Chapter 6
EXTRACTION, ISOLATION, PURIFICATION
AND CHARACTERISATION
OF ISOLATED COMPOUNDS
(a) Extraction

Moderately coarse powder of the aerial parts of *Trianthema portulacastrum* Linn. (5.0 kg) was extracted with petroleum ether (40-60°) in Soxhlet to remove the oily and fatty material. The extract was concentrated and the residue was preserved for further study. After extraction with petroleum ether, the exhausted drug was taken out in open to evaporate the solvent. After drying it was packed again in the Soxhlet and then extracted successively with benzene, chloroform, ethyl acetate, alcohol and water. Each time the drug was dried before next extraction.

Isolation of pure compound from alcoholic extract of *Trianthema portulacastrum* by column chromatography:

The alcoholic extract was found to be most active as hepatoprotective so it was selected for isolation of compounds by column chromatography. The benzene: ethyl acetate (5:3) solvent system gave four spots (Rf value 0.18, 0.37, 0.64, 0.90) in TLC analysis.

Silica gel (BDH) was weighed and transferred to a clean dry beaker. The beaker was covered with watch glass and kept in an oven at 100° C for 3 hours and then cooled in a dessicator.

A glass column (internal diameter 2.8 cm) was cleaned, washed and dried. A piece of glass wool (previously kept overnight dipped in benzene) was introduced with the help of a glass rod to the base of the column. The column was then placed in vertical position with the help of clamps and column stand. The activated silica gel was then poured into the column and it was gently tapped by a rubber cork fixed at the end of glass rod.

The alcoholic extract was dissolved in minimum quantity of benzene and ethyl acetate. Small amount of silica gel equal to the weight of extract
was then added to the mixture, stirred with a glass rod and dried in air. Completely dried mixture was introduced into the column with the help of a glass tube to prevent the sticking of the mixture to the wall of the column. Another piece of glass wool was finally inserted with the help of a glass rod. The column was eluted with benzene : ethanol (5:3) solvent system. The rate of elution was maintained at 25-30 drops/min. In all 50 fractions were collected. All the fractions were tested by TLC and those fractions which were giving single spots and same Rf value were mixed together and distilled. By distillation, three-fourth of the solvent was distilled off and the remaining was evaporated in air.

Table 39

Column chromatography

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Eluent</th>
<th>No. of spots</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-8</td>
<td>Benzene: Ethyl acetate</td>
<td>Blank</td>
<td>-</td>
</tr>
<tr>
<td>9-20</td>
<td>(5:3)</td>
<td>One</td>
<td>-</td>
</tr>
<tr>
<td>21-32</td>
<td>&quot;</td>
<td>One</td>
<td>TP1</td>
</tr>
<tr>
<td>33-38</td>
<td>&quot;</td>
<td>Blank</td>
<td>-</td>
</tr>
<tr>
<td>39-45</td>
<td>&quot;</td>
<td>One</td>
<td>TP2</td>
</tr>
<tr>
<td>46-50</td>
<td>&quot;</td>
<td>Blank</td>
<td>-</td>
</tr>
</tbody>
</table>
(b) Extraction

The extraction of moderately coarse powder of tuberous roots of *Cyperus rotundus* (5.0 kg) was done in the same manner as *T. portulacastrum*. And isolation of compounds from the alcoholic extracts was made using Alcohol: chloroform (1.5:0.5) solvent system.

Table 40

Column chromatography

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Eluent</th>
<th>No. of spots</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>Alcohol: chloroform</td>
<td>Blank</td>
<td>-</td>
</tr>
<tr>
<td>6-16</td>
<td>(1.5:0.5)</td>
<td>One</td>
<td>CR1</td>
</tr>
<tr>
<td>17-24</td>
<td>&quot;</td>
<td>Blank</td>
<td>-</td>
</tr>
<tr>
<td>25-33</td>
<td>&quot;</td>
<td>One</td>
<td>CR2</td>
</tr>
<tr>
<td>34-50</td>
<td>&quot;</td>
<td>Blank</td>
<td>-</td>
</tr>
</tbody>
</table>

Purification of TP1: (Fraction 21-32)

As these fractions gave spots of same Rf value on TLC they were mixed together and evaporated. The compound was purified by dissolving in absolute ethanol. This substance was a pure compound as revealed by single spot obtained by TLC analysis (Benzene:Ethyl acetate ::5:3)

Purification of TP2: (Fraction 39-45):

As these fractions gave spots of same Rf value on TLC, they were mixed together and evaporated. The compound was purified by dissolving in absolute alcohol. This substance was a pure compound as revealed by single spot by TLC (Benzene:Ethyl acetate ::5:3)
Purification of CR1; (Fraction 6-16):

All these fractions gave spots of same Rf value on TLC, they were mixed together and evaporated. The compound was purified by dissolving in absolute ethanol. This substance was a pure compound as revealed by single spot obtained by TLC (Alcohol:Chloroform::1.5:0.5).

