and Araucaria brasiliensis ridolfiana are two forms of this species Araucaria cookii (Cook's) L. Awl shaped, short densely imbricated around the frondose branches. Described by Bailey (1919) as having "a somewhat curious habit, even when growing alone, of shedding their branches for five sixths or more of their height, and then replacing them by a smaller and more bushy growth. So that the tree at a distance presents a very columnar appearance, the resemblance being increased by the summit being crowned with a mass of foliage somewhat like a capital" height 200ft. New Caledonia 1851 syn Araucaria columnaris.

Araucaria cunninghamii (Cunningham's) L. on the sterile branches needle shaped, obscurely quadrangular, rigid, acute, on the fertile branches shorter, stouter, closely appressed bright green, upper branches ascending lower ones horizontal height 100ft Moreton Bay. This fine species we have found to be quite hardly on the South West Coast of England.

Araucaria cunninghamii (milkygreen) A very handsome variety with silvery glaucous leaves.

Araucaria excelsa (lofty) The Norfolk Island pine L. awl-shaped, curved, sharply acuminate bright green, densely packed on the frondose deltoid, horizontal, conservatory species attaining to a height of 105 ft and circumference of 20ft or more. This is especially desirable in a small state. There are several
varieties known the best being Araucaria excelsa glauca, having lighter green and very glaucous foliage and Araucaria excelsa robusta which is larger in all its parts.

8. Araucaria goldiae-anu (Goldiae) allied to Araucaria ruelli. produced in whorls, pendulous, dark green, varying in size New Caledonia. Most distinct and elegant for conservatory decoration.

9. Araucaria imbricata (imbricated). The monkey puzzle fl. male and female, catkins on separated trees, the males are six or seven in a cluster, penduculate, yellow, and oval with numerous scales, imbricated, long and recurred at the points, the female catkins are oval with numerous wedge-shaped scales with narrow along brittle points, they are produced at the ends of the branches, cones when fully ripe globular, from 3 inch to 4 inch in diameter, dark brown. The branches are horizontal, inflexed, and ascending at the extremities, and are produced in whorls, Lovate, lanceolate, sessile, thickened at the base stiff, leathery, straight, somewhat keeled shaped below and strongly mucronate at the apex; verticillate, with seven or eight in whorl, imbricate, and closely encircling the branches concave, glabrous shining marked with longitudinal lines, dotted on both sides height 50ft to 100ft chilli 1796. A well known hardy tree, of striking aspect, and indispensable to Arboreta and shrubberies.

10. Araucaria rulei (Rule's) male cones oblong, obtuse
female cones oval, L.oblong, lanceolate, with a prominent dorsal nerve, more closely appressed, and less sharply pointed than in the foregoing species, imbricated in four rows. Branches horizontal, branchlets often quite pendulous h. 50ft. Papuan Archipelago.

11. Araucaria rulei (elegant) L.smaller; whorls of branches closer together; branchlets more slender an elegant form, and from its comparative dwarf and graceful habit should be very grown.

These are some of the species of Araucaria.

Phytochemical and Pharmacological studies reported:

The plant Araucaria bidwilli Hook belonging to the family araucariaceae distributed commonly at high altitudes also occurs in some parts of Nilgiris. The leaves of the plant are being used to treat insomnia by the Lahu, a group of tribal people in Northern Thailand. This was the only ethnomedical information available on Araucaria bidwilli,(Anderson, 1986).

The 95% ethanolic extracts of leaves were also tested for moulting activity (insect) and found to be inactive (Hoffmeister, et al., 1967)

The leaves were screened for phytochemical constituents and the presence of the following flavanoids has been reported in the literature by Ilyas and others (1978).

The flavones were amentoflavone, cupressuflavone, 7,7'-di-o-methyl cupressuflavone, 7-o-methyl cuppresuflavone, agathisflavone, 7,7'-di-o-methyl-agathisflavone, 7-o-methyl
Oleo-resin exuding from the bark of
ARAUCARIA BIDWILLI
agathisflavone, 7-o-methyl agathisflavone, bilobetin and hinokiflavone.

