CHAPTER-V

Chemical characterisation of the unsaponifiable fraction of *Mahua* Oil
CHEMICAL CHARACTERISATION OF UNSAPONIFIABLE FRACTION OF MAHUA OIL

Fats and Oils are chemically composed of neutral triglycerides (90\%), minor amounts of free fatty acids (FFA) and the unsaponifiable non-glyceride fraction which includes phytosterols, tocopherols, triterpene alcohols, pigments and vitamins. The nature of the fatty acids attached to the glycerol moiety differs in various fats and oils. Fatty acids may be saturated (SFA), monounsaturated (MUFA) and Polyunsaturated (PUFA).

Oils and fats occupy a premier position in the agricultural and industrial economy of a nation. India’s present production of edible oils is 6.4 million tons. Until the early eighties, India enjoyed an envious position in the field of vegetable oils, but with expanding population and rising living standards, the country has declined progressively from one of the net exporters to the world’s largest importers, spending about Rs.4102 crores of foreign exchange reserves towards imports. Hence, it is proposed to explore alternative sources of edible oils to minimise the imports of oil and reduce the drain on foreign exchange. The government has launched the Technology Mission on Oilseeds (TMO) in March 1986, whose specific objective was to accelerate the process of self-reliance in vegetable oils and free the country from the recurring burden of imports. As a result of these efforts, the oilseeds situation improved dramatically. The unconventional oils of high potential availability and those which are already existing as by-products of an industry like ricebran, mango kernel etc were given high priority by the TMO. These unconventional oils are different
from common conventional oils in having unusual chemical composition with unknown effects. Therefore, they need to be evaluated for safe edibility before recommendation for human use. The National Institute of Nutrition has evaluated several unconventional oils for safe edibility by the use of a specially designed protocol\(^5\).

Studies on nutritional and toxicological evaluation of Mahua oil at the National Institute of Nutrition indicated antifertility effect in male rats\(^6\). On withdrawal of Mahua oil from the diet, the male animals regained their fertility. These results indicate temporary male sterility on feeding Mahua oil to rats. No other adverse toxicological effects were found. The hydrogenated vegetable oil containing 30% Mahua oil indicated it to be safe and the antifertility effect was not shown probably due to low concentration of the unsaponifiable matter\(^7\).

The available literature indicated that the composition of the saponifiable fraction of the Mahua oil does not possess any major biological effects related to the antifertility nature. Hence the present study is planned with an objective to investigate, isolate and indentify the minor components of Mahua oil which could have the antifertility /male sterility effect.

*Mahua* (syn. *Madhuca, Bassia*) belongs to the family *sapotaceae*, included in order *Ebenales*. The family of *sapotaceae* is divided into three subfamilies *Sideroxyloideae, Achradoideae* (*Mimusopoideae*) and *Madhucoideae*. There are over 600 tropical species grouped in about forty genera and its members are important for their durable timber, edible fruits and fatty seed kernels\(^8\).
The common species of *Madhuca* are *M. malbarica*, *M. bourdillonii*, *M. longifolia*, *M. butyraceae* and *M. latifolia*. The two major species of *Madhuca* found in India are *Madhuca indica* (Syn. *Bassia latifolia, Madhuca latifolia*) and *Madhuca longifolia* (Syn. *Bassia longifolia*). These two species are so closely related that no distinction is made in the trade of their seed or fat. *Mowrah* is the widely accepted local name for the fat from both sources.

*Madhuca latifolia* is a medium sized to large size deciduous tree found in Uttar Pradesh, Madhya Pradesh, Gujarat and Andhra Pradesh. *Madhuca longifolia* is a large evergreen tree commonly found in South India and the monsoon forests of the western ghats from Konkan southwards. The tree, its flowers and seeds have been very gainfully used in our economy for a long time.

