ISOLATION AND STRUCTURAL ELUCIDATION OF THE BIOACTIVE COMPOUNDS

6.1. Selected Plant for Compound Isolation by Column Chromatography

*Sarcochlamys pulcherrima* (Urticacaceae), are small trees and shrubs, (2-6) m height. Branchlets and petioles are dense, pubescent, stipules triangular-ovate, 8-10 mm; petiole 2-6 cm; leaf blade lanceolate, 12-22(-29) × 3-6(-9) cm, thinly leathery, adaxial surface dark green, abaxial surface grayish tomentose. Cymose panicles 7-9 cm; glomerules 2-4 mm in diam. Male flowers subsessile. Female flowers 0.3-0.5 mm. Fl. Apr-Jun, fr. Jun-Sep. The species is often dioecious, white flower, tropical, distributed in North-East India, China and Thailand. The aerial parts of *Sarcochlamys pulcherrima* is the used in folk medicine in Assam and Manipur against malaria, diabetes, diarrhea, fever, purgative, stomach pain, skin rashes, antibacterial, antifungal, Anti-inflammatory, carminative, digestive and used in dying fabrics and the bioactivity study of the leaf extracts shows *S. pulcherima* as a good antimicrobial and antioxidant plant source. However, there had been no adequate phytochemical and biological investigation report made from elsewhere. Hence, the species was selected to carryout isolation and identification of some of the bioactive (especially antimicrobial and antioxidant) compounds.

6.2. Material and methods

6.2.1. Extraction, fractionation, isolation

Leaves of *Sarcochlamys pulcherrima* Gaudich was shade dried, grounded to powder and soaked in MeOH in a round bottom flask for a week with frequent shaking (cold maceration). The extracts were filtered using a muslin cloth to remove the fibres
Isolation and structural elucidation of the bioactive compounds

and dirt. The extract was dried by removing the solvent using rotary vacuum evaporator
and finally by speedvac.

The powered extracts were fractionated using silica Column chromatography
and through bioactivity test of the fractions, the active fractions were identified. The
active fractions Ethylacetate: Hexane fraction (1:1, v/v), (F₁), 200 g dry wt., MeOH:
EthylAcetate (1:1, v/v), (F₂), 250 g dry wt. were further fractionated for the isolation of
the bioactive compounds.

Fraction F₁ (200 g) was subjected to dione column followed with Si (CC) eluted
stepwise by Hexane-DCM (95:5, 90:10, 85:15, 80:20, 75:25, 65:35, 50:50, 40: 60, 30:
70, 20: 80, 10: 90, 0:1) to isolate the compound (I) (290 mg), confirmed by TLC under
Hex-DCM (1:9).

Fraction (F₂) after subjecting to dione column chromatography it was further
eluted stepwise under DCM-Acetone (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:1) v/v,
followed by PrepTLC eluted by isocratic EtOAc-DCM-MeOH (10:6:3:0.1) v/v,
followed by washing of the separated spots with acetone using silica cartridge to obtain
the compound (II) 250 mg. The compounds were identified using ¹H-NMR, ¹³C-NMR,
GHMBC, GHMQC, HRESIMS spectral data analyses, as well as by comparison with
the reported values (Breitmaier et al., 1986; Lawrence et al.,2004; Kalegari et al.,2011).

