EXTRACTION AND ISOLATION OF α -AMYRIN ACETATE FROM THE FRUITS OF FICUS RACEMOSA.

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ABSTRACT

Ficus racemosa Linn belongs to the family Moraceae is popular in indigenous system of medicine like ayurveda, siddha, unani and homoeopathy. It has been known to possess diverse pharmacological activities. In the traditional system of medicine various plant parts such as bark, root, leaves, fruits and latex are used in diarrhea, dysentery, piles, wound healing, skin disorders, diabetes, ulcers, menorrhagia, visceral obstructions, bilious affections, as a hypoglycemic, antiperiodic, anti-hemorrhagic, carminative, astringent, antioxidant and also as anticancer agents. The research describes a new method for the extraction and isolation of α-Amyrin Acetate from the fruits of Ficus racemosa. This would be of immense value in the study and preparation of α-Amyrin Acetate derivatives and analogs.

KEYWORDS: α-Amyrin Acetate, Cluster Fig Tree, Ficus racemosa, Gular Fig, Moraceae.

INTRODUCTION:

Ficus racemosa (syn. Ficus glomerata) (Figure 1) is a species of plant in the Moraceae family. It is popularly known as the Cluster Fig Tree or Gular Fig. This is native to Australia, South-East Asia and the Indian Subcontinent. It is unusual in that its figs grow on or close to the tree trunk.¹,²,³

It has been used in Ayurveda, the ancient system of Indian medicine for various disorders including diabetes, liver disorders, diarrhea, inflammatory conditions, hemorrhoids, respiratory and urinary diseases.⁴

SCIENTIFIC CLASSIFICATION OF FICUS RACEMOSA:

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliophyta
Order : Rosales
Family : Moraceae
Genus : Ficus
Species : racemosa

DISTRIBUTION AND DESCRIPTION:

The plant grows all over India in many forests and hills from outer Himalayan ranges, Punjab, Khasia mountain, Chota Nagpur, Bihar, Orrisa, West Bengal, Rajasthan, Deccan and common in South India. It is
frequently found around the water streams and is also cultivated. The tree is medium, tall, growing 10-16m in height. The rich green foliage provides a good shade. The leaves are dark green, 7.5-10 cm long, glabrous; receptacles small subglobose or piriform, in large clusters from old nodes of main trunk. They have a pleasant smell resembling that of cider apples. The bark is rusty brown with a thickness from 0.5-2 cm according to the age of trunk or bark. The fruits receptacles are 2-5 cm in diameter, pyriform, in large clusters, arising from main trunk or large branches. The fruits resemble the figs and are green when raw, turning orange, dull reddish or dark crimson on ripening. The udumbara flower is enclosed within a fig-like fruit structure. In Buddhist mythology, the flower was said to bloom only once every 3,000 years.

TRADITIONAL USES:
All parts of *Ficus racemosa* are medicinally important in traditional system of medicine in India and have been used extensively in biliary disorders, jaundice, dysentery, diabetes, and diarrhea and in inflammatory conditions. Experimental studies have demonstrated the anti-inflammatory, hepatoprotective, hypoglycemic and hypolipidemic effects of *Ficus racemosa*.

ROOT: It is used in dysentery, pectoral complaints, and diabetes, applied in mumps, other inflammatory, glandular enlargements and hydrophobia. In Sri Lankan indigenous system of medicine, it is used in the treatment of skeletal fracture.

BARK: It is highly efficacious in threatened abortion and also recommended in urological disorders, diabetes, leprosy, dysentery, asthma and piles, antiseptic, antipyretic and vermicidal and the decoction of bark is used in the treatment of various skin diseases, ulcers.

LEAF: Leaves are useful in dysentery and diarrhea. The infusion of bark and leaves is also employed as mouth wash to spongy gums and internally in dysentery, menorrhagia, effective remedies in glandular swelling, bronchitis, abscess, chronic wounds, cervical adenitis, haemorrhage and haemoptysis. Tender leaves are used in bilious affection and also to improve skin complexion. A decoction of leaves is a good wash for wound and ulcer.

FRUIT: Tender fruits are astringent, stomachic, refrigerant, dry cough, loss of voice, disease of kidney and spleen, astringent to bowel, styptic, tonic, useful in the treatment of leucorrhoea, blood disorders, burning sensation, fatigue, urinary disorders, leprosy, menorrhagic epistaxis, intestinal worms and carminative. They are useful in miscarriage, menorrhagia, spermatorrhoea, epididymitis, cancer, myalagia, scabies, haemoptysis, intrinsic hemorrhage, excessive thirst, visceral obstructions. It is believed to be a good remedy for visceral obstructions and extract of the fruit is used in leprosy, diarrhoea, circulatory and respiratory disorders and menorrhagia.

LATEX: It is aphrodisiac and administered in hemorrhoids, diarrhea, diabetes, alleviates the edema in adenitis, parotitis, or orchitis, traumatic swelling, toothache and vaginal disorders.

