BIOACTIVE COMPOUNDS FROM SEED AND SEED COAT OF CYNOMETRA IRIPA A MANGROVE SPECIES

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ABSTRACT

The bioactive volatile compounds from plant samples are analyzed by using GC-MS technique. Plant extract of mangrove species Cynometra iripa Kostel. are analysed for bioactive compounds. Five major bioactive volatile compounds have been identified in the seeds and ten in seed coat viz. falcarinol, Butyric acid-pentadecyl ester, stigmast-4-en-3-one, Beta-sitosterol, Vitamin E cholesta-4, 6-diene-3-01, (3-beta), 3,5-di-ter-butyl-4-hydroxy benzaldehyde, 1- Eicosanol, Distearyl sulphide, 1,2-Benzenedicarboxylic acid butyl 2- ethylhexyl ester, 2,5-di-ter-butyl-1,4-benzoquinine, Squalene, Progesterone, Beta-carotene, Cholesta-4,6-diene-3-ol, (3 beta). These compounds are found to be useful as bactericides, insecticides, fungicides and anti cancerous. Cynometra iripa seeds are thus rich in volatile compounds.

Keywords: Cynometra iripa, Mangrove, Bioactive Volatile Compounds, GC-MS Technique, Fungal, Microbial, Bioresource.

INTRODUCTION

Cynometra iripa Kostel. is a typical mangrove species that occur along west coast of Maharashtra. Seeds are used in the traditional medicinal treatment for leprosy and scabies. Only traditional uses have been reported. The species reported both in Sindhudurg & Ratnagiri districts of Maharashtra1. Antibacterial activity and phytochemical screening from different parts of C. iripa are carried out4. Seasonal variation in organic constituents from different parts of C. iripa are carried out5. Salt Tolerance in seedlings of the species is studied6. Fatty acid profile of the C. iripa from seeds and leaves are also carried out6. The habitat of the species along west coast of Maharashtra has studied7. The Isolation and characterization of starch from mangroves Aegiceras corniculatum (l.) Blanco and Cynometra iripa Kostel. have been studied7. The present attempt was determine the possible chemical constituents from Cynometra iripa seed and seed coat by using GC-MS technique.

MATERIALS AND METHODS

The methanolic extracts obtained from C. iripa seed and seed coat was subjected to Gas Chromatography and Mass Spectroscopy for the determination of bioactive volatile compounds. GC-MS analysis of the samples were carried out using Shimadzu Make QP-2010 with non polar 60 M RTX 5 MS column-Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 40°C and held for 3 min. and the final temperature of the oven was 480°C with rate at 10°C min. sup. 1. 2 ml sample was injected with split less mode. Mass spectra were recorded over 35-650 amu range with electron impact ionization energy 70 ev. The total running time for a sample was 45 min. The chemical components from the methanolic extracts of fruits were identified by comparing the retention time of chromatographic peaks using Quadra pole detector with NISI Library to relative, retention indices. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS.

RESULTS

Mass spectra analysis of bioactive volatile compounds of Cynometra iripa seed and seed coat are shown in Table-1 & Table-2. While the mass fragments are presented in Fig. 1 & Fig. 2. It is clear from the table that seed & seed coat of C. iripa are rich in bioactive volatile compounds. Among these volatile compounds...
some represents class of monoterpenes, alkenes and alcohols. The volatile constituents are found which play a prominent role as plant protection agents and are explored for their insecticidal, pesticidal and herbicidal properties\(^{16}\).

**Uses of Bioactive Volatile Compounds of seeds:**

**Beta-sitosterol:** Molecular formula: \(C_{29}H_{50}O\)

It is found in seeds 2.92%. It is phytosterol with chemical structures similar to that of cholesterol. They are hydrophobic and soluble in alcohols. Alone and in combination with similar phytosterols, beta-sterol reduces blood levels of cholesterol, and is sometimes used in treating hypercholesterolemia, leima, beta-sterols inhibit cholesterol absorption in the intestine. Positive effects have shown on male hair loss in combination with saw palmetto\(^{12}\). It is used in Europe for the treatment of prostatic carcinoma\(^{17}\) and breast cancer \(^{2}\) although the benefits are still being evaluated in the US.

**Butyric acid, 2-pentadecyl ester:** Molecular Formula: \(C_{19}H_{38}O_2\)

Butyric acid is used in the preparations of various butanoate esters. Low molecular weight esters of butyric acid, such as methyl butanoate, have mostly pleasant aromas or tastes. As a consequence, they find use as food and perfume additives. Content of Butyric acid, 2-pentadecyl ester is 0.63% in seeds.

