

**CHEMICAL CONSTITUENTS FROM THE AERIAL PARTS OF *ARTEMISIA MARITIMA* L.**Irshad Ahmad Lone<sup>1,3</sup>, Mohammad Ali<sup>2\*</sup>, M. Sarwar Alam<sup>1</sup>, Showkat Rasool Mir<sup>2</sup> and Shahnaz Sultana<sup>2,4</sup><sup>1</sup>Department of Chemistry, School of Chemical and Life Sciences, Jamia Hamdard (Hamdard University), New Delhi – 110 062, India.<sup>2</sup>Phytochemistry Research Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard (Hamdard University), New Delhi - 110 062, India.<sup>3</sup>Present Address: Department of Chemistry, Faculty of Science, Govt. Degree College, Baramulla-Kupwara National Highway, Handwara-193002, Jammu & Kashmir, India.<sup>4</sup>Present Address: College of Pharmacy, Jazan University, Jazan, Saudi Arabia.**\*Corresponding Author: Mohammad Ali**

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**ABSTRACT**

*Artemisia maritima* L. (family Asteraceae) is a stout, slender, much branched shrub. It is used to treat ague, inflammation, intermittent fever, griping, jaundice, ophthalmia, stomach ache, toothache and scorpion sting. The plant exhibits several pharmaceutical properties. The present study was designed to isolate and characterize chemical constituents from the aerial parts of *A. maritima*. Phytochemical investigations of a defatted ethanolic extract of aerial parts resulted in the isolation of a naphthalene diol identified as 8-ethoxynaphthalene-1,2-diol (**1**), a phenyl alkene benzyl characterized as 19,27-dimethyl tetratriacont-(Z)-7-ene (**2**), a phenolic derivative, viz., (Z)-4-(hexa-1', 3'-dienyl) pyrogallol (**3**), a sesamol piperonylic acid derivative viz., 3,4-methylenedioxy phenol 5, 2'-methylene 3',4'-methylenedioxy benzoic acid (**4**) and a biflavanone diglycoside recognized as 7-methoxy-3',4'-dihydroxyflavanonyl-5-oxy-(5→4''')-5'',3'''-dihydroxy-7''-methoxyflavanone-4'-α-D-lucopyranosyl-(6a→1b)-α-D-rhamnopyranoside (**5**). All these chemical constituents are reported for the first time from this plant.

GC-MS analysis of a petroleum ether fraction of the alcoholic extract of the aerial parts showed the presence of three aliphatic diols, eight aliphatic triols and one monohydroxy alcohol. The major constituents detected were *n*-hexatriacont-18-en-17-ol (12.7 %), *n*-cosan-1,4,11-triol (12.4 %), *n*-cosan-1,6,18-triol (10.8%) and *n*-cosan-1,10,17-triol (10.7 %). Two alcoholic constituents detected in small amounts were *n*-henecosan-1,7-diol (2.7 %) and *n*-cosan-1,5,12-triol (2.6 %). Except *n*-hexatriacont-18-en-17-ol, all the alcoholic constituents were saturated in nature.

**KEYWORDS:** *Artemisia maritima*, aerial parts, ethanolic extract, phytoconstituents, isolation, characterization.**INTRODUCTION**

*Artemisia maritima* L., syn. *A. pseudogallica* (Rouy) A.W.Hill, *A. salina* Willd. (family Asteraceae), known as sea wormwood and old woman, is a native to European countries including France, the United Kingdom, Italy, Beldium, Germany, Denmark, Sweden, Bulgaria and Russia. The species is reported from north-western India from Kumaon to Kashmir.<sup>[1]</sup> It is a shrub with stout, branched rootstock; stems up to 1 m high, slender, much branched; leaves white, 2-pinnatisect, linear, small, with numerous segments; flower heads homogamous, numerous ellipsoid, oblong or ovoid, in spike-shaped clusters in the axil of small linear leaf; receptacles are naked.<sup>[1,2]</sup> The plant is alexiteric, antiperiodic, aromatic, febrifuge, laxative, bitter tonic and vulnerary, used to treat ague, inflammation, intermittent fever, griping, jaundice, ophthalmia,

stomach ache, toothache, scorpion sting. The plant is poisonous if taken in large quantities. The seeds are anthelmintic, aphrodisiac, appetiser, bitter, stomachic and thermogenic, useful to cure abdominal pain, indigestion and mucus diarrhoea. Powder of the leaves and flowers is ingested with castor oil to expel round worms. The flower heads juice is lapped to counteract snake bite and scorpion sting.<sup>[3,4]</sup> The plant exhibits several pharmaceutical properties, including anthelmintic, antimicrobial, anti-inflammatory, bronchodilatory and spasmolytic activities.<sup>[5-7]</sup>

The plant contained artemin, santonin, 1-keto-6β, 7α, 1βH-selin-4(5)-en-6, 12-olide, vulgarin, maritim and martimin.<sup>[4,8,9]</sup> The plant essential oils were composed of 1,8-cineole (44.22%), camphor (9.16%) and borneol (10.94%), chrysanthenone, camphor,

borneol, bornyl acetate, terpinen-4-ol, sabinene and germacrene D.<sup>[6, 10-14]</sup> Keeping in view the various therapeutic values of the plants and the development of ecofriendly, biodegradable and safer herbal preparations, aerial parts of *A. maritima* collected from Kashmir valley were extracted with ethanol. The concentrated ethanolic extract was used for the isolation of chemical constituents. Structures of the isolated phytoconstituents were established using detailed spectral studies.

## MATERIALS AND METHODS

The protocols of all methodologies (procedures, experimental designs and analysis assays) were adopted from the earlier published work.<sup>[15,16]</sup>

### General procedures

The melting points were measured by means of a thermoelectrically operated Perfit apparatus and are uncorrected. UV spectra were recorded on Lambda Bio 20 spectrophotometer (Perkin Elmer, Schwerzenbach, Switzerland) in methanol. The IR spectra were recorded on Jasco FT/IR-5000 Spectrometer (FTS 135, Hong Kong) using KBr pellet. The <sup>1</sup>H (400 MHz) and <sup>13</sup>C (100 MHz) NMR spectra were scanned on Bruker DRX Spectrometer (Rheinstetten, 2 Germany) using CDCl<sub>3</sub> or DMSO-d<sub>6</sub> as a solvent and TMS (Fluka analytical, Sigma-Aldrich, Netherland) as an internal standard. Mass spectrometric detection was carried out on (Q-TOF-ESI) (Waters Corp., UK) instrument with a +ve FAB MODE technique.