The plant Araucaria bidwillii also yields a yellow coloured oleoresin and presence of following diterpenes was reported in the literature.

The diterpenes were: - Methyl-ent-8B-hydroxy-labd-E-13en-15 oate (1.2%), ent-8B-15-labd-E-13-ene-diol (1.3%), Methyl-ent-8a-hydroxy-labd-E-13-en-15 oate (1.0%), ent-8a-hydroxy labd-E-en-15 oic acid (1.6%), ent-15-Acetoxy-labda-8-E-13-diene (0.8%), ent-labda-8-E-13-dien-15-ol (0.7%).
c) Ailanthus Excelsa Roxb

Family: Simaroubaceae

Ailanthus excelsa, a tree has wide application in the traditional medicine system of the tribals. The barks and the leaves of the tree are used as a bitter tonic, carminative and febrifuge. The tribals use the bark as a expectorant and antispasmodic. They also claim that an extract of the bark when administered to the women, produces permanent antifertility effect.

Morphology and Habitat (Kirtikar and Basu, 1984)

A tree 18-24 M high, leaves unequally or equally pinnate, usually 20-30cm, but sometime reaching 60-90cm long, the younger tomentose, the older more or less so glabrous; leaflets 8-14 pairs alternate or subopposite, very variable in shape, 10-15cm long coarsely and irregularly toothed or sublofate, very unequal at the base; petioles 2.5cm long. Flowers in large lax often much branched panicles; pedicels long, slender lobes ovate triangular; petals 4mm long, ovate-lanceolate glabrous, refused filaments glabrous about half as long as the anthers. Samara 3.8-5.5cm long by 1-1.3cm broad lanceolate, alute both ends, redding brown, twisted near the base many nerved, the nevers reticulatem, above the seed, otherwise nearly parallel seed solitary in the centre of the samara. Distribution: Indigenous in the Indian peninsula and often planted in various parts of India. Common in many parts of
AILANTHUS EXCELSA ROXB

Family: Simaroubaceae
India, U.P., Bihar, Bombay, Western peninsula, Carnatic
Coramandal coast.

Synonyms: Sanskrit - Aralu, Atarusha mada, Mahanimba
Tamil - Agal, naru, peruppi, pi
Telugu - Pedda, Paddamandu, Poddam nu
Hindi - Limbade, Maharukka

Constituents (Bhatia et al., 1985):

Bark contains an important bitter principle known as allantic acid. It is wax like, reddish brown, easily soluble in alcohol: water, ether etc. It is related to Quassain probably identical with condrin and samaderin obtained from other members of this species.

Uses: Bitter tonic, carminative and febrifuge. Bark is expectorant and antispasmodic (Kirtikar and Basu, 1984).

Bark and the leaves in infusion form are reputed as tonic in debility after childbirth. Especially useful in dyspepsia, bronchitis and asthma. Allantic acid is given as tonic and in dyspepsia with constipation.

Phytochemical and Pharmacological studies reported:

Sukumar (1984) had reported the isolation and identification of b-sitosterol, b-sitosterol glucoside, campesterol and octacosamol in Ailanthus excelsa and some plants.

Khosa et al., (1985) had reported the spectral details of the Quassanoid 13:18 dehydroexcelsin, isolated from the root-bark of Ailanthus excelsa.
The isolation and spectral details of a new quassinoid, excelsin \((C_{25}H_{26}O_9, \text{M.P. 268-270°C})\) obtained from the root-bark of *Ailanthus excelsa* has been reported by Bhatia, et al., (1985). The presence of a new quassinoid \(1:12\) deoxy-\(13\)-formyl allanthiol \((C_{25}H_{34}O_8, \text{M.P. 282-300°C})\) and \(\beta\)-sitosterol have been reported in the *A. excelsa* bark by Bhatia et al., (1983).

