Algerian Journal of Natural Products 5:2 **(2017)** 515-523

OPEN ACCESS

Online ISSN: 2353-0391

Algerian Journal of Natural Products

www.univ-bejaia.dz/ajnp

Type of the Paper (Article)

New aliphatic constituents from the aerial parts of *Artemisia annua* L.

Vijender Singh[1], Mohammed Ali [2,*], Archana Malik[3] and Shahnaz Sultana[2,4]

[1]School of Pharmacy, Sharda University, Plot No. 32-34, Knowledge Park – III, Greater Noida - 201306, Uttar Pradesh, India
[2] Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110062, India
[3]Ram-Eesh College of Pharmacy, Plot No. 3, Knowledge Park – I, Kasna Road, Greater NOIDA, Gautam Budh Nagar – 201310, Uttar Pradesh, (India)
[4] College of Pharmacy, Jajan University, Jajan, Saudi Arabia.

**Author to whom correspondence should be addressed; E-mail: maliphyto@gmail.com*

Received: 29/10/2016 /Accepted: 15/12/2017 DOI: https://doi.org/10.5281/zenodo.1117151

Abstract: *Artemisia annua* L. (Asteraceae) is an aromatic annual herb, up to 2 m in height, found in temperate Asia, especially China and naturalized in many countries of the world. The plant is prescribed against fever, malaria, skin diseases, jaundice, malignant ulcers and haemorrhoids. Phytochemical investigation of the aerial parts of *A. annua* led to the isolation of alkyl alcohols, fatty acid esters, alkyl glucoside and fatty acids characterized as *n*-nonadecan-2β-ol (isononadecanol, **1**), *n*-docosan-9β-ol (isodocosanol, **2**), 1-octacosanol (*n*-octacosanyl alcohol, **3**), *n*-heptadecanyl *n*-octadec-9,12-dienoate (*n*-heptadecanyl linoleate, **4**), *n*-octadecanyl *n*-octadec-9,12,-dienoate (*n*-octadecanyl linoleate, **5**), *n*-nonacosanyl *n*-octadec-9, 12-dienoate (*n*-nonacosanyl linoleate, **6**), *n*-cos-(Z)-10-enoic acid (*cis*-cos-10-enoic acid, **7**), *n*-cos-(Z)-9-enoic acid (*cis*-cos-9-enoic acid**, 8**) and *n*-heptadecanyl-ß-D-glucopyronoside (*n*-heptadecanyl glucoside, **9**). The structures of all phytoconstituents, isolated for the first time from *A. annua* have been elucidated on the basis of spectral data analysis and chemical reactions.

Keywords: *Artemisia annua L.; aerial parts; aliphatic phytoconstituents; structures elucidation.*

I. Introduction

Artemisia annua L. (Asteraceae), known as sweet wormwood, sweet sagewort or annual wormwood (Chinese: qinghao), is an aromatic annual herb up to 2 m in height with yellow flowers. It is a native to temperate Asia, especially China but naturalized throughout the world including Argentina, Bulgaria, France, Hungary, India, Italy, Romania, Spain and USA. Currently, it is the source for the production of artemisinin and semi-synthetic artemisinin derivatives (including dihydroartemisinin, artesunate, artemether and arteether) which are cadinane-type sesquiterpenic lactones used for the production of combination therapies for treatment of malaria [1,2] (Klayman et al. 1984; Mueller et al., 2000). The plant is highly pollinated and the seeds exhibited a great variation in maturity, biomass and the quantity of artemisinin. Artemisinin (Qinghaosu) presently is the most potent and efficacious compound against chloroquine and quinine-resistant *Plasmodium falciparum* and other malaria-causing parasites. Beside antimalarial effects, *A. annua* exhibited antibacterial, anti-inflammatory, angiotensin converting enzyme inhibitory, cytokinin-like and antitumor effects [3] (Abidin et al., 2003). In China, an aqueous preparation of the dried herb is prescribed against fever, malaria, skin diseases, jaundice, malignant ulcers and haemorrhoids. It is effective against pathogenic 'shu', a summer heat syndrome characterized by headache, fever, dry mouth, irritability, profused sweating and full pulse. *A. annua* is one of the important ingredient in several Ayurvedic formulations. World Health

Organization shows high interest with the active constituent Artemisinin and its chemical derivatives. *A. annua* is included in the official Pharmacopoeia of China as qinghao and in the drug directories of India, Japan and Vietnam.