Column chromatography was performed on silica gel (Qualigens, Mumbai, India) with 60-120 mesh particle size and petroleum ether, chloroform, methanol and other solvents used were purchased from Merck Specialties (E. Merck Pvt. Ltd., New Delhi, India). The purity of the isolated compounds was checked on precoated TLC plates with Silica gel 60 F<sub>254</sub> (Merck, 0.25 mm) and the spots were visualized by exposure to iodine vapours or under UV radiations and spraying with ceric sulphate solution.

### Collection of plant material

The aerial parts of *Artemisia maritima* were collected from Kultura, Kupwara, Kashmir valley and authenticated by Dr. H.B. Singh, Scientist and Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (CSIR), New Delhi. A voucher specimen is preserved in the herbarium of the Phytochemistry Research Laboratory, Jamia Hamdard, New Delhi.

### Extraction and isolation

The powdered shade-dried aerial parts of the plant (2.0 kg) were extracted exhaustively with ethanol (95%) in a Soxhlet apparatus. The ethanolic extract was concentrated to dryness under reduced pressure in a rotary evaporator to yield light brown mass (516.0 g). A portion of this extract (500.0 g) was treated with petroleum ether to dissolve fatty materials (95.3 g). This

fraction was dried under reduced pressure to yield a dark green semi-solid mass. All these extracts were stored at 4 °C until use.

### Isolation of chemical constituents from the defatted ethanolic fraction

The defatted dark brown ethanolic mass (350 g) was dissolved in a minimum quantity of methanol and adsorbed on silica gel (60-120 mesh) for preparation of a slurry. It was dried in air and chromatographed over silica gel for column packed in petroleum ether. The column was eluted with petroleum ether, petroleum ether - chloroform (9:1, 3:1, 1:1, 1:3, v/v), chloroform and chloroform - methanol (99:1, 49:1, 19:5, 9:1, 17:3, 4:1 7:3, 1:1, v/v). Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R<sub>f</sub> values were combined and crystallized with solvents. The isolated compounds were recrystallized to get pure compounds.

### 8-Ethoxynaphthalene-1,2-diol (1)

Elution of the column with petroleum ether - chloroform (1:1) mixture afforded light brown crystals of **1**, recrystallized from acetone; 93 mg, m. p. 188 - 189 °C; R<sub>f</sub> 0.54 (ethyl acetate - toluene, 3:17); UV λ<sub>max</sub> (MeOH): 248, 305 nm (log ε 2.6, 5.8); IR ν<sub>max</sub> (KBr): 3510, 3412, 2927, 2850, 1560, 1458, 1385, 1279, 994 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.96 (1H, d, J = 9.6 Hz, H-3), 7.58 (1H, dd, J = 8.4, 1.6 Hz, H-7), 6.91 (1H, d, J = 8.4 Hz, H-6), 6.88 (1H, d, J = 1.6 Hz, H-9), 6.27 (1H, d, J = 9.6 Hz, H-4), 4.06 (2H, m, H<sub>2</sub>-1'), 1.33 (3H, t, J = 6.8 Hz, H<sub>3</sub>-2'); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 160.26 (C-1), 161.64 (C-2), 144.24 (C-3), 112.55 (C-4), 111.96 (C-5), 100.94 (C-6), 129.05 (C-7), 155.84 (C-8), 129.36 (C-9), 112.14 (C-10), 63.91 (C-1'), 14.33 (C-2'); FAB MS m/z (rel. int.): 204 [M]<sup>+</sup> (C<sub>12</sub>H<sub>12</sub>O<sub>3</sub>) (15.3), 189 (100), 175 (23.8).

### Benzyl 19,27-dimethyl tetratriacont-(Z)-7-ene (2)

Elution of the column with petroleum ether - chloroform (1:3) gave pale yellow crystals of **2**, yield 76.4 mg, R<sub>f</sub> 0.93 (petroleum ether - chloroform, 9:1), m. p. 127-129 °C; UV λ<sub>max</sub> 222, 275 nm (log ε 3.1, 5.4); IR ν<sub>max</sub> (KBr): 2918, 2845, 1635, 1551, 1435, 1260, 1183, 995, 826, 722 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.47 (2H, m, H-2, H-6), 7.06 (2H, m, H-3, H-5), 6.87 (1H, m, H-4), 5.76 (1H, d, J = 6.8 Hz, H-7), 5.27 (1H, m, w<sub>1/2</sub> = 7.1 Hz, H-8), 2.06 (2H, m, H<sub>2</sub>-9), 1.51 (1H, m, H-19), 1.37 (1H, m, H-27), 1.28 (10H, brs, 5 x CH<sub>2</sub>), 1.22 (6H, brs, 3 x CH<sub>2</sub>), 1.18 (22H, brs, 11 x CH<sub>2</sub>), 1.07 (4H, m, H<sub>2</sub>-26, H<sub>2</sub>-28), 0.91 (3H, d, J = 6.1 Hz, Me-35), 0.88 (3H, d, J = 6.4 Hz, Me-36), 0.82 (3H, t, J = 6.8 Hz, Me-34); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 140.15 (C-1), 138.57 (C-2), 128.13 (C-3), 126.06 (C-4), 125.78 (C-5), 138.57 (C-6), 122.06 (C-7), 112.69 (C-8), 31.76 (C-9), 29.03 (C-10), 29.17 (C-11), 28.67 (C-12), 28.69 (C-13 to C-18), 36.42 (C-19), 28.65 (C-20 to C-25), 30.92 (C-26), 36.09 (C-27), 30.92 (C-28), 29.17 (C-29), 28.35 (C-30), 26.08 (C-31), 23.45 (C-32), 22.68 (C-33), 13.10 (C-34), 18.75 (C-35), 21.68 (C-36); +ve FAB MS m/z (rel. int.): 496 [M]<sup>+</sup> (C<sub>36</sub>H<sub>64</sub>)

(11.7), 393 (31.4), 369 (9.8), 271 (22.5), 253 (56.2), 243 (27.1), 225 (23.4), 127 (70.8), 103 (27.3), 99 (18.1).