Ogura et al., (1977) reported the anticancer activity observed from the extracts of the root-bark of the *A. excelsa* which is attributed to 2-principle components allanthinone and glaucarubinone. A third component which exhibited anti 9-KB cancer activity was a mixture of glamacarubol-15 is ovalerate and 13,18-dehydro-glaucarubol-15 is ovalerate.

A new triterpene, \(3S, 24S, 2S\) trihydroxy tirucall-7-ene was isolated from *A. excelsa* root-bark and its structure determined by chemical and spectral data. This was reported by Sherman and Mary (1980).

Suroor Khan, (1980) had reported the structure of 13,18 dehydroxy excelsin, a new quassinoid isolated from *A. excelsa* was determined by spectral analysis of it and its methyl ester. Glaucarubol was also isolated from an alcoholic extract of the bark.

\(\beta\)-sitosterol and vitexin were isolated from *A. excelsa* was reported by Kapoor et al., (1971). From the root bark of *A. excelsa*, 4 alkaloids were obtained. Three of these, canthin-6-one, 1-methoxy canthin-2-one and 5-methoxy canthin-6-one are known. The fourth alkaloid is new and from analysis of
spectral data it was deduced to be 8-hydroxy canthin-6-one as reported by Cordell et al., (1978).

The isolation of a few C_{20} quassanoids from the ethanol extract of the bark of A. excelsa is described. One of the quassainonoids was identified as gaucabolin and was reported by Suroor Khan et al., (1978).

Sitosterol, 2,6 dimethoxy benzoquinone and melathin have been isolated from the light petroleum extract and an unidentified bitter principle (m.p. 215-20°C) from the ethanol extract of the bark of A. excelsa was reported by Mahendra Kumar Jain (1964).

Chemical examination of the quassainoid contents of A. excelsa has led to the isolation of a new quassainoid designated as excelsin. Its structure has been arrived at on the basis of spectral and chemical evidence as reported by Suroor Khan, et al., (1980).

Bhakuni et al., (1980) had reported the antispasmodic activity of unspecified type tested with ethanol: water (1:1) extract of the stem bark of A. excelsa in guinea pig ileum preparation. They have also reported the hypotensive activity of the ethanol (50%) extract of A. excelsa on dog at a dose of 50mg/kg.

d) Pristimera grahami (Synonyms: Hippocratae grahami; Reissantia grahami)

Family: Hippocrateaceae

Lianes or stragglers. Leaves decussate, coriaceous; stipules caducous. Cymes axillary, sometimes in panicle. Flowers small. Calyx lobes 5, imbricate. Petals 5, imbricate, entire
Reissantia grahami (Wight) Ding Hou Hippocrates grahamii Wight
1 inflorescence; 2 branchlet; 3 flower; 4 sepal; 5 petal; 6 & 7 stamens (bud);
8 stamens with pistil; 9 & 10 pistil. l.s. & t.s. (RUT 27213)

PRISTIMERA GRAHAMI

Family: Hippocratesaceae
or lacinate. Disc extrastaminal, cupular, thick. Stamens 3, inserted on top of disc; filaments erect or recurved, base dilated, flat; anthers transversely dehiscent. Ovary immersed in disc at base, globose, 3-celled; ovules 2 or more per cell; style stout; stigma simple. Fruit samaroid.

Inflorescence 75cm, green. Petals lacinate. Leaves 3.5cm across, margin entire, obtuse to rotund at base.