6.2.2. Identification of the Compounds (I and II)

Ultraviolet spectrophotometric and IR analysis: IR spectra were obtained using
a Bruker Tensor 27 IR spectrometer. UV spectra were recorded on Varian Cary-50 Bio
UV-Visible spectrophotometer. The ¹H, ¹³C and ²D NMR spectra were recorded on a
Bruker 500 MHz spectrometer and Bruker BioSpin AG 2014 NMR Spectroscopy,
Ascend™ 500 Bruker, Avance III HD. High resolution mass spectra (HRESIMS) were obtained using HPLC-ESI-ToF G1969A, Agilent Technologies, Palo Alto, CA, USA. Column chromatography (CC) was performed on flash chromatography silica gel (porosity 60Å°, particle size 40-63μm, 230-400 mesh, bulk density 0.4g/ml, pH ranges 6-8, residual water <7%, Sorbtech catalog 40930-25), Sephadex™ LH-20 (Mitsubishi Kagaku, Tokyo, Japan), and Solid phase extraction (SPE) Silica cartridge (Strata™, Phenomex, 20mg). Analytical Thin Layer Chromatography (TLC) was conducted on Silica XG TLC plates w/UV 254, aluminum backed, 200μm, (20x20) cm (Catalog#: 4434126, Lot#:070219P, Sorbtech, USA). Preparative Thin Layer Chromatography (PTLC) was conducted on glass support Silica XG, 500μM. Agilent Mass Hunter, MestReNova, and ChemDraw softwares were also used for analyzing the NMR and Mass data. The isolated compounds were dissolved in deuterated CD₃OD for reading the NMR spectra. NMR spectra (¹H, ¹³C, C-Dept, GHMBC and GHMQC) of compound I (Fig. 1 - Fig. 5). NMR spectra (¹H, ¹³C, C-Dept, GHMBC and GHMQC) of compound II (Fig. 6 - Fig. 10).
Fig. 1. $^1$H NMR Spectra for compounds (I)
Fig. 2 $^{13}$C NMR Spectra for compounds (I)
Fig 3. Carbon-13 DEPT NMR Spectra for compound I
Fig 4. 2D Carbon-13 NMR Spectra (GHMBC) for compound (I)
Fig 5. 2D Carbon-13 NMR Spectra (GHMQC) for compound (I)
Isolation and structural elucidation of the bioactive compounds

NMR Spectra for compound (II)

Fig 6. $^1$H NMR Spectra for compounds (II)
\textbf{13C NMR (1D Spectra)}

\begin{figure}[h]
\centering
\includegraphics[width=\linewidth]{fig7}
\caption{13C NMR Spectra for compounds (II)}
\end{figure}
Fig 8. Carbon-13 DEPT NMR Spectra for compound II
Fig 9. 2D Carbon-13 NMR Spectra (GHMBC) for compound (II)
Fig 10. 2D Carbon-13 NMR Spectra (GHMQC) for compound (II)
Fig 11. HRESIMS of Compound (II) under +ve mode
### Table 6.1. 1H– 13C-NMR Data of compound I and II

<table>
<thead>
<tr>
<th>Position</th>
<th>Compound I</th>
<th></th>
<th></th>
<th></th>
<th>Compound II</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>δH</td>
<td>δC</td>
<td>δH</td>
<td>δC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>159</td>
<td>159.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>136</td>
<td>136.3</td>
<td></td>
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<tr>
<td>4</td>
<td>179.4</td>
<td>179.6</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5</td>
<td>158.6</td>
<td>158.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6.16 (d, J = 1.7 Hz, H-6)</td>
<td>100.4</td>
<td>6.20 (d, J = 2 Hz, H-6)</td>
<td>99.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>167.8</td>
<td>165.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>6.32 (d, J = 3 Hz, H-8)</td>
<td>95.1</td>
<td>6.37 (d, J = 2 Hz, H-8)</td>
<td>94.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>163</td>
<td>163.2</td>
<td></td>
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<td></td>
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<td>10</td>
<td>105.3</td>
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<tr>
<td>1'</td>
<td>122.9</td>
<td>121.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2'</td>
<td>6.91 (d)</td>
<td>116.3</td>
<td>6.95 (s, J = 1.5 Hz, H-2')</td>
<td>109.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3'</td>
<td>146.5</td>
<td>146.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4'</td>
<td>149.9</td>
<td>137.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5'</td>
<td>6.9 (d, J = 8.5 Hz, H-5')</td>
<td>116.8</td>
<td>146.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6'</td>
<td>7.30 (d, J = 8 Hz, H-6')</td>
<td>122.8</td>
<td>6.95 (s, J = 1.5 Hz, H-6')</td>
<td>109.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1''</td>
<td>5.34 (s, J = 3.8 Hz, H-1'')</td>
<td>103.5</td>
<td>5.31 (d, J = 1.2 Hz, H-1'')</td>
<td>103.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2''</td>
<td>3.76, (br d, J = 8 Hz, H-2'')</td>
<td>71.9</td>
<td>4.22,(dd, J= 2 Hz, 2 Hz, H-2'')</td>
<td>71.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3''</td>
<td>3.41 (dd, J = 3.5 Hz, 3.5 Hz, H-3'')</td>
<td>72.1</td>
<td>3.51 (m, H-3'')</td>
<td>72.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4''</td>
<td>3.34 (m, H-4'')</td>
<td>73.2</td>
<td>3.35 (s, J = 2, H-4'')</td>
<td>73.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5''</td>
<td>3.32 (m, H-5'')</td>
<td>72</td>
<td>3.79, (dd, J = 3 Hz, 3 Hz, H-5'')</td>
<td>72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6''</td>
<td>0.94 (d, J= 6 Hz, Me = 6'')</td>
<td>17.7</td>
<td>0.96 (d, J = 6.5, H-6'')</td>
<td>17.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.5 (s, OH - 3' and 4', D2O exchange)</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