#### 4-(Hexa-1', 3'-dienyl) pyrogallol (3)

Elution of the column with chloroform yielded light brown crystals of **3**, recrystallized from acetone; 86 mg, m. p. 197 - 198 °C;  $R_f$  0.32 (diethyl ether - acetone, 1:1); UV  $\lambda_{max}$  (MeOH): 210, 266 nm ( $\log \epsilon$  4.1, 2.8); IR  $\nu_{max}$  (KBr): 3515, 3470, 3250, 2912, 2855, 1626, 1508, 1409, 1274, 988  $cm^{-1}$ ;  $^1H$  NMR ( $CDCl_3$ ):  $\delta$  7.93 (1H, d,  $J = 9.6$  Hz, H-5), 7.52 (1H, d,  $J = 9.4$  Hz, H-6), 6.71 (1H, d,  $J = 2.1$  Hz, H-1'), 6.23 (1H, dd,  $J = 2.0$ , 1.8 Hz, H-2'), 6.03 (1H, dd,  $J = 1.8$ , 5.6 Hz, H-3'), 5.88 (1H, m,  $w_{1/2} = 7.2$  Hz, H-4'), 2.51 (2H, m, H<sub>2</sub>-5'), 1.06 (3H, t,  $J = 6.4$  Hz, H<sub>3</sub>-6');  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  160.41 (C-1), 161.26 (C-2), 155.46 (C-3), 144.48 (C-4), 129.63 (C-5), 113.07 (C-6), 129.68 (C-1'), 125.21 (C-2'), 123.15 (C-3'), 111.36 (C-4'), 55.01 (C-5'), 18.51 (C-6'); FAB MS  $m/z$  (rel. int.): 206 [ $M$ ]<sup>+</sup> ( $C_{12}H_{14}O_3$ ) (2.1), 191 (21.8), 125 (14.6), 81 (18.3).

#### Sesamol 5,2'-methylene piperonylic acid (4)

Elution of the column with chloroform - methanol (49:1) mixture furnished yellow crystals of **4**, recrystallized from acetone; 98.4 mg, m. p. 233 - 235 °C;  $R_f$  0.56 (petroleum ether - acetone, 9:1); UV  $\lambda_{max}$  (MeOH): 248, 277 nm ( $\log \epsilon$  3.2, 5.6); IR  $\nu_{max}$  (KBr): 3450, 3275, 2921, 2853, 1680, 1635, 1569, 1432, 1315, 1150, 991  $cm^{-1}$ ;  $^1H$  NMR ( $CDCl_3$ ):  $\delta$  6.44 (1H, d,  $J = 1.6$  Hz, H-2), 6.14 (1H, d,  $J = 1.6$  Hz, H-6), 3.79 (2H, brs, O-CH<sub>2</sub>-O), 2.44 (2H, brs, H<sub>2</sub>-7), 7.98 (1H, d,  $J = 8.8$  Hz, H-5'), 7.05 (1H, d,  $J = 8.8$  Hz, H-6'), 3.30 (2H, brs, O-CH<sub>2</sub>-O);  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  164.18 (C-1), 103.72 (C-2), 163.25 (C-3), 162.27 (C-4), 128.30 (C-5), 103.49 (C-6), 55.52 (C-7), 94.01 (O-CH<sub>2</sub>-O), 122.77 (C-1'), 128.30 (C-2'), 161.41 (C-3'), 157.30 (C-4'), 114.54 (C-5'), 114.54 (C-6'), 181.76 (C-7'), 98.85 (O-CH<sub>2</sub>-O); FAB MS  $m/z$  (rel. int.): 316 [ $M$ ]<sup>+</sup> ( $C_{16}H_{12}O_7$ ) (1.6), 179 (5.3), 137 (9.8), 135 (77.1), 121 (21.6), 107 (23.9).

#### Bis-(5,3',4'-trihydroxy-7-methoxyflavanone)-4'-O-glucorhamnoside (5)

Elution of column with chloroform-methanol (9:1) mixture produced green crystals of **5**, yield 94.6 mg, m. p. 278 - 280 °C,  $R_f$  0.61 (ethyl acetate - methanol - *n*-butanol, 2:3:10); UV  $\lambda_{max}$  (MeOH): 284, 329 nm ( $\log \epsilon$  4.1, 1.5);  $\lambda_{max}$  (MeOH +  $AlCl_3$ ): 310, 333 nm;  $\lambda_{max}$  (MeOH +  $AlCl_3$  + HCl): 308, 333 nm; IR  $\nu_{max}$  (KBr): 3510, 3460, 3350, 3225, 2920, 2845, 1690, 1645, 1525, 1425, 1388, 1250, 1180, 1095  $cm^{-1}$ ;  $^1H$  NMR ( $DMSO-d_6$ ):  $\delta$  7.03 (1H, d,  $J = 2.8$  Hz, H-2'), 6.95 (1H, d,  $J = 8.4$  Hz, H-5'), 6.91 (1H, d,  $J = 8.4$  Hz, H-6'), 6.21 (1H, d,  $J = 2.0$  Hz, H-8), 6.19 (1H, d,  $J = 2.0$  Hz, H-6), 5.45 (1H, dd,  $J = 2.8$ , 13.2 Hz, H-2), 3.09 (1H, dd,  $J = 2.8$ , 17.1 Hz, H<sub>2</sub>-3ax), 2.82 (1H, dd,  $J = 2.8$ , 17.1 Hz, H<sub>2</sub>-3eq), 6.28 (1H, d,  $J = 3.0$  Hz, H-8''), 6.24 (1H, d,  $J = 3.0$  Hz, H-6''), 5.35 (1H, dd,  $J = 2.8$ , 12.8 Hz, H-2''), 3.81 (1H, dd,  $J = 11.2$ , 12.8 Hz, H<sub>2</sub>-3''ax), 2.85 (1H, dd,  $J = 2.8$ , 14.4 Hz, H<sub>2</sub>-3''eq), 7.01 (1H, d,  $J = 3.0$  Hz, H-2'''), 6.97 (1H, d,  $J = 7.9$  Hz, H-5'''), 6.93 (1H, dd,  $J = 3.0$ , 7.9 Hz, H-6''').

5.39 (1H, d,  $J = 4.8$  Hz, H-1a), 4.71 (1H, m, H-2a), 4.63 (1H, m, H-3a), 3.50 (1H, m, H-4a), 4.96 (1H, m, H-5a), 3.32 (2H, d,  $J = 7.2$  Hz, H<sub>2</sub>-6a), 5.28 (1H, d,  $J = 5.2$  Hz, H-1b), 4.78 (1H, dd,  $J = 4.8$ , 5.2 Hz, H-2b), 4.63 (1H, m, H-3b), 3.68 (1H, m, H-4b), 4.90 (1H, m, H-5b), 1.15 (3H, d,  $J = 5.0$  Hz, Me-6b), 3.80 (3H, brs, OMe), 3.83 (3H, brs, OMe);  $^{13}C$  NMR ( $DMSO-d_6$ ):  $\delta$  78.41 (C-2), 48.67 (C-3), 197.08 (C-4), 163.01 (C-5), 99.34 (C-6), 165.07 (C-7), 95.49 (C-8), 162.50 (C-9), 110.05 (C-10), 130.09 (C-1'), 111.95 (C-2'), 146.37 (C-3'), 147.86 (C-4'), 114.09 (C-5'), 117.84 (C-6'), 78.39 (C-2''), 41.99 (C-3''), 197.03 (C-4''), 162.96 (C-5''), 99.31 (C-6''), 165.02 (C-7''), 95.52 (C-8''), 161.45 (C-9''), 110.05 (C-10''), 130.01 (C-1'''), 111.90 (C-2'''), 146.35 (C-3'''), 147.96 (C-4'''), 114.02 (C-5'''), 117.75 (C-6'''), 100.56 (C-1a), 72.01 (C-2a), 70.32 (C-3a), 68.28 (C-4a), 76.20 (C-5a), 65.97 (C-6a), 96.31 (C-1b), 72.92 (C-2b), 70.69 (C-3b), 69.52 (C-4b), 75.44 (C-5b), 17.81 (C-6b), 55.69 (OMe), 55.64 (OMe); +ve FAB MS  $m/z$  (rel. int.): 894 [ $M$ ]<sup>+</sup> ( $C_{44}H_{46}O_{20}$ ) (3.1), 309 (5.3), 301 (6.8), 284 (11.2), 249 (32.5), 166 (15.3), 163 (15.3), 147 (10.4).

#### GC-MS analysis of the petroleum ether fraction

The chemical characterization of the green viscous petroleum ether extract was carried out by gas chromatography coupled with mass spectroscopy (GC-MS). A Shimadzu-17A gas chromatograph interfaced with mass selective detector equipped with a DB-5 capillary column (30 m x 0.32 mm i.d.; 0.25  $\mu$ m film thickness) packed with 5% phenyl polydimethyl siloxane was used for separation of the components. Helium at a flow rate of 1.2 mL/min (constant flow mode) was used as a carrier gas. A volume of 2  $\mu$ L of sample extracts was injected in a split less mode. The injection port was set at 320 °C and temperature of oven was initially set at 70 °C for 5 minutes. The oven temperature was subsequently ramped to 205 °C at a rate of 5 °C/min for 5 minutes, 280 °C at a rate of 5 °C/min for 5 minutes and finally to 300 °C at a rate of 5 °C/min for 5 minutes. The maximum oven temperature was set at 320 °C. The mass spectrometer was operated in an electron ionization (EI) mode within the mass range of 60-900 amu with 0.6 scan times (min). The MS transfer line temperature and ion source temperature were kept at 320 °C and 350 °C, respectively, with an electron multiplier voltage of 1 KV.

#### Identification of constituents

The mass spectra were interpreted using X caliber software and the fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using the NIST, MAINLIB and REPLIB built-in libraries attached to the GC-MS instrument, the published mass spectral data and the interpretation of MS fragmentation patterns. The constituent percentages were measured based on the peak area.

## RESULTS AND DISCUSSION

Compound **1** responded positive tests for phenols, showed UV absorption maxima at 248 and 305 nm for naphthols and had IR absorption bands for hydroxyl groups ( $3510, 3412\text{ cm}^{-1}$ ), and aromaticity ( $1560\text{ cm}^{-1}$ ). Its molecular ion peak was established at  $m/z$  204 on the basis of mass and  $^{13}\text{C}$  NMR spectra consistent with a molecular formula of a naphthalene diol linked with an ethoxy group,  $\text{C}_{12}\text{H}_{12}\text{O}_3$ . The ion peaks generated at  $m/z$  189  $[\text{M} - \text{Me}]^+$  and 175  $[\text{M} - \text{C}_2\text{H}_5]^+$  supported the linkage of an ethyl function linked with the naphthol unit. The  $^1\text{H}$  NMR spectrum of **1** exhibited four one-proton deshielded doublets at  $\delta$  7.96 ( $J = 9.6\text{ Hz}$ ), 6.91 ( $J = 8.4\text{ Hz}$ ), 6.27 ( $J = 9.6\text{ Hz}$ ) and 6.88 ( $J = 1.6\text{ Hz}$ ) assigned to aromatic ortho-coupled H-3, H-6 and H-4 protons, and meta-coupled H-9, respectively, a one-proton doublet at  $\delta$  7.58 ( $J = 8.4, 1.6\text{ Hz}$ ) due to ortho-, meta-coupled H-7, a two-proton multiplet at  $\delta$  4.06 ascribed to oxymethylene  $\text{H}_2\text{-1}'$  and a three-proton triplet at  $\delta$  1.33 ( $J = 6.8\text{ Hz}$ ) accounted to primary C-2' methyl protons. The  $^{13}\text{C}$  NMR spectrum of **1** showed signals for ten aromatic carbons of naphthalene unit between  $\delta$  161.64 – 100.94, oxymethylene carbon at  $\delta$  63.91 (C-1') and methyl carbon at  $\delta$  14.33 (C-2'). The DEPT spectrum of **1** showed the presence of five methine, one methyl, five quaternary and one methylene carbons. The  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of **1** exhibited correlations of H-3 with H-4; H-4, H-6 and H-9 with H-7; and  $\text{H}_2\text{-1}'$  with  $\text{H}_3\text{-2}'$ . The HMBC spectrum of **1** displayed interactions of H-3 and H-4 with C-2; H-6, H-7, H-9 and  $\text{H}_2\text{-1}'$  with C-8; and  $\text{H}_3\text{-2}'$  with C-1'. These evidences led to formulate the structure of **1** as 8-ethoxynaphthalene-1,2-diol, a new naphthalene diol (Fig. 1).