Commercial Significance

*Mahua* tree yields a constructional timber, used for building purposes, agricultural implements and for railway sleepers etc. *Mahua* berries are eaten raw or cooked. The berries are also eaten by cattle, sheep, goats, monkeys and parrots and are also medicinal. *Mahua* leaves are astringent and are used in embrocations. The bark contains 17% tannin and is used in dyeing and tanning. The flowers are rich in sugar (73 percent) and next to cane molasses constitute the most important raw materials for alcohol fermentation.
The seeds have a high potential availability and around 400,000 tonnes of oil is produced every year. Seeds contain concave kernels (about 75%) and the kernels contain 50% pale yellow semi-solid fat. The fat can be obtained in screw press, hydraulic press and ghani. The oil yield in a ghani is 20 to 30 percent, in an expeller 34 to 37 percent. Both expelled and solvent extracted oils are available commercially. Mahua oil is used in the manufacture of soaps. Refined oil is used in the manufacture of lubricating greases, fatty alcohols and as a raw material for the production of stearic acid.

Biological Significance of Madhuca species

The plants belonging to Madhuca possess a wide variety of medicinal uses in the Indian system of medicine. The bark is used for rheumatism, ulcers, itches, bleeding and spongy gums, tonsilitis and diabetes mellitus. It is given to horses for stomach-ache. The roots are applied to ulcers. The flowers have mild laxative activity. The flowers of M. malbarica are used in kidney complaints. The decoction of flowers of M. latifolia is useful to cure coughs. The flowers are also used in the treatment of piles.

Mahua oil is used in skin diseases, rheumatism and headache. It is laxative and is useful in habitual constipation, piles and haemorrhoids and also as an emetic.
The seed kernel of *M. longifolia* contain saponins which are found to have anti-inflammatory and antiulcerogenic activity\(^{11}\). Mowric acid obtained from *M. logifolia* was found to be a blood coagulating agent resembling digitalis in its action on heart\(^{12}\). The saponins present in the seed of *M. butyraceae* were found to possess anti-inflammatory\(^{13}\) and spermicidal activities\(^{14}\).

**Chemical Constituents of Madhuca species**

Most of the plants of this species have furnished triterpenoids, flavonoids, saponins, sterols, amino acids and their glycosidic and fatty acid ester derivatives.

The details of chemical constituents isolated from different parts of the *Madhuca* species are given in Table-V.I.
TABLE-V.1