**Falcarnol:** Molecular formula: \(C_{17}H_{24}O\)

Falcarnol is a natural pesticide and fatty alcohol found in carrots, red ginseng and ivy. Content of Falcarnol is 1.17% in seed. It protects roots from fungal diseases, such as liquorice rot that causes black spots on the roots during storage. Falcarnol is thought to reduce the risk of developing cancer, as a research team from the University of Newcastle upon Tyne and Danish Universities found in February 2005 in a study on rats\(^{11}\). Seeds of *Cynometra iripa* have sufficient % of Falcarnol.

**Vitamin E:** Molecular formula: \(C_{29}H_{50}O_2\)

Content of Vitamin E is 0.87% in seeds. It is antiageing analgesic, antiidiabetic antilinflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective, antiulcerogenic, vasodilator, antispasmodic, antobronchitic, anticoronary\(^{10}\).

**Stigmast-4-ene-3-one:** Molecular formula: \(C_{29}H_{48}O\)

It is a steroid compound. It is an antitumor promoting and cytotoxic constituent. Content of Stigmast-4-ene-3one is 31.90% in the seeds. The content proves the value as best medicinal resource as mentioned above.

### Table 1: Mass spectral analysis of bioactive volatile compounds of *Cynometra iripa* seeds.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of Molecule</th>
<th>RT</th>
<th>Molecular weight</th>
<th>Area</th>
<th>Peak Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>butyric acid 2-pentadecyl ester</td>
<td>10.75</td>
<td>298</td>
<td>137994</td>
<td>0.63</td>
</tr>
<tr>
<td>2.</td>
<td>falcarnol</td>
<td>14.62</td>
<td>244</td>
<td>255293</td>
<td>1.17</td>
</tr>
<tr>
<td>3.</td>
<td>stigmast-4-ene-3-one</td>
<td>45.71</td>
<td>412</td>
<td>6953000</td>
<td>31.90</td>
</tr>
<tr>
<td>4.</td>
<td>beta-sitosterol</td>
<td>47.86</td>
<td>414</td>
<td>636564</td>
<td>2.92</td>
</tr>
<tr>
<td>5.</td>
<td>vitamin E</td>
<td>49.23</td>
<td>430</td>
<td>188994</td>
<td>0.87</td>
</tr>
</tbody>
</table>

![Figure 1: GC-MS Spectra of Seed of Cynometra iripa](image-url)
Uses of Bioactive Volatile Compounds of seed coat:
3, 5-Di-tert-butyl-4-hydroxybenzaldehyde: Molecular formula: C(15)H(12)O
It has an antioxidant activity. The antioxidant activity of these 3, 5-Di-tert-butyl-4-hydroxybenzaldehyde derivatives in stripped corn oil at 79.5° ±1.0C was measured on the basis of length of the induction period. Content of 3, 5-Di-tert-butyl-4-hydroxybenzaldehyde is 3.63% in seed coat.

1, 2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester: Molecular formula: C(20)H(28)O
It is used as ingredients in the industries of agricultural, foodstuff, metal processing, cosmetics, lube additive, pharmaceutical, rubber, textile, perfume and flavouring as well as synthetic detergent. In seed coat content is 6.27%. This oily liquid can be used in various industries.

1-Eicosanol: Molecular formula: C(30)H(58)O
1-Eicosanol is emulsifiers and emollients to make skin smoother and prevent moisture loss. It is used to improve rub-out of formulas and to control viscosity and dispersion characteristic in cosmetics, personal care products and pharmaceutical ingredients. Content of 1-Eicosanol is 4.83% in seed coat.

1, 2-Benzenedicarboxylic acid mono (2-ethylhexyl) ester:
Molecular formula: C(14)H(22)O
It is used as a plasticizer for polyvinyl chloride polyvinyl acetate, rubbers, cellulose plastics and polyurethane. It is also used in PVC floorings and wall coverings, expanded leather, PVC foams, films, sealing and adhesive systems based on polyurethane or polysulphide. Content is 6.27% present in seed coat.

Distearil sulfide: Molecular formula: C(36)H(64)S
It is used as antioxidant. It is used in rubber, oil, soap, lubricants and polyolefin and antioxidants. This is not colouring materials, pollution, suitable for producing white truck products. The content of Distearil sulfide is 5.77% in seed coat.

2,5-di-tert-butyl-1,4-benzenquinone: Molecular formula: C(14)H(20)O
It is a potent and selective inhibitor of liver microsomal Ca(2+) sequestration which may be a useful tool in studies of Ca(2+) fluxes in intact cells and tissues. It is used as inhibitor, stabilizer, antioxidant, etc. And it is also used as the intermediates for pigments, dyestuffs, medicines. Seed coat contains 2.24% 2, 5-di-tert-butyl-1,4-benzenquinone.

