Chapter - 8

Isolation and characterization of active components from bark of Mussaenda frondosa L.
The Chloroform and methanol extracts of *Mussaenda frondosa* L. bark exhibited considerable antifungal, analgesic and wound healing activity. In view of these findings the chloroform and methanol extracts of the bark of *Mussaenda frondosa* L. was subjected to column chromatography, four fractions were obtained from chloroform extract and three fractions were obtained from methanol extract. All the four fractions of chloroform and three fractions of methanol extract were investigated for antimicrobial activity, Fraction-4 of chloroform and fraction-3 of methanol extract exhibited maximum activity against all tested dermatophytes. The MIC value was less than that of the standard drug ketoconazole. Hence Fraction-4 of chloroform and fraction-3 of methanol were taken up for further purification using column chromatography.

Compound obtained from chloroform extract is labeled as **Compound-1** and Compound obtained from methanol extract is labeled as **compound-2**.

**Characterization of isolated compounds**

The isolated compounds were characterized by physical parameter, i.e. melting point and spectral studies.

1. **Melting point**
   Melting points were recorded in an open capillary tube and is uncorrected.

2. **Infrared spectra**
   The infrared spectrum of the compounds were recorded in KBr in the range of 4000-400 cm\(^{-1}\) on Unicam FTIR (Research Spectrometer Series).

3. **Nuclear Magnetic Resonance Spectra (\(^1\)H NMR and \(^{13}\)C NMR)**
   The magnetic resonance spectra of the compounds were recorded on AV 400 FT NMR Spectrophotometer (400 MHz) using TMS as an internal standard. Samples were prepared by dissolving the compounds in CDCl\(_3\) and DMSO. The chemical shifts are expressed in \(\delta\) ppm.

4. **Mass spectra**
   The mass spectrum was recorded in Auto Spec E\(_i\) mass Spectrometer at 70 Ev ionisation energy with direct inlet system.
Characterization of compound- 1

1. Melting point
   The melting point of compound-1 was found to be -70°C.

2. Infrared spectrum
   The IR spectrum of compound-1 (Fig 8.1) exhibited the following bands.
   IR cm$^{-1}$ (KBr):
   - A broad band at 2922 cm$^{-1}$.
   - 1712 cm$^{-1}$.
   IR spectrum of compound-1 exhibited a broad band at 2922 cm$^{-1}$ revealed the presence of a C-H stretching and peak at 1741 cm$^{-1}$ indicated the presence of carbonyl group of carboxylic acid.

3. Proton magnetic resonance spectrum
   $^1$H NMR spectrum (Fig. 8.2) of compound-1 exhibited the following peaks.
   - $\delta$ 0.87
   - $\delta$ 1.25
   - $\delta$ 2.34
   The $^1$H NMR Spectrum exhibited a signal at $\delta$ 0.87 for a terminal methyl group. The strong singlet at $\delta$ 1.25 is indicated the presence of long chain methylene groups. The signal at $\delta$ 2.34 is due to a methylene group attached to carbonyl group.

4. $^{13}$C Magnetic Resonance Spectrum
   The data obtained by $^1$H NMR spectrum was complemented by recording $^{13}$C NMR (Fig 8.3).

<table>
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<tr>
<th>Position</th>
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<tr>
<td>28</td>
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The signal at $\delta$ 14.10 due to the presence of terminal methyl group. The strong signals at $\delta$ 24.75, 25.18, 28.17, 29.70, 31.95, 33.99 and 38.79 were assigned to long chain methylene carbons. The signals at $\delta$ 179.19 confirmed the presence of carbonyl function of terminal carboxylic group.

5. Mass spectrum

The mass spectrum confirmed the molecular weight of compound is 424, as revealed by the appearance of molecular ion peak [M+H]$^+$ at m/z 425 (Fig 8.4).

The base peak at m/z 409, is in accordance with the expected mass fragmentation pattern of the compound.

\[ \text{IUPAC name: } 18-\text{(17-Carboxyheptadecan-3-yloxy)octadeca-9,12-dienoic acid} \]

Based on the spectral studies the following structure has been assigned to the compound

Thus, in the present investigation, isolation and identification of acid is reported for the first time from the bark of *Mussaenda frondosa* L.
Fig. 8.3
**LCMS-2010A DATA REPORT**

**SHIMADZU**

- **User**: Admin
- **Sample**: B4
- **Inj. Volume**: 5.000
- **Data Name**: C:\LCMSSolution\User\Data\B4-APCI-POS1.qld
- **Method Name**: C:\LCMSSolution\User\Method\Copy of JAY-4-APCI.qlm

**MS Spectrum**

**Fig. 8.4**
Characterization of compound-2

1. Infrared spectrum

The IR spectrum of compound-2 (Fig 8.5) exhibited the presence of a broad band of hydroxyl group (OH) and carbon-carbon double bond (C=C).

IR cm⁻¹ (KBr): 3414 cm⁻¹ (OH)
  1599 cm⁻¹ (C=C)

2. Proton magnetic resonance spectrum

¹H NMR spectrum (Fig. 8.6) of compound-2 exhibited the following peaks.

- δ 7.05- 6.75
- δ 3.56

The NMR spectrum of the compound-2 exhibited a cluster of signals at δ 7.05 (m, 2H, H-3,5), δ 6.95 (m, 2H, H-2,6) and δ 6.75 (m, 1H, H-4) indicating the presence of benzene ring. The intense signal at δ 3.56 is due to the glycoside protons.

3. Carbon-13 Nuclear Magnetic Resonance

The data obtained by ¹H NMR spectrum was complemented by recoding ¹³C NMR (Fig. 8.7).

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<td>6''</td>
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The $^{13}$C NMR shows six signals in the aromatic region at $\delta$ 145.82 (C-1), 121.50 (C-3), 121.41 (C-5), 116.07 (C-2'), 115.95 (C-6) and 114.89 (C-4) for an aromatic ring. The anomeric carbons exhibited their signals at 104.23 (C-1') and 91.97 (C-2'). The two sets of signals 82.72 (C-4''), 76.90 (C-2'), 74.56 (C-3'), 70.20 (C-5'), 62.35 (C-6') and at 77.38 (C-4''), 75.99 (C-2''), 73.02 (C-3''), 70.08 (C-5''), 60.74 (C-6'') suggests two glycosidic groups in the compound.

4. Mass spectrum

The structure assigned to compound-2 was further ascertained by the mass spectra of the compound (Fig. 8.8).

The mass spectrum of compound-2 exhibited the molecular ion peak at m/z [418+Na]$^+$. The other major peaks appearing in its mass spectrum at m/z 341 and 93 were in accordance with expected mass fragmentation pattern of the compound.
2-(4,5-Dihydroxy-2-hydroxymethyl-6-phenoxy-tetrahydro-pyran-3-yloxy)-6-hydroxymethyl-tetrahydropyran-3,4,5-triol

Thus, in the present investigation identification of a glycoside is reported for the first time from the methanol extract of bark of *Mussaenda frondosa* L.
Fig. 8.5
LCMS-2010A DATA REPORT
SHIMADZU

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Inj. Volume: 5.000
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Method Name: C:\LCMSsolution\User\Method\Copy of JAY-4-APCI.qlm

MS Spectrum

Fig. 8.8