Liane to 20m; branchlets sometimes coiled, glabrous. Leaves ovate-oblong or elliptic, 6.5-9 x 4-4.5cm, coriaceous, nerves prominent below, glabrous, base subacute to rotund, margin entire, apex acute to obtuse; petiole sometimes coiled, to 1.5cm; stipules triangular ovate, 0.5mm. Inflorescence dense and massive to 75cm; peduncle ca. 15 cm; bracts ovate, somewhat produced at base; barcteoles 3-5 clustered, imbricate, 0.5mm; pedicel to 3.5mm. Flowers 3 mm across. Calyx-lobes 5, ovate 1mm, lacinate, Petals 5, green, oblong or obovate-oblanceolate, 3mm, margins inflexed, lacinate. Disc 5-angular. Stamens 3; filaments 0.8mm, patillose, flat dilated. Ovary 3-celled, 0.5mm; ovules ca. 6 per cell; style 0.5 mm; stigma obscure.

Only from Guthirayans from deep inside the shola.
Extensive Gnetum-like liane. Flowers (March).

Fruits not seen. Not mentioned in Fl. Madras.

Distribution: Peninsula and N.E. India, Upper Burma, Thailand and Malaysia (Ding Hou, 1964).


Tamil - Odankodi, Morasarakodi

Straggler to 4(6)m; branchlets sometimes coiled, glabrous.

Leaves ovate or obovate-elliptic, 2.5-4 x 1-2cm, thin-coriaceous, nerves obscure above prominent below, glabrous, base attenuate or cuneate, margin serrate, apex abruptly acute-acuminate; petiole to 1cm. Cymes 4-10cm, rusty usually with supplementary branchlets in dichotomies; peduncle to 3 cm; bracts triangular; pedicel to 2mm. Flowers 1mm across. Calyx-lobes 5, ovate 0.3mm, papillose or erose. Petals 5, rusty to yellow, oblong, 1mm. papillose, mid region callose. Disc cupular, thick. Stamens 3; filaments that, subulate, to 0.5mm. ovary 3-celled; ovules 2 per cell; style stout; stigma obscure.

Foothill scrub jungles to lower slopes, to 500(750)m.

Common.

Flowers with two peak during February-April and July-October.

Distribution: Widely distributed, but scattered in India, Sri Lanka, Burma, Thailand, Indo-China, S.China (Yunnan, Hainan), Malaysia (Ding Hou, l.c.).
Phytochemical and Pharmacological studies reported:

The survey of the literature shows that no information relating to the biological activities of this plant is available. However, Fernandes et al., (1975) have reported the presence of triterpenes, erythrodiol and pristemerin from the leaf and the root bark respectively.
B. MATERIALS AND METHODS

a) Phytochemical investigations

1) Preparation of Extracts and Fractions:

i) Santolina chamaecyparissus:

The fresh samples of plant were collected and subjected to steam distillation. The plants were distilled for a period when it was observed that there was no further increase in the yield for half an hour continuously. The amount of volatile oil obtained was calculated and subjected to phytochemical tests.

ii) Araucaria bidwilli:

The oleoresin collected from plant was subjected to cold maceration with petroleum ether and methonal successively for 24 hours and the extracts were then evaporated to dryness under reduced pressure. These extract were then subjected to phytochemical investigation.

The leaves of the plant were extracted with petroleum ether and methonal in a Soxhlet's apparatus successively. The extract was filtered, concentrated and the solvent was removed finally by distillation and then dried in vacuum. The extracts were then subjected to phytochemical investigation.

iii) Ailanthus excelsa:

The air dried plant materials, leaves and stem bark, were powdered and subjected to extraction with methanol for 24 hours. The extracts were then concentrated and dried under vacuum and then subjected to phytochemical investigations.
iv) Pristimera grahami:

The air dried plant materials, leaves and stem bark were subjected to continuous extraction for 24 hours with methanol. The extracts were then concentrated and dried under vacuum and then subjected to phytochemical investigation.

2) Chemical test:

The various plant extracts and distillates were subjected to chemical tests of identification for the various constituents.