<table>
<thead>
<tr>
<th>S No</th>
<th>Name of the plant &amp; Part of the plant</th>
<th>Compound</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>V. longifolia</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>3β-Caproxy-olean-12-en-28-ol (5 X&lt;sub&gt;c&lt;/sub&gt;)</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Seed kernel</td>
<td>Proto basic acid (5 X&lt;sub&gt;a&lt;/sub&gt;)</td>
<td>16, 17</td>
</tr>
<tr>
<td>2</td>
<td><em>V. latifolia</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>β-sitosterol (5 I), stigmasterol (5 II), oleanolic acid (5 IX&lt;sub&gt;b&lt;/sub&gt;)</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3β-palmitoxy-olean-Δ&lt;sup&gt;12&lt;/sup&gt;-ene-28-ol (5 IX&lt;sub&gt;d&lt;/sub&gt;)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>erythrodiol (5 IX&lt;sub&gt;a&lt;/sub&gt;)</td>
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<tr>
<td></td>
<td></td>
<td>quercetin (5 IV)</td>
<td>19</td>
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<td></td>
<td></td>
<td>quercetin-3-galactoside (5 IV&lt;sub&gt;b&lt;/sub&gt;)</td>
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<tr>
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<td></td>
<td>myrcetin (5 V)</td>
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<td></td>
<td>myrcetin-3-o-L-rhamnoside (5 V&lt;sub&gt;b&lt;/sub&gt;)</td>
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<td>myrcetin-3-arabinoside (5 V&lt;sub&gt;b&lt;/sub&gt;)</td>
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<td>protobassic acid (5 X&lt;sub&gt;a&lt;/sub&gt;)</td>
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<td></td>
<td>Trunk bark</td>
<td>betulinic acid caprylate (5 VIII&lt;sub&gt;b&lt;/sub&gt;)</td>
<td>15</td>
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<tr>
<td></td>
<td></td>
<td>oleanolic acid caprylate (5 IX&lt;sub&gt;e&lt;/sub&gt;)</td>
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<tr>
<td></td>
<td></td>
<td>lupeol acetate (5 VIII)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>β-amyrrn acetate (5 IX&lt;sub&gt;b&lt;/sub&gt;)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>α-spinasterol (5 III)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>erythrodiol glucoside (5 IX&lt;sub&gt;a&lt;/sub&gt;)</td>
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<td>Mesocarp of the fruits</td>
<td>α-amyrrn acetate (5 VI&lt;sub&gt;b&lt;/sub&gt;)</td>
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<td>β-amyrrn acetate (5 IX&lt;sub&gt;b&lt;/sub&gt;)</td>
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<td>3β-caprylic ester of erythrodiol (5 IX&lt;sub&gt;c&lt;/sub&gt;)</td>
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<td>3β-capryloxy oleanolic acid (5 IX&lt;sub&gt;a&lt;/sub&gt;)</td>
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<td>3</td>
<td><em>V. butyraceae</em></td>
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<td>Leaves</td>
<td>butyracic acid (5 X&lt;sub&gt;b&lt;/sub&gt;)</td>
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<tr>
<td></td>
<td>Flowers</td>
<td>friedelin (5 VII)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>erythrodiol monocaprylate(5 IX&lt;sub&gt;c&lt;/sub&gt;)</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β-amyrrn acetate (5 IX&lt;sub&gt;b&lt;/sub&gt;)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>β-sitosterol (5 I), α-spinasterol (5 III)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bark and fruit pulp</td>
<td>3β-palmitoxy oleanol (5 IX&lt;sub&gt;d&lt;/sub&gt;), oleanolic acid palmitate (5 IX&lt;sub&gt;e&lt;/sub&gt;)</td>
<td>24</td>
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<tr>
<td></td>
<td>kernel</td>
<td>betulinic acid palmitate (5 VIII&lt;sub&gt;b&lt;/sub&gt;), friedelin (5 VII), α-amyrrn (5 VI&lt;sub&gt;b&lt;/sub&gt;)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Seed</td>
<td>butyraceol (5 XI)</td>
<td>25</td>
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<td></td>
<td></td>
<td>Mi-saponin A, (5 XII&lt;sub&gt;c&lt;/sub&gt;)</td>
<td></td>
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<td></td>
<td>16β-hydroxy-Mi-saponin A (5 XII&lt;sub&gt;d&lt;/sub&gt;)</td>
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<td></td>
<td></td>
<td>butyroside A (5 XII&lt;sub&gt;a&lt;/sub&gt;)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>butyroside B (5 XII&lt;sub&gt;b&lt;/sub&gt;)</td>
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</tbody>
</table>
Rham