Purification of CR2; (Fraction 25-33):

As these fractions gave spots of same Rf value on TLC, they were mixed together and evaporated. The compound was purified by dissolving in absolute ethanol. This substance was a pure compound as revealed by single spot obtained by TLC (Alcohol:Chloroform::1.5:0.5).

Characterisation of the compounds:

The compounds isolated from the column chromatography were purified by recrystallisation and the purity was confirmed by obtaining single spot on TLC. The physicochemical tests and spectral analysis were also then performed on the individual purified compounds for their characterisation.

Compounds from the aerial parts of *Trianthema portulacastrum*:

Two compounds named TP1 and TP2 were isolated from the alcoholic extract of the aerial parts of *T. portulacastrum*.

(a) Compounds TP1:

Physical properties:

(i) Description: A brownish-black coloured solid compound.
(ii) Melting point: 168-171°C
(iii) Solubility: Soluble in ethanol, partially soluble in chloroform and insoluble in water.
Test for elements and functional groups:

(i) Lassaigne's Test: This test indicated the presence of nitrogen and absence of sulphur and halogens.

(ii) Functional group Test: The compound was tested for the presence of various functional groups and the following functional groups were found to be present

(a) Free carboxylic acid group
(b) Alcoholic –OH group
(c) A keto group
(d) Secondary amino group

Elemental Analysis Data: C-45.4%, H-6.0%, N-7.9% and O-40.7% (by difference) Table 41

Empirical Formula: C₇H₁₁NO₄

UV spectrum: The compound gives sharp absorption peaks at λ_max 236nm and 290nm.

IR Spectral Data (KBr): The IR spectrum (Fig. 40) showed maxima at 3500 (OH stretching), 2925 (CH stretching), 1716 (C=O stretching), 1629 (C-C multiple bond stretching), 1486 (CH₂ symmetrical stretching, 1086 (C=O stretching of ketones), 1053 & 981 (CH bending) cm⁻¹.

PNMR Spectral Data: The PNMR spectrum (Fig. 41) showed the peaks of δ values at 8.39 (OH), 1.25 & 1.5 ppm (secondary and tertiary hydrogen atoms).

Mass Spectral Data: The mass spectrum (Fig. 42) showed a molecular ion peak at m/e 173 M⁺ with a base peak at 142 (M-CH₂OH). The other prominent peaks are at 156 (M-OH), 106, 118 (CH₂) and 17, 45, 56 and 72.

Molecular Formula: C₇H₁₁NO₄.
Major fragments of compound TP1

From the above data and literature\(^{367-370}\), the compound TP1 from the aerial parts of \emph{T. portulacastrum} is proposed to have the following probable structure: (Fig. 43)

**Fig. 43: 2-Hydroxymethyl-3-carboxyl-4-keto piperidine (TP1)**

\(\text{(b) Compound TP2:}\

\textbf{Physical properties:}

(i) Description: The compound TP2 is white coloured solid compound.

(ii) Melting point: The compound decomposes at 210-214\(^\circ\)C.

(iii) Solubility: Freely soluble in ethanol and methanol, insoluble in water.
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(SICART)  
CHARUTAR VIDYA MANDAL  
Vallabh Vidyanagar  

Table 41  

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample ID</th>
<th>C%</th>
<th>H%</th>
<th>N%</th>
<th>AVERAGE</th>
<th>C%</th>
<th>H%</th>
<th>N%</th>
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<td>50.62</td>
<td>4.78</td>
<td>0.305</td>
<td>49.9</td>
<td>4.9</td>
<td>0.32</td>
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<td></td>
<td></td>
<td>51.30</td>
<td>5.21</td>
<td>0.34</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>47.95</td>
<td>5.0</td>
<td>0.32</td>
<td></td>
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<td></td>
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<tr>
<td>2</td>
<td>TP1</td>
<td>45.44</td>
<td>6.0</td>
<td>7.86</td>
<td>45.4</td>
<td>6.0</td>
<td>7.9</td>
<td></td>
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</table>
Fig. 42: Mass spectrum of TP1
Test for Elements and Functional groups:

(i) Lassaigne's Test: This test indicated the absence of nitrogen, sulphur and halogens.

(ii) Functional group Test: The compound was tested for the presence of various functional groups. The following groups were found to be present:
   (a) Free carboxylic acid group
   (b) A keto group
   (c) Alcoholic OH group
   (d) Test for sterols: Positive

Elemental Analysis Data: C-70.19%, H-9.29%, O-20.52%. Table 42

Empirical Formula: C_{27}H_{43}O_{6}.

UV Spectrum: The compound gives sharp absorption peaks at $\lambda_{\text{max}}$ 242nm and 287nm.

IR Spectral Data (KBr): The IR spectrum (Fig. 44) showed maxima at 3452 (OH stretching), 2919, 2850 (CH stretching), 1739 (C=O stretching), 1462 (CH$_2$ symmetrical stretching), 1090 (C=O stretching of ketones), 1260 (Ch bending), 722 (CH deformation) cm$^{-1}$.

PNMR Spectral Data: PNMR (Fig. 45) gives $\delta$ values at 0.9, 1.3 and 1.5 ppm indicating the presence of primary, secondary and tertiary hydrogens.

Mass Spectrum Data: The mass spectrum (Fig. 46) showed a molecular ion peak at m/e 463 M$^+$ with a base peak at 393. A number of peaks at an interval of 14 indicates the presence of CH$_2$ groups in the compound.

Molecular Formula: C_{27}H_{43}O_{6}.

From the above data and literature, the compound TP2 isolated from the aerial parts of *T. portulacastrum* is proposed to have the following probable structure: (Fig. 47)
Table 42

**CHN Test Results**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample ID</th>
<th>C%</th>
<th>H%</th>
<th>N%</th>
<th>AVERAGE</th>
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<td></td>
<td>C%</td>
<td>H%</td>
<td>N%</td>
<td>C%</td>
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<tr>
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<td>CR2</td>
<td>74.46</td>
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<td>77.98</td>
<td>9.98</td>
<td>0.03</td>
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<tr>
<td>2.</td>
<td>TP2</td>
<td>68.42</td>
<td>8.34</td>
<td>0.00</td>
<td>70.19</td>
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<tr>
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<td></td>
<td></td>
<td>71.95</td>
<td>9.87</td>
<td>0.00</td>
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Fi. 45: NMR spectrum of

**F2 - Acquisition Parameters**
- Date: 20020809
- Time: 15:00
- INSTRUM: DPX-200
- PROBNO: 5 mm Dual 13C/29
- SOLVENT: CDC13
- NS: 32
- DS: 0
- SMS: 3612.7 Hz
- FLORES: 0.055126 Hz
- AB: 9.0702324 sec
- MG: 332.5
- DW: 138,400 usec
- TE: 6.00 usec
- DI: 1,000,000 usec

**F2 - Processing parameters**
- SI: 32,768
- SF: 200.1748016 MHz
- MDW: Ek
- SSB: 0
- LB: 0.30 Hz
- GB: 0
- PC: 1.00

**10 NMR g parameters**
- C1: 21.00 cm
- CV: 45.00 cm
- FIP: 10,000 ppm
- F1: 200.7 Hz
- F2P: 0.500 ppm
- F2: 100.09 Hz
- PHCH: 0.50000 ppm/cm
- NZCM: 100 0.5975 Hz/cm

**Integral**

<table>
<thead>
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<th>ppm</th>
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<th>0.016</th>
<th>0.010</th>
<th>0.008</th>
<th>0.006</th>
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<td>4</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Data : SFU02458.D00
Scan # : 85  B.G. Scan # : 200
Mass Peak # : 64  Ret. Time : 1.600
Base Peak : 393.20 (496922)

![Mass spectrum of TP2](image)

Fig. 46: Mass spectrum of TP2
Major fragments of TP2

From the above data and literature, the compound TP2 isolated from the aerial parts of *T. portulacastrum* is proposed to have the following probable structure: (Fig.47)

Fig. 47 : 3,11,16-Trihydroxy-22-oxo-26-cholestanolic acid (TP2)
Compounds from the tuberous roots of *Cyperus rotundus*.

Two compounds named CR1 and CR2 were isolated from the alcoholic extract of the tuberous roots of *Cyperus rotundus*.

**(a) Compound CR1:**

**Physical properties:**

(i) **Description:** The CR1 is brownish coloured solid compound.

(ii) **Melting point:** 201-204°C.

(iii) **Solubility:** Soluble in ethanol and methanol, insoluble in benzene and water.

**Test for Elements and Functional groups:**

(i) **Lassaigne's Test:** This test indicated the absence of nitrogen, sulphur and halogens.