* Test for alkaloids: The test for alkaloids was carried out using the following reagents and tests:
  (i) Mayer's  (ii) Dragendorff's  (iii) Haver's  
  (iv) Wagners

* Test for glycosides and sugars: The test for glycosides and sugars was carried out using the following reagents:
  tests:
  (i) Molisch's  (ii) Fehling's  (iii) Barford's  
  (iv) Benedict's  (v) Legal's  (vi) Borntrager's

* Test for phenolic compounds and tannins: The test for phenolic compounds and tannins was carried out using the following reagents:
  (i) Ferric chloride  (ii) Gelatin  (iii) Lead acetate  
  (iv) Aqueous bromine.

* Test for saponins: The test for saponins was carried out using the following tests:
  (i) Haemolysis  (ii) Foam test
Test for proteins and amino acids: The test for proteins and amino acids was carried out using the following reagents and tests.

(i) Millon's  (ii) Biuret  (iii) Ninhydrin

These chemical tests on the various extracts of plant materials showed the presence of various phytochemical constituents and were tabulated (Table 16).

b) Antifungal studies:

1) in vitro studies: (Two fold serial dilution technique)

The antifungal activity of the various plant extracts, obtained as described above, was studied against 14 strains of C. albicans.

The various drugs at doses ranging from 500 μg/ml to 62.5 pg/ml of the broth, dissolved in dimethyl sulphoxide (1mg/ml of DMSO) was tested for its antifungal activity against the above fungi, 24 hours cultures of which containing $10^5 - 10^6$ cfu/ml were used to seed the broth.

The procedure involving these studies is already elaborated in Chapter II of this thesis.

2) in vivo studies: Experimental vaginal candidosis:

This was carried out as described by Wildfeuer (1974) and as also explained in the earlier pages (Chapter II) of this thesis.

C) Results and Discussion:

1) in vitro studies:

Antifungal studies with the various plant extracts against the 14 strains of C. albicans was studied as described...
Table 16: Investigation of various plant extracts

<table>
<thead>
<tr>
<th>Plant</th>
<th>Extract</th>
<th>Chemical test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Alkaloid</td>
</tr>
<tr>
<td>S.chamaecrypissus</td>
<td>Volatile oil distillate</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Pet.ether ext.of oleoresin</td>
<td>-</td>
</tr>
<tr>
<td>A.bidwilli</td>
<td>Methanolic ext.of oleoresin</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Pet.ether ext.of leaf</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Methanolic ext.of leaf</td>
<td>-</td>
</tr>
<tr>
<td>A.Excelsa</td>
<td>Methanolic ext.of leaf</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Methanolic ext.of stem bark</td>
<td>+</td>
</tr>
<tr>
<td>P.grahami</td>
<td>Methanolic ext.of stem bark</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Methanolic ext.of leaf</td>
<td>-</td>
</tr>
</tbody>
</table>

+, Present; -, Absent
earlier. The results revealed that among the various natural products tested, the volatile oil distillate of Santolina chammaecryparissus possessed antifungal activity in vitro thus becoming a potential candidate for in vivo studies (Table 17). The minimum inhibitory concentration of the volatile oil of Santolina chammaecryparissus was 250 µg/ml of the broth. The other extracts did not show any significant antifungal activity at the dose levels tested.

These data were further subjected to statistical treatment and found that the treatment mean value of 103.57 was observed with the volatile oil distillate of Santolina chammaecryparissus, which concurs with our above observation.

2) In vivo studies: Experimental vaginal candidosis:

In vivo anti candidal activity of volatile oil distillate of Santolina chammaecryparissus was studied in mice against experimental vaginal candidosis.

The results indicate, comparable activity of the drug (Santolina chammaecryparissus) to that of the standard drug, clotrimazole (Table 18) in effectively controlling the experimental vaginal candidosis in mice.

To confirm the data presented in Table 18, it was subjected to statistical analysis. The drug (Santolina chammaecryparissus) controls vaginal candidosis significantly with 4% formulation mixture.