5. V
H

5. V_a
Rham

5. V_b
Ara

5. V_I_a
OH

5. V_I_b
O – C – CH_3

5. VII

5. VIII
-O – C – CH_3
-CH_3

5. VIII_a
-O – C – C_{15}H_{31}
-COOH

5. VIII_b
O – C – C_7H_{15}
-COOH
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<th>5.IX</th>
<th>(-\text{OH})</th>
<th>(\text{CH}_3)</th>
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<tr>
<td>5.IX(_a)</td>
<td>(-\text{OH})</td>
<td>(-\text{CH}_2\text{OH})</td>
</tr>
<tr>
<td>5.IX(_b)</td>
<td>(-\text{OH})</td>
<td>(-\text{COOH})</td>
</tr>
<tr>
<td>5.IX(_c)</td>
<td>(\text{O}) (-\text{C}) (-\text{C}<em>7\text{H}</em>{15})</td>
<td>(-\text{CH}_2\text{OH})</td>
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<tr>
<td>5.IX(_d)</td>
<td>(\text{O}) (-\text{C}) (-\text{C}<em>{15}\text{H}</em>{31})</td>
<td>(-\text{CH}_2\text{OH})</td>
</tr>
<tr>
<td>5.IX(_e)</td>
<td>(\text{O}) (-\text{C}) (-\text{C}<em>7\text{H}</em>{15})</td>
<td>(-\text{COOH})</td>
</tr>
<tr>
<td>5.IX(_f)</td>
<td>(\text{O}) (-\text{C}) (-\text{C}<em>{15}\text{H}</em>{31})</td>
<td>(-\text{COOH})</td>
</tr>
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<td>5.IX(_g)</td>
<td>(\text{O}) (-\text{Glu})</td>
<td>(-\text{CH}_2\text{OH})</td>
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<td>5.IX(_h)</td>
<td>(\text{OCOCH}_3)</td>
<td>(-\text{CH}_3)</td>
</tr>
<tr>
<td>5.IX(_i)</td>
<td>(\text{OH})</td>
<td>(-\text{COOCH}_3)</td>
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5. XII

<table>
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<tr>
<th></th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
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<tr>
<td>5.XIIa</td>
<td>Glc</td>
<td>H</td>
<td>Ara (2→1) Rha (4→1) Xyl (3→1) Api</td>
</tr>
<tr>
<td>5.XIIb</td>
<td>Glc</td>
<td>OH</td>
<td>Ara (2→1) Rha (4→1) Xyl (3→1) Api</td>
</tr>
<tr>
<td>5.XIIC</td>
<td>Glc</td>
<td>H</td>
<td>Ara (2→1) Rha (4→1) Xyl (3→1) Rha</td>
</tr>
<tr>
<td>5.XIId</td>
<td>Glc</td>
<td>OH</td>
<td>Ara (2→1) Rha (4→1) Xyl (3→1) Rha</td>
</tr>
</tbody>
</table>
The survey of literature revealed that there are not many studies on *Mahua* oil. The physicochemical parameters and fatty acid composition of *Mahua* oil\(^{(6)}\) are presented in Table V.2 and Table V.3.

**TABLE-V.2**

**PHYSICOCHEMICAL PARAMETERS OF MAHUA OIL**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Value</th>
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<tbody>
<tr>
<td>1.</td>
<td>Iodine value</td>
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<tr>
<td>2.</td>
<td>Saponification value</td>
<td>202.4</td>
</tr>
<tr>
<td>3.</td>
<td>Acid value</td>
<td>7.5</td>
</tr>
<tr>
<td>4.</td>
<td>Unsaponifiable matter (%)</td>
<td>3.2</td>
</tr>
</tbody>
</table>

**TABLE-V.3**

**FATTY ACID COMPOSITION OF MAHUA OIL**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fatty acids</th>
<th>Oil % weight (GLC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>C16:0 (Palmitic)</td>
<td>18.6</td>
</tr>
<tr>
<td>2.</td>
<td>C18:0 (Stearic)</td>
<td>15.4</td>
</tr>
<tr>
<td>3.</td>
<td>C18:1 (Oleic)</td>
<td>41.8</td>
</tr>
<tr>
<td>4.</td>
<td>C18:2 (Linoleic)</td>
<td>24.2</td>
</tr>
</tbody>
</table>
The true nature of the chemical constituents of the unsaponifiable fraction of *Mahua* oil are not studied in detail except for few reports\(^8,27\) which indicated the presence of an unsaturated hydrocarbon, illipene \((C_{32}H_{56} \text{ or } C_{64}H_{106} \text{ or } C_{65}H_{108})\) and a sterol. The sterol was found to be isomeric with \(\beta\) and \(\gamma\)-sitosterols, but possessed a higher specific rotation \([\alpha]_D^{20} \text{ -57.9}^0\) in chloroform and lower m.p 121-122\(^0\) than any of the isomeric sterols (\(\beta\) and \(\gamma\)).