Compound **2** showed UV absorption maximum at 275 nm for an aromatic ring and IR absorption bands for unsaturation ( $1635\text{ cm}^{-1}$ ), aromatic ring ( $1551\text{ cm}^{-1}$ ) and long aliphatic chain ( $722\text{ cm}^{-1}$ ). On the basis of mass and  $^{13}\text{C}$  NMR spectra its molecular ion peak was determined at  $m/z$  496 consistent with a molecular formula of a phenyl alkene,  $\text{C}_{36}\text{H}_{64}$ . The ion peaks arising at  $m/z$  103  $[\text{C}_8 - \text{C}_9\text{ fission}, \text{C}_6\text{H}_5\text{-CH=CH}, \text{C}_8\text{H}_7]^+$  and 393  $[\text{M} - 103]^+$  indicated that phenyl ethene was present at one of the terminal of the aliphatic chain. The ion fragments generated at  $m/z$  243  $[\text{C}_{18} - \text{C}_{19}\text{ fission}, \text{C}_{18}\text{H}_{27}]^+$ , 253  $[\text{M} - 243]^+$ , 271  $[\text{C}_{19} - \text{C}_{20}\text{ fission}, \text{C}_{20}\text{H}_{31}]^+$  and 225  $[\text{M} - 271]^+$  suggested the existence of one of the secondary methyl group at C-19. The ion fragments produced at  $m/z$  369  $[\text{C}_{26} - \text{C}_{27}\text{ fission}, \text{C}_{27}\text{H}_{45}]^+$ , 127  $[\text{M} - 369]^+$  and 99  $[\text{C}_{27} - \text{C}_{29}\text{ fission}, \text{C}_7\text{H}_{15}]^+$  supported the location of another secondary methyl group at C-27. The  $^1\text{H}$  NMR spectrum of **2** displayed two two-proton multiplets at  $\delta$  7.47 and 7.06 and a one-proton multiplet at  $\delta$  6.87 assigned to aromatic H-2, H-6, H-3, H-5 and H-4 protons, respectively. A one-proton doublet at  $\delta$  5.76 ( $J = 6.8\text{ Hz}$ ) and a one-proton multiplet at  $\delta$  5.27 with half-width of 7.1 Hz were ascribed correspondingly to cis-oriented vinylic H-7 and H-8 protons. Two one-proton multiplets at  $\delta$  1.51 and 1.37 were due to methine H-19

and H-27 protons, respectively. Two three-proton doublets at  $\delta$  0.91 ( $J = 6.1\text{ Hz}$ ) and 0.88 ( $J = 6.4\text{ Hz}$ ) and a three-proton triplet at  $\delta$  0.82 ( $J = 6.8\text{ Hz}$ ) were accounted to secondary C-35 and C-36 and primary C-34 methyl protons, respectively. The methylene protons appeared as multiplets at  $\delta$  2.06 ( $\text{H}_2\text{-9}$ ) and 1.07 ( $\text{H}_2\text{-26}$  and  $\text{H}_2\text{-28}$ ) and as singlets at  $\delta$  1.28 (10H), 1.22 (6H) and 1.18 (22H).

The  $^{13}\text{C}$  NMR spectrum of **2** exhibited signals for aromatic carbons between  $\delta$  140.15 – 125.78, vinylic carbons at  $\delta$  122.06 (C-7) and 112.69 (C-8), methyl carbons at  $\delta$  13.10 (C-34), 18.75 (C-35) and 21.68 (C-36) and other methine and methylene carbons from  $\delta$  36.42 to 22.68. The DEPT spectrum of **2** showed the presence of nine methine, three methyl, one quaternary and other methylene carbons. The  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of **2** exhibited correlations of H-3, H-5, H-6 and H-7 with H-2; H-7 and  $\text{H}_2\text{-9}$  with H-8;  $\text{H}_2\text{-18}$ ,  $\text{H}_2\text{-20}$  and  $\text{H}_3\text{-35}$  with H-19;  $\text{H}_2\text{-26}$ ,  $\text{H}_2\text{-28}$  and  $\text{H}_3\text{-36}$  with H-27; and  $\text{H}_2\text{-33}$  and  $\text{H}_2\text{-32}$  with  $\text{H}_3\text{-34}$ . The HMBC spectrum of **2** displayed interactions of H-2, H-3, H-6, H-7 and H-8 with C-1;  $\text{H}_2\text{-18}$ ,  $\text{H}_2\text{-20}$  and  $\text{H}_3\text{-35}$  with C-19;  $\text{H}_2\text{-26}$ ,  $\text{H}_2\text{-28}$  and  $\text{H}_3\text{-36}$  with C-27; and  $\text{H}_2\text{-33}$  and  $\text{H}_2\text{-32}$  with C-34. On the basis of the aforementioned spectral data analysis the structure of **2** has been elucidated as benzyl 19,27-dimethyl tetratriacont-(Z)-7-ene, a new phenyl alkene (Fig. 1).

Compound **3**,  $[\text{M}]^+$  at  $m/z$  206 ( $\text{C}_{12}\text{H}_{14}\text{O}_3$ ), gave positive tests for phenols, showed UV absorption maximum at 266 nm for aromatic compounds and had IR absorption bands for hydroxyl groups ( $3515, 3470, 3250\text{ cm}^{-1}$ ), unsaturation ( $1626\text{ cm}^{-1}$ ) and aromaticity ( $1508\text{ cm}^{-1}$ ). The ion peaks generated at  $m/z$  191  $[\text{M} - \text{Me}]^+$ , 125  $[\text{C}_4 - \text{C}_1\text{ fission}, \text{C}_6\text{H}_2(\text{OH})_3]^+$  and 81  $[\text{M} - 125]^+$  indicated the linkage of a hexa-dienyl unit with a three hydroxyphenyl (pyrogallol) unit. The  $^1\text{H}$  NMR spectrum of **3** displayed two one-proton deshielded doublets at  $\delta$  7.93 ( $J = 9.6\text{ Hz}$ ) and 7.52 ( $J = 9.4\text{ Hz}$ ) assigned to aromatic ortho-coupled H-5 and H-6 protons, respectively. A one-proton doublet at  $\delta$  6.71 ( $J = 2.1\text{ Hz}$ ), two one-proton doublets  $\delta$  6.23 ( $J = 2.0, 1.8\text{ Hz}$ ) and 6.03 ( $J = 1.8, 5.6\text{ Hz}$ ) and a one-proton multiplet at  $\delta$  5.88 with half-width of 7.2 Hz were ascribed correspondingly to cis- (Z-) oriented vinylic H-1', H-2', H-3' and H-4' protons. A two-proton multiplet at  $\delta$  2.51 was due to methylene  $\text{H}_2\text{-5}'$  linked to a vinylic carbon. A three-proton triplet at  $\delta$  1.06 ( $J = 6.4\text{ Hz}$ ) was accounted to primary C-6' methyl protons. The  $^{13}\text{C}$  NMR spectrum of **3** had signals for six aromatic and four vinylic carbons between  $\delta$  161.26 – 111.36, the methylene carbon at  $\delta$  55.01 (C-5') and methyl carbon at  $\delta$  18.51 (C-6'). The DEPT spectrum of **3** showed the presence of six methine, one methyl, four quaternary and one methylene carbons. The  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of **3** exhibited correlations of H-6 and H-1' with H-5; H-1', H-2', H-4' and  $\text{H}_2\text{-5}'$  with H-3';  $\text{H}_3\text{-6}'$  and  $\text{H}_2\text{-5}'$  with H-4'. The HMBC spectrum of **3** displayed interactions of H-5 and H-6 with C-4; H-5, H-1' and H-2' with C-1;

