Chapter 4

Phytochemical Investigation of the Pods of *Acacia farnesiana* (L.) Willd.
4.1 Introduction to the Plant Family:

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
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</thead>
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<tr>
<td>Sub kingdom</td>
<td>Phanerogams</td>
</tr>
<tr>
<td>Division</td>
<td>Magnoliophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida</td>
</tr>
<tr>
<td>Family</td>
<td>Fabaceae</td>
</tr>
<tr>
<td>Sub - Family</td>
<td>Mimosaceae</td>
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<tr>
<td>Genus</td>
<td>Acacia</td>
</tr>
<tr>
<td>Species</td>
<td><em>Acacia farnesiana (L.) Willd.</em></td>
</tr>
<tr>
<td>Local Name</td>
<td>Dev Babhul, Vedi Babhul</td>
</tr>
</tbody>
</table>

The species *Acacia farnesiana (L.) Willd.*, belongs to Family Fabaceae, sub-Family Mimosaceae and the genus *Acacia*.

**Family – Fabaceae**

Plants belonging to Fabaceae family are mostly herbs also some of them are shrubs and trees found in both temperate and tropical areas. Fabaceae comprises the third largest families of flowering plants, after Orchidaceae and Asteraceae numbering more than 700 genera and near about 20,000 species [1]. This family is commonly known as the legume, pea or bean family. The family is also known for its large and economically important flowering plants.

The most important commercial species of this family includes *Glycine max* (Soya bean), *Pisum sativum* (garden pea), *Arachis hypogaea* (ground nut) and *Medicago sativa* (alfalfa). The name Fabaceae comes from the genus *Faba*. The term ‘Faba’ comes from Latin and appears to simply meaning “bean”. Leguminosae is
also the older name considered for the family and refers to the fruit of these plants, which are called legumes [2]. Plants of this family includes trees, shrubs, herbaceous plants perennial or an annual that are easily recognized by their legumes and their compound stipulated leaves.

**Sub-Family-Mimosaceae:**

The members of the sub-family Mimosaceae mostly belongs to tropical and subtropical regions especially in arid and semi-arid climates. The Mimosaceae plants include climber, trees, shrubs, deciduous trees. They constitute trees and shrubs comprising of about 60 genera and near about 2000 species. This family is characterized by flowers with small petals and numerous prominent stamens. Mimosaceae members also have nitrogen fixing bacteria associated with the root nodule that is common to the Fabaceae members. Economically the plants of this family are widely used as a source of timber and the gum Arabic, as well as ornamentals and animal fodder.

**Genus – Acacia:**

The genus acacia belongs to the Mimosaceae family. There are near about 1300 species of genus Acacia found throughout the world. Most of the species of genus acacia are native to India, Pakistan, Australia, Africa and America [3]. Acacia trees dominate in many parts of the arid and semi-arid region, parts of Sub-Saharan Africa and reported multiple uses. These include use in food, medicine, fodder aside from being resistant to diseases and harsh climatic conditions [4]. Until recently, approximately 1300 species of Mimosaceae worldwide were classified as Acacias of whom about 960 species are native to Australia, remaining belonging to tropical to warm-temperate regions of Europe, Africa, Southern Asia and the Americas.
However taxonomists divided Acacia into five separate genera viz. Acacia, Vachellia, Senegalla, Acaciella and Mariosousa [5].

**Species – Acacia farnesiana (L.) Willd:**


*Acacia farnesiana* is native to West Indies; now occurring throughout India. Bark of the plant acts as astringent, demulcent, anthelmintic, anti-dysenteric, anti-inflammatory used in stomatitis, ulcers, swollen gums, dentalcaries, bronchitis, skin diseases. Essential oil from the pods acts as direct muscle relaxant, cardiac depressant and sedative. The plant as a whole is useful as various plant parts are used in insanity, epilepsy, delirium and convulsion. The plant acts as an antiseptic agent for curing sores, gums and loose teeth. The flowers are the source of Cassie perfume [7].

Sweet Acacia takes its common name from the unmistakable fragrance of its bright yellow ball flowers. Sweet Acacias like other desert natives have slender, white to grey thorns along the branches. These thorns are conspicuous, readily visible and pose little risk to Pedestrians. They are frequently used in street and sidewalk plantings as well as in parking lots. Their abundant shade and moderate stature contributes to their use incourtyards, patios, seating areas and near building entries. If desired, thorns on lower branches are easily removed with hand pruners. Mature trees
are adapted to full sun, well-draining soils and infrequent deep irrigation. They will thrive in both desert and lawn plantings [8].

It is a medium sized shrub with many branches. The leaves are alternate, bipinnately compound with two to six pairs of pinnae, with 10 to 25 pairs of narrow leaflets 3 to 5 mm in length. Its bright yellow or orange flowers, produced over a period of two to four months, depending on the locality, are very fragrant and are used in the perfume industry in France and other countries. Herbal remedies are important resources in traditional medicine. In India and most part of the world the inhabitants of the rural areas still use medicinal plants as an alternative to resolve their primary health problems. Ethno-botanical information gives a number of species widely used in the country to treat ailments related to the inflammatory process. In traditional system of medicine barks of *A. nilotica*, *A. leucophloea* and *A. farnesiana* are mainly used for the treatment of diarrhea, liver disorders and inflammation [9-10].