**PRESENT WORK**

The present investigation deals with the isolation and characterisation of the unsaponifiable fraction of *Mahua* oil, which may be responsible for the antifertility effect\(^6\) reported from the National institute of Nutrition, Hyderabad.

*Mahua* oil used in this investigation was obtained from Girijan Cooperative Corporation, Hyderabad.

The two species of *Mahua, Madhuca indica* (syn. *Bassia latifolia, Madhuca latifolia*) and *Madhuca longifolia* (Syn *Bassia logifolia*) are so closely related that no distinction is made in the trade of their seed or fat\(^9\). Mowrah is the widely accepted local name for fat from both sources.
The commercially available oil used in the present investigation may be a mixture of oils from seeds of both the plants (*Madhuca latifolia* and *Madhuca longifolia*).

The unsaponifiable fraction of oils contain hydrocarbons, sterols, triterpene alcohols, tocopherols, carotenoids and other materials which are peculiar to certain oils.

**PREPARATION OF UNSAPONIFIABLE FRACTION**

The unsaponifiable fraction of the oil was obtained by saponification of the oil with alcoholic potassium hydroxide that is refluxing the oil with IN alcoholic potassium hydroxide followed by dilution with water and extraction with diethylether (1%).

The TLC examination of the unsaponifiable fraction showed its complex nature. Hence it was subjected to column chromatographic separation.

**CHROMATOGRAPHIC SEPARATION OF THE UNSAPONIFIABLE FRACTION**:

The unsaponifiable fraction (5g) was adsorbed over a column of silica gel and eluted with petroleum ether, benzene, benzene-ethylacetate (9:1), benzene-ethylacetate (8:2), and ethylacetate.
Dammar-12,25-diene-3β-ol (5.XV)
The petroleum ether eluate afforded an oily substance (1 g). It was not homogeneous on TLC indicating the complex nature. The separation was not affected as their Rf values were very close.

The benzene eluate furnished a colourless solid (compound A), homogeneous on TLC (1.5 g).

The benzene - ethylacetate (9:1) eluate afforded a colourless solid (0.5 g) (compound B), homogeneous on TLC.

The benzene-ethylacetate (8:2) eluate did not furnish any solid. The ethylacetate eluate yielded a dark yellow coloured sticky semisolid (1.5 g), which was not homogeneous on TLC and could not be separated because of its complex nature.

EXAMINATION OF COMPOUND A

Compound-A gave a positive Liebermann-Burchard test for triterpenoids. The mass spectrum of compound-A gave a molecular ion peak at \( M^+ 426 \) and a base peak at \( m/z 218 \). IR spectrum showed an absorption band around 3330 cm\(^{-1}\) indicating the presence of an hydroxyl function. The \(^1\)H NMR showed signals at \( \delta 5.2(t,1H) \), \( \delta 3.25(m,1H) \), \( \delta 4.65(d,2H) \), seven signals in the methyl region between 0.7 and 1.7 for seven methyl protons.
Dammar-12,25-diene-3β-yl-acetate (5.XV)
Dammar-12,25-diene-3β-yl-acetate (S.XV)
Dammar-12,25-diene-3β-yl-acetate (5.XV).
Dammar-12.25-diene-3β-ol (5.XV).
On acetylation the compound-A gave a monoacetate. The IR spectrum of the acetate showed a strong ester carbonyl absorption band at 1730 cm\(^{-1}\). Mass spectrum of the acetate gave a molecular ion peak at \(M^+\) 468 indicating that it is a monoacetate. The \(^1\)H NMR showed signals at \(\delta\) 5.2 (t, 1H), \(\delta\) 4.65 (d, 2H), \(\delta\) 4.5 (m, 1H; 3α-H) and \(\delta\) 2.05 (s, 3H; O-C-CH\(_3\)).

Since the oxygen function at position 3 is ubiquitous with steroids and triterpenoids, the compound-A is a 3β-hydroxy triterpenoid with a molecular formula \(C_{30}H_{50}O\).