and H<sub>3</sub>-6' and H<sub>2</sub>-5' with C-4'. The HSQC spectrum of **3** indicated that aromatic protons H-5 ( $\delta$  7.93) and H-6 ( $\delta$  7.52) interacted with their respective carbons C-5 and C-6 signals, vinylic protons from H-1' to H-4' interacted with their relative carbon signals, methylene protons at  $\delta$  2.51 (H<sub>2</sub>-5') interacted with C-5' at  $\delta$  55.01 and methyl H<sub>3</sub>-6' protons at  $\delta$  1.06 interacted with C-6' signal at  $\delta$  18.51. On the basis of these evidences the structure of **3** was elucidated as (Z)- 4-(hexa-1', 3'-dienyl) pyrogallol, a new phenolic derivative (Fig. 1).

Compound **4**, named sesamol 5,2'-methylene piperonylic acid, [M]<sup>+</sup> at  $m/z$  316 (C<sub>16</sub>H<sub>12</sub>O<sub>7</sub>), gave positive tests for phenols, produced effervescence with sodium bicarbonate solution, showed UV absorption maximum at 277 nm for aromatic compounds and IR absorption bands for a hydroxyl group (3450 cm<sup>-1</sup>), carboxylic function (3275, 1680 cm<sup>-1</sup>) and aromaticity (1635, 1568 cm<sup>-1</sup>). The ion peaks produced at  $m/z$  137 [C<sub>5</sub> - C<sub>7</sub> fission, C<sub>7</sub>H<sub>5</sub>O<sub>3</sub>]<sup>+</sup>, 179 [M - 137, C<sub>9</sub>H<sub>7</sub>O<sub>4</sub>]<sup>+</sup>, 135 [179 - CO<sub>2</sub>]<sup>+</sup>, 121 [135 - CH<sub>2</sub>]<sup>+</sup> and 107 [121 - CH<sub>2</sub>]<sup>+</sup> showed the presence of 3,4-methylenedioxyphenol and 3', 3'-methylenedioxybenzoic acid, both linked to methylene carbon. The <sup>1</sup>H NMR spectrum of **4** displayed four one-proton deshielded doublets at  $\delta$  6.44 (J = 1.6 Hz) and 6.14 (J = 1.6 Hz), and at  $\delta$  7.98 (1H, d, J = 8.8 Hz) and 7.05 (1H, d, J = 8.8 Hz) assigned to aromatic meta-coupled H-2 and H-6, and ortho-coupled H-5' and H-6' protons, respectively. A two-proton singlet at  $\delta$  2.44 was ascribed to methylene H<sub>2</sub>-7 protons. Two two-proton singlets at  $\delta$  3.79 and 3.30 were attributed to dioxomethylene protons. The <sup>13</sup>C NMR spectrum of **4** exhibited signals for twelve aromatic carbons between  $\delta$  163.25 - 103.49, methylene carbon at  $\delta$  55.52 (C-7), dioxomethylene carbons at  $\delta$  94.01 and 98.85, and carboxylic carbon at  $\delta$  181.76 (C-7). The DEPT spectrum of **4** showed the presence of three methylene, four methine and nine quaternary carbons. The <sup>1</sup>H-<sup>1</sup>H COSY spectrum of **4** exhibited correlations of H-2 and H<sub>2</sub>-7 with H-6; and H-5' with H-6'. The HMBC spectrum of **4** displayed interactions of H-2 and O-CH<sub>2</sub> with C-3; H-6, H<sub>2</sub>-7 with C-5; and H-6' and H-5' with C-4'. The HSQC spectrum of **4** showed correlations aromatic protons H-2, H-6, H-5' and H-6' ( $\delta$  6.44, 6.14, 7.98 and 7.05) with their respective aromatic carbon signals, methylene protons at  $\delta$  2.44 (H<sub>2</sub>-7) and 3.79 and 3.30 (2 x O-CH<sub>2</sub>-O) with their corresponding carbon signals at  $\delta$  55.52 (C-7), 94.01 and 98.85. On the basis of above discussion the structure of **4** was characterized as 3,4-methylenedioxy phenol 5, 2'-methylene 3',4'-methylenedioxy benzoic acid, a new sesamol piperonylic acid derivative (Fig. 1).

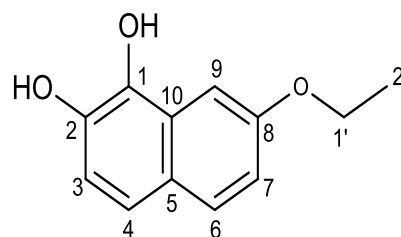
Compound **5**, named bis-(5,3',4'-trihydroxy-7-methoxyflavanone)- 4'-O-glucorhamnoside, responded positively to flavonoid and glycosidic tests and showed UV absorption maxima at 284 and 329 typical for a flavanone derivative. A bathochromic shift of band at 329 nm was not noticed on addition of aluminium

chloride and aluminium chloride with hydrochloric acid ruling out the presence of a chelated hydroxyl functions at C-4' and C-4'''. Its IR spectrum demonstrated characteristic absorptions for the conjugated carbonyl functions (1690 cm<sup>-1</sup>), aromaticity (1525 cm<sup>-1</sup>) and hydroxyl groups (3510, 3460, 3350, 3225 cm<sup>-1</sup>). On the basis of its mass and <sup>13</sup>C NMR spectra the molecular ion peak of **5** was determined at  $m/z$  894 consistent with a molecular formula of a bisflavonoid diglycoside, C<sub>44</sub>H<sub>46</sub>O<sub>20</sub>. The important ion peaks generated at  $m/z$  301 [C<sub>5</sub> - O fission, C<sub>16</sub>H<sub>13</sub>O<sub>6</sub>]<sup>+</sup>, 284 [C<sub>5</sub> - O, C<sub>4</sub>' - O fission, C<sub>16</sub>H<sub>12</sub>O<sub>5</sub>]<sup>+</sup>, 309 [M - 301 - 284, C<sub>12</sub>H<sub>21</sub>O<sub>9</sub>]<sup>+</sup>, 163 [C<sub>4</sub>' - O, C<sub>6a</sub> - O fission, C<sub>6</sub>H<sub>11</sub>O<sub>5</sub>]<sup>+</sup> and 147 [C<sub>1b</sub> - O fission, C<sub>6</sub>H<sub>11</sub>O<sub>4</sub>]<sup>+</sup> indicated the presence of two flavanone units linked each other and attachment of a gluco-rhamnoside moiety to the C-4' carbon of ring B. An ion peak produced at  $m/z$  166 [C<sub>6</sub>H<sub>2</sub>O(OMe)(OH)(C=O)]<sup>+</sup> due to retro-Diels-Alder fragmentation supported the presence of the glycosidic unit at one of the flavanone unit.