It is a drought-hardy, fire resistant species that does not tolerate frost and grows well in areas receiving between 500 and 750 mm of rain fall with a dry season of 4 to 6 months [11]. Its best growth occurs on well-drained soils. It tolerates heavy clays to a variety of soil conditions, including saline soils, at elevations up to 2,000 m. A light demanding species, sweet acacia often forms dense thickets on disturbed sites and is associated with numerous other shrub and tree species in secondary thorn woodlands, shrublands, and dry forests in its tropical and sub tropical American range. It is susceptible to attack by a number of insect species, leaf, stem, and root pathogens, though none appear to pose a serious threat to the species [12].

In Marathwada it is distributed in all parts of the region [13]. The people from Marathwada use pods of the plant for medicinal purposes in cough and cold. The
mature pods are swallowed by removing the seeds inside from it. In Marathi the plant is known as Dev Babhul and in Marathwada especially in Latur and Osmanabad districts it is known as Vedi Babhul. The plant species is not famous among the people of the Marathwada region and also in India as compared to the global scenario of the plant.

In Papua New Guinea *Acacia farnesiana* is considered as food plant where seed is considered as edible portion with 8.1% moisture with energy value 1522 KJ, protein content 36.6% with Iron and Zinc 6.0 mg and 0.6 mg respectively and also the plant is medicinally important in Tripura tribes of Bangladesh [14-15]. The photograph of the plant is shown in Fig. 4.1.
Fig. 4.1 Photograph of *Acacia farnesiana* (L.) *Willd.*
4.2 The chemistry of genus *Acacia* – A review:

Phytochemical investigations of the seeds of *Acacia farnesiana* (L.) *willd* dates back to 1962 when a novel non protein amino acid viz. N-Acetyl-L-Djenkolic acid was isolated by R. Gmelin et al [16]. N-acetyl-L-djenkolic acid appears to be present also in seed extracts of *Acacia horrida* Willd, *Acacia karroo* Hayne and *Mimosa acanthocarpa* Benth., which all give paper chromatograms very similar to that of *A. Farnesiana*.

\[
\text{CH}_3
\]

\[
\text{C}=\text{O}
\]

\[
\text{COOH-}\text{C-CH}_2\text{-S-CH}_2\text{-S-CH}_2\text{-C-COO}^-
\]

\[
\text{NH}_3^+ \quad \text{H}
\]

(1). N-acetyl-L-djenkolic acid

\((+)-\text{Mollisacacidin} \ [\ (+)-7,3',4'-\text{trihydroxy-2,3-trans-flavan-3,4-trans-diol, leucofisetinidin}]\) is the predominant monomeric heartwood component of the black wattle, *Acacia mearnsii*. The isomeric leucofisetinidins (2, 3) \((\pm)-\text{Molisacacidin}\) have been isolated and fully characterized by S. E. Drewes and A.H. Ilsley [17].
Based on the examinations by paper chromatography of the flavonoid content of the heartwoods and barks of sixty one species of Acacia native to Australia, a phytochemical survey was done by Mary D. Tindale and D.G. Roux and showed broad sub division of the flavonoid into four groups depending on variations of the phenolic hydroxyl patterns viz- 3’,4’,7 – trihydroxy; 4’,7 – dihydroxy; 3’,4’,7,8 – tetrahydroxy or 4’,7,8 trihydroxy flavonoid [18].

A group of 3’, 4’, 7, 8 – tetra hydroxyl flavonoids from the heartwood of Acacia nigrescens was isolated by T.G. Fourie et al [19]. Nigrescin, the first optically active 2 – hydroxyl – 2 – benzylcoumaranone and a (+) – 2,3 – trans – flavan – 3, 4 – cis –diol representing a new member of the natural 3’, 4’, 7, 8 – tetrahydroxy flavonoid was isolated along with chlacone, flavanone, flavanol and dihydroflavonol analogues, the flavan – 3, 4 – diols (-) – melacacidin , (-) – isomelacacidin and protocatachuic acid.

T.G. Fourie et al further sub-divided the isolated flavonoids in to 2,3-cis-3,4-cis (-) melacacidin (4); 2,3-cis-3,4-trans (-) isomelacacidin (5); (+) – 2,3-trans-3,4-cis-flavandiols (6); (±) – dihydroflavanol (7); flavanol (8); (±) – flavanone (9); chalcone (10) and 2 – hydroxy -2 – benzyl coumaran – 3- one (11).
(4). 2,3-cis-3,4-cis (-) melacacidin  

(5). 2,3-cis-3,4- trans (-) isomelacacidin

(6). (+) - 2,3-trans-3,4- cis (-) flavandiols  

(7). (±) dihydro flavanol

(8). flavanol  

(9). (±) flavanone
Acacia jacquemontii is a bushy, thorny shrub with sweet scented yellow flowers from the genus acacia distributed over various parts of India. Phytochemical investigation of this species has been reported by K.C. Joshi et al. and reported two novel Cassane diterpenoids (12-13) from the roots of Acacia jacquemontii along with n-triacontanoic acid, tectol, β-amyrin and β-sitosterol [20].
Two acylated triterpenoids bisglycosides viz. Acaciaside A and Acaciaside B from *Acacia auriculiformis cunn* was reported by Shashi B. Mahato et al [21]. These bis glycosides were defined to be 3-O-[(β-D-glucopyranosyl (1→6) {α-L-arabinopyranosyl (1→2)}-β-D-glucopyranosyl]-21-O-{(6’S)-2’-trans-2’,6’-dimethyl-6’-O-β-D-glucopyranosyl-2’,7’-octadienoyl} acacic acid 28-O-α-L-rhamnopyranosyl (1→6) [β-D-xylpyranosyl (1→2)]-β-D-glucopyranoside (14) and 3-O-[(β-D-glucopyranosyl (1→6) {α-L-arabinopyranosyl (1→2)}-β-D-glucopyranosyl]-21-O-{(6’S)-2’-trans-2’,6’-dimethyl-6’-O{β-D-xylpyranosyl (1→2)-β-D-glucopyranosyl} – 2’,7’ - octadienoyl} acacic acid 28-O-α-L-rhamnopyranosyl (1→6) [β-D-xylpyranosyl (1→2)]-β-D-glucopyranoside (15).
A novel diterpene glycoside farnesiaside (16) along with farnesian (17) from the seeds of *Acacia farnesiana* was isolated by Niranjan P. Sahu et al [22]. The farnesiaside isolated from the seeds bears a novel skeleton of diterpene.
(16). Farnesiaside $R = \text{Beta-D-glucose}$

(17). Farnesian $R = \text{H}$

A lactone of acacic acid (18), sapogenin B (19) and machaerinic acid along with nortriterpene, acacidiol (20) has been reported from the pods of *Acacia sinuata* in addition to a genin, acacigenin (21) by Anjaneyulu et al [23-25]. The phytosterols $\alpha$-Spinasterol (22) and stigmast-7-enol have been characterized from a number of species of Acacia genus viz. *A. auriculiformis*, *A. maidenii*, *A. mearnsii*, *A. melanoxylon*, *A. obtusifolia* and *A. sparisflora* by Clark-Lewis et al, Mahato et al [26-27]. A triterpenoids trisaccharide, acaciaside (23) and a phytosterol $\alpha$-Spinasterol have been reported from *A. auriculiformis* by Mahato et al [27].

Eade et al extracted three triterpene glycosides namely myrtifoliosides A (24), myrtifoliosides B (25) and C from the leaves of *Acacia myrtidolia* [28]. Recently several saponic triterpene glycosides from *Acacia victoriae* have been studied for their ability to decrease tumor cell proliferation and to induce apoptosis by Hanausek et al, Haridas et al and Mujoo et al [29-31].
(18). Acacic Acid

(19). Sapogenin-B

(20). Acacidiol

(21). Acacigenin-B
Diosmetin, farnisin and farnisin diacetate (26-28) was isolated by Niranjan P. Sahu et al. [32] from the seeds of *Acacia farnesiana*. Farnisin is 7, 3’ – dihydroxy – 4’ – methoxyl flavone.
Elfranco Malan isolated two partially methylated flavonols 7’, 8’, 3’, 4’ – tetrahydroxy – 3 – methoxyl flavone (29) and 7, 8, 4’ – trihydroxy – 3, 3’ – dimethoxy flavone (30) from the heartwood of *Acacia nigrescens* [33].

David S. Seigler et al studied the cyanogenesis in *Acacia farnesiana*, it has been reported that the species is both cyanogenic and acyanogenic. Study suggested that the cyanogens that are present in the species are linamarian (31) and lotaustralin. The amount of cyanide is also known to vary in a single specimen sampled at different times of the year [34].
Cyano-glucosides have been isolated from the leaves of *Acacia sutherlandii.*

The novel, non-cyanogenic, glycoside 1 – cyano -2-β-D-glucopyranosyloxymethyl – (Z) – prop – 1 – en – 3 – ol which has been given the trivial name sutherlandin (32) reported by Wendy K. Swenson et al [35].

![Chemical structure of Linamarin](image1)

**(31). Linamarin**

![Chemical structure of Sutherlandin](image2)

**(32). Sutherlandin**

A diol glucoside, 2 - β-D-glucopyranosyloxy – 2 – methyl propanol (33) was reported by Brimer et al [36] from the species of genus Acacia viz. *Acacia sieberiana* var. *woodii.*

![Chemical structure of 2-Beta-D-glucopyranosyloxy - 2 - methyl propanol](image3)

**(33). 2-Beta-D-glucopyranosyloxy - 2 - methyl propanol**
Survey of Australian species of subgenus *phyllodinae* was examined of which forty five species were found to be positive for cyanogenic potential was reported by Maslin et al [37-38]. The cyanogenic glycosides of these plants reported are (R) - Prunasin (34) and/ or Sambunigrin (35) derived from phenyl alanine.

![Chemical structures](image)

(34). (R) - Prunasin

(35). Sambunigrin.

It is observed that many members of the Mimosaceae are particularly rich source of the amino acids. The survey made by K. Ito and L. Fowden and co-workers observed that the free amino acids are present in seeds of about forty species of Acacia. This survey indicated the presence of pipecolic acid and 4- and 5- hydroxyl pipecolic acids as a common feature of the amino acid pattern of Acacia seeds. Albizziine and β-acetyl-α-β-diamino-propionic acid and the various sulphur containing amino acids accumulated in many seeds of acacia species [39].

The seeds of *Acacia angustissima* contain the non- protein amino acids 2 – amino – 4- acetyl amino butyric acid, 2,4–di amino butyric acid and 2-amino-6N-oxalylureidopropionic acid (36) reported by Evans et al [40]. Willardiine (37) and S-
[β – Carboxyisopropyl]-L-cysteine (38) was reported from the seeds of *A. willardiana* and *A. millefolia* by Gmelin [41] and Gmelin and Hietala [42]. The presence of S-carboxyethylcysteine, S-[β– Carboxyisopropyl]-L-cysteine, Albizziine (39) and α-amino-β-acetylaminopropionic acid (40) was reported by Evans et al [43] in subgenus *Phyllodineae*. (-) pipecolic acid (41) and trans-(−)-4-hydroxypipecolic acid (42) occur in *Acacia mearnsii*.
The presence of various fatty acids in the seed oil of different species of Acacia genus was reported in the survey made by D. S. Seigler. Oleic acid, Linoleic acid, palmitic acid, stearic acid, palmitoelic acid, linolenic acid are some of the fatty acids that are reported in *A. caven*, *A. farnesiana*, *A. giraffae*, *A. lenticularis*, *A. leucophloea*, *A. macrothyrsa*, *A. modesta*, *A. nilotica*, *A. planifrons*, *A. seyal*, *A. sieberiana*, *A. tortilis* and *A. Arabica* [44].

The seeds of *Acacia farnesiana*, *A. lenticularis*, *A. nilotica* and *A. tortilis* contained coronaric acid (43) and vernolic acid (44) in the seeds of *Acacia catechu*, *A. melifera* and *A. sinuate* was reported by Banerji et al, Brown et al, Chowdhury et al, and Jamal et al [45-48].

\[
\text{(42). trans-4-hydroxypipecolic acid}
\]

\[
\text{HO} 
\begin{array}{c}
\text{H} \\
\text{H} \\
\text{COOH}
\end{array} 
\]

\[
\text{(43). Coronaric acid}
\]

\[
\text{O} 
\begin{array}{c}
\text{H} \\
\text{H} \\
\text{COOH}
\end{array} 
\]

\[
\text{(44). Vernolic acid}
\]
4.3 Experimental:

Chemical Examination of Pods of *Acacia farnesiana* (L.) Willd.

Extract:

Mature pods of *Acacia farnesiana* (L.) Willd was collected from Latur District of Marathwada region (M.S.) India, in January 2010. The plant material was identified by Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. A voucher specimen of the plant was deposited in the Herbarium of the Botany department.

The matured pods of *Acacia farnesiana* (L.) Willd was allowed to shade dry for 20 days and powdered. The seeds from the mature pods of *Acacia farnesiana* were removed and deeseeded part of the pods was powdered. The powder (3.5 kg.) of the pods of *Acacia farnesiana* (L.) Willd sequentially extracted with Petroleum ether, Ethyl acetate, Chloroform, and Methanol by following maceration extraction process for seven days each with occasional shaking. The resulting extracts was then concentrated under reduced pressure by using rotary vacuum evaporator (Model Superfit India) to obtain a residue. The Petroleum ether extract showed nine prominent spots on thin layer chromatography (TLC). Solvent system used for TLC was Petroleum ether: Ethyl acetate (5: 5). The extraction process is depicted in Scheme III.
Scheme III

Powder of Pods of Acacia Farnesiana (L.) Willd (3.5 Kg.)

- Extracted with 7.5 Lit. of Petroleum ether. Macerated for 7 days
  - Petroleum Ether Extract
    - Green Solid
      - (11.5 gm)
      - Extracted with 7.5 Lit. of Ethyl Acetate for 7 days
        - Ethyl Acetate Extract
          - Greenish Semisolid
            - (115 gm)
            - Extracted with 7.5 Lit. of Chloroform for 7 days
              - Chloroform Extract
                - (10 gm)
                - Extracted with 7.5 Lit. of Methanol for 7 days
                  - Methanol Extract
                    - (Dark Red Viscous Liquid)
                      - (500 gm)
                      - Residue Discarded

- Residue
  - Extracted with 7.5 Lit. of Ethyl Acetate for 7 days
    - Ethyl Acetate Extract
      - Greenish Semisolid
        - (115 gm)
        - Extracted with 7.5 Lit. of Chloroform for 7 days
          - Chloroform Extract
            - (10 gm)
            - Extracted with 7.5 Lit. of Methanol for 7 days
              - Methanol Extract
                - (Dark Red Viscous Liquid)
                  - (500 gm)
Chromatographic Separation of the Petroleum Ether Extract:

The petroleum ether extract (11.5 gm.) was chromatographed on a silica gel (60-100 mesh) column (150 × 15 cm) and eluted with a step-wise gradient of Petroleum Ether : Ethyl acetate, with a total volume of 50 ml of the solvent system (50, 49:1, 47.5:2.5, 46.25:23.75, 45: 5, 43.75: 6.25, 42.5:7.5, 41.25:8.75, 40:10, 35:15, 30:20, 50 by volume). A total of 30 fractions of 20 ml each were collected, and tested for the composition of each fraction. The grouping was done according to their composition determined by thin layer chromatography. Spots were detected by using various detecting techniques such as UV chamber, Iodine Chamber, spraying with 10% methanol-sulphuric acid spray reagent and then heating on a hot plate. The grouping of the eluents is depicted in Table 4.1.

Table 4.1

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Fraction Number</th>
<th>Group Number</th>
<th>Constituents of Fraction</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1-4</td>
<td>I</td>
<td>2</td>
<td>A, B</td>
</tr>
<tr>
<td>2.</td>
<td>5-9</td>
<td>II</td>
<td>1</td>
<td>C</td>
</tr>
<tr>
<td>3.</td>
<td>10-11</td>
<td>III</td>
<td>1</td>
<td>D</td>
</tr>
<tr>
<td>4.</td>
<td>12</td>
<td>IV</td>
<td>1</td>
<td>E</td>
</tr>
<tr>
<td>5.</td>
<td>13-14</td>
<td>V</td>
<td>1</td>
<td>F</td>
</tr>
<tr>
<td>6.</td>
<td>15-19</td>
<td>VI</td>
<td>1</td>
<td>G</td>
</tr>
<tr>
<td>7.</td>
<td>20-25</td>
<td>VII</td>
<td>1</td>
<td>H, I</td>
</tr>
</tbody>
</table>
Re-Column of Group I:

Repeated column chromatography was performed for the group I by using the solvent system Petroleum ether: Ethyl acetate by using (7:3) solvent composition. The silica gel used for the column was (100-200 mesh) with column (150 × 15 cm). First pure petroleum ether was eluted in the column and then with the selected composition of the solvent system. Total 20 fractions of 10 ml each were collected by testing the TLC for each fraction. The fractions 1-8 showed similar nature and hence mixed together and allowed the solvent to evaporate to get the solid compound A. The remaining fractions were collected and tested for TLC, fractions with similar composition was collected together and the solvent was allowed to evaporate which gave a solid residue designated as compound B.

Re-Column of Group VII:

Repeated column chromatography was performed for the group VII by using the solvent system Chloroform: Methanol with composition of the mixture (7:3). The silica gel used for the column (100-200 mesh) with column (150 × 15 cm). Total volume of the solvent system used for the re-column 100 ml. Total fractions collected for the re-column of this group was ten of 10 ml each. Each fraction collected was subjected to TLC testing and the fractions of similar composition were grouped together. In all two grouping of this re-column was made and allowed to evaporate the solvent to get the solids H and I. The quantity of the compound I was very less (5 mg) hence no further characterization of this compound was undertaken.
Physical and Spectral Characterization of the compounds isolated from Pods of *Acacia farnesiana (L.) Willd.*

**Compound A.**

**Physical State:**
White solid

**Yield:**
20 mg.

**M.P.**
60 °C

**UV $\lambda_{max}$ (CH$_3$OH):**
Transparent

**IR (KBr) $\nu_{max}$:**
2917, 2849, 1460, 1375, 723, 513, 479, 467, 447 cm$^{-1}$.

**$^1$H-NMR (400 MHZ, CDCl$_3$) $\delta$:**
0.94 (3H, S), 1.28 – 1.32 (br, S)

**ESI-MS m/z:**
408

**Compound B.**

**Physical State:**
White crystals

**Yield:**
30 mg.

**M. P.**
66 °C
UV $\lambda_{\text{max}}$(CH$_3$OH):

Only terminal absorption

IR (KBr) $\nu_{\text{max}}$:

2917, 2850, 1459, 1375, 831, 723, 527, 487 cm$^{-1}$.

$^1$H-NMR (400 MHz, CDCl$_3$) $\delta$:

0.92 (3H, S,), 1.26 – 1.33 (br, S)

ESI-MS m/z:

422

Compound C.

Physical State:

Yellowish solid

Yield:

30 mg.

M. P.

260 °C

UV $\lambda_{\text{max}}$(CH$_3$OH):

260 nm

IR (KBr) $\nu_{\text{max}}$:

3736, 2589, 1701, 1630, 1493, 1454, 1262, 1212, 801, 764, 702 cm$^{-1}$.

$^1$H-NMR (400 MHz, CDCl$_3$) $\delta$:

11.00 (1H, S), 7.0 (1H, S), 5.2 (1H, S)

ESI-MS m/z:

170
Compound D.

Physical State:
White solid

Yield:
15 mg.

M. P.
70 °C

UV $\lambda_{\text{max}}$(CH$_3$OH):
Terminal absorption.

IR (KBr) $\nu_{\text{max}}$:
3336, 2919, 2851, 2662, 1708, 1461, 1374, 1170, 1101, 967, 722, 523, 486, 442 cm$^{-1}$.

$^1$H-NMR (400 MHZ, CDCl$_3$) $\delta$:
0.84 (t, J=6.4 HZ), 1.23 – 1.30 (br, S).

ESI-MS m/z:
284

Compound E.

Physical State:
White solid

Yield:
10 mg.

M. P.
120 °C

UV $\lambda_{\text{max}}$(CH$_3$OH):
298, 296 nm.
IR (KBr) $\nu_{\text{max}}$:  
3396, 1712, 1457, 1169, 1086, 721, cm$^{-1}$.

$^1$H-NMR (400 MHZ, CDCl$_3$) $\delta$:  
7.48 (2H, t, J=7.6 Hz), 7.62 (1H, t, J=7.4 Hz), 8.10 (2H, d, J=7.4 Hz).

ESI-MS m/z:  
122

**Compound F.**

**Physical State:**  
Amorphous Solid

**Yield:**  
30 mg.

**M. P.**  
70 °C

**UV $\lambda_{\text{max}}$(CH$_3$OH):**  
Only terminal absorption

IR (KBr) $\nu_{\text{max}}$:  
3435, 2921, 2853, 1709, 1459, 1374, 1259, 1167, 1089, 1017, 796, 720, 518, 485, 456 cm$^{-1}$.

$^1$H-NMR (400 MHZ, CDCl$_3$) $\delta$:  
0.88 (t, 6.4 HZ), 1.22 – 1.29 (br, S).

ESI-MS m/z:  
256
Compound G.

Physical State:
White solid

Yield:
20 mg.

M. P.
270 °C

UV $\lambda_{\text{max}}$(CH$_3$OH):
220 nm

IR (KBr) $\nu_{\text{max}}$:
3300, 2922, 2854, 1710, 1493, 1454, 1130 cm$^{-1}$.

$^1$H-NMR (400 MHZ, CDCl$_3$) $\delta$:
12.96 (1H, S, ), 10.82 (1H, S,), 9.46 (1H, S), 7.50 (1H, dd, J=9.4 Hz), 7.40 (1H, d, J=2 Hz), 7.06 (1H, d, J=9.0 Hz), 6.72 (1H, S), 6.46 (1H, d, J=2Hz), 6.20 (1H, d, J=2 Hz), 3.86 (3H, S).

ESI-MS m/z:
302
**Compound H.**

**Physical State:**

Yellowish solid

**Yield:**

20 mg.

**M. P.**

266\(^0\)C

**UV \(\lambda_{max}(\text{CH}_3\text{OH})\):**

340, 235 nm

**IR (KBr) \(v_{max}\):**

3736, 2922, 2855, 1712, 1449, 1374, 1163, 1086, 1013, 833, 746 cm\(^{-1}\).

**\(^1\)H-NMR (400 MHZ, CDCl\(_3\)) \(\delta\):**

10.76 (1H, S), 9.40 (1H, S), 6.66 (1H, S), 3.86 (3H, S), 6.94 (1H, m), 7.50 (1H, dd, \(J=8.2\) Hz), 7.42 (1H, d, \(J=2\) Hz), 7.10 (1H, d, \(J=8.0\) Hz), 7.90 (1H, d, \(J=8.4\) Hz).

**ESI-MS m/z:**

284
4.4 Result and Discussion:

Structure of Compound A:

The sub-fraction obtained from the pure petroleum ether soluble fraction of the plant (11.5 gm.) following the maceration process of the pods of *Acacia farnesiana* was subjected to column chromatography. The solvent system used for the column chromatography was Petroleum ether: Ethyl acetate with step wise gradient. Elution of the column yielded a dark solid (70 mg) of the substance. The solid obtained showed two spots on the TLC plate.

The solid thus obtained was subjected to repeated column chromatography which yielded two compounds designated as compound ‘A’ (20 mg) and compound ‘B’ (30 mg). Compound A was isolated as a white solid with a melting point of 60°C. The molecular formula of the compound was deduced as C$_{29}$H$_{60}$ from its GC-MS spectrum (Fig. 4.2) which showed m/z at 408.

The IR spectrum of the compound exhibited the absorption band at 2917 cm$^{-1}$, 2849 cm$^{-1}$ indicating the C-H stretching in the alkanes. The absorption band at 1460 cm$^{-1}$ purely exhibited the C-H bending for alkane moiety. The band at 1375 cm$^{-1}$ indicated the C-H bending in methyl group. The IR spectrum of Compound ‘A’ is shown in (Fig. 4.3). The compound showed only terminal UV absorption confirming the nature of the compound as aliphatic hydrocarbon.

The $^1$H-NMR spectrum of compound ‘A’ exhibited the signal at $\delta$ 0.94 indicating the presence of methyl group in the compound. The signal at $\delta$ 1.20 – 1.30 indicating broad singlet for the methylene protons in the same environment. The $^1$H-NMR spectrum of compound ‘A’ is shown in (Fig. 4.4). The presences of methyl and methylene carbon atoms were confirmed in the compound from $^{13}$C-NMR spectrum. The $^{13}$C-NMR spectrum of compound ‘A’ is shown in (Fig. 4.5).
Based on the above observations and the reported literature data suggests that the compound ‘A’ is higher alkane i.e. Nonacosane isolated first time from the deseeded part of the pods of *Acacia farnesiana* species which is new source for the compound.

1. Nonacosane
Fig. 4.2  GC-MS Spectrum of Nonacosane
Fig. 4.3  IR Spectrum of Nonacosane
Structure of Compound B:

Re-column of the group-I yielded a white solid (30 mg) designated as compound ‘B’, the melting point of the yielded compound was observed at 66°C. The molecular formula of the compound ‘B’ was found to be C_{30}H_{62} from its molecular ion peak [M+] at m/z – 422 in GC-MS spectrum of the compound shown in (Fig. 4.6). The molecular formula of the compound shows zero degrees of unsaturation.

The IR spectrum of compound ‘B’ showed absorption band at 2917 cm\(^{-1}\) and 2850 cm\(^{-1}\) respectively indicating the C-H stretching in the alkane moiety. The absorption band at 1459 cm\(^{-1}\) and 1375 cm\(^{-1}\) purely indicates the C-H bending in alkanes. The C-C stretching in alkanes is exhibited by the band in the absorption range of 800 cm\(^{-1}\) to 1200 cm\(^{-1}\). The IR spectrum of compound ‘B’ is shown in (Fig. 4.7).

The \(^1\)H-NMR spectrum of compound ‘B’ is depicted in (Fig. 4.8). The observation of \(^1\)H-NMR spectrum of compound ‘B’ exhibited the singlet for 3H at \(\delta\) 0.92 showing the presence of methyl protons in the compound. The broad singlet signal at \(\delta\) 1.26 – 1.33 shows the presence of methylene protons in the same environment in the compound. The UV absorption study of compound ‘B’ exhibited only terminal absorption indicating the presence of aliphatic higher alkane.

The \(^{13}\)C-NMR spectrum of compound B showed the similar nature of carbons as was observed in the compound ‘A’. The \(^{13}\)C-NMR spectrum of the compound ‘B’ leads to the conclusion that it is also a class of hydrocarbon compound. The \(^{13}\)C-NMR spectrum of compound ‘B’ is shown in (Fig. 4.9).

All the physical and spectral characterization of compound ‘B’ was in good agreement with the data reported for a triacontane. This compound is also isolated
first time from the pods of the *A. farnesiana* species and is thus a new natural source of the compound.