PMR spectrum of compound-A showed a signal at \(\delta\) 4.65 (d, 2H) due to vinylidene protons and another at \(\delta\) 5.2 (t, 1H) for the presence of a trisubstituted double bond. \(^{13}\)C NMR spectrum of the compound confirmed the presence of a vinylidene group (\(\delta\) 150.959 and \(\delta\) 109.687 for two vinylidene carbons) and a trisubstituted double bond (\(\delta\) 121.663 and \(\delta\) 145.122 for the two trisubstituted double bonded carbons). Therefore the compound -A is a 3β-hydroxy triterpenoid with two double bonds, having the molecular formula \(C_{30}H_{50}O\). The molecular formula \(C_{30}H_{50}O\) fits with a monohydroxy pentacyclic triterpenoidal structure with one double bond or a monohydroxy tetracyclic triterpenoidal structure with two double bonds. The \(^1\)H NMR and \(^{13}\)C NMR spectral data clearly indicate the presence of two double bonds. Therefore the compound-A will have tetracyclic carbon skeleton with two double bonds, a trisubstituted double bond and a vinylidene double bond. Hence, it is imperative to assign the
position of above two double bonds in the structural elucidation of Compound 'A'.

**Position of the trisubstituted double bond**

Compound-A showed a $^1$H NMR signal at $\delta$ 5.2 (t, 1H) for methenic protons and $^{13}$C NMR showed signals at $\delta$ 121.663 and $\delta$ 145.122 for the two trisubstituted double bonded carbons. The position of the trisubstituted double bond is fixed at $\Delta^{12}$ by comparing the reported $^1$H NMR and $^{13}$C NMR spectral data of $\beta$-amyrin$^{28}$ (5.IX) which contains the trisubstituted double bond at $\Delta^{12}$ which, is in agreement with $^1$H NMR and $^{13}$C NMR spectral data of compound-A.

<table>
<thead>
<tr>
<th></th>
<th>$^1$H NMR Spectral Values ($\delta$)</th>
<th>Carbon Number</th>
<th>$^{13}$C NMR Spectral Values ($\delta$)</th>
<th>Ref</th>
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</thead>
<tbody>
<tr>
<td>Compound-A (5.XV)</td>
<td>5.2 (t,1H)</td>
<td></td>
<td>121.663</td>
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<tr>
<td></td>
<td></td>
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<td>145.122</td>
<td></td>
</tr>
<tr>
<td>$\beta$- amyrin (5.IX)</td>
<td>5.2 (t,1H)</td>
<td>12</td>
<td>122.7</td>
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<tr>
<td></td>
<td></td>
<td>13</td>
<td>145.6</td>
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</tbody>
</table>

The prominent RDA fragment at m/z 218 in the mass spectrum of compound-A (5.XV) further confirmed the position of trisubstituted double bond at $\Delta^{12}$. (chart I)
Position of the vinylidene double bond

The position of vinylidene double bond is fixed at $\Delta^{25}$ by comparing the $^{13}$C NMR values ($\delta$150.959 and $\delta$109.689 for the two vinylidene carbons) of compound A with the reported $^{13}$C NMR spectral values of 24-methyl-lanost-9(11), 25-diene-3-one$^{29}$ (5.XIII) ($\delta$150.1 and $\delta$109.4 for C-25 and C-26 carbons respectively) which are in close agreement.

This is further confirmed by the mass spectral fragment at m/z 69 (chart I). The fragment at m/z 69 is due to the rearrangement of C-25/C-26 double bond to C-24/C-25 followed by allylic cleavage. 3\beta-acetoxy dammar-20,25 -diene-24-ol$^{30}$ (5.XIV) which contains a double bond at $\Delta^{25}$ gave a mass spectral fragment at m/z 69.