The <sup>1</sup>H NMR spectrum of **5** showed an ABX system of resonances as one-proton double doublets at  $\delta$  5.45 (J = 2.8, 13.2 Hz), 3.09 (1H, dd, J = 13.2, 17.1 Hz) and 2.82 (J = 2.8, 17.1 Hz) characteristic of oxymethine H-2 and methylene H<sub>2</sub> -2 axial and H<sub>2</sub> -2 equatorial protons, respectively, of a flavanone moiety. Another ABX system of signals as one-proton double doublets at  $\delta$  5.35 (J = 2.8, 12.8 Hz), 3.81 (J = 11.2, 12.8 Hz) and 2.85 (J = 2.8, 14.4 Hz) were assigned to oxymethine H-2'' and methylene H<sub>2</sub> -3'' axial and H<sub>2</sub> -3'' equatorial protons, respectively, of a second flavanone moiety. Four one-proton doublets at  $\delta$  7.03 (J = 2.8 Hz), 6.21 (J = 2.0 Hz), 6.19 (J = 2.0 Hz, H-6) and 6.95 (J = 8.4 Hz) and a one-proton double doublet at  $\delta$  6.91 (J = 2.8, 8.4 Hz) were ascribed to meta-coupled H-2', H-8, and H-6, ortho-coupled H-5' and ortho-, meta-coupled H-6' protons, respectively. Another deshielded signals as doublets at  $\delta$  6.28 (J = 3.0 Hz), 6.24 (J = 3.0 Hz), 7.01 (J = 3.0 Hz), 6.97 (J = 7.9 Hz) and as a double doublet at  $\delta$  6.93 (J = 3.0, 7.9 Hz) were attributed correspondingly to meta-coupled H-8'', H-6'' and H-2'', ortho-coupled and H-5''' and meta-, ortho-coupled H-6''' protons. Two one-proton doublets at  $\delta$  5.39 (J = 4.8 Hz) and 5.28 (J = 5.2 Hz) were accounted to anomeric alpha-oriented H-1a and H-1b, respectively. The oxy-methine sugar protons appeared as one-proton multiplets between  $\delta$  4.96 - 3.50. A two-proton doublet at  $\delta$  3.32 (J = 7.2 Hz) was associated with the oxymethylene H<sub>2</sub>-6a. A three-proton doublet at  $\delta$  1.15 (J = 5.0 Hz) was due to secondary methyl H<sub>3</sub> -6b of rhamnose unit. Two three-proton singlets at  $\delta$  3.80 and 3.83 were assigned to the methoxy protons.

The <sup>13</sup>C NMR spectrum of **5** displayed signals for two each flavanone and sugar units including carbonyl carbons at  $\delta$  197.08 (C-4) and 197.03 (C-4''), oxymethine carbons at  $\delta$  78.41 (C-2) and 78.39 (C-2''), methylene carbons of the flavanone units at  $\delta$  48.67 (C-3) and 41.99 (C-3''), aromatic carbons between  $\delta$  165.07 - 95.49, methoxy carbons at  $\delta$  55.69 and 55.64, anomeric

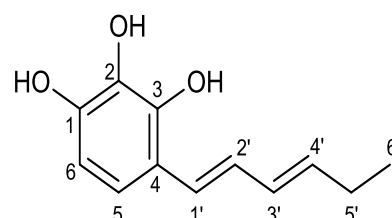
carbons at  $\delta$  100.56 (C-1a) and 96.31 (C-1b), methyl carbon at  $\delta$  17.81 (C-6b) and other sugar carbons between  $\delta$  76.20 – 65.97. The multiplicity of each carbon was determined by DEPT spectrum of **5** which exhibited the presence of three methyl, three methylene and twenty two methine carbons. The presence of C-5 carbon signal in the deshielded region at  $\delta$  163.01 supported linkage of another flavanone unit at C-5 carbon. The existence of the oxymethylene H<sub>2</sub>-6a proton signal at  $\delta$  3.32 and carbon signal of C-6a at  $\delta$  65.97 in the deshielded region indicated the attachment of the second sugar unit at C-6a carbon. The <sup>1</sup>H-<sup>1</sup>H COSY spectrum of **5** exhibited correlations of H-6 and OMe with H-8; H<sub>2</sub>-3, H-2' and H-6' with H-2; H-6'' and OMe with H-8''; H-3'', H-2''' and H-6''' with H-2''; H-5' and H-2a with H-1a; H<sub>2</sub>-6a and H-2b with H-1b; and H<sub>3</sub>-6b and H-4b with H-5b. The HMBC spectrum of **5** displayed interactions of H-6, H-8 and OMe with C-7; H-2, H<sub>2</sub>-3, H-2' and H-6' with C-1'; H-6'' and H-8'' with C-7''; H-2'', H<sub>2</sub>-3'', H-2''' and H-6''' with C-1'''; H-5' and H-1a with C-4'; H<sub>2</sub>-6a, and H-2b with C-1b; and H<sub>3</sub>-6b and H-4b with C-5b. The HSQC spectrum of **5** showed correlations flavanone protons H-2, H<sub>2</sub>-3, H-2'' and H<sub>2</sub>-3'' signals with their respective carbon signals, anomeric proton signals H-1a ( $\delta$  100.56) and H-1b ( $\delta$  5.28) with the anomeric carbons signals, methylene H<sub>2</sub>-6a signal at  $\delta$  3.32 with the carbon signal at  $\delta$  65.97 (C-6a), and methyl Me-6b signals at  $\delta$  1.15 with the carbon signal at  $\delta$  17.81 (C-6b). The <sup>1</sup>H and <sup>13</sup>C NMR spectral values of the flavanone unit were compared with related flavanone-type molecules.<sup>[17,18]</sup> Acid hydrolysis of **5** yielded D-glucose, R<sub>f</sub> 0.18 (*n*-butanol-acetic acid-water, 4:1:5) and D-rhamnose, R<sub>f</sub> 0.86 (*n*-butanol-acetic acid-water, 4:1:1.6). On the basis of these evidences the structure of **5** was formulated as 7-methoxy-3',4'-dihydroxyflavanonyl-5-oxy-(5→4'')-5'',3'''-dihydroxy-7''-methoxyflavanone-4'- $\alpha$ -D-glucopyranosyl-(6a→1b)- $\alpha$ -D-rhamnopyranoside, a new biflavanone diglycoside.



