

## Chemical Investigation of *Annona squamosa* (Stem bark)

RAKESH RANJAN<sup>1</sup>, SUBRA SINGH<sup>1</sup>, SEEMA TIWARI<sup>2</sup> and K.K. SINGH<sup>2</sup>

<sup>1</sup>Department of Medicinal Chemistry, Institute of Medical Sciences  
Banaras Hindu University, Varanasi - 221 005 (India).

<sup>2</sup>Department of Chemistry, Udai Pratap P.G. College, Varanasi - 221 005 (India).

(Received: June 25, 2010; Accepted: August 08, 2010)

### ABSTRACT

4, 9-Dihydroxy -3, 8-dimethoxy- benzo [4, 5] furo [3, 2-C] chromen -6- one, 6, 7'-dihydroxy -3-methoxydihydroflavonol, 5,7- dihydroxy -4'-methoxy isoflavone, 7-hydroxy -4'-methoxy isoflavone, 7,3'-dihydroxy -4'-methoxy isoflavone, 4', 5, 7-trihydroxy isoflavone, 2'- hydroxy genistein have been isolated for the first time from the stem bark of *Annona squamosa* and identified by spectroscopic data.

**Key words:** *Annona squamosa*, Spectroscopic data.

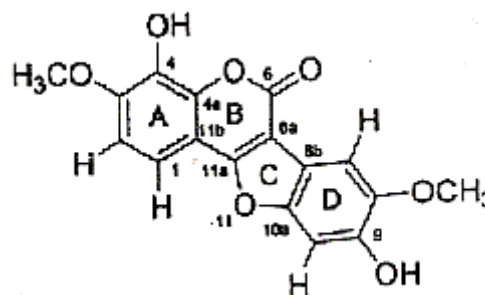
### INTRODUCTION

*Annona squamosa* (family Annonaceae) is native of Africa. It is also cultivated in India<sup>1</sup>. The decoction of the leaves of this plant is given for cholera<sup>2</sup>. In continuation to earlier work<sup>3</sup> we investigated the stem bark of *Annona squamosa* and isolated seven phenolic compounds for the first time from this source. Earlier workers isolated a wide variety of compounds viz. Amino acids<sup>4</sup>, terpenes<sup>5</sup>, sesquiterpenes<sup>6</sup>, diterpenes<sup>7</sup>, fats and oils<sup>8</sup>, steroids<sup>9</sup>, vitamins<sup>10</sup>, bezyltetrahydroisoquinoline<sup>11</sup>, proaporphines<sup>12</sup>, aporphines<sup>13</sup>, oxoaporphines<sup>14</sup>, and a large number of acetogenins<sup>15</sup>. The compounds are provisionally designated as AO-1 (I) to AO-7 (VII) and were characterised on the basis of their detailed spectroscopic analysis.

#### Isolation and Characterization of Compounds

The stem bark of *Annona squamosa* were purchased from United Chemicals & Allied work, Clive Row-10, Kolkatta, India. The stem bark were milled by conventional method. Defatted milled stem bark of *Annona squamosa* (5 kg) were extracted with methanol (15 l) for 16 hours. The methanolic extract (63.8 g) was then fractionated into four parts according to the increasing polarity of solvents viz,

n-hexane chloroforms, ethylacetate and n-butanol. Chloroform soluble portion was chromatographed over SiO<sub>2</sub> gel column and eluted with solvents of increasing polarity. The silica gel column chromatography of ethyl acetate soluble portion (see experimental) yielded seven compounds compound I to VII by elution with hexane-ethyl acetate 95 : 5 upto ethyl acetate by gradual increasing of the polarity of solvents.

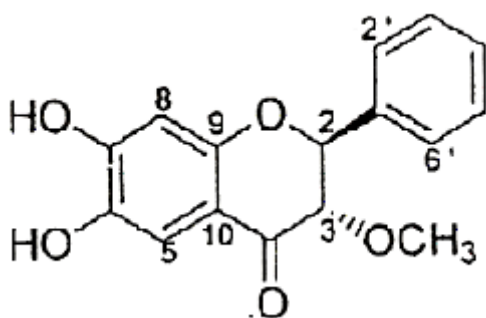


#### AO-1 (I)

White crystals of AO-1 (I) MF C<sub>17</sub>H<sub>12</sub>O<sub>7</sub> (M+ 328), m.p. 310°C responded positive to FeCl<sub>3</sub> test. Its IR absorption bands showed the presence of