Squalene: Molecular formula: C(30)H(56)
Squalene has been proposed to be an important part of the mediterranean diet as it may be a chemo preventative substance that protects people from cancer. Squalene is a hydrocarbon and a triterpene and is a natural and vital part of the synthesis of cholesterol, steroid hormones, and Vitamin D in the human body(4-8). Squalene is used in cosmetics, and more recently as an immunologic adjuvant in vaccines. Seed coat contains 5.77% Squalene which can be utilized as medicine, cosmetics.

Progesterone: Molecular formula: C(27)H(44)O
It is steroid hormone involved in the female menstrual cycle, pregnancy and embryogenesis of humans and other species. In Juglans regia progesterone has been detected(5). Progesterone like steroid is found in Dioscorea mexicana. It is a plant that is part of the yam family native to Mexico. It contains a steroid called dioxygenin that is taken from the plant and is converted into progesterone(5). Seed coat contains 4.96% progesterone.

β-Carotene: Molecular formula: C(40)H(56)
β-carotene is an organic compound and classified as a terpenoid. It is a strongly coloured red-orange pigment abundant in plants and fruits. It is a precursor (inactive form) of vitamin A (en.wikipedia.org/wiki/beta-carotene). Plant carotenoids are the primary dietary source of pro-vitamin A worldwide, with β-carotene as the most well-known pro-vitamin A carotenoid. 3.53% of β-carotene is found to be in seed coat.

Cholesta-4, 6-diene-3-ol (3-beta): Molecular formula: C(27)H(44)O
It is a 28 carbon sterol synthesized by several unicellular algae (phyto-plankton) and some terrestrial plants, e.g. oil seed rape. This compound has frequently been used as a biomarker for the presence of marine algal matter in the environment. 4.3% content is present in seed coat.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Name of Molecule</th>
<th>RT</th>
<th>Molecular Weight</th>
<th>Area</th>
<th>Peak Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1-Eicosanol</td>
<td>16.34</td>
<td>298</td>
<td>300753</td>
<td>4.83</td>
</tr>
<tr>
<td>2.</td>
<td>3,5-di-tert-butyl-4-hydroxybenzaldehyde</td>
<td>15.750</td>
<td>234</td>
<td>226077</td>
<td>3.63</td>
</tr>
<tr>
<td>3.</td>
<td>1,2-Benzenedicarboxylic acid butyl 2-ethylhexyl ester</td>
<td>20.390</td>
<td>334</td>
<td>390492</td>
<td>6.27</td>
</tr>
<tr>
<td>4.</td>
<td>Distearil sulfide</td>
<td>21.310</td>
<td>538</td>
<td>359581</td>
<td>5.77</td>
</tr>
<tr>
<td>5.</td>
<td>2,5-di-tert-butyl-1,4-benzoquinone</td>
<td>21.740</td>
<td>220</td>
<td>139729</td>
<td>2.24</td>
</tr>
<tr>
<td>6.</td>
<td>1,2-Benzenedicarboxylicacid mono (2-ethylhexyl) ester</td>
<td>38.710</td>
<td>278</td>
<td>748273</td>
<td>12.01</td>
</tr>
<tr>
<td>7.</td>
<td>Squalene</td>
<td>44.660</td>
<td>410</td>
<td>359617</td>
<td>5.77</td>
</tr>
<tr>
<td>8.</td>
<td>Progesterone</td>
<td>45.690</td>
<td>314</td>
<td>307633</td>
<td>4.94</td>
</tr>
<tr>
<td>9.</td>
<td>Beta carotene</td>
<td>46.450</td>
<td>536</td>
<td>220122</td>
<td>3.53</td>
</tr>
<tr>
<td>10.</td>
<td>Cholesta-4, 6-diene-3-ol (3-beta)</td>
<td>48.070</td>
<td>384</td>
<td>268250</td>
<td>4.31</td>
</tr>
</tbody>
</table>

Table 2: Mass spectral analysis of bioactive volatile compounds of Cynometra iripa seed coat.
DISCUSSION

This is the first attempt to analyze volatile compounds from *Cynometra iripa* seed and seed coat. The compounds found are likely to be responsible for the special odour and taste. This comparison will lead to identification of novel flavour and odour compounds and be a step towards understanding the chemistry behind the *Cynometra iripa* seed and seed coat.

CONCLUSION

The results of this attempt have proved the resource value of *Cynometra iripa* as cosmetics and pharmaceuticals. The identified compounds are already in use as bactericides, insecticides, fungicides too. GC-MS analysis has proved the potential of *Cynometra iripa* as a ‘bioresource’. Further value based attempts are under progress.

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