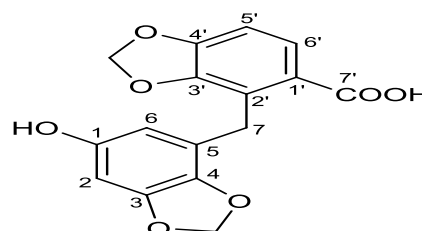
**8-Ethoxy naphthalene-1,2-diol. (1)**



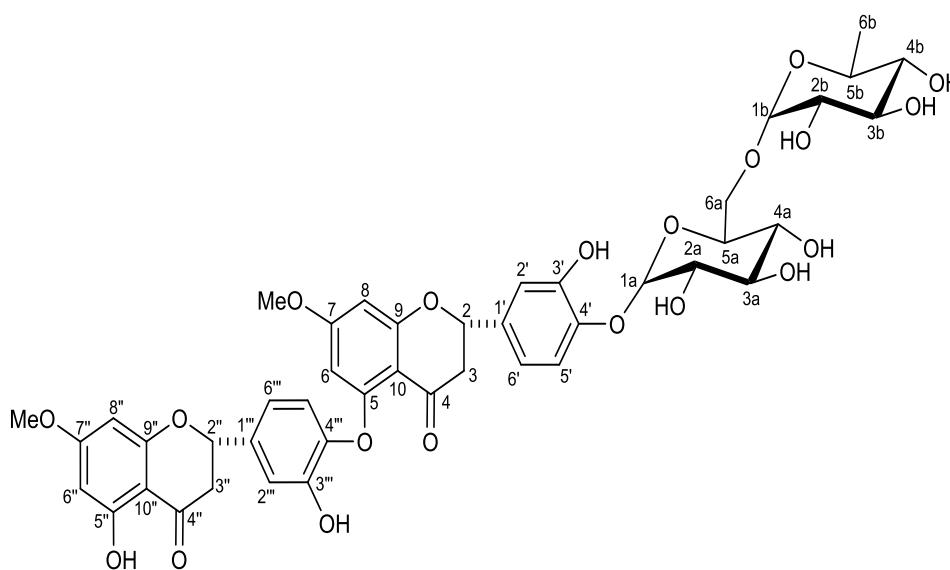
**Benzyl 19,27-dimethyl tetratriacontan-(Z)-7-ene. (2)**



**4-(Hexa-1',3'-dienyl) pyrogallol. (3)**



**Sesamol 5,2'-methylene piperonylic acid. (4)**



**Bis-(5,3',4'-trihydroxy-7-methoxy-flavanone)-4'-O-glucorhamnoside (5)**

**Fig. 1: Chemical constituents 1 – 5 isolated from the aerial parts of *Artemisia maritima*.**

### Chemical composition of the petroleum ether fraction

The chemical composition of the petroleum ether fraction of the ethanolic extract of the aerial parts of *A. maritima*, their retention times and percentage areas are tabulated in Table 1. The components are arranged in order of GC elution on DB-1 column. All the identified components were long chain aliphatic alcohols present in the range of 2.6 – 12.7 %. There were three aliphatic diols (15.0 %), eight aliphatic triols (72.3) and one monohydroxy alcohol (12.7). *n*-Hexatriacont-18-en-17-ol (12.7 %) was the predominant alcoholic constituent followed by *n*-cosan-1,4,11-triol (12.4 %), *n*-cosan-1,6,18-triol (10.8%) and *n*-cosan-1,10,17-triol (10.7 %). Two alcoholic constituents, viz., *n*-henecosan-1,7-diol (2.7 %) and *n*-cosan-1,5,12-triol (2.6 %) were detected in small amounts. Except *n*-hexatriacont-18-en-17-ol, all the alcoholic constituents were saturated in nature.

**Table 1. Chemical composition of the petroleum ether extract of the aerial parts of *Artemisia maritima*.**

S.No.	Components	RT	% Area
1.	<i>n</i> -Henecosan-1,7-diol	19.07	2.7
2.	<i>n</i> -Tricosan-1,9-diol	19.20	6.0
3.	<i>n</i> -Tricosan-1,18-diol	19.27	6.3
4.	<i>n</i> -Cosan-1,12,18-triol	19.40	8.6
5.	<i>n</i> -Cosan-1,10,18-triol	19.47	9.4
6.	<i>n</i> -Cosan-1,6,18-triol	19.55	10.8
7.	<i>n</i> -Cosan-1,8,18-triol	19.65	8.7
8.	<i>n</i> -Cosan-1,10,11-triol	19.71	9.1
9.	<i>n</i> -Cosan-1,4,11-triol	19.77	12.4
10.	<i>n</i> -Cosan-1,10,17-triol	19.82	10.7
11.	<i>n</i> -Hexatriacont-18-en-17-ol	23.30	12.7
12.	<i>n</i> -Cosan-1,5,12-trio	23.72	2.6

RT = Retention time

### CONCLUSION

Phytochemical investigations of a defatted ethanolic extract of aerial parts afforded naphthalene diol 8-ethoxynaphthalene-1,2-diol (1), benzyl 19,27-dimethyl tetratriacont-(Z)-7-ene (2), (Z)-4-(hexa-1',3'-dienyl) pyrogallol (3), 3,4-methylenedioxy phenol 5, 2'-methylene 3',4'-methylenedioxy benzoic acid (4), and 7-methoxy-3',4'-dihydroxyflavanonyl-5-oxy-(5→4'')-5'',3'''-dihydroxy-7''-methoxyflavanone-4'-α-D-glucopyranosyl- (6a→1b)-α-D-rhamnopyranoside (5). GC-MS analysis of the petroleum ether fraction of the alcoholic extract of the aerial parts showed the presence of *n*-hexatriacont-18-en-17-ol, *n*-cosan-1,4,11-triol, *n*-cosan-1,6,18-triol and *n*-cosan-1,10,17-triol as major constituents. This work has enhanced understanding about the phytoconstituents of the undertaken plant. All these chemical constituents are reported for the first time from this plant and can be used for quality control of the plant.

### ACKNOWLEDGEMENTS

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