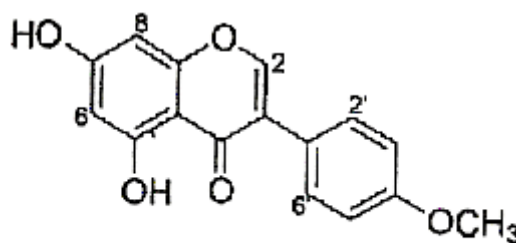
unsaturated lactone (1716 & 1253  $\text{cm}^{-1}$ ), hydroxyl group (3350  $\text{cm}^{-1}$ ) and aromatic functionality (1608 and 1427  $\text{cm}^{-1}$ ). The  $^1\text{H}$  NMR spectrum of AO-1 (I) showed the presence of one AB system [ $\delta$  7.54 (1H, d,  $J=8.6$  Hz, H-1)] and [ $\delta$  7.00 (1H, d,  $J=8.6$  Hz, H-2)] and one AX system [ $\delta$  7.42, 1H, s and 7.20, 1H, s]. In addition to four proton signals the spectrum also showed two singlets ( $\delta$  4.02 and 3.99, 3H, each) for two methoxyl group and two exchangeable signal at  $\delta$  9.96 and 9.07 (1H, each) for two hydroxyl group.

The  $^{13}\text{C}$  NMR, nOe studies in  $^1\text{H}$  NMR and UV spectral studies bathochromic shift with ( $\text{AlCl}_3$  and  $\text{H}_3\text{BO}_3$ ) has indicated the structure of AO-1 (I) as 4, 9-dihydroxy - 3, 8 - dimethoxy - benzo [4, 5] furo [3, 2-C] chromen - 6 - one<sup>16</sup>.



#### AO-2 (II)

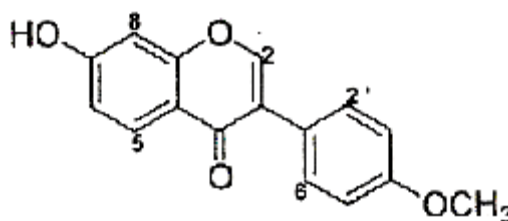
Greenish crystals, m.p. 210-211°C. IR absorption bands showed hydroxyl group (3333  $\text{cm}^{-1}$ ) aromatic ring (1515 and 1741  $\text{cm}^{-1}$ ) and a carbonyl group (1653  $\text{cm}^{-1}$ ) in its molecule, UV absorption bands at  $\lambda_{\text{max}}$  216, 235 276 and 341 nm and bathochromic shift of band II with MeOH + NaOAc suggested the presence of 7-OH group, addition of boric acid also showed bathochromic shift of band II, thus confirming *o*-dihydroxyl group.  $^1\text{H}$  NMR spectrum has suggested ring B as unsubstituted [ $\delta$ 7.55 (2H, m, H-2' & H-6') and ( $\delta$  7.45, 3H, m, H-3', H-4' and H-5')] while two singlets at  $\delta$  7.19 and 6.43, 1H each was assigned to H-5 and H-8. The only methoxy singlet at  $\delta$  3.80 was assigned to methoxyl at C-3 position. Other two nonaromatic hydrogens at  $\delta$  5.14 and 4.53, dd where assigned to H-2 and H-3 respectively. Thus compound was identified as 6, 7- dihydroxy -3- methoxydihydro flavonol.



#### III

#### AO-3 (III)

$\text{C}_{16}\text{H}_{12}\text{O}_5$  ( $\text{MH}^+$  at 285), white needles from methanol, m.p. 215-216°C. The UV data and the UV with shift reagents suggested it to be 5, 7- dihydroxy flavonoid. Its isoflavone nature was deduced by characteristic signal for H-2 at  $\delta$  8.19 (1H, s) and C-2 at  $\delta$  15.5 ppm. The  $^1\text{H}$  NMR data AX system ( $\delta$  6.28 and 6.42,  $^1\text{H}$  d, each  $J= 1.9$  Hz) and  $\text{A}_2\text{B}_2$  system ( $\delta$  7.5 and 6.98 2H each d,  $J= 8.8$  Hz) along with two exchangeable hydrogens and a methoxy singlet ( $\delta$  3.91) has allowed us to assign the structure of AO-3 (III) as 5, 7- dihydroxy -4'- methoxy isoflavone commonly known as biochanin<sup>17</sup>.