500 MHz, and 600 MHz respectively for compound 1 and 2 respectively, CD3OD; chemical shifts in ppm relative to TMS; coupling constants (J) in Hz.

Note: s= singlet, d=doublet, dd= double doublet, m= multiplet, J= coupling constant
6.3. Results and Discussions

**Quercetin 3-O-α-rhamnoside or Quercitrin (I):** A light green amorphous powder. UV $\lambda_{\text{max}}$ (MeOH) nm: 261, 301, 356. IR $\nu_{\text{max}}$ (KBr) cm$^{-1}$: 3500-3200 (− OH), 1700 (C=O), 1600, 1500, 1450 (ring structure) and 1360 (C=C), 1150, 1000 (ether), 1050 (− OH). The proton and carbon NMR spectra both 1D and 2D were taken, the chemical shift and the carbon-proton correlation and proton of one carbon correlating with the proton of neighbouring carbon were shown in table 6.1.

$^1$H NMR (CD$_3$OD) $\delta$: 0.94 (d, $J = 6$ Hz, Me = 6$''$), 3.32 (m, H-5$''$), 3.34 (m, H-4$''$), 3.41 (dd, $J = 3.5$ Hz, 3.5 Hz, H-3$''$), 3.76, (br d, $J = 8$ Hz, H-2$''$), 5.34 (s, $J = 3.8$ Hz, H-1$''$), 6.16 (d, $J = 1.7$ Hz, H-6), 6.32 (d, $J = 3$ Hz, H-8), 6.9 (d, $J = 8.5$ Hz, H-5$'$), 7.30 (d, $J = 8$ Hz, H-6$'$), 7.33, (d, $J = 2$ Hz, H-2$'$), 6.91 (d), 8.5 (s, OH - 3$'$ and 4$'$, D$_2$O exchange).

$^{13}$C NMR (CD$_3$OD), Bruker 500 MHz, $\delta$: 179.4 (C-4), 167.8 (C-7), 163 (C-9), 159 (C-2), 158.6 (C-5), 149.9 (C-4$'$), 146.5 (C-3$'$), 136 (C-3), 122.9 (C-1$'$), 122.8 (C-6$'$), 116.8 (5$'$), 116.3 (2$'$), 105.3 (C-10), 103.5 (C-1$''$), 100.4 (C-6), 95.1 (C-8), 73.2 (C-4$''$), 72 (C-5$''$), 72.1 (C-3$''$), 71.9 (C-2$''$), 17.7 (C-6$''$). The chemical formula is C$_{21}$H$_{20}$O$_{11}$, the ESIMS of [M+Na]$^+$ was obtained as 471.3689, the calculated [M+Na]$^+$ being 471.3698, therefore the difference was 1.90 ppm which is negligibly small therefore, the compound’s molecular weight should be 448.38 g/mol. The molecular weight developed from the structure developed from the IR, NMR spectrometric datas and molecular weight obtained from High resolution mass spectra (HRESIMS) show the similarities and were difference less than 50ppm and the compound is confirmed as “Quercetin 3-O-α-rhamnoside” referring to $^{13}$C spectral data in literature (Breitmaier
et al., 1986) and is a first-time report from Sarchoclymes pulcherima however reported from different other plants (Lawrence et al., 2004 and Kalegari et al., 2011).