The statistical studies further supported the fact that the infection was maximally controlled on the 9th day of the treatment. The interaction between drug and the periods shows that 4% formulation mixture of the drug effectively and maximally controls the infection by 9th day of the treatment.
### Table 17: In vitro evaluation of antifungal activity of various extracts against various strains of Candida albicans using two fold serial dilution technique

<table>
<thead>
<tr>
<th>S1 No.</th>
<th>Name of the compound</th>
<th>Minimum inhibitory concentration in μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ca5</td>
</tr>
<tr>
<td>2.</td>
<td>Araucaria bidwilli Pet. ether ext. Oleoresin</td>
<td>500</td>
</tr>
<tr>
<td>3.</td>
<td>Araucaria bidwilli MeOH.ext. of Oleoresin</td>
<td>500</td>
</tr>
<tr>
<td>4.</td>
<td>Araucaria bidwilli Pet.ether ext.of leaf</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Araucaria bidwilli MeOH.ext. of leaf</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Ailanthus excelsa MeOH.ext. of stem bark</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Ailanthus excelsa MeOH.ext. of leaf</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Pristimera grahami MeOH.ext. of stem bark</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>Pristimera grahami MeOH.ext.of leaf</td>
<td>-</td>
</tr>
</tbody>
</table>

Broth used: Sabouraud's Dextrose broth  
Volume of broth in assay tube: 1.0 ml  
Inoculum strength: $10^3 - 10^6$ cells/ml  
Temperature at which incubated: 37°C  
Solvent used: DMSO  
Observations recorded after: 24 Hrs.
Table 18: Effect of volatile oil distillate of Santolina chamaecyparissus against C. albicans in experiment
Vaginal candidosis in mice

<table>
<thead>
<tr>
<th>Sl NO</th>
<th>Drug concentration/groups</th>
<th>Colony forming units</th>
<th>Result</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3rd day</td>
<td>6th day</td>
<td>9th day</td>
</tr>
<tr>
<td>1</td>
<td>S. chamaecyparissus 2%</td>
<td>1.1x10^4</td>
<td>7x10^3</td>
<td>4x10^3</td>
</tr>
<tr>
<td>2</td>
<td>S. chamaecyparissus 4%</td>
<td>3.7x10^3</td>
<td>2.4x10^3</td>
<td>1x10^3</td>
</tr>
<tr>
<td>3</td>
<td>Clotrimazole 2%</td>
<td>3.6x10^3</td>
<td>2.0x10^2</td>
<td>1.5x10^2</td>
</tr>
<tr>
<td>4</td>
<td>Solvent control</td>
<td>5.76x10^5</td>
<td>5.8x10^5</td>
<td>7.48x10^5</td>
</tr>
</tbody>
</table>

Animal used: Swiss albino mice
Candida strain used: Ca27
Strength of inoculum: 10⁷ - 10⁸ cells/ml
Sterile diluent: Distilled water + Tween 80
Standard drug: 2% Clotrimazole in PEG 200

Media used: SDA + Chloramphenicol 0.05 mg/ml
Incubation Temperature: 37°C
Incubation period: 24 hours
Solvent used: PEG(Polyethylene Glycol) 200
Concilia Ion: The yeast like fungi are resistant to most antibacterial agents and there are few available antifungal agents useful in treatment of fungal disease. There is a need to develop a wider variety of antifungal compounds that are most effective and less toxic. In search of this goal, the studies with the extracts of various medicinal plants against C. albicans was carried out.

The antifungal activity of the various plant extracts against 14 strains of C. albicans studied in vitro and in vivo, has projected the volatile oil distillate of Santolina chammaecryparissus as a very effective antifungal drug.

The usefulness of medicinal plants in general and especially volatile oils as antifungal agents has already been emphasised by Abraham et al., (1986) and Deans et al., (1989). Giner et al., (1986 a,b) and Rios et al., (1989) have described the anti-inflammatory activity of the drug Santolina chammaecryparissus.

The presently studied and recommended antifungal activity of the said drug along with its reported anti-inflammatory activity makes it a very promising drug to be recommended for further studies, including clinical studies.