MATERIALS AND METHODS:

PLANT MATERIAL: The fruit of *Ficus racemosa* Linn. (Moraceae) were collected from local area of Lucknow & the authentication was done by the Botany division of Central Drug Research Institute, Lucknow, Uttar Pradesh, India.

EXTRACTION: Powdered plant material of *Ficus racemosa* (2 kg) were placed in glass percolator with 95% ethanol (25 litres) and allowed to stand at room temperature for 24 hours. The percolate was collected and this process was repeated for four times. The combined percolate was filtered, concentrated under vacuum using rotary evaporator at 40°C & weighed. The weight of extract was found to be 1200 gm.

SUB FRACTIONATION OF ETHANOLIC EXTRACT: The Ethanolic extract was fractionated between chloroform and water. The chloroform soluble fraction was separated using separating funnel and concentrated under reduced pressure. The weight of chloroform soluble fraction concentrated was 60 gm. The aqueous fraction was partitioned with ethyl acetate in separating funnel and ethyl acetate soluble fraction was concentrated under reduced pressure using rotary evaporator at 40°C. The weight of ethyl acetate fraction was found to be 10gm. Aqueous fraction was again fractionated with butanol and its weight was found to be 20gm. The water soluble fraction was evaporated to dryness under reduced pressure in a rotatory evaporator and weighted.

RESULT:

α-Amyrin acetate was obtained as white crystalline solid (Figure 2), mp 222-227°C and gave positive Lieberman-Burchard test for triterpenoid. Its ESI-MS spectrum exhibited molecular ion signal at m/z 469 [M+H]+ corresponding to molecular formula C32H52O2 consistent with the 1H and 13C NMR spectral data. The 1H NMR spectrum (experimental section) displayed eight C-tertiary methyl signals at δ 1.06-0.79 (3H each, s), 0.87 (6H, s), 2.04 (3H, s), 4.51 (1H, m) and an olefinic proton at δ 5.12
(1H, t, J=3.74 Hz), characteristic of a typical $\Delta^{12}$ oleanane skeleton. The $^{13}$C NMR showed thirty two carbon signals in the molecule. Multiplicity assignment with the aid of DEPT experiment revealed the presence of one quaternary carbon of ester at $\delta_C$ 171.40, nine methylenes, ten methylenes, five methines including one oxymethines at $\delta_C$ 81.39 and one olefinic methine at $\delta_H$ 124.75 and seven quaternary carbons including one at $\delta_C$ 140.05. On the basis of these data, this compound was identified as $\alpha$-amyrin acetate which was confirmed by comparison of its physicochemical data with that reported in the literature.

**DISCUSSION:**

Chloroform soluble fraction was taken for isolation of compounds. The chloroform soluble fraction was dissolved in the minimum volume of mixture of methanol and observed over silica gel (#60-120, gm) to prepare slurry. The slurry was first dried on rotary evaporator & completely dried slurry was packed for gross column chromatography over the bed of silica gel (#60-120, gm). Hexane-Ethyl acetate solvent was taken for gross column chromatography. The column was eluted gradiently with increasing polarity e.g. 1%, 2%, 3% & so on Hexane-Ethyl acetate & then mixture of ethyl acetate-methanol to afford 42 fractions. Each 2 liter of eluent was collected and concentrated. Depending upon the similar TLC patterns, the fractions were pooled together and rechromatographed on silica gel (#60-120). (Table 1)

**REFERENCES:**


**Table 1: Gross column chromatography of chloroform fraction.**

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Eluent</th>
<th>Weight(gm)</th>
</tr>
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<tbody>
<tr>
<td>1-2</td>
<td>Hexane</td>
<td>1</td>
</tr>
<tr>
<td>3-4</td>
<td>Hexane-Ethyl acetate(99:01)</td>
<td>1.5</td>
</tr>
<tr>
<td>5-11</td>
<td>Hexane-Ethyl acetate(95:05)</td>
<td>2gm $\alpha$-amyrin acetate</td>
</tr>
<tr>
<td>12-14</td>
<td>Hexane-Ethyl acetate(94:06)</td>
<td>2</td>
</tr>
<tr>
<td>15-18</td>
<td>Hexane-Ethyl acetate(90:10)</td>
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</tr>
<tr>
<td>19-23</td>
<td>Hexane-Ethyl acetate(88:12)</td>
<td>8</td>
</tr>
<tr>
<td>24-27</td>
<td>Hexane-Ethyl acetate(85:15)</td>
<td>10</td>
</tr>
<tr>
<td>28-30</td>
<td>Hexane-Ethyl acetate(80:20)</td>
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<tr>
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<td>Hexane-Ethyl acetate(75:25)</td>
<td>10</td>
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<tr>
<td>36</td>
<td>Hexane-Ethyl acetate(60:40)</td>
<td>8</td>
</tr>
<tr>
<td>37</td>
<td>Hexane-Ethyl acetate(50:50)</td>
<td>10</td>
</tr>
<tr>
<td>38</td>
<td>Washing with Methanol</td>
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