(ii) **Functional group Test:** The compound was tested for the presence of various functional groups. The following functional groups were found to be present

(a) Free carboxylic acid group

(b) Phenolic OH group

(c) Alcoholic OH group

(d) **Test for sterols:** Positive.

**Elemental Analysis Data:** C-49.9%, H-4.9%, N-0.32%, O-44.88%. Table 41

**Empirical Formula:** C$_3$H$_4$O.

**UV Spectrum:** The compound gives $\lambda_{\text{max}}$ at 199nm, 240nm and 255nm.

**IR Spectral Data (KBr):** The IR Spectrum (Fig. 48) showed maxima at 3403 (OH stretching), 2926 (CH stretching), 1611 (C-C multiple bond stretching), 1284 (CH bending), 1600-2000 (overtones for aromaticity), 1523 (double bond), 768 (CH bending).
PNMR Spectrum Data: The δ values at 1.5 ppm indicates the presence of secondary and tertiary hydrogens and at 7.5 ppm indicates aromatic hydrogens. (Fig. 49)

Mass Spectral Data: The mass spectrum (Fig. 50) showed a molecular ion peak at m/e 336 with a base peak at 298. The other peaks are 318 (M-H₂O), 45 (COOH), 149,132 (OH). A number of peaks at an interval of mass 14 indicated the presence of CH₂ groups.

Molecular Formula: C₁₈H₂₄O₆.

From the above data and literature, the compound CR₁ isolated from the rhizomes of C. rotundus is proposed to have the following probable structure: (Fig. 51)

Fig. 51: 3,7,11-Trihydroxy-1,3,5(10) triene-16-hydroxymethyl 17-carboxyl gonane (CR₁)
Sophisticated Instrumentation Center for Applied Research and Testing
SICART-CVM
Vallabh Vidyanagar-388120

FTIR Spectra

Date: 06/24/2000 Time: 4:37.24 PM

Fig. 10. IR spectra of Cd1.
**ACQUISITION COMMENT**

Sample: CR1
Solvent: CDCl3
Conc.: N/A
Ref.:
Tube D.: 5mm
Operator: JPT
Date: 13/2/2K3

Fi. 49: NMR spectrum of CR1
Fig. 50: Mass spectrum of CR1
(b) Compound CR2:

Physical properties:

(i) Description: A brownish-yellow semisolid compound.

(ii) Melting point: 185-188°C.

(iii) Solubility: Soluble in ethanol, Insoluble in ether and water.

Test for Elements and Functional groups:

(i) Lassaigne's Test: This test indicated the absence of nitrogen, sulphur and halogens.

(ii) Functional group Test: The compound was tested for the presence of various functional groups. The following functional groups were found to be present

(a) Free carboxylic acid group

(b) A carbonyl group

(c) Unsaturation

(d) Test for flavonoids: Positive.

Elemental Analysis Data: C-76.35%, H-10.89%, O-12.76%. Table 42

Empirical Formula: C_{16} H_{9} O_{4}.

UV Spectrum: The compound gives \( \lambda_{\text{max}} \) at 195nm, 200nm and 245nm.

IR Spectral Data (KBr): The IR spectrum (Fig. 52) showed maxima at 3418 (OH stretching), 2916 (CH stretching), 1711 (C=O stretching), 1454, 1376 (CH bending), 1600-2000 (overtones for aromaticity), 1247 (OH bending) cm\(^{-1}\).

PNMR Data: The \( \delta \) values at 1.2 and 1.4 indicates the presence of secondary and tertiary protons and at 7.4 indicates the presence of aromatic protons. (Fig. 53)
(CH bending), 1600-2000 (overtones for aromaticity), 1247 (OH bending) cm⁻¹.

**PNMR Data**: The δ values at 1.2 and 1.4 indicates the presence of secondary and tertiary protons and at 7.4 indicates the presence of aromatic protons. (Fig. 53)

**Mass Spectrum Data**: The mass spectrum (Fig. 54) showed a molecular ion peak at m/e 265 M⁺ with a base peak at 219. The peak at 191, 163 (C=O), 135,123 (C), and at 257, 233 (C₂H₄).

**Molecular Formula**: C₁₆H₉O₄.

![Molecular Structure](image)

**Major fragments of CR2**

From the above data and literature, the compound CR2 isolated from the rhizomes of *C. rotundus* is proposed to have the following probable structure: (Fig. 55)

![Proposed Structure](image)

**Fig. 55: 2-(2-Propenoic acid) Xanthone (CR2)**
Fig. 54: Mass spectrum of CR2