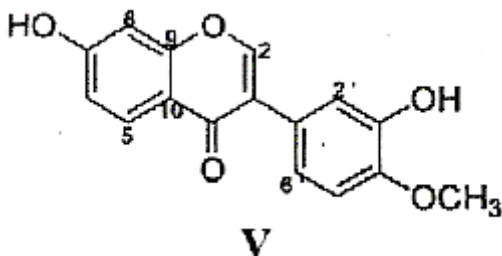


#### IV

#### AO-4 (IV)

$\text{C}_{16}\text{H}_{12}\text{O}_4$  ( $\text{M}^+$  268) white crystal, m.p. 258°C showed in its IR spectrum absorption bands at 3424  $\text{cm}^{-1}$  (hydroxyl group) and 1624  $\text{cm}^{-1}$  (conjugated carbonyl group). The isoflavone nature of AO-4 (IV) was established by  $^1\text{H}$  NMR signal at  $\delta$  8.17 (1H, s). The  $^1\text{H}$  NMR spectrum also showed one ABX system in A ring [  $\delta$  8.07 (1H, d,  $J=9.2$  Hz)  $\delta$  6.99 (1H, dd,  $J= 9.2$  and 2.1 Hz and  $\delta$  6.90 (1H, d,  $J=2.1$  Hz)] and an  $\text{A}_2\text{B}_2$  system in B ring [ $\delta$  7.56 (2H, d,  $J=8.7$  Hz) and  $\delta$  6.97 (2H, d,  $J=8.7$  Hz)]. The position of hydroxyl and methoxyl groups were established by study of shift reagents in UV spectrum. On the

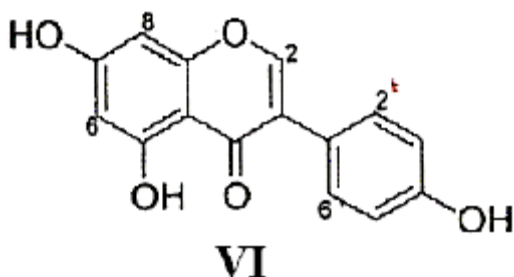
basis of these data and its comparison with reported literature data<sup>18, 19</sup> AO-4 (IV) was identified 7-hydroxy -4'- methoxy isoflavone commonly known as formonentin.



#### AO-5 (V)

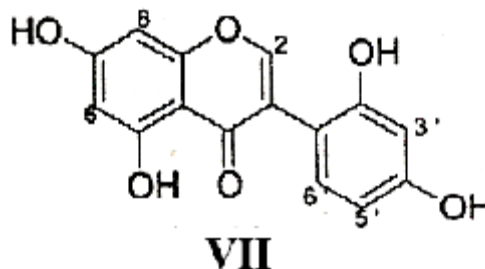
$C_{16}H_{12}O_5$  (MH<sup>+</sup> 285), colourless needles from methanol, m.p. 241-245°C. IR spectrum showed absorption bands for hydroxyl (3420  $cm^{-1}$ ) carbonyl (1624  $cm^{-1}$ ) and aromatic (1580  $cm^{-1}$ ) functionalities. The UV spectrum in methanol showed  $\lambda_{max}$  224, 247 and 288 nm while in NaOAc band II showed a bathochromic shift which confirmed the presence of hydroxyl group at C-7 position in flavonoid.

<sup>1</sup>H NMR spectrum of AO-5 (V) confirms the isoflavone nature ( $\delta$  8.83, 1H s,  $\delta$  c 153.2 s). The <sup>1</sup>H NMR spectrum also showed two ABX system in the molecule one in ring A [ $\delta$  8.03 (1H, d, J = 8.7 Hz)  $\delta$  7.02-6.97 (1H, m) and  $\delta$  6.92 (1H, d, J=2 Hz)] for H-5, H-6 and H-8 respectively and the other ABX system of B ring at  $\delta$  7.09 1H and 7.02, 6.92, 2H, m). The spectrum also showed the presence of a methoxy singlet at  $\delta$  3.84. The compound was established as 7, 3' - dihydroxy -4'- methoxy isoflavone by nOe studies, the irradiation of methoxy signal at  $\delta$  3.84 enhanced the signal for H-5'. This compound AO-5 (V) was thus identified as calicosin by comparison of the spectral data reported in literature<sup>20</sup>.



#### AO-6 (VI)

$C_{15}H_{10}O_5$  (MH<sup>+</sup> at m/z 271), yellow needles m.p. 301-302°C. IR showed absorption bands for hydroxyl (3430  $cm^{-1}$ ) and carbonyl (1650  $cm^{-1}$ ) functionalities. UV absorption maximum in methanol was found at 262 and 337 nm. Use of shift reagents has suggested the presence of phenolic group at 5 and 7 position. The presence of isoflavonic 2-H was settled by <sup>1</sup>H NMR signal ( $\delta$  8.31, 1H, s) and A<sub>2</sub>B<sub>2</sub> system in B ring ( $\delta$  7.22 and 6.81, 2H, d, each, J=8.6 Hz) and AX system in A ring ( $\delta$  7.13 and 6.21, 1H, d each J=1.8 Hz) Thus settled the structure of AO-6 (VI) as 4', 5, 7 trihydroxy isoflavone a commonly known isoflavone by comparison with reported data<sup>21,22</sup>.



#### AO-7 (VII)

$C_{15}H_{10}O_6$  (MH<sup>+</sup> at M/z 287), m.p. 270-273°C. The IR absorption bands at  $\nu_{max}$  3350 and 1655  $cm^{-1}$  and UV absorption maxima ( $\lambda_{max}$  258 and 315 nm) taken in consideration with molecular formula has suggested it to 2', 4', 5, 7- tetrahydroxy isoflavone. This is found to be in full agreement with <sup>1</sup>H NMR data which showed AX system in A ring and ABX system in B ring along with characteristic 2-H. The compound was readily recognised as 2'-hydroxy genistein, by comparison with reported spectroscopic data in literature<sup>23</sup>.

### EXPERIMENTAL

The melting point were measured on a Yazawa hot stage microstage apparatus and are uncorrected. Optical rotations were measured on JASCO DIP-360 Polarimeter (cell length 5 cm). UV absorption spectra were recorded on JASCO UV/visible spectrophotometer (model no. 7800) while IR on JASCO FT-IR 5300 spectrometer. <sup>1</sup>H and <sup>13</sup>C NMR data of compounds I, II, III, IV, V, VI, VII recorded in CDCl<sub>3</sub> at 300 MHz and 75 MHz respectively.

**Compound I**

White light crystals, m.p. 310°C, EI-MS : m/z 328 [M]<sup>+</sup>, IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup> 3350, 2928, 1716, 1601, 1520, 1427, 1253 and 745, UV  $\lambda_{\max}$  nm (MeOH) 208, 244, 345; (MeOH + NaOAc) 210, 245, 347; (MeOH + NaOAc/ H<sub>3</sub>BO<sub>3</sub>) 208, 244, 347, (MeOH + NaOMe) 209, 245, 378, (MeOH + AlCl<sub>3</sub>) 209, 247, 345, (MeOH + AlCl<sub>3</sub>/ HCl) 208, 244, 344

<sup>1</sup>H NMR :  $\delta$  9.96 (1H, s, 9-OH); 9.07 (1H, s, 4-OH); 7.54 (1H, d, J=8.6 Hz, C-1H); 7.42 (1H, s, 4-OH) 7.20 (1H, s, C-10-H); 7.00 (1H, br, d, J=8.6 Hz, C-2-H); 4.02 (3H, s, 3-OCH<sub>3</sub>); 3.99 (3H, s, 8-OH-<sub>3</sub>)

<sup>13</sup>C NMR:  $\delta$  161.0 (C-6); 159.0 (C-3); 157.0 (C-10a); 154.6 (C-11a); 149.8 (C-4); 147.2 (C-8); 147.1 (C-9); 135.4 (C-4a); 116.6 (C-1); 114.4 (C-6b); 114.1 (C-2); 105.5 (C-6a); 102.6 (C-11b); 102.2 (C-7); 99.4 (C-10); 61.0 (3-OCH<sub>3</sub>); 56.4 (8-OCH<sub>3</sub>)

**Compound II**

Greenish crystals, m.p 210-211°C, [ $\alpha$ ]<sub>D</sub><sup>22</sup> + 13.3 (methanol + CHCl<sub>3</sub>, c, 0.06), FAB-MS m/z 287, IR (KBr)  $\nu_{\max}$  3333, 1653, 11515, 1471, 830 and 766 cm<sup>-1</sup>, UV  $\lambda_{\max}$ nm (MeOH) 341, 276, 235, 216; (MeOH + NaOAc) 346, 255, 218; (MeOH + AlCl<sub>3</sub>) 377, 238, 218, (MeOH + AlCl<sub>3</sub> + HCl) 339, 236, 219

<sup>1</sup>H NMR :  $\delta$  7.55 (m, C-H -2') 7.55 (m, C-H-6'); 7.48-7.41 (m, C-H-3'); 7.48-7.41 (m, C-H-4'); 7.48-7.41 (m, C-H-5'); 7.19 (1H, s, C-H-5); 6.43 (1H, s, C-H-8) 5.68 (1H, br d, 1  $\times$  OH); 5.14 (1H, d, J=11.6 Hz, C-H-2); 4.53 (1H, dd, J=3.7, 11.6 Hz, C-H-3); 3.80 (3H, s, 3-OCH<sub>3</sub>)

<sup>13</sup>C NMR :  $\delta$  192.5 (C-4); 157.5 (C-9); 155.3 (C-7); 144.3 (C-1'); 137.9 (C-6) 128.8 (C-2'); 128.5 (C-3'); 128.5 (C-5'); 128.5 (C-6'); 128.3 (C-4'); 110.0 (C-10); 107.6 (C-8); 103.6 (C-5); 84.0 (C-3); 72.9 (C-2); 56.2 (3-OCH<sub>3</sub>)

**Compound III**

White needles, m.p 215-216°C, FAB-MS m/z 285 [M+ H], 569 [2M+ H], IR (KBr)  $\nu_{\max}$  3500, 1724, 1687, 1606, 1594, 1455, 1383, 1246, 1181, 1043 cm<sup>-1</sup>, UV  $\lambda_{\max}$ nm MeOH : 261, 330 sh; MeOH + NaOAc : 272, 327; MeOH + AlCl<sub>3</sub> + HCl) 273, 310 sh, 373

<sup>1</sup>H NMR :  $\delta$  12.85 (1H, s, 5-OH); 8.19 (1H, s, C-H-2); 7.54 (2H, d, J=8.8 Hz, C-H-2') 7.54 (2H, d, J=8.8 Hz, C-H-8'); 6.98 (2H, d, J=8.7 Hz C-H-3'); 6.98 (2H, d, J=8.7 Hz, C-H-5'); 6.42 (1H, d, J=2.1, C-H-8); 3.91 (3H, s, 4-OCH<sub>3</sub>)

<sup>13</sup>C NMR :  $\delta$  180.5 (C-4); 165.1 (C-7); 163.9 (C-5); 160.7 (C-4'); 159.1 (C-9); 154.5 (C-2); 131.1 (C-6'); 124.1 (C-3); 123.7 (C-1'); 114.4 (C-3'); 114.4 (C-5'); 106.1 (C-10); 99.1 (C-6); 94.4 (C-8); 56.3 (4-OCH<sub>3</sub>)

**Compound IV**

White crystals, m.p. 240°C, EI-MS m/z 284 [M]<sup>+</sup>, IR (KBr)  $\nu_{\max}$  3500, 1724, 1687, 1606, 1594, 1455, 1383, 1246, 1181, 1043 cm<sup>-1</sup>, UV  $\lambda_{\max}$ nm MeOH : 261, 330 sh; MeOH + NaOAc : 272, 327; MeOH + AlCl<sub>3</sub> + HCl : 273, 310 sh, 373

<sup>1</sup>H NMR :  $\delta$  9.72 (1H, s, 7-OH); 8.17 (1H, s, H-2, C-H-2); 8.07 (1H, d, J=8.7 Hz, C-H-5); 7.56 (2H, d, J=8.7 Hz, C-H-2') 7.56 (2H, d, J=8.7 Hz, C-H-6'); 6.99 (1H, dd, J=9.2 Hz, 2.1 Hz, C-H-6); 6.97 (2 H, d, J=8.7 Hz, C-H-3'); 6.97 (2H, d, J=8.7 Hz, C-H-5') 3.83 (3H, s, 4'-OCH<sub>3</sub>)

<sup>13</sup>C NMR :  $\delta$  162.7 (C-7); 159.1 (C-4'); 157.6 (C-9); 152.6 (C-2); 147.8 (C-4); 130.3 (C-6'); 130.0 (C-2'); 127.2 (C-5); 124.4 (C-1'); 123.5 (C-3); 116.8 (C-10); 115.1 (C-6); 113.5 (C-3'); 113.5 (C-5'); 102.1 (C-8); 56 (4' - OCH<sub>3</sub>)

**Compound V**

Colourless crystals, m.p. 245-247°C, FAB-MS m/z 285 [M+H]<sup>+</sup>, IR (KBr)  $\lambda_{\max}$  3420, 1624, 1580, 1510, 1470, 1381, 1023, 853 cm<sup>-1</sup>, UV  $\lambda_{\max}$  nm MeOH : 288, 247, 224; MeOH + NaOAc : 327, 255, 221; NaOAc + boric acid : 288, 247, 225

<sup>1</sup>H NMR :  $\delta$  9.10 (1H, br. hump, 4' OH) 8.33 (1H, s, C-H-2); 8.03 (1H, d, J=8.7 Hz, C-H-5); 7.09 (1H, C-H-2'); 7.02-6.97 (C-H-6); 7.02-6.97 (C-H-5'); 7.02-6.97 (C-H-6') 6.92 (1H, d, J=2.0 Hz, C-H-8); 3.84 (3 H, s, 7- OCH<sub>3</sub>)

<sup>13</sup>C NMR :  $\delta$  177.8 (C-4)  $\delta$  162.6 (C-4'); 157.5 (C-3'); 153.2 (C-2); 147.6 (C-7); 146.1 (C-10); 127.4 (C-5); 124.7 (C-9); 123.5 (C-1') 119.8 (C-2'); 116.7 (C-4); 116.5 (C-5') 115.3 (C-6); 112.0 (C-6'); 55.7 (7-OCH<sub>3</sub>)

**Compound VI**

Bright yellow needles, m.p. 301-302°C, FAB-MS m/z 271 [M+H]<sup>+</sup>, IR (KBr)  $\nu_{\text{max}}$  3430, 2920, 1650, 1617, 1571, 1510, 1465, 1240, 1188, 1170  $\text{cm}^{-1}$ , UV  $\lambda_{\text{max}}$  nm MeOH : 337, 262

<sup>1</sup>H NMR:  $\delta$  12.94 (1H, s, 5-OH)  $\delta$  9.56 (1H, br. hump, 7- OH); 8.31 (1H, s, C-H-2); 7.72 (2H, d, J=8.4 Hz, C-H-2'); 7.72 (2H, d, J=8.4 Hz, C-H-2'') 7.72 (2H, d, J=8.4 Hz, C-H-6') 7.13 (1H, d, J=1.8 Hz, C-H-6); 6.81 (2H, d, J=8.4 Hz, C-H-3'); 6.81 (2H, d, J=8.4 Hz, C-H-5'); 6.21 (1H, d, J = 1.8, C-H-8)

<sup>13</sup>C NMR:  $\delta$  181.3 (C-4); 164.7 (C-7); 163.7 (C-5); 158.8 (C-9); 158.2 (C-4'); 154.0 (C-2); 130.9 (C-2''); 130.9 (C-6'); 123.8 (C-3); 122.9 (C-1'); 115.8 (C-3'); 115.8 (C-5'); 106.0 (C-10); 99.7 (C-6); 94.3 (C-8)

**Compound VII**

White needles, m.p. 270-273°C, FAB-MS m/z 287 [M+H]<sup>+</sup>, IR (KBr)  $\nu_{\text{max}}$  3350, 1655, 1575, 1500, 1464, 1234, 1178, 1104  $\text{cm}^{-1}$ , UV  $\lambda_{\text{max}}$  nm MeOH : 315 (sh), 258, MeOH + AlCl<sub>3</sub> : 315 (sh), 268; MeOH + AlCl<sub>3</sub> + HCl : 315 (sh), 268

<sup>1</sup>H NMR:  $\delta$  12.97 (1H, s, 5-OH);  $\delta$  9.29 (2H, br. d, 2  $\times$  OH) 8.13 (1H, s, C-H-2); 6.95 (1H, d, J=8.4 Hz, C-H-6'); 6.36 (1H, d, J=1.5, C-H-6); 6.34 (1H, d, J=2.1, C-H-3'); 6.25 (1H, d, J=8.4, 2.1 Hz, C-H-5'); 6.20 (1H, d, J=1.5 Hz, C-H-8)

<sup>13</sup>C NMR:  $\delta$  180.5 (C-4); 164.2 (C-5); 161.2 (C-7); 158.6 (C-4'); 157.7 (C-9); 156.5 (C-2'); 155.3 (C-2); 132.2 (C-6'); 120.5 (C-3); 108.7 (C-1'); 106.3 (C-5'); 104.5 (C-10) ; 102.7 (C-3'); 98.9 (C-6) 93.7 (C-8)

**REFERENCES**

- The Wealth of India : Raw Materials, A Dictionary of Indian Raw Materials and Industrial products, Council of Scientific and Industrial Research, New Delhi, **1**: 80-81(1948).
- Glossary of India Medicinal Plants - CSIR New Delhi, India, p. 25 (1956).
- Rupprecht, J.K., Hui, Y.H. and McLaughlin, J.L., *J. Nat. Prod.*, **53**: 237 (1990).
- Touche, A., Desconclois, J.F., Jacquemin, H., Lelievre, Y. and Forgacs, P. *Plant. Med. Phytother.* **15**: 4 (1981).
- Luciana Alves, Rodrigues Dos Santos, Maria Amelia, Diamatino Boaventura, Lucia Pinheiro, Santos Pimenta, *Biochemical Syntematics and Ecology* **34**: 78-82 (2006).
- Xiao-Xi Liu, Elsa Pilorinon and Jerry L McLaughlin *Tetrahedron Letters* **40**: 399-402 (1999).
- Alassane Wele, Celine Landon, Henri Labbe Francoise Vovelle, Yanjun Zhang and Bernard Bodo, *Tetrahedron* **6**: 405-414 (2004).
- Kawashima, A., Kishimoti, M. Morimoti, S., Akyama, T., Maejima, A., Kawada, I., Japanese Patent JPO841068, 1996, *Chem. Abstr.* **124**: P298921C (1996).
- Ishikawa, T., Sekine, K. Japanese Patent JPO1122871, 2001, *Chem. Abstr.*, **134**: P344563f (2001).
- Lion Corp. Japanese Patent JP 2000191542-A, 10 (2000)..
- Xu, S., Zhang, G., Song, S., Meng, S., Cao, Y, Xu, B., Lu, J. Cinese Patent CN 1370537, 2002, *Chem. Abstr.* **139**: P255374F (2003).
- Sassa, S., Sakamoto, S., Zhou, YF., Mori, T., Kikuchi, T., Shinoda, H. *In-Vivo* **15**: 25 (2001).
- Xie, H., Wu, T., Huang, L., Li, C. *Zhongguo Zhong Yao Za Zhi* **22**: 238 (1997).
- Rao, S.V. Ramachandran, K. and Zaher, S.H., *J.I.C.S. Ind. and News Ed.* **18**: 215 (1955).
- Ghanekar, RRV and Ayyar, P.R. *J. Indian Inst. Sci.*, **10A**: 28 (1927)
- Farnsworth, N.R. Blomster, R.N. Quimby, M.W. and Schermerhorn, J.W., *The Lynn Index Monograph*, **3**: 60 (1974).
- Oliveros - Belardo, L., *Lloydia*, **38**: 537 (1075).
- Rao, R.V.K. Murty, N., Rao, J.V.L.N. and Seshagiri, *Indian J. Pharm. Sci.* **40**: 170 (1978).
- Bohlmann, F. and Rao, N., *Chem. Ber.*, **106**: 841 (1973).
- Hernandez. M., *Agronomia (Cuba)*, **3**: 58

- (1943).
21. Yang, T.-H. and Chan, C.-M. *J. Cem. Soc. (Taipei)*, **17**: 243 (1970).
  22. Balbaa, S.L. Haggag, M.Y. and Taha, K.F. *Egypt. J. Pharm. Sci.* **18** : 1 (1979).
  23. Jolad, S.D. Hoffmann, J.J., Schram, K.H., Cole, J.R., Tempesta, M.S., Kriek, G. R. and Bates, R.B. *J. Org. Chem.*, **47**: 3151 (1982).
  24. Lieb, F., Nonfon, M., Wachendorff - Neuman, V. and Wendisch D., *Planta Med.* **56**: 317 (1990).
  25. Kawazu, K., Alcantero, J.P. and Kobayashi, A., *Agric. Biol. Chem.*, **53**: 2719 (1989).