The prominent coumarins identified from *A. annua* were aesculetin, iso-fraxidin, scopoletin, scopolin and tomentin. The main phenolic components of *A. annua* were quercetin glucoside, flaviolin, rhamnetin, chrysoplenol D, pillion and chlorogenic acid. In addition, other phenolic compounds such as 2,4-dihydroxy-6-methoxy-acetophenone, 5-nonadecyl-3-O-methyletherresorcinol, caffeic acid derivatives, 2,2,6-trihydroxychromene and 2,2-dihydroxy-6-methoxychromene have also been isolated from *A. annua* [4-12] (Zheng et al. 1994; Mohamed et al. 2010; Ferreira et al. 2010; Lai et al. 2007; Brown , 1992; Bhakuni et al. 2001; Elford et al. 1887; Rice-Evans et al. 1996; Zhao et al, 2014). Apart from artemisinin, other terpenoidal lactones were reported in the aerial parts of the plant [13] (Anonymous, 2001). The plant also contained phytol, flavones, chrysoplenetin, chrysosplenol-D, friedelin, casticin, chrysosplenetin, sterols, essential oils, and anthraquinones [12 - 15]. (Anonymous, 2001; Carbonara et al. 2012; Zhao et al, 2014; Cavar et al. 2012). The major constituents of the essential oils were reported camphor (up to 48%), germacrene D (up to 18.9%), artemisia ketone (up to 68%) and 1,8 cineole (up to 51.5%) [16] (Bilia et al., 2014). The root essential oil was consisted mainly of *cis*-arteannuic alcohol (25.9%), β-farnesene (6.7%), β-maaliene, β-caryophyllene, its oxide and 2-phenylbenzaldehyde [17] (Goel et al. 2007a). The stem essential oil was rich in sesquiterpenes [18] (Goel et al. 2007b). The paper describes isolation and characterization of aliphatic constituents from the aerial parts of *A. annua*.

II. MATERIAL AND METHODS

II.1. General

Melting points were determined on a Perfit melting point apparatus and are uncorrected. IR spectra were obtained on a Perkin Elmer spectrum RX-1 model. The ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were scanned by Bruker DRX-300 spectrospin NMR instrument using TMS as internal standard and coupling constants (J values) are expressed in Hertz (Hz). Mass spectra were recorded by affecting electron impact ionization at 70 eV on a Jeol SX-102 mass spectrometer equipped with a direct inlet prob system. The *m/z* values of the more intense peaks are mentioned and the figures in brackets attached to each *m/z* values indicated relative intensities with respect to the base peak. For column chromatography, silica gel (60-120 mesh, Merck, Mumbai, India) was used and thin-layer chromatography was performed on silica gel G 60 F_{254} precoated TLC plates (Merck, Mumbai, India). The spots were visualized by exposure to iodine vapours and UV radiations (254 and 366 nm) and spraying with ceric sulphate solution.

II. 2. Plant material

The aerial parts of the plant were obtained from M /S Tanz.Art, Bezoekadres Hoofdweg, 678, 2130 Hoofdweg, Netherlands and authenticated by Dr. Sushil Kumar, Scientist Emeritus, National Institute of Plant Genome Research, Aruna Asif Ali Road, New Delhi – 110067.

II.3. Extraction and isolation

The air-dried powder of the aerial part (2.0 kg) was exhaustively extracted with ethanol (95%) in a Soxhlet apparatus. The ethanolic extract was evaporated under reduced pressure to get a brown viscous mass (220 g, 1.1% yield). The dried extract was dissolved in minimum quantity of methanol and added to silica gel (60-120 mesh) to prepare a slurry. It was air-dried, powdered and loaded on a silica gel column prepared in petroleum ether. The column was eluted with petroleum ether (b. p. 60-80°C), 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 and 1:1, v/v). Various fractions were collected separately and matched by TLC to check homogeneity. Similar fractions having the same R_f values were combined and crystallized. The isolated compounds were recrystallized to get pure compounds. The following compounds were isolated:

II. 4. Isononadecanol (1)

Elution of the column with petroleum ether-chloroform (3:1) gave colourless crystals of 1 recrystallized from acetone-methanol (1:1), 118 mg (0.55%yield), R_f : 0.55 (petroleum ether-chloroform-methanol, 1:9:1), m. p. 89 - 90^{o}C. IR υ_{max} (KBr): : 3433, 2920, 2851, 1437, 1317, 1021, 953, 709 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 3.66 (1H, m, $w_{1/2}$ = 15.3 Hz, H-2α), 2.30 (2H, m, CH_2), 2.03 (2H, m, CH_2), 1.60 (2H, m, CH_2), 1.25 (26H, brs, 13 × CH_2), 0.87 (3H, d, J = 6.3 Hz, Me-1), 0.85 (3H, t, J = 6.1 Hz, Me-19). ^{13}C NMR ($CDCl_3$): δ 64.16 (C-2), 37.85 (CH_2), 32.05 (CH_2), 29.69 (12 × