Chapter 3

Phytochemical Investigation of *Merremia emarginata* (Burm.f.) Hall.
3.1 Introduction to the Plant Family:

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
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<tr>
<td>Sub kingdom</td>
<td>Phanerogams</td>
</tr>
<tr>
<td>Division</td>
<td>Magnoliophyta</td>
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<tr>
<td>Class</td>
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<tr>
<td>Family</td>
<td>Convolvulaceae</td>
</tr>
<tr>
<td>Species</td>
<td><em>Merremia emarginata</em> (Burm.f.) Hall.</td>
</tr>
<tr>
<td>Local Name</td>
<td>Mooshakarni, Undir kani (Marathi)</td>
</tr>
</tbody>
</table>

*Merremia emarginata* (Burm.f.) Hall belongs to Convolvulaceae family and the genus *Merremia*.

**Family – Convolvulaceae**

The Convolvulaceae is commonly known as the morning glory family containing about 60 genera and probably 1650 species. The members of this family are well known as showy garden plants as well as troublesome weeds for example - bindweed hence this family is also known as Bindweed family. Most of the species are herbaceous vines, trees, herbs and shrubs distributed in tropical and subtropical regions. Convolvulaceae members can be recognized by their funnel shaped, radially symmetrical corolla, having five petals and five fused sepals. The stems of the plants from this family are usually winding, leaves are simple and alternate without stipules.
Genus – Merremia:

The genus *Merremia* comprises about 60 species. This genus shows its importance in folk medicine. The genus *Merremia* presents interesting phytochemical features such as the Occurrence of terpenoids, phenolics, flavonoids and alkaloids. Tropane and pyrrolidine alkaloids from the genus have shown significant biological activities. One of the species of this genus, *Merremia tridentata* (L.) Hallier.f. is the perennial herb with elongate stems belonging to the family Convolvulaceae, and is distributed in the tropical parts of the world. The plant is used in traditional medicine to cure many diseases such as arthritis, skin infections, inflammation, fever, diabetes, diarrhoea, urinary disorders, and also used to improve hair growth [1].

Species - *Merremia emarginata* (Burm.f.) Hall.

Convolvulaceae and is prevalent throughout India, Malaysia and tropical Africa. It is an uncultivated food crop used as a green leaf vegetable by poor people in India [2]. The Plant is annual herb 12–25 cm tall, stem glabrous, simple or branched at the top. The species frequently found on sandy banks of river and in fields with fertile soil. Flowering and fruit season of the plant is from November to February. In Marathwada generally it is distributed in all districts of Marathwada especially in Aurangabad, Nanded and Osmanabad [3].

Leaves of the plant are used as a vegetable and also in Ayurvedic medicine system. It mainly grows in rainy and winter season. In Marathi (local) it is known as Undir kani meaning leaves of this plant resembles like ear of the mouse. Charaka, Sushruta and Vagbhatta the great Indian medicinal practitioners have attributed the importance of the plant in Ayurveda. The plant is traditionally used as diuretic and for cough, headache, neuralgia and rheumatism [2]. In vitro antioxidant and antimicrobial activities of *Merremia emarginata* using thio-glycolic acid-capped
cadmium telluride quantum dots was studied by Ramesh Kumar A.et al because this plant has very high flavonoid and phenol content [4].

Powder of leaves is used as a snuff during epileptic seizures, juice acts as purgative and the root is having diuretic, laxative, and applied in the disease of the eyes and gums [5]. Anticancer activity of *Merremia emarginata* (burm.f.)Hall., against human cervical and breast carcinoma was reported by Purushoth et al and significant results were obtained justifying the use of plant in traditional system of medicine [6].

A significant decrease in blood glucose, serum urea and serum creatinine and significant increase in body weight, insulin and protein level were observed in diabetic rats treated with *Merremia emarginata*. Treatment with *Merremia emarginata* resulted in a significant reduction of HbA1C and an increase in total hemoglobin level. The activities of carbohydrate metabolizing enzymes such as hexokinase were significantly increased whereas glucose-6-phosphatase, fructose-1, 6-bisphosphatase were significantly decreased by the administration of *Merremia emarginata* in diabetic rats [7]. The photograph of *Merremia emarginata* (Burm. f.)Hall . is shown in fig 3.1.
Fig. 3.1 Merremia emarginata (Burm.f.) Hall.
3.2 The chemistry of genus Merremia– A review:

The genus Merremia is included in the family Convolvulaceae comprising of the major group of angiosperms (flowering plants). This genus comprises of 293 plant species having medicinal importance [8]. The phytochemical investigation of *Merremia mammosa*, an Indonesian medicinal plant was reported by Isao Kitagawa et al [9] showing four new ionophoric resin-glycosides of which the structures of two major resin-glycosides, named Mammoside B (1) and Mammoside H1(2) have been studied.
(2) Mammoside $H_1$

Prunasin-6'-Malonate (3), A Cyanogenic glucoside from the leaves of *Merremia dissecta* have been reported by Adolf Nahrstedt et al [10].

(3) Prunasin - 6 - Malonate
Flavonoids, an important class of phenolics featuring the linkage of two benzene rings by a chain of three carbon atoms so as to form pyran or pyrone ring, play a predominant role in plant physiology and serve as light screens, antioxidants, enzyme inhibitors, precursors of toxic substances, and pigments [11]. Flavonoids have been isolated from the aerial parts of the plant of Merremia genus, species Merremia tridentata (L.) Hallier.f., which include Diosmetin (4), luteolin–7–o–β–d–glucoside (5), Luteolin (6), diosmetin–7–o–β–d–glucoside (7). [12].

(4) Diosmetin

(5) Luteolin 7-O-beta-D-glucoside

(6) Luteolin
Phytochemical investigation on flavonoid sulphates of *Ipomoea Regnellii Meisn* and *Ipomoea reticulata O’Donell* was studied by Petra Mann et al [13]. Kaempferol 3-Sulfate (8), Kaempferol 7-OMe-3-Sulfate (9) and Kaempferol 4’,7-OMe-3-sulfate (10), Quercetin 3,7-di OMe-4’-sulfate (11), Quercetin 3’,4’,7-tri OMe-3-sulfate (12) were reported from *Ipomoea regnellii* and *Ipomoea reticulata* respectively.
(8). Kaempferol 3-Sulfate: \( R_1 = \text{SO}_3\text{Na} \quad R_2 = \text{OH} \quad R_3 = \text{H} \quad R_4 = \text{OH} \)

(9). Kaempferol 7-OMe-3-Sulfate: \( R_1 = \text{SO}_3\text{Na} \quad R_2 = \text{OCH}_3 \quad R_3 = \text{H} \quad R_4 = \text{OH} \)

(10). Kaempferol 4',7- di OMe-3-Sulfate: \( R_1 = \text{SO}_3\text{Na} \quad R_2 = \text{OCH}_3 \quad R_3 = \text{H} \quad R_4 = \text{OCH}_3 \)

(11). Quercetin 3,7- di OMe-4'-Sulfate: \( R_1 = \text{OCH}_3 \quad R_2 = \text{OCH}_3 \quad R_3 = \text{OH} \quad R_4 = \text{OSO}_3\text{Na} \)

(12). Quercetin 3',4',7- tri OMe-3-Sulfate: \( R_1 = \text{SO}_3\text{Na} \quad R_2 = \text{OCH}_3 \quad R_3 = \text{OCH}_3 \quad R_4 = \text{OCH}_3 \)

The glycol resins represent an important chemotaxonomic marker of the Convolvulaceae family. Extensive studies of *Ipomoea tricolor* from this family have been studied by Moustapha Bah and Rogelio Pereda-Miranda [14] and isolated tricolorins A - E (13-17).
(13). Tricolorin A : $R_1 = OH, R_2 = H, R_3 = mba$

(14). Tricolorin B : $R_1 = OH, R_2 = H, R_3 = iba$

(15). Tricolorin C : $R_1 = OH, R_2 = H, R_3 = nla$

(17). Tricolorin E : $R_1 = H, R_2 = OH, R_3 = mba$

\[ mba = C_2H_5-CH-CH3 \]

\[ iba = H_3C-CH-CO \]

\[ nla = \]

(16). Tricolorin D
The glycol resins isolated from the Convolvulaceae species are responsible for purgative properties showing the medicinal importance of the family. The tuber of *Merremia mammosa (Lour.) Hallier f.*, is said to be useful as medicinal plant in Indonesia and is used for treating diabetes and illness involving the throat and respiratory system. An extensive study of *Merremia mammosa* was done by Isao Kitagawa et al. [15] and isolated five new resin glycosides named merremosides a, b, c, d and e (18-22) along with Mammoside B and Mammoside H₁.
(23). Tuguajalapins I: $R_1=\text{Palmitoyl}$ $R_2=\text{H}$
(24). Tuguajalapins II: $R_1=\text{H}$ $R_2=\text{Palmitoyl}$
(25). Tuguajalapins III: $R_1=\text{Palmitoyl}$ $R_2=\text{H}$
(26). Tuguajalapins IV: $R_1=\text{Stearoyl}$ $R_2=\text{H}$
(27). Tuguajalapins V: $R_1=\text{H}$ $R_2=\text{Stearoyl}$
(28). Tuguajalapins VI: $R_1=\text{Stearoyl}$ $R_2=\text{H}$
(29). Tuguajalapins VII: $R_1=\text{H}$ $R_2=\text{arachidoyl}$
(30). Tuguajalapins VIII: $R_1=\text{arachidoyl}$ $R_2=\text{H}$
(31). Tuguajalapins IX: $R_1=\text{H}$ $R_2=\text{H}$
(32). Tuguajalapins IX: $R_1=\text{H}$ $R_2=\text{H}$

Convolvulaceae family is known to produce a wide variety of alkaloids such as ergolines, pyrrolidines and the closely related tropane alkaloids. Some *Ipomoea* species contain unique indolizine alkaloids of the ipalbidine type and ipobscurine type serotonin-hydroxycinnamic acid conjugates [17-18]. Pyrrolizidine alkaloids and loline alkaloids were detected in the tropical bindweed, *Ipomoea hederifolia* and *Agyreia mollis* respectively [19-20]. Further, the pyrrolizidine alkaloids of *Ipomoea hederifolia* were studied by Kristina Jenett-Siems et al [21] and isolated novel ipangulines (33-35).

![Ipanguline D10](image1.png) ![Ipanguline B2](image2.png)
Isolation of alkaloids from *Merremia dissecta* roots was achieved by Kristina Jenett-Siems et al. [22] reporting the four unknown alkaloids Merresectine A (36), Merresectine B (37), Merresectine C (38), and Merredissine (39) along with Datumetine (40). The Merresectine A and Merresectine B have been also isolated from the roots of *Merremia quinquefolia* and *Merremia cissoides* respectively.

(36). Merresectine A : \( R_1 = \text{H} \quad R_2 = \text{OCH}_3 \quad R_3 = \text{H} \)

(40). Datumetine : \( R_1 = \text{CH}_3 \quad R_2 = \text{OCH}_3 \quad R_3 = \text{H} \)
(37). Merresectine B: \( R = \text{Beta-D-glucose} \)

(38). Merresectine C: \( R = H \)

(39). Merredissine

Recently Pentasaccharide resin glycosides from \textit{Ipomoea cairica} and their cytotoxic activities was studied by Bangwei Yu et al [23] and succeeded in the isolation of six partially acylated Pentasaccharide resin glycosides, Cairicosides A-F (41-46) from the aerial parts of \textit{Ipomoea cairica}. Cairicosides are a group of macro lactones of simonic acid A, partially acylated with different organic acids.
(41). Cairicoside A: $R_1 = \text{mba} \quad R_2 = \text{CA} \quad R_3 = \text{H} \quad R_4 = \text{Deca}$

(42). Cairicoside B: $R_1 = \text{mba} \quad R_2 = \text{H} \quad R_3 = \text{CA} \quad R_4 = \text{Deca}$

(43). Cairicoside C: $R_1 = \text{mba} \quad R_2 = \text{CA} \quad R_3 = \text{H} \quad R_4 = \text{mba}$

(44). Cairicoside D: $R_1 = \text{mba} \quad R_2 = \text{CA} \quad R_3 = \text{H} \quad R_4 = \text{Octa}$

(45). Cairicoside E: $R_1 = \text{mba} \quad R_2 = \text{H} \quad R_3 = \text{H} \quad R_4 = \text{Deca}$
(46). Cairicoside E: $R_1 = \text{mba}$  $R_2 = \text{H}$  $R_3 = \text{H}$  $R_4 = \text{Dodeca}$

mba = 2S-methylbutanoyl
CA = trans-cinnamoyl
Deca = n-decanoyl
Octa = N-octanoyl
Dodeca = n-decanoyl

Allelopathic effects on Arabidopsis seed germination of phenolic compounds isolated from *Merremia umbellate* sub sp. *Orientalis* was studied by Jian Yan et al [24] and reported salicylic acid derived new natural product, SA 2-O-β-D-(3’,6’-dicaffeoyl)-glucopyranoside (47) along with known phenolic compounds from the species.
(47). SA 2-O-Beta-D-(3',6'-dicaffeoyl)-glucopyranoside.
3.3 Experimental:

Chemical Examination of *Merremia emarginata* (Burm.f.) Hall.

Extract:

*Merremia emarginata* (Burm.f.) Hall., was collected from Osmanabad District of Marathwada region (M.S.) India, in December 2009. 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 whole plant *Merremia emarginata* (Burm.f.) Hall., was shade dried and powered. The powder (1kg.) of *Merremia emarginata* (Burm.f.) Hall., was sequentially extracted with Petroleum ether, Hexane, Chloroform, Acetone and Ethanol by following maceration process for three days each with successive shaking. The resulting extracts was then concentrated under reduced pressure by using rotary vacuum evaporator (Model Superfit India) to obtain a residue. The Chloroform extract showed eight prominent spots on thin layer chromatography (TLC). Solvent system used for TLC was Hexane: Ethyl acetate (5: 5). The extraction process is depicted in Scheme II.
Scheme II

*Merremia emarginata (Burm.f.) Hall.*

Powder (5 kg)

- Extracted with 10 Lit. of Petroleum ether. Macerated for 3 days

**Petroleum Ether Extract**

(18.5 gm)

- Residue

**Hexane Extract**

(15 gm)

- Extracted with 10 Lit. of Hexane for three days.

**Chloroform Extract**

(30 gm)

- Residue

**Acetone Extract**

(10 gm)

- Extracted with 10 Lit. of Ethanol for three days.

**Ethanol Extract**

*Syrupy Liquid*

- Residue

- Discarded
Chromatographic Separation of the Chloroform Extract:

The chloroform extract (30 gm.) was chromatographed on a silica gel (60-100 mesh) column (150 × 15 cm) and eluted with a step wise gradient of Hexane : Ethyl acetate (100, 90:10, 85:15, 80:20, 75:25, 70:30, 100 by volume). A total of 115 fractions of 10 ml each were collected, and the similar ones were poured to eight groups. 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 3.1.

Table 3.1

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Eluent</th>
<th>Fraction Number</th>
<th>Group Number</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Hexane</td>
<td>1-20</td>
<td>I</td>
<td>A</td>
</tr>
<tr>
<td>2.</td>
<td>Hexane: Ethyl Acetate 95:05</td>
<td>21 - 35</td>
<td>II</td>
<td>B</td>
</tr>
<tr>
<td>3.</td>
<td>Hexane: Ethyl Acetate 90:10</td>
<td>36 - 50</td>
<td>III</td>
<td>C</td>
</tr>
<tr>
<td>4.</td>
<td>Hexane: Ethyl Acetate 85:15</td>
<td>51 - 60</td>
<td>IV</td>
<td>D</td>
</tr>
<tr>
<td>5.</td>
<td>Hexane: Ethyl Acetate 80:20</td>
<td>61 - 75</td>
<td>V</td>
<td>E</td>
</tr>
<tr>
<td>6.</td>
<td>Hexane: Ethyl Acetate 75:25</td>
<td>76 - 85</td>
<td>VI</td>
<td>F</td>
</tr>
<tr>
<td>8.</td>
<td>Ethyl Acetate</td>
<td>100 - 115</td>
<td>VIII</td>
<td>H</td>
</tr>
</tbody>
</table>
Group I:
The fractions 1 – 20 obtained from hexane fraction were concentrated under reduced pressure and a yellowish solid was obtained (20 mg.). The Rf value of the compound was found to be 0.78. This compound was designated as compound A.

Group II:
The fractions 21 – 35 obtained by the elution of Hexane: Ethyl acetate solvent system (95: 05) and were concentrated under reduced pressure and a yellow solid was obtained (20 mg.). This solid was designated as compound B.

Group III:
The fractions 36 – 50 obtained by the elution of Hexane: Ethyl acetate (90: 10) showed one prominent spot on TLC. These fractions were mixed together and allowed to concentrate under reduced pressure to obtain a creamy white solid (30 mg.). This compound was designated as compound C.

Group IV:
The fractions 51 – 60 were concentrated in a vacuum to obtain a yellow solid (10 mg.). This compound was designated as compound D.

Group V:
Fractions 61 – 75 were concentrated under reduced pressure that showed one prominent spot on thin layer chromatography gave a greenish solid (40 mg.) and designated as compound E.

Group VI:
Fractions 76 – 85 on concentration under reduced pressure gave a white solid (20 mg.). This solid showed one prominent spot on TLC with an Rf value 0.90.
Group VII:
Fractions 86 – 99 were collected together and concentrated under reduced pressure to get a white amorphous solid. The solid was subjected to TLC showed one prominent spot on it. The compound was thus designated as compound G.

Group VIII:
Fractions from 100 – 115 were collected together by the elution of pure ethyl acetate and concentrated under reduced pressure gave a single spot on TLC. The fractions after concentration gave pale yellow colored solid designated as compound H.
Physical and Spectral Characterization of the compounds isolated from Chloroform extract of *Merremia emarginata* (Burm. f.) Hall.

**Compound A.**

**Physical State:**
Yellowish Solid

**Yield:**
20 mg.

**MP:**
328 °C

**UV*λ*max** (CH$_3$OH):
258, 266, 348 nm.

**IR (KBr) ν*max*:**
3144, 1616, 1174, 879, 802, 729 cm$^{-1}$.

**$^1$H-NMR (400 MHZ, CDCl$_3$) δ:**
5.0 (4H, S), 5.95 (2H, d), 6.51 (1H, S), 6.60 (1H, S), 6.69 (1H, S), 6.71 (1H, S).

**ESI-MS m/z:**
302

**Compound B.**

**Physical State:**
Yellow Solid

**Yield:**
20 mg.

**MP:**
224°C
UV $\lambda_{\text{max}}$ (CH$_3$OH):
290, 320 nm

IR (KBr) $\nu_{\text{max}}$:
3437, 1739, 1599, 1518, 1460, 1415, 1271, 1226, 1157, 1116, 1074, 823, 702 cm$^{-1}$.

$^1$H-NMR (400 MHZ, CDCl$_3$) $\delta$:
5.0 (1H, S), 6.51 (1H, S), 6.69 (1H, S), 6.60 (1H, S), 6.41 and 7.61 (1H, dd, J = 11.5 Hz), 11.0 (1H, S),

ESI-MS m/z:
180

Compound C.

Physical State:
White Solid

Yield:
30 mg.

MP:
149$^0$C

UV$\lambda_{\text{max}}$ (CH$_3$OH):
209 nm, 240

IR (KBr) $\nu_{\text{max}}$:
3440, 2962, 2850, 2380, 1598, 1520, 1460, 1420, 1270, 1226, 1157, 1074, 823, 704 cm$^{-1}$
1H-NMR (400 MHz, CDCl3) δ:
0.73 (3H, S), 0.96 (3H, S), 1.02 (3H, d, J=6.6Hz), 0.86 (3H, d, J=6.2 Hz), 0.76 (3H, d, J=6.5 Hz), 0.78 (3H, d, J=7.5), 5.02 (1H, dd, J=15.6Hz, J=8.5Hz), 5.22 (1H, dd, J=16.0 Hz, J=8.5 Hz), 5.33 (1H, S), 3.19 (1H, dd, J=12.2 Hz, J=6.1Hz).

ESI-MS m/z:
468

**Compound D.**

**Physical State:**
Amorphous Solid

**Yield:**
10 mg.

**MP:**
250°C

**UVλ_{max} (CH₃OH):**
320 nm

**IR (KBr) ν_{max}:**
3529, 3387, 2926, 2854, 1600, 1512, 1448, 1410, 1330, 1271, 1126,1080, 1037, 825, 775, 705, 621, 542, 518 cm⁻¹.

1H-NMR (400 MHz, CDCl3) δ:
3.20 (dd, J=11.7 HZ, J=5.4HZ), 3.28 and 3.52 (d, J=11.2 HZ), 2.90 (ddd, J=13.8 HZ, J=9.8 HZ, J=4.8 HZ), 4.88 and 4.63 (1H each, S), 2.0 (1H each, S), 0.68, 0.86, 0.88, 0.92, 1.11, and 1.16 (3H each, S).

**ESI-MS m/z:**
442
Compound E.

Physical State:
Greenish solid

Yield:
40 mg.

MP:
168-172°C

\textbf{UV} \lambda_{\text{max}} (\text{CH}_3\text{OH}): 
290 nm, 310 nm

\textbf{IR (KBr)} \nu_{\text{max}}:
3440, 3080, 3017, 2942, 2843, 1692, 1658, 1590, 1518, 1460, 1380, 1270, 1178, 
1155, 1036, 853, 805 cm$^{-1}$.

\textbf{\textsuperscript{1}H-NMR (400 MHz, DMSO)} \delta:
6.64, 6.58, 6.70 (1 H, br, S), 5.0 (1H, S), 3.73 (3H, S), 7.61 (1H, d), 6.41 (1H, d), 11.0 
(1H, S).

\textbf{ESI-MS m/z:}
412

Compound F.

Physical State:
White Solid

Yield:
20 mg.

MP:
70°C
UV$\lambda_{\text{max}}$ (CH$_3$OH):
290 nm

IR (KBr) $\nu_{\text{max}}$:
2924, 2852, 1462, 1383, 1039, 910, 875, 731, 453 cm$^{-1}$.

$^1$H-NMR (400 MHZ, DMSO) $\delta$:
0.96 (3H, S), 1.33 (2H, br, S), 1.29 (2H, br, S).

ESI-MS m/z:
436

Compound G.

Physical State:
White Solid

Yield:
10 mg.

MP:
72$^0$C

UV$\lambda_{\text{max}}$ (CH$_3$OH):
290

IR (KBr) $\nu_{\text{max}}$:
2917, 2849, 1460, 1375 cm$^{-1}$.

$^1$H-NMR (400 MHZ, CDCl$_3$) $\delta$:
0.89 (3H, S), 1.33 (2H, S), 1.29 (2H, S).

ESI-MS m/z:
426
Compound H.

Physical State:
Pale yellow Solid

Yield:
40 mg.

MP:
210-212 °C

UVλ_max (CH₃OH):
272 nm, 240 nm, 268nm

IR (KBr) ν_max:
3400, 1639, 1535, 1462, 1373, 1246, 1026, 981 cm⁻¹.

¹H-NMR (400 MHZ, CDCl₃) δ:
7.72 (d, J=2.2 HZ, H-2), 7.02 (d, J=8.5 HZ, H-5), 7.74 (dd, J=2.01, 8.31 HZ, H-6),
3.93 (3H, S), 5.0 (1H, S).

ESI-MS m/z:
168
3.4 Result and Discussion:

**Structure of Compound A:**

The sub fraction obtained from the chloroform soluble fraction of the plant (30 gm.) following maceration extraction process was subjected to column chromatography. The column was eluted with hexane and with increasing polarities of hexane – ethyl acetate mixtures. Elution of the column with pure hexane yielded a solid compound (20 mg) designated as compound ‘A’ with melting point of 328 °C. The ESI-MS spectrum of compound ‘A’ showed the [M+] ion at m/z- 302, which was in agreement with the molecular formula C_{15}H_{10}O_{7} with ten degrees of unsaturation.

The UV spectrum of compound ‘A’ showed characteristic absorption at 254, 266 and 348 nm which are characteristic absorption peaks of flavonoid moiety. The IR spectrum of compound ‘A’ displayed no intense bands in the region of 1100-1000 cm\(^{-1}\) showing absence of glycoside band or any type of sugar linkage. The compound ‘A’ shows no absorption value at 2950 cm\(^{-1}\) and 2850 cm\(^{-1}\) of C-H stretching in CH\(_2\) or CH\(_3\) moiety showing the absence of methyl or methylene group in the compound. Compound ‘A’ exhibited the absorption band in the range of 3400-3100 cm\(^{-1}\) showing the presence of phenolic –OH group in the compound. The band at 1620 cm\(^{-1}\) exhibited C=O stretching attached to heterocyclic ring. The absorption band at 1170 cm\(^{-1}\) was in confirmation with the C-O stretching in the compound.

The \(^1\)H – NMR spectrum of compound ‘A’ exhibited the signal at \(\delta\) 5.0 indicating the aromatic hydroxyl group linkage in the compound. The signal at \(\delta\) 5.95 indicates the doublet of aromatic protons. The signal at \(\delta\) 6.52, 6.62, 6.68 showing singlet of aromatic protons present in different environment.

The \(^1\)H-NMR, UV, and IR absorption data along with other physical data of compound A suggests that compound ‘A’ is Luteolin which is previously isolated.
from the *Merremia tridentata* [12] species and reported first time from the species which is new source.

![Structure of Compound B](image)

(1) Luteolin.

**Structure of Compound B:**

The sub fraction eluted with Hexane: Ethyl acetate (95:05) yielded a yellow solid with melting point at 224 °C; this compound was designated as compound ‘B’. The molecular formula of the compound was deduced as C$_9$H$_8$O$_4$ based on its ESI-MS spectrum at m/z- 180 [M$^+$] base peak. The compound with molecular formula C$_9$H$_8$O$_4$ exhibited six degrees of unsaturation. The UV absorption spectrum of compound ‘B’ was observed at 328 nm indicating the presence of olefinic and carbonyl moiety in the compound.

The IR spectrum of compound ‘B’ exhibited the absorption band at 3437 cm$^{-1}$ showing the presence of hydroxyl group in the compound. The band at 1739 cm$^{-1}$ indicates the (C=O) stretching in carboxylic acid. The absorption band at 1074 cm$^{-1}$ exhibits (C-O) stretching for phenolic compound. The band at 1210-1320 cm$^{-1}$ is for
(C-O) for carboxylic acid group. The band at 823 cm\(^{-1}\) shows para di-substituted aromatic ring present in the compound.

The \(^1H\)-NMR spectrum of compound ‘B’ exhibited signal at \(\delta\) 6.50, 6.60, and 6.68 indicating the aromatic protons in the compound. The signal at \(\delta\) 5.2 indicates the phenolic hydroxyl proton in the compound. The olefinic protons exhibited signal at \(\delta\) 7.11 and 6.11 respectively. The signal at \(\delta\) 11.2 was for one H singlet in hydroxyl group of carboxylic acid. The \(^{13}C\)-NMR spectrum of compound ‘B’ exhibited four quaternary and five methine carbons present in the compound. Based on the above physical and spectral characterization and the literature [25-26] reported that the isolated compound ‘B’ is Caffeic acid. The presence of Caffeic acid in \textit{Merremia emarginata} was detected by A. Ramesh Kumar et al [27] which is now confirmed.

(2) Caffeic Acid.
**Structure of Compound C:**

Compound ‘C’ was isolated by column chromatography of the Chloroform extract. Elution of the column with 10% ethyl acetate in hexane gave compound ‘C’ showing melting point 163 °C. The ESI-MS spectrum of Compound ‘C’ showed the molecular ion peak [M⁺] at m/z of 413 indicating a molecular formula of C_{29}H_{48}O with six degrees of unsaturation.

The IR spectrum displayed a broad band at 3457 cm⁻¹ for (-OH) stretching in hydroxyl group. Stretching at 2926 cm⁻¹ and 2864 cm⁻¹ exhibits (C-H) stretching in alkanes. The absorption at 1415 cm⁻¹ to 1599 cm⁻¹ exhibits the (C=C) stretching in olefin. The band at 1074 cm⁻¹, 1116 cm⁻¹, 1157 cm⁻¹ exhibits (C-O) stretching in C-OH linkage.

The ¹H-NMR of compound ‘C’ exhibited two singlets at δ 0.72 and 0.98 assigned to two methyl protons respectively. The signal at δ 1.01 (3H, d, J=6.6Hz), 0.84 (3H, d, J=6.2 Hz), 0.78 (3H, d, J=6.5 Hz), and 0.78 (3H, d, J=7.5 Hz) were assigned to the four methyl protons, respectively. The signal at δ 5.02 (1H, dd, J=15.6 Hz, J=8.5 Hz), 5.22 (1H, dd, J=16.0Hz, J=8.5 Hz) and 5.31 exhibited the olefinic proton resonance. A doublet doublet at 3.17 (1H, dd, J=12.2, 6.1 Hz) was assigned to the proton of C-3.

The ¹³C-NMR data of Compound ‘C’ exhibited ten methylene, ten methine, three quaternary and six methyl carbons respectively. All the physical and spectral characterization of Compound ‘C’ was found to be identical with the literature values reported for stigmasterol which is discussed in previous chapter. Compound ‘C’ was isolated first time from *Merremia emarginata* (Burm.f.) Hall. The presence of Stigmasterol in *Merremia emarginata* (Burm.f.) Hall. was also reported by Purushoth
Prabhu T. et al [28] by analyzing Ethanolic extract by GC-MS study with retention time 27.384 with peak area 1.16%.

(3) Stigmasterol

Structure of Compound D:

The sub fractions eluted with Hexane: Ethyl acetate (85:15) yielded amorphous yellowish solid with yield (10 mg) designated as compound ‘D’. The compound ‘D’ exhibited physical constant at 250 °C. The molecular formula of the compound was deduced as C\textsubscript{30}H\textsubscript{50}O\textsubscript{2} from its ESI-MS spectrum at m/z 442. The compound with molecular formula C\textsubscript{30}H\textsubscript{50}O\textsubscript{2} corresponds to six degrees of unsaturation. The other fragments at 234, 203, 189 etc. shows the characteristic pattern of pentacyclic triterpene skeleton in the compound.

The IR spectrum of compound ‘D’ exhibited absorption band in the range of 3500-3000 cm\textsuperscript{-1} exhibiting the alcoholic hydroxyl group in the compound. The absorption band at 2926 cm\textsuperscript{-1} and 2854 cm\textsuperscript{-1} shows the C-H stretching in the methyl and methylene group. The absorption band in the range of 1220-1550 cm\textsuperscript{-1} shows C-
O-H bending in alcoholic compound thus confirming the alcoholic –OH group in the compound. The absorption band at 1080 cm\(^{-1}\) indicates C-O stretching in alcohols. The absorption band at 1380 cm\(^{-1}\) represents C-H stretching in methyl group.

The \(^1\)H-NMR spectrum of compound ‘D’ showed double doublets at \(\delta\) 3.28 and 3.52 (\(J=11.2\) HZ) for methylene protons attached to hydroxyl group. A signal at \(\delta\) 3.20 double doublet (\(J=11.9\) HZ, \(J=5.4\) HZ) assigned to the proton geminal to a hydroxyl group attached to an aliphatic ring. The tertiary methyl’s showed the signal at \(\delta\) 0.68, 0.86, 0.88, 0.92, 1.11 and 1.16 respectively. The signal at \(\delta\) 4.88 and 4.63 exhibited the broad singlets indicating a terminal isopropenyl group in the compound.

The \(^{13}\)C-NMR spectrum of compound ‘D’ exhibited six methyl, twelve methylene, six methine and six carbons. All the above physical and spectral data compared with the data base showed that the compound is betulin which is reported first time from the plant and is a new source of the compound.

(4) Betulin
Structure of Compound E:

Elution of the column with Hexane: Ethyl acetate (80:20) yielded a greenish solid with 40 mg weight showing melting point in the range of 168-172 °C; this compound was designated as compound ‘E’. The ESI-MS spectrum of compound ‘E’ exhibited the molecular ion peak [M+] at m/z of 194 indicating the molecular formula of the compound as C_{10}H_{10}O_4.

The IR spectrum of compound ‘E’ displayed a sharp band at 3440 cm\(^{-1}\) indicating the presence of O-H stretching for phenolic compound. Stretching at 2942 cm\(^{-1}\) and 2843 cm\(^{-1}\) exhibits the C-H stretching for methyl and methylene protons in alkanes. The absorption band in the range of 1460 cm\(^{-1}\) to 1599 cm\(^{-1}\) exhibits the (C=C) stretching in olefins. The band at 1036 cm\(^{-1}\), 1155 cm\(^{-1}\) and 1178 cm\(^{-1}\) shows (C-O) stretching in C-OH linkage. The absorption band at 1380 cm\(^{-1}\) and 1280 cm\(^{-1}\) exhibited the C-H bending in methyl and C-O stretching for phenolic compound.

The \(^1\)H-NMR spectrum of compound ‘E’ exhibited signal at \(\delta\) 6.64, 6.57 and 6.69 for aromatic protons. The signal at \(\delta\) 5.1 was in good agreement with the phenolic –OH group present in the compound. The signal at \(\delta\) 11.2 exhibits the –COOH group in the compound. The signal at \(\delta\) 7.61 and 6.41 is indicative of the doublet doublet protons of olefinic moiety.

Lastly the UV absorption spectra of compound ‘E’ exhibited the absorption at \(\lambda_{\text{max}}\) 215, 290 and 310 nm respectively which is characteristic absorption of Ferulic acid synonym of (E)-3-(4-hydroxy-3-methoxy phenyl) acrylic acid. Thus all the physical and spectral study of compound ‘E’ showed that the isolated compound is Ferulic acid which is isolated first time from Merremia emarginata (Burm,f.) Hall., and is a new source for the compound. The presence of Ferulic acid in the species was also reported by A. Ramesh Kumar et al [27].
(5). (E)-3-(4-hydroxy-3-methoxyphenyl)acrylic acid

Structure of Compound F:

Compound ‘F’ was isolated as a white solid with a melting point of 70°C. The molecular formula of the compound was deduced as C$_{31}$H$_{64}$ from its GC-MS spectrum at m/z – 436 [M$^+$]. The GC-MS spectrum of compound ‘F’ is shown in Fig. 3.2. The IR spectrum of the compound exhibited the absorption band at 2924 cm$^{-1}$ and 2852 cm$^{-1}$ indicating the C-H stretching in alkane moiety. The absorption band at 1462 cm$^{-1}$ purely exhibited the C-H bending for alkanes. The band at 1383 cm$^{-1}$ indicated the C-H bending in methyl group. The absorption band in the range of 800-1200 cm$^{-1}$ exhibited the C-C stretching in paraffin’s. The IR spectrum of compound ‘F’ is shown in Fig 3.3. The UV absorption of compound ‘F’ showed absorption near 290 nm corresponding to the higher alkanes.

The $^1$H-NMR spectrum signals at $\delta$ 0.97 exhibited for methyl groups present in different positions. The signal at $\delta$ 1.33 and $\delta$ 1.29 exhibited the broad singlet for methylene group in the compound. The $^1$H-NMR spectrum of compound F is shown in Fig. 3.4. The $^{13}$C-NMR spectrum of compound ‘F’ exhibited the presence of five types of carbons in the compound. The presence of two methyl and twenty nine
methylene groups were confirmed in the compound. The $^{13}$C-NMR spectrum of compound ‘F’ is shown in Fig. 3.5.

All the physical and spectral characterization of compound ‘F’ was in good agreement with the literature for Hentriacontane, an alkane compound. This compound, Hentriacontane was isolated first time and is a new compound isolated from this genus.
Fig. 3.2 GC-MS Spectrum of Hentriacontane
Structure of Compound G:

The compound ‘G’ was obtained from the elution of the column with Hexane: Ethyl acetate (70:30) with melting point of 72 °C. The molecular formula of the compound was found to be C_{33}H_{68} from its molecular ion peak at m/z- 464. The GC-MS spectrum of compound ‘G’ is shown in Fig. 3.6. The molecular formula of the compound shows zero degree of unsaturation.

The IR spectrum of the compound ‘G’ exhibited the absorption band at 2917 cm^{-1} and 2849 cm^{-1} respectively indicating the C-H stretching in the alkane moiety. The absorption band at 1460 cm^{-1} and 1375 cm^{-1} purely indicates the C-H bending in alkanes. The IR spectrum of compound ‘G’ is shown in Fig. 3.7. The UV absorption of compound ‘G’ was observed at 290 nm.

The $^1$H-NMR spectrum of compound ‘G’ showed signal at $\delta$ 0.95 indicating the presence of methyl protons in the compound. The $^1$H-NMR spectrum of compound ‘G’ exhibited the similar pattern like that of compound F showing the broad singlet for methylene protons. The $^1$H-NMR spectrum of compound ‘G’ is shown in Fig. 3.8.

The $^{13}$C-NMR spectrum of compound ‘G’ showed similar nature of carbon atoms as in compound F; the $^{13}$C-NMR spectrum of compound ‘G’ is shown in Fig. 3.9. The physical and spectral characterization of compound ‘G’ was in good agreement with the data reported for Tritriacontane, a higher alkane. This compound is reported for the first time from this plant and is thus a new source of higher alkane.

(7) Tritriacontane
Fig. 3.6 GC-MS spectrum of Tritriacontane
Fig. 3.8  H-NMR spectrum of Tritriacontane
Structure of Compound H:

Compound ‘H’ (40 mg) was isolated as a pale yellow solid with a melting point in the range of 210-212 °C. The molecular formula of the compound was deduced as C₈H₈O₄ from its ESI-MS spectrum at m/z- 168 as [M⁺] ion peak. The molecular formula of compound ‘H’ shows four degrees of unsaturation indicating the presence of benzene skeleton in the compound.

The absorption band at λ_{max} 270 nm in the UV spectrum confirmed the presence of a benzoic acid moiety having substituents at meta and para positions. The IR spectrum of compound ‘H’ displayed bands at 3400 -2600 cm⁻¹ and 1660 cm⁻¹ respectively indicating the presence of a conjugated carboxylic acid.

In the ESI-MS, the base peak at m/z – 168 along with other peaks at 183 and 155 were due to the loss of hydroxyl and methoxyl groups from the molecular ion peak. The ¹H-NMR spectrum of compound ‘H’ displayed two doublets in the aromatic region at δ 7.72 and 7.02 (J=2.2 HZ and J=8.5 HZ respectively) were assigned to the C-2 and C-5 proton doublets. One signal appearing as a doublet doublet at δ 7.74 (J=2.2 HZ and J=8.3 HZ) was assigned to the C-6 proton. The signal at δ 3.93 indicating three proton singlets was due to the methoxyl protons. The signal appearing at δ 5.0 was in conformity with the aromatic hydroxyl protons.

All the physical and spectral characterization of compound ‘H’ was in good agreement with those reported for vanillic acid [29-31]. The presence of vanillic acid in Merremia emarginata was also reported by A. Ramesh Kumar et al [27] through UPLC-ESI-MS in positive ion mode with retention time 2.0 showing the presence of vanillic acid which is now confirmed.
(8) Vanillic Acid
References:


