

**RESEARCH ARTICLE****GLYCOSIDIC COMPOUND AND NOVEL LIPIDS FROM THE LEAVES OF  
*ARTABOTRYS ODORATISSIMUS* (R.BR)****Rohini Ojha, B. K. Mehta and Darshana Mehta**Natural Products Research Division, School of Studies in Chemistry and Biochemistry,  
Vikram University, Ujjain 456010, M.P., India**ARTICLE INFO****Article History:**Received 29<sup>th</sup> August, 2017

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**ABSTRACT**

New glycosidic compounds were isolated from the ethanolic extracted from leaves of Artabotrys odoratissimus (R.Br) by using ethanol. The six known compounds, two new rhmnopyranosyl compound, one pyranocoumarin compound and three aliphatic hydrocarbon were the new alcoholic compound identified by spectral (IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR spectra, mass spectrum, elemental analysis) and chemical analysis. The rhmnopyranosyl compounds was identified as 22,23-dihydro-5-stigmastene-3-β-D-glucopyranoside, [→ 3-α-L-rhm- (1→] and one coumarin compound 7-hydroxy-8-(3"-methylene acetoxy-1"-oxo butenyl)-6'-6'-dimethyl pyrano (2', 3':5, 6)-coumarin and aliphatic were identified as Octatricontane, 11-methyl hexacontan-1, 45, 60-triol, Octaeicosandioate. These are novel compounds and being reported first time by us.

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**INTRODUCTION**

Artabotrys odoratissimus R.Br commonly known as Kantili champa or Nag champa belongs to family Annonaceae. The plant is used medicinally in various ailments. A decoction of leaves is given for the treatment of cholera in some of the islands in Malaya Archipelago (Jain, 1968). Leaves are used to treat some antifungal and antimicrobial iseases. The antifertility activity of A. odoratissimus plant has been reported in albino rats (Chakrabarti, 1968). The different extracts of the plant have shown antifertility, antimicrobial, antifilarial and antihelminthic activities (Trivedi, 1971). The present study reports the isolation and structural elucidation of six new compounds (Fig. 1) isolated from the leaves of Artabotrys odoratissimus.

**Experimental****General procedures**

Melting points (mp) are uncorrected. <sup>1</sup>H NMR was recorded on 300 MHz Varian XL spectrometer, <sup>13</sup>C NMR spectra were recorded on Varian XL 75 MHz s pectrometer, IR spectra were

recorded in KBr disk on Perkin Elmer-377 spectrometer, EIMS on Jeol-JMS D 300 mass spectrometer. All chemical shifts (d) are given in ppm and Me4Si was used as internal standard. The carbon type (CH<sub>3</sub>, CH<sub>2</sub>, and CH) was determined by DEPT experiments. Chemicals are of analytical-reagent grade and column chromatography was carried out on alumina grade III and TLC on silica gel G (CDH/Glaxo laboratories). Spots were visualized by exposure to iodine vapor or by spraying with H<sub>2</sub>SO<sub>4</sub>-vanillin solution followed by heating at 105 °C for 5 min.

**Plant material**

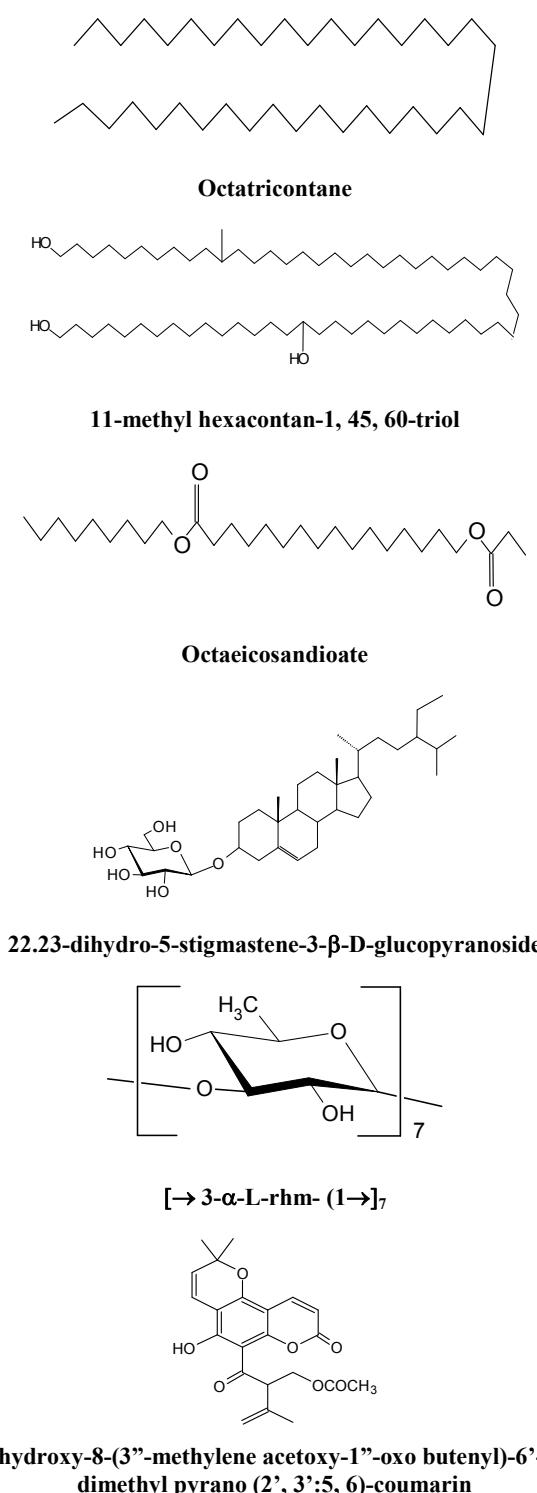
The leaves (8 kg) of A. odoratissimus were collected from the gardens of Ujjain city and University campus and were identified by the authorities of the Institute of Environment Management and Plant Science, Vikram University, Ujjain. voucher specimen was deposited in the herbarium of the School of Studies in Botany, Vikram University, Ujjain, India.

**Extraction and isolation**

The leaves (8 kg) were shade dried, cleaned, coarsely powdered and extracted with n-hexane, benzene, benzene: acetone and ethanol in soxhlet-extractor for 72 h. Removal of solvent under reduced pressure afforded solid extracts.

\*Corresponding author: Rohini Ojha, B. K. Mehta,

Natural Products Research Division, School of Studies in Chemistry and Biochemistry, Vikram University, Ujjain 456010, M.P., India.



**Figure 1. Chemical structures of Compounds**

The yield of n-hexane extract [150g] and ethanol extract was quite good but the benzene: acetone extract was poor. Since the yield of hexane and ethanol extract was quite good it was taken for the isolation of active compounds by repeated column chromatography. The three aliphatic compounds are isolated from hexane extract by repeated column chromatography on alumina grade III. The column was eluted by gradient elution in increasing order of polarity like hexane, benzene was eluted by gradient elution in increasing order of polarity like hexane, benzene, EtOAc and methanol. The fractions were collected in bulk and monitored by TLC. The residue (6.8 g) of hexane fraction was rechromatographed on alumina on the basis of increasing order of polarity of eluents.

The column was successively eluted with hexane, hexane: benzene and methanol and their mixtures of increasing polarity. Fractions (a, b and c) (hexane, hexane: benzene v/v 8:2 and 3:7 v/v) were purified and identified as Octatricontane, 11-methyl hexacontan-1,45,60-triol, Octaeicosadioate. The ethanol extract was analyzed by TLC and group test analysis, which revealed the presence of several spots and positive test for glycoside and flavonoids. To separate the extract was subjected to column chromatography on silica gel as adsorbent. The column was eluted with different solvents in their increasing order of polarity. The methanol fraction of ethanol extract was rechromatographed on silica on the basis of increasing order of polarity of eluents. The column was successively eluted with EtOAc: MeOH (1:1v/v) and methanol and their mixtures of increasing polarity. The Fractions EtOAc: MeOH (1:1v/v) was rechromatographed on silica on the basis of increasing order of polarity of eluents. Then fraction (a) (MeOH: EtOAc 7:3 v/v) and the pure form by rechromatographed on silica gel on fraction (a) (MeOH:EtOAc 8:2 v/v) was identified as 23-dihydro-5-stigmastene-3-β-D-glucopyranoside.

The methanol fraction was rechromatographed on silica on the basis of increasing order of polarity of eluents. Then fraction (b and c)(CHCl<sub>3</sub>:MeOH 6:4 v/v) and the pure form by rechromatographed on silica gel on fraction (b and c) (MeOH:EtOAc 5:5 v/v) and (MeOH:EtOAc 5:5 v/v) was purified and identified as [→ 3-α-L-rhm- (1→7] and 7-hydroxy-8-(3"-methylene acetoxy-1"-oxo butenyl)-6'-6'-dimethyl pyrano (2', 3':5, 6)-coumarin. Presence of this a steroid at glycoside was analyzed by IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR and mass spectrometry and compared with the literature data<sup>14</sup>. Fractions (a and b) afforded a mixture of rhmnopyranosyl 5 and pyrano coumarin compound 6 and other impurities. Fraction (c) was further rechromatographed by eluent MeOH: EtOAc (5:5 and 9:1 v/v) to give 5 and 6 in pure form respectively,

### Compound 1

Octatricontane (1). Mass m/z (% intensity) M<sup>+</sup> 534(4.2), 517(4.8), 501(8.2), 486(4.7), 485(28.2), 483(4.4), 463(18.2), 445(13.3), 429(30.0), 415(14.8), 413(40.0), 397(4.1), 391(14.8), 377(6.0), 375(21.2), 363(14.0), 347(22.0), 337(14.9), 327(23.7), 317(28.2), 316(20.1), 301(100.0), 289(76.9), 279(42.0), 261(19.2), 247(28.6). C<sub>38</sub>H<sub>78</sub> (15 mg CDCl<sub>3</sub>), mp 147 °C. Isolated from hexane fraction. On TLC examination it showed a single homogenous spot using hexane: diethylether: (9:0.5:1, v/v). IR (KBr) tmax: 2956, 2918, 1455, 1378, 1122, 1063 and 762- 719 cm<sup>-1</sup>. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, TMS) 0.88 (t, 6H, -2CH<sub>3</sub>, J=7.5 Hz), 1.25 (s, 72H, -36CH<sub>2</sub>).

### Compound 2

11-methyl hexacontan-1, 45, 60-triol (2) mass m/z (% intensity) M<sup>+</sup> 890, 889(40.1), 887(20.0), 810(11.0), 752(12.3), 95(4.0), 646(21.0), 599(6.0), 596(6.0), 51(4.0), 549(3.8), 496(3.2), 439(12.0), 391(12.0), 383(10.2), 339(7.0), 317(14.0), 289(24.0), 279(4.0), 229(7.2), 229(7.2), 176(21.2), 154(100.0), 136(89.9), 127(62.3), 111(75.2), 95(80.2), C<sub>60</sub>H<sub>122</sub>O<sub>3</sub> (30 mg, methanol) m.p. 185 °C. Isolated from hexane: benzene (8:2, v/v) fraction, TLC hexane:benzene (7:3 v/v) as solvent system, it showed single clear spot. IR (KBr) tmax: 3459, 2917, 2849, 1471, 1020 and 730-719 cm<sup>-1</sup>.

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, TMS): 0.98 (s, 3H, -CH<sub>3</sub>), 3.51 and 3.63 (m, 4H, -CH<sub>2</sub>), 3.73 (m, 1H, -CH), 1.96 (s, 1H, -OH) 1.29 (s, 114H, -57CH<sub>2</sub>). The formation of abundant fragment at m/z 646 formed by  $\alpha$  cleavage indicated the position of hydroxyl group in the chain at 45. Based on the above spectral evidences the compound 2 was characterized as 11-methyl hexacontan-1, 45, 60-triol and being reported first time by us.

### Compound 3

Octaeicosandoate (3) EIMS m/z (% intensity) M<sup>+</sup> 454(10.0), 439(10.2), 426(5.0), 417(9.8), 416(14.0), 409(9.8), 392(11.2), 365(18.9), 363(32.2), 361(24.0), 343(5.0), 336(28.0), 317(45.5), 314(61.4), 292(18.8), 289(100.0), 287(78.0), 264(30.0), 262(76.2), 260(56.0), 242(29.0), 227(13.0), 215(60.2), 213(95.0), 205(8.2), 188(16.5), 186(21.0). C<sub>28</sub>H<sub>54</sub>O<sub>4</sub>, (25 mg CDCl<sub>3</sub>; MeOH) m.p. 210 °C. Isolated from hexane: benzene (3:7, v/v) fraction, TLC hexane: benzene (9:1, v/v) as solvent system, it showed single clear spot. IR (KBr) tmax: 2923, 2853, 1730, 1638, 1384, 1066, and 831, 668 cm<sup>-1</sup>. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, TMS):  $\delta$  1.43 (br., 6H, -2CH<sub>3</sub>, J=7.5 Hz), 4.25 (t, CH<sub>2</sub>OCO, J = 8 Hz), 2.54 (t, 2H, -CH<sub>2</sub>OCO. J = 8Hz), 1.25 (s, 44H, 22CH<sub>2</sub>).

### Compound 4

22,23-dihydro-5-stigmastene-3- $\beta$ -D-glucopyranoside (4) FABMS m/z (% intensity) 414(1.22) (M<sup>+</sup>), 398(50.0), 396(42.4), 384(20.0), 307(42.0), 289(25.4), 279(10.1), 273(5.01), 215(5.2), 154(96.6), 149(56.5), 137(92.3), 138(92.4), 119(30.0), 107(48.6), 91(64.5), 83(48.0). C<sub>35</sub>H<sub>60</sub>O<sub>6</sub>, (aglycone C<sub>29</sub>H<sub>50</sub>O), (30 mg Pyridine), mp 255 °C. Isolated from EtOAc: MeOH (1:1v/v) fraction of ethanolic extract. On TLC examination it showed a single homogenous spot using MeOH: CHCl<sub>3</sub> (2:8, v/v). IR (KBr) tmax: 3439, 2974, 2841, 1633, 1458, 1384, 1077, 1048 and 468 cm<sup>-1</sup>. <sup>1</sup>H NMR (300 MHz, Pyridine, TMS): 85.25 (d, 1H,-CH=C), 83.95 (m. 1H, C-3), 80.66 (s, 3H,Me-18), 80.93 (s, 3H, Me-19), 81.06 (d, 3H, J=7Hz, Me-21), 80.84 (d, 3H, J=6.5Hz, Me-26), 80.88 (d, 3H, J=6.5 Hz, Me-27) and 80.99 (d, 3H, J=7.5 Hz, Me-29). <sup>13</sup>C NMR spectrum, 5 MHz, Pyridine, ppm): 140.5, 36.5, 42.12 (-C), 78.2, 121.5, 31.80, 49.99, 56.4, 55.8, 45.6, 36.0, 29.1 (-CH<sub>2</sub>), 11.7, 18.8, 18.6, 19.6, 19.0, 11.7 (-CH<sub>3</sub>) and 102.2, 74.9, 78.1. 71.3, 78.2 and 62.5 ppm (glucose of compound 4).

### Compound 5

[→ 3- $\alpha$ -L-rhm- (1→] <sub>7</sub> (5) FABMS m/z (% intensity): M<sup>+</sup>1208(1.9), 1170(0.8), 1127(2.0), 1042(3.2), 1000.3(2.0), 958(3.21), 873(4.8), 788(8.2), 703(8.2), 618(8.2), 533(22.0), 448(21.1), 363(24.8), 147(64.2), 147(64.2), 107(100). C<sub>42</sub>H<sub>72</sub>O<sub>29</sub>, (30 mg methanol), mp 300 °C. Isolated from EtOAc:MeOH (1:1 v/v) fraction of ethanolic extract. On TLC examination it showed a single homogenous spot using CHCl<sub>3</sub>: MeOH (4:6, v/v). IR (KBr) tmax: 3470, 2940, 2892, 2361, 1633, 1430, 1384, 1048 and 1077 cm<sup>-1</sup>. <sup>1</sup>H NMR (300 MHz, MeOH, TMS): 4.84, 3.98, 3.64, 3.71, 4.00 and 1.35. <sup>13</sup>C NMR spectrum (75 MHz, MeOH, ppm): 104.2, 71.6, 83.6, 73.9, 74.1 and 18.2. Finally the molecule is characterized as [→ 3- $\alpha$ -L-rhm- (1→] <sub>7</sub> is being reported first time by us from this plant.

### Compound 6

7-hydroxy-8-(3"-methylene acetoxy-1"-oxo butenyl)-6'-6' dimethyl pyrano (2', 3':5, 6) coumarin (6) FABMS m/z (%)

intensity): M<sup>+</sup> 421(1.9), 396(10.8), 378(2.0), 364(3.2), 329(2.0), 307(12.2), 299(25.8), 283(11.2), 261(8.2), 242(8.2), 228(22.0), 176(41.1), 169(20.8), 154(64.2), 136(61.2), 121(5.00) 107(6.0), 105(5.0). C<sub>22</sub>H<sub>22</sub>O<sub>7</sub>, (30 mg methanol), mp 250 °C. Isolated from MeOH: EtOAc (9:1 v/v) fraction of ethanolic extract. On TLC examination it showed a single spot using CHCl<sub>3</sub>: MeOH (4:6, v/v). IR (KBr) tmax: 3464, 2360, 1654, 1412, 1383, 1177 and 1051 837, 463 cm<sup>-1</sup>. <sup>1</sup>H NMR Spectrum (300 MHz, CDCl<sub>3</sub>,δ): 6.38, 7.79 (d, d H-3 and H-4 protons) 12.80 (-OH). 1.60, 1.62 (s) and 5.48 and 7.04 (d), 2.01 and 4.01(s, H8) 5.36, 5.34 (d), 4.20, 4.36 (d), 2.13 (s). <sup>13</sup>C NMR spectrum (75 MHz, CDCl<sub>3</sub>, ppm): 159.7, 114.0, 138.0, 162.6, 116, 128, 28.45, 27.8, 143, 114, 20.2, 210.0, 73.7, 170.1 and 20 ppm.

## RESULT AND DISCUSSION

The natural compounds were identified mainly by their IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR and Mass spectrometry analysis including a comparison with the literature data. The mass spectrum Octatricontane 1 indicated the molecular ion peak at m/z 534 suggesting its molecular formula C<sub>38</sub>H<sub>78</sub>. IR spectrum showed absorption bands for long chain aliphatic hydrocarbon. The long chain aliphatic nature of compound was showed by 2956, 2918, 1455, 1378, 1122, 1063 and 762–719 cm<sup>-1</sup>. <sup>1</sup>HNMR spectrum showed triplet (J=7.5Hz) at  $\delta$  0.88 for the six protons of terminal methyl groups. The rest of the methylene protons merged into a singlet at  $\delta$  1.25 (Mc Lafferty, 1994). Thus on the basis of the above evidences the compound is identified as Octatricontane and being reported first time by us.

Table.1 <sup>1</sup>H NMR and <sup>13</sup>C NMR data of nucleus of compound 5 → 3- $\alpha$ -L-rhm- (1→)

Pro.No.	<sup>1</sup> H	<sup>13</sup> C
H-1	4.84	104.2
H-2	3.98	71.6
H-3	3.64	83.6
H-4	3.71	73.9
H-5	4.00	74.1
-CH <sub>3</sub>	1.35	18.2

Table no.2. <sup>1</sup>H NMR and <sup>13</sup>C NMR data of compound 6

Pro.No.	<sup>1</sup> H	<sup>13</sup> C
2		159.7
3	6.38,d	114.0
4	7.79, d	138.0
4a		99.98
5		156.0
6		104.2
7		162.6
8		104.8
8a		158.7
4'	7.04, d	116.0
5'	5.48, d	128.0
6'		86.0
7'	1.61, s	27.8
8'	1.62, s	28.4
1"		210.8
2"	4.01, d	49.1
3"		143.0
4"	5.36, 5.34,br. S	114.0
5"	2.01, s	20.2
6"	4.28 dd, 4.23 dd	73.7
-CO	2.13	170.0
-CH <sub>3</sub>	12.80	205.0
-OH		

The mass spectral analysis of 11-methyl hexacontan-1, 45, 60-triol 2 gave the molecular formula as  $C_{60}H_{122}O_3$ . IR spectrum showed bands at  $3459\text{ cm}^{-1}$  for the presence of hydroxyl group. Bands at  $2917$ ,  $2849$  and  $1471\text{ cm}^{-1}$  were due to  $-\text{CH}$  stretching and bending vibrations, where as bands at  $1020$ , and  $730$ - $719\text{ cm}^{-1}$  revealed the long chain aliphatic nature of the molecule. Thus the IR spectrum indicated the compound may be a long chain aliphatic alcohol (Chitti, 1993; Ripperger, 1992).  $^1\text{H}$  NMR spectrum showed multiplets at  $\delta$   $3.51$  and  $3.63$  for the four terminal methylene protons of methyl alcohol and a multiplet at  $\delta$   $3.73$  was due to carbinolic proton. A broad singlet at  $\delta$   $0.98$  for the three methyl protons present in side chain in the molecule.

The hydroxyl proton resonated at  $\delta$   $1.96$  as a singlet for three protons. The methylenes  $\beta$  to  $-\text{OH}$  group resonated at  $\delta$   $1.60$  as broad peak. The rest of the methylene protons merged into a singlet at  $\delta$   $1.29$  (Filchet et al., 2008; Igarashi et al., 2000). Based on the above spectral evidences the compound 2 was characterized as 11-methyl hexacontan-1, 45, 60-triol and being reported first time by us. The mass spectrum of Octaeicosadioate 3 indicated the molecular ion peak at  $m/z$   $454$  suggesting its molecular formula  $C_{28}H_{54}O_4$ . IR spectrum showed strong  $1730\text{ cm}^{-1}$  was due to the ester group. Band at  $2923$ ,  $2853\text{ cm}^{-1}$  for the  $-\text{CH}$  stretching vibration. Bands at  $1730\text{ cm}^{-1}$  for the presence of the ester group in the molecule. Other bands at  $1638$ ,  $1384$ ,  $1066$ ,  $831$ ,  $668\text{ cm}^{-1}$  confirm the aliphatic ester nature of the compound (Manjul et al., 2004; Mc Lafferty, 1994; Chitti, 1993; Ripperger et al., 1992). The  $^1\text{H}$  NMR showed a broad peak at  $\delta$   $1.43$  for three protons of terminal methyl adjacent to ester group and the methylene resonated at  $\delta$   $2.54$ . Two triplets at  $\delta$   $4.25$  and  $2.54$  were assigned to methylene ( $\text{CH}_2\text{OCO}$ ,  $\text{CH}_2\text{COO}$ ) protons adjacent to ester group. The peak at  $\delta$   $1.46$  was assigned to methylene protons  $\beta$  to ester group. Remaining methylene protons were resonated at  $\delta$   $1.37$  as an intense singlet (Mc Lafferty, 1994; Chitti, 1993). Based on the above spectral evidences the compound 3 was characterized as Octaeicosadioate and being reported first time by us.

The unknown natural compound glucosylated  $\beta$ -sitosterol 4 was identified as 22, 23-dihydro-5-stigmastene-3- $\beta$ -D-glucopyranoside by IR,  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR and mass spectral analysis. The molecular ion peak for the aglycone was observed at  $m/z$   $414$  and the molecular formula was  $C_{29}H_{50}O$ . The fragmentation was typically that of sitosterol molecule. The diagnostically important peaks were obtained at  $m/z$   $398$ ,  $396$ ,  $384$ ,  $307$ ,  $273$ ,  $255$  and  $213$  (255-ring D-fission) (Naqvi, 1973; Osman et al., 1975). These fragments suggested the aglycone of compound 4 to be a C-29 sterol with one double bond, one hydroxyl group and a C-10 saturated side chain. The IR spectrum revealed the presence of hydroxyl group in the molecule, (as broad band in the region  $3439\text{ cm}^{-1}$  and bands at  $1048$  and  $1077\text{ cm}^{-1}$ ). The bands at  $2974$ ,  $2892$ ,  $2841$  and  $1458\text{ cm}^{-1}$  were due to  $-\text{CH}$  stretching and bending vibration. A band at  $1633\text{ cm}^{-1}$  indicated unsaturation in the molecule. Band at  $1384\text{ cm}^{-1}$  revealed the presence of isopropyl group in the molecule. Thus the IR spectrum gave evidence that the molecule may be a steroid or terpenoidal type (Bellamy, 1984; Silverstein, 1984). The NMR spectrum indicated the molecule to be a steroid at glycoside. The complexity of the signals in the region  $83.9$  to  $5.2$  indicated the presence of one or more sugar moieties in the compound. One proton doublet at  $\delta$   $5.34$  was attributed to the double bond at C-5 –C-6

position in the ring ‘B’ of the steroidal nucleus (Greca, 1990). There was no unsaturation observed in the side chain. A multiplet centered at  $\delta$   $3.9$  was attributed to carbinolic proton at C-3 and the deshielding was characterized for the glucosylation of the steroidal nucleus at C-3 position. The singlet at  $\delta$   $0.65$  and at  $0.93$  was attributed to the angular methyls at C-18 and C-19 position. The rest of the four secondary methyls at position C-21, C-26, C-27, C-29 appeared as doublet at  $\delta$   $1.06$ ,  $0.84$ ,  $0.88$ ,  $0.97$  respectively. The presence of one sugar was observed, as only one anomeric proton was appeared in the spectrum at  $\delta$   $5.04$  ( $J=7.6\text{Hz}$ ) (Alembert et al., 2002). The sugar attached at C-3 was characterized to be glucose by acid hydrolysis followed by paper chromatography. The protons of sugar resonated at  $\delta$   $5.04$ ,  $4.05$ ,  $4.32$ ,  $4.28$ ,  $3.98$  and  $4.40$  ( $J=2.5$ ,  $11.9\text{ Hz}$ ). The PMR spectra clearly indicated the aglycone of AO-R to be a sitosterol type with a glucose unit attached at C-3 position. The  $^{13}\text{C}$  NMR spectrum revealed the aglycone to be a C-29 carbon skeleton with the presence of one double bond at  $121.6$  and  $140.9\text{ ppm}$ . The deshielding observed for the C-3 carbon ( $78.1\text{ ppm}$ ) was justified for the place of glycosilation at this position<sup>17</sup>. The chemical shift values of C-2 ( $31.8\text{ ppm}$ ) C-3 ( $78.1\text{ ppm}$ ) and C-4 ( $29.8\text{ ppm}$ ) revealed the presence of a  $\beta$ -oriented glucosyl moiety at C-3 position, by comparison with the same signals of aglycone<sup>10</sup>. The  $^{13}\text{C}$  NMR values are listed in the table no.1 Finally the molecule is characterized as 22, 23-dihydro-5-stigmastene-3- $\beta$ -D-glucopyranoside is being reported first time by us from this plant.

The mass spectrum of compound 5 indicated the molecular ion peak at  $m/z$   $1209$  suggesting its molecular formula  $C_{42}H_{72}O_{29}$ . The molecular ion peak at  $m/z$   $1209$  [ $M^+ 2\text{Na}^+$ ] showed the presence of seven rhmnopyranosyl units. The mass spectrum reveals abundant fragments at the regular interval of mass  $169$  at  $m/z$   $1039$ ,  $870$ ,  $701$ ,  $532$  by the loss of  $363$  (two units). Thus it indicates that the 7-rhmnpopyranose units linked to each other with a glycosidic linkage at 1 and 3 position forming 7 units of  $\rightarrow 3\text{-}\alpha\text{-L-rhm-}$  ( $1\rightarrow$ <sup>18</sup>). IR spectrum showed a strong absorption band at  $3470\text{ cm}^{-1}$  showed the presence of  $-\text{OH}$  group. The characteristic stretching and bending vibrations were obtained at  $2940$ ,  $1430$  and  $1384\text{ cm}^{-1}$  due to  $-\text{CH}$ ,  $\text{CH}_2$  and  $-\text{CH}_3$  moieties. A band at  $1076\text{ cm}^{-1}$  was due to C-O-C linkage in the molecule (Dobson, 1999).