2. Triacontane
Fig. 4.6 GC-MS Spectrum of Triacontane
Fig. 4.7 Infra-red Spectrum of Tricontane
Fig. 4.9 $^1$C-NMR Spectrum of Triacontane
Structure of Compound C:

Compound ‘C’ (30 mg) was isolated as a yellowish solid with a melting point of 260 $^{0}$C. The molecular formula of the compound was deduced as C$_7$H$_6$O$_5$ from its ESI-MS spectrum at m/z- 170 [M$^+$/ molecular ion peaks. The molecular formula of the compound ‘C’ shows six degrees of unsaturation indicating the basic benzene skeleton in the compound.

The absorption band at $\lambda_{\text{max}}$ 260 nm in UV spectrum confirmed the presence of benzoic acid moiety in the compound. The IR spectrum of compound ‘C’ exhibited the absorption band in the range of 3500-2500 cm$^{-1}$ and 1701 cm$^{-1}$ indicating the presence of carboxylic acid group in the compound. The bands at 1454 cm$^{-1}$, 1493 cm$^{-1}$ and 1630 cm$^{-1}$ indicates the C=C stretching in the aromatic compounds. The band at 1262 cm$^{-1}$ and 1212 cm$^{-1}$ exhibits the C-OH stretching in the compound. Other bands at 801 cm$^{-1}$, 764 cm$^{-1}$ and 702 cm$^{-1}$ showing the o-, m- and p- substitution pattern in the compound.

The $^1$H-NMR spectrum of compound ‘C’ exhibited the signal at $\delta$ 11.00 for the carboxylic acid proton. The signal at $\delta$ 7.0 exhibited the aromatic protons whereas the signal at $\delta$ 5.2 exhibited the C-OH protons attached to the aromatic ring. The $^{13}$C-NMR spectrum of the compound exhibited the similar pattern like Gallic acid.

Based on all above data and physical and spectral characterization, the compound isolated was found to be Gallic acid which is reported in various plant resources. The Gallic acid thus isolated is considered as a part of Naringenin7-O-β-(4”,6”'-digalloylglucopyranoside) in the form of sugar moiety isolated from the pods of Acacia farnesiana by Barakat et al [49].
(3). Gallic acid

Structure of Compound D:

The sub-fraction eluted with Petroleum ether: Ethyl acetate from the group III yielded a white solid with a melting point $40^\circ\text{C}$ and was designated as compound ‘D’. The molecular formula of the compound from its ESI-MS showed the molecular ion peak $[M^+]$ at $m/z = 284$ suggesting it to be $\text{C}_{18}\text{H}_{36}\text{O}_2$ corresponding to one degree of unsaturation.

The IR spectrum of compound ‘D’ exhibited the band at $3336 \text{ cm}^{-1}$ and $1708 \text{ cm}^{-1}$ for carbonyl stretching indicating the presence of carboxylic acid group moiety in the compound. The band at $2919 \text{ cm}^{-1}$ and $2851 \text{ cm}^{-1}$ indicates the C-H stretching in methyl and methylene groups respectively. The absorption band in the range of 800 – 1200 cm$^{-1}$ exhibits the C-C stretching in the alkanes. The C-H stretching in the methyl and methylene moiety is observed at 1461 cm$^{-1}$.

The $^1\text{H-NMR}$ spectrum of compound ‘D’ showed a broad signal between $\delta 1.23 – 1.30$ and a triplet at $\delta 0.84$ which shows that the compound is a long chain fatty acid. All the physical and spectral characterization of the compound was compared with the literature value. The compound thus identified as stearic acid.
whose presence was detected in the same species earlier by different workers. Thus the compound ‘D’ isolated was stearic acid.

\[
\text{HO} \quad \begin{array}{c}
\text{O} \\
\end{array}
\]

4. stearic acid

**Structure of compound E:**

The compound ‘E’ was isolated from the Petroleum ether: Ethyl acetate fractions collected from group IV. White solid with the yield (10 mg) having melting point 120 °C was isolated from these fractions of the group IV. The molecular formula of the compound was found to be C\(_7\)H\(_6\)O\(_2\) from its ESI-MS spectrum at m/z – 122 of molecular ion peak [M\(^+\)]. The characteristic peak at m/ - 77 was observed due to the loss of –CO\(_2\) moiety from the compound. The molecular formula of the compound ‘E’ suggests the five degrees of unsaturation in the compound.

The IR spectrum of compound ‘E’ exhibited the absorption bands at 3396 cm\(^{-1}\) and 1712 cm\(^{-1}\) indicating the presence of carboxylic acid group in the compound. The band at 1457 cm\(^{-1}\) indicates the aromatic C=C stretching. The absorption band at 712 cm\(^{-1}\) exhibits the C-H bending for mono-substituted aromatic compound. The band at 1086 cm\(^{-1}\) exhibited the presence of C-O absorption in the compound. The UV absorption of compound ‘E’ exhibited the aromatic nature of the compound.
The $^1$H-NMR spectrum of compound ‘E’ exhibited the signal at $\delta$ 7.48 (J=7.8 HZ) and $\delta$ 8.12 (J=7.4 HZ) for the aromatic region exhibiting two triplets and one doublet. The carboxylic acid proton exhibited the signal at $\delta$ 10.0. All the physical and spectral characterization of the compound compared with the literature suggested that it is a well-known compound i.e. benzoic acid which is isolated first time from the pods of the species.

![Structure of Benzoic Acid](image)

5. Benzoic Acid

Structure of Compound F:

The compound ‘F’ was isolated as amorphous solid with a melting point of 70°C. The molecular formula of the compound was deduced as C$_{16}$H$_{32}$O$_2$ from its m/z – 256 in ESI-MS spectrum which corresponds to two degrees of unsaturation.