The literature survey indicated that there is no report of a monohydroxy tetracyclic triterpenoid with a double bond at $\Delta^{12}$. This shows that the compound A is isolated from the nature for the first time. Hence the compound A is Dammar-12, 25 - diene-3\beta-ol: (5.XV).
Chart-I

\[ \text{CH}_n \]

\[ \text{RDA fragmentation} \]

\[ m/z \ 189 \]

\[ m/z \ 207 \]

\[ M^+ \ 426 \]

\[ m/z \ 69 \]

\[ m/z \ 218 \]

\[ - \text{CH}_3 \]

\[ m/z \ 203 \]
EXAMINATION OF COMPOUND B

Compound B was homogeneous on TLC. It gave a positive Liebermann-Burchard test for triterpenoids, m.p: 230-235°C \([\alpha]^{20}_D + 74.5^0\) (1.0, CHCl₃). The mass spectrum of compound B gave a molecular ion peak at \(M^+ 442\) and a base peak at \(m/z 203\). IR spectrum showed an absorption around 3350 cm\(^{-1}\) indicating the presence of a hydroxyl function. The \(^1\)H NMR showed signals at \(\delta 5.25\) (1H t, \(C=CH\)), \(\delta 3.5\) (bm, 3α-H, 28-CH₂), seven signals in the methyl region between \(\delta 0.8-1.2\) for seven methyl protons.

The spectral and physical data of compound B were found to be identical in all respects with the literature values of dihydroxy triterpene erythrodiol\(^{31}\). The identity of compound B was further confirmed by comparison with erythrodiol obtained by the reduction of methyl oleanolate with Lithium aluminium hydride (LAH). Hence the compound is identified as erythrodiol (5.IXₐ).
EXPERIMENTAL

The commercially available Mahua oil is obtained from the Girijan Cooperative Corporation, Hyderabad.

PREPARATION OF THE UNSAPONIFIABLE FRACTION OF MAHUA OIL

The oil (100 gms) was taken in a 2 litre round bottomed flask and refluxed for two hours with alcoholic potassium hydroxide (1 litre). The alcohol was then distilled off and cold water (1 litre) was added to the residue. It was taken in a separating funnel and extracted with diethyl ether (3x500 ml). The combined ether extract was washed three times with water, dried over anhydrous sodium sulphate overnight. On removal of the solvent a dark yellow coloured semisolid was obtained. (Unsaponifiable matter, 1 gm).

The TLC examination of the unsaponifiable fraction showed its complex nature.

CHROMATOGRAPHIC SEPARATION OF THE UNSAPONIFIABLE FRACTION

The unsaponifiable matter (5 g) was adsorbed over a column of silicagel (150 gms, finer than 200 mesh) and eluted successively with
petroleum ether (1 litre), benzene (1.5 litre), Benzene-ethylacetate (9:1; 1 litre), Benzene-ethyl acetate (8:2; 500 ml) and ethyl acetate (1 litre). The solvent from each of the above eluents was removed.

**Petroleum ether eluate:**
- Physical state: oily substance (1g)
- Colour: Pale yellow
- TLC examination: non-homogeneous

The oily substance could not be characterised because of its complex nature.

**Benzene eluate:**

**Compound A (5:XV) (1.5g), crystallised from alcohol.**
- Physical state: shiny solid
- Colour: colourless
- m.p.: 155-160$^0$
- $\scriptstyle{[\alpha]}_D^{20}$: +65$^0$ (C 1.0, CHCl$_3$)

**Spectral Data:**

**IR (cm$^{-1}$):** 3330 cm$^{-1}$ (-OH group), 1370 and 1360 (gemdimethyl)

**$^1$H NMR (δ ppm):** 5.2 (t, 1H trisubstituted double bond) 3.25 [m, 1H, 3α- H], 4.65 (d, 2H, H-26). seven signals in the methyl region between 0.7 and 1.7 for seven methyl protons.
$^{13}$C NMR ($\delta$ ppm): 40.013 (C-1), 23.467 (C-2), 79.959 (C-3), 38.530 (C-4), 55.120 (C-5), 18.311 (C-6), 35.524 (C-7), 40.671 (C-8), 50.381 (C-9), 37.999 (C-10), 21.430 (C-11), 121.663 (C-12), 145.122 (C-13), 48.575 (C-14), 32.596 (C-15), 29.788 (C-16), 50.521 (C-17), 16.045 (C-19), 47.910 (C-20), 26.098 (C-21), 25.922 (C-22), 34.671 (C-23), 42.880 (C-24), 150.959 (C-25), 109.687 (C-26), 18.311 (C-27), 15.045 (C-28), 28.029 (C-29), 16.744 (C-30).