The  $^1\text{H}$  NMR spectrum showed characteristic signals of an oligosaccharide. The NMR spectrum reveals the presence of oligosaccharide with an anomeric proton at  $\delta$   $4.84$ , identified it as the rahmnopyranosyl (Voutquinne et al., 2005). The signal at  $\delta$   $4.02$  for the H-4 indicates the equatorial position of C-4. The rhmnose methyl resonated at  $\delta$   $1.35$  and the other signals are being given in table no.-2.  $^{13}\text{C}$  NMR showed peak at  $104.2\text{ ppm}$  assigned to anomeric carbon. The signal at  $83.2$  was deshielded, showed the presence of interglycosidic linkage. The peak at  $18.2\text{ ppm}$  is due to rhmnose methyl. The assignment of  $^{13}\text{C}$  is shown in table no.-2 (Agrawal, 1992). Finally the molecule is characterized as  $[\rightarrow 3\text{-}\alpha\text{-L-rhm-}$  ( $1\rightarrow$ )<sub>7</sub> is being reported first time by us from this plant. The mass spectrum of compound 6 indicated the molecular ion peak at  $m/z$   $398$  suggesting its molecular formula  $C_{22}H_{22}O_7$ . The IR spectrum exhibited the characteristic signal of carbonyl ( $1745\text{ cm}^{-1}$ ), unsaturation ( $1612\text{ cm}^{-1}$ ) and hydroxyl ( $3464\text{ cm}^{-1}$ ) functionalities (Negi et al., 2005). The absorption band for acetylated group was showed by  $1654\text{ cm}^{-1}$ . The isopropyl group was showed by signal at  $1383\text{ cm}^{-1}$  and C-O-C linkage at

1051 cm<sup>-1</sup>. The <sup>1</sup>H NMR spectral data showed a characteristic signals as of a pair of AB type doublets at δ 6.38 and 7.79 which was assigned to H-3 and H-4 protons of a coumarin nucleus. It showed a chelated hydroxyl proton at δ 12.80 as singlet assigned to 7-OH. The two *gem* dimethyl singlet at δ 1.60 and 1.62 and at δ 5.48 and 7.04 as doublets were assigned to the presence of chromene ring substituted at 5 and 6 position (Ito et al., 2005). The presence of one isopropyl acyl group at the H-8 position was confirmed by the signals at δ 2.01 and 4.01 in addition to doublets at δ 5.36 and 5.34 revealed the presence of unsaturated protons. The presence of doublets at δ 4.20 and 4.36 in addition to the singlet at δ 2.13 for the acetylated methyl suggest the presence of methylene acetoxy moiety present at H-2" position. The absence of signal at H-1" and presence of chelated hydroxyl group at H-7 position suggest the presence of oxo or keto group at H-1" position<sup>25</sup>. The <sup>13</sup>C NMR spectrum confirmed the presence of structure containing a 7- oxygenated coumarin nucleus.

The signal at 159.7 ppm was assigned to the lactonic carbonyl at C-2. The signal at 114.0 and 138.0 ppm were assigned to C-3 and C-4 position respectively. The deshielded signal at 162.6 ppm was assigned to chelated hydroxyl present at C-7 position. The presence of 6'-6"-dimethyl chromene ring placed at C-5 and C-6 position in the basic coumarin nucleus was assigned at 156.0 and 104.2 ppm respectively (Mahidol et al., 2002). The signals at 116 and 128 ppm were assigned to C-4' and C-5' of the chromene ring. The *gem*- dimethyl signals were observed at 28.45 and 27.8 ppm which assigned to C-7' and C-8' position. The presence of acetylated isopropyl group was confirmed by the signal at 143, 114 and 20.2 ppm were assigned to C-3", C-4" and C-5" respectively (Tosa et al., 1997; Patra et al., 1981). The presence of oxo group at C-1" position was observed at 210.0 ppm. The presence of CH<sub>2</sub>OAc group was confirmed at position C-2" due to signal at 73.7, 170.1 and 20 ppm. This data confirmed the presence of side chain at C-8 position as 1-oxo, 2-methylene acetoxy-3-methyl-3butenyl moiety (Tosun et al., 2005). Rest of the carbon signals were given in the table no.2. Finally on the basis of above evidences the molecule is characterized as 7-hydroxy-8-(3"-methylene acetoxy-1"-oxo butenyl)-6'-6"-dimethyl pyrano(2', 3':5, 6)-coumarin being reported first time by us from this plant.

## Conclusion

From the survey of the literature to the best of our knowledge all the three compounds were novel and being reported first time by us from *A. odoratissimus* (leaves) and further examination of the constituents of this plant is currently in progress.

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