The IR spectrum of compound ‘F’ exhibited the band at 3435 cm$^{-1}$ and 1709 cm$^{-1}$ for carbonyl group stretching indicating the presence of aliphatic carboxylic acid group in the compound. The band at 2921 cm$^{-1}$ and 2853 cm$^{-1}$ indicates the stretching in C-H in methyl and methylene group respectively. The band at 1459 cm$^{-1}$ and 1374 cm$^{-1}$ exhibits the C-H bending in methyl and methylene group. The absorption band in the range of 800 – 1200 cm$^{-1}$ indicates the C-C stretching in the compounds. The band at 1259 cm$^{-1}$ indicates the C-O stretching in carboxylic acids.
The $^1$H-NMR spectrum of compound ‘F’ showed a broad signal at $\delta$ 1.22 – 1.29 and a triplet at $\delta$ 0.88 indicating the nature of the protons in the compound as aliphatic long chain fatty acid. The compound ‘F’ was identified as Palmitic acid by the comparison of all the physical and spectral data reported for the above mentioned acid. The compound thus isolated from the selected species has been reported by Gunstone F.D. [50] from different plant sources.

\[ \text{(6). palmitic acid} \]

**Structure of Compound G:**

The fractions collected from group – VI were mixed together and the solvent was allowed to evaporate in rotary vacuum evaporator. A white solid (20 mg) having melting point 270 $^\circ$C was obtained which is designated as compound ‘G’. The molecular formula of the compound was deduced as C$_{16}$H$_{12}$O$_6$ from its ESI-MS spectrum for [M$^+$] ion peak at m/z 300. The other peaks observed were 301 for [M+1] and 302 for [M+2].

The IR spectrum of compound ‘G’ exhibited the band at 3300 cm$^{-1}$ indicating the presence of hydroxyl group in the compound. The band at 2922 cm$^{-1}$ and 2854 cm$^{-1}$ indicating the C-H stretching in the compound. The absorption band at 1710 cm$^{-1}$ indicates the carbonyl stretching in the compound. The absorption band at 1454 cm$^{-1}$
186 cm\(^{-1}\) and 1493 cm\(^{-1}\) indicating the C=C stretching in the aromatic ring. The absorption band at 1130 cm\(^{-1}\) showing the presence of C-O-C stretching for the ether linkage in the compound.

The \(^1\)H-NMR spectrum of compound ‘G’ exhibits the signal at δ 12.96, 10.82, and 9.44 exhibiting the presence of hydroxyl proton in the compound. The signal at δ 7.50, 7.40 and 7.06 exhibits the aromatic protons. The other signal at δ 6.72, 6.46 and 6.20 also exhibits the aromatic protons present in the compound. The signal at δ 3.86 indicates the singlet for –OCH\(_3\) protons in the compound. The \(^13\)C-NMR spectrum of compound ‘G’ exhibits the nature of carbons as methyl, methine and quaternary respectively which is identical with the reported data [51].

All the physical and spectral characterization of the compound is found to be identical with the data reported for Luteolin 4’-methyl ether which is reported earlier from the same plant by N. P. Sahu et al [32].

(7). Luteolin 4’ Methyl Ether.
Structure of Compound H:

The sub-fractions eluted with Petroleum ether: Ethyl acetate and pure Ethyl acetate were grouped together and the solvent is allowed to evaporate in rotary vacuum evaporator. A yellowish solid (20 mg) with melting point 266 °C was obtained which is designated as compound ‘H’. The molecular formula of the compound was found to be C_{16}H_{12}O_{5} from its ESI-MS spectrum at m/z – 284 for [M⁺] ion peak. The other peaks observed were 285 and 286 for [M+1] and [M+2] peak.

The IR spectrum of compound ‘H’ exhibited the band at 3336 cm⁻¹ indicating the presence of hydroxyl group in the compound. The absorption band at 2922 cm⁻¹ and 2855 cm⁻¹ were observed for C-H stretching. The absorption in the range of 1712 cm⁻¹ indicates the carbonyl group stretching in the compound. The absorption band at 1449 cm⁻¹ exhibits the C=C stretching in aromatic compound. The absorption band at 746 cm⁻¹ and 833 cm⁻¹ indicates the C-H bending in O- and P- di substituted aromatic compound. The band at 1374 cm⁻¹ indicates the C-H bending in methyl group. The UV absorption band of compound ‘H’ exhibited at 340 nm and 235 nm respectively indicating carbonyl moiety in the compound.

The ¹H-NMR spectrum of compound ‘H’ exhibited the signal at δ10.76, 9.40 indicating the hydroxyl protons present in the compound. The signal at δ 6.66 indicates a proton singlet and the signal at δ 3.86 showing the singlet for –OCH₃ protons. The signal at δ 7.50, 7.42 and 7.10 exhibits the aromatic protons present in the compound. The ¹³C-NMR spectrum of compound ‘H’ exhibited the similar nature of carbon atoms as was found in the compound ‘G’ which indicates the similarity of the compounds except the signal for one hydroxyl group carbon atom.
All the physical and spectral data of compound ‘H’ matches with the data reported for 7, 3’-dihydroxy – 4’-methoxy flavone which is reported by N. P. Sahu et al [32] from the same plant species.

(8). 7,3'-dihydroxy-4'-Methoxy Flavone.
References:


51. Mabry T. J., Markham K. R. and Chari V. M., $^{13}$C-NMR spectra of flavonoids; *the flavonoids, Advances in Research*; Editor J.B. Harborne, T.J. Mabry, Chapman and Hall, London, 1985, PP. 51