**Myricetin 3-O-α-rhamnoside (II)**

A light green amorphous powder. UV λ\text{max} (MeOH) nm: 350, 308, 262. Mass obtained from ESIMS [M+H]\(^+\), [M+2H]\(^+\) and [2M+Na]\(^+\) (experimental values) are 465.1028 (21.43%), 466.1050 (3.21%) and 951.1775 (4.39%) respectively (Fig. 11); while from the molecular formula C\(_{21}\)H\(_{20}\)O\(_{12}\), the calculated mass [M+H]\(^+\), [M+2H]\(^+\) and [2M+Na]\(^+\) (calculated values) are 465.10323, 466.11106 and 951.1806 (calculated values), the difference therefore is 0.92ppm, 13 ppm and 3.26 ppm respectively which is <50 ppm. The molecular weight of the compound is 464.0954 g/mol.

R \nu\text{max} (KBr) cm\(^{-1}\): 3450-3200 (–OH), 1700 (C=O), 1600, 1500, 1450 (ring structure) and 1360 (C=C), 1150, 1000 (ether), 1050 (–OH).

\(^1\)H NMR (CD\(_3\)OD) \(\delta\): 0.96 (d, \(J = 6.5\), H-6\(^\prime\)), 3.79, (dd, \(J = 3\) Hz, 3 Hz, H-5\(^\prime\)), 3.35 (s, \(J = 2\), H-4\(^\prime\)), 3.51 (m, H-3\(^\prime\)), 4.22, (dd, \(J = 2\) Hz, 2 Hz, H-2\(^\prime\)), 5.31 (d, \(J = 1.2\) Hz, H-1\(^\prime\)), 6.95 (s, \(J = 1.5\) Hz, H-6\(^\prime\)), 6.95 (s, \(J = 1.5\) Hz, H-2\(^\prime\)), 6.37 (d, \(J = 2\) Hz, H-8), 6.20 (d, \(J = 2\) Hz, H-6). \(^{13}\)C NMR (CD\(_3\)OD), Bruker 500 MHz, \(\delta\): 179.6 (C-4), 165.9 (C-7), 163.2 (C-9), 159.4 (C-2), 158.5 (C-5), 137.9 (C-4\(^\prime\)), 146.8 (C-3\(^\prime\)), 136.3 (C-3), 121.8 (C-1\(^\prime\)), 109.5 (C-6\(^\prime\)), 146.8 (5\(^\prime\)), 109.5 (2\(^\prime\)), 105.8 (C-10), 103.6 (C-1\(^\prime\)), 99.8 (C-6), 94.6 (C-8), 73.3 (C-4\(^\prime\)), 72 (C-5\(^\prime\)), 72.1 (C-3\(^\prime\)), 71.9 (C-2\(^\prime\)), 17.7 (C-6\(^\prime\)). The chemical formula is C\(_{21}\)H\(_{20}\)O\(_{12}\), and molecular weight is 464.378 g/mol. The structure, developed from the IR, NMR spectrometric datas was calculated for the molecular weight and compared with the molecular weight obtained from High resolution mass spectra.
Isolation and structural elucidation of the bioactive compounds (HRESIMS). The difference in the molecular weight was less than 50 ppm and confirmed as “Myricetin 3-O-α- rhamnoside or myricetin” and is another first-time reported compound from *Sarchoclymes pulcherima* however reported from different other plants (David *et al*., 1996).

![Structure of the isolated compound](image)

(1) \( R_1 = R_2 = R_3 = \text{OH} \)
(2) \( R_1 = R_2 = \text{OH}; R_3 = \text{H} \)

Myricetin-3-O-α-L- rhamnoside (I)
Quercetin - 3 -O-α-L- rhamnoside (II)

**Fig. 12.** Structure of the isolated compound
REFERENCES