Mass:

m/z 426 (M$^+$), 218 (base peak), 203, 189, 69. (Chart I)

[Found: C, 82.50; H, 11.72; C$_{30}$H$_{50}$O requires C, 84.50; H, 11.73%]

Acetate of Compound A (5.XV$_a$)

Compound A gave a monoacetate (AC$_2$O/pyridine) crystallised from alcohol

Physical state - solid
Colour - colourless
m.p. - 160-165°C
$[\alpha]_{D}^{20}$ - +58.5° (C 1.0, CHCl$_3$)
Spectral Data :

\[
\text{IR (cm}^{-1}\text{)} : 1730 (\text{O-C-CH}_3), 1370 \text{ and } 1360 \text{ (gemdimethyl)}.
\]

\[
^1\text{H NMR } \delta \text{ (ppm)} : 5.2 \text{ (t, 1H, trisubstituted double bond)}, 4.65 \text{ (d, 2H, H-26)}, 4.5 \text{ (m, 1H, 3α- H)}, 2.05 \text{ (s, 3H, OC-CH}_3\text{)}, 0.7-1.7 \text{ (7 signals for seven methyl protons)}.
\]

\[
\text{Mas : m/z 468(M)}, 218 \text{ (RDA fragment), 203, 189, 69, 43}.
\]

[Found : C, 81.50, H, 11.05 C\textsubscript{32}H\textsubscript{52}O\textsubscript{2} requires C, 82.05; H, 11.11%]

Benzene - ethylacetate (9:1) eluate :

Compound B (5.IX\textsubscript{a}) (0.5g) crystallised from alcohol.

Physical state - solid

Colour - colourless

m.p. - 230-235\textdegree C

\[
[\alpha]_D^{20} - +74.5^0 \text{ (C 1.0, CHCl}_3\text{)}
\]

Spectral data :

\[
\text{IR. (cm}^{-1}\text{)} : 3350 (-\text{OH group}), 1370 \text{ and } 1360 \text{ (gem dimethyl group)}
\]

\[
^1\text{H NMR (δ ppm)} : 5.25 \text{ (1H, t, C=CH}_-\text{)}, 3.5 \text{ (bm, 3α-H, 28-CH}_2\text{)}, 0.8-1.2 \text{ (7XCH}_3\text{)}
\]
Mass : m/z 442(M⁺), 234 (RDA fragment), 203 (base peak), 189.

[Found : C, 81.2; H, 11.20; C₃₀H₅₀O₂ requires C, 81.45; H, 11.31]

Preparation of authentic sample of erythrodiol (5.IXₐ)

Reduction of methyl oleanolate (5.IX₁) with Lithium Aluminium hydride (LAH). To a stirred solution of methyl oleanolate (1 gm) in dry ether (200 ml) LAH (3g) was added in small quantities at a time during the course of one hour. After continuous stirring for 18 hours, the excess reagent was decomposed by addition of 200 ml of 10% hydrochloric acid. The organic layer was separated and washed with water. The dried ether solution on evaporation yielded erythrodiol (5.IXₐ) (600 mg). It crystallised from ethanol as colourless needles, m.p. 235°C, [α]²⁰ D + 76° [C 1.0, CHCl₃].

Benzene - ethylacetate (8:2) eluate : no residue

Ethylacetate eluate :

Physical state - Sticky semisolid (1.5g)
Colour - dark yellow
TLC examination - non-homogeneous

The sticky solid could not be separated because of its complex nature.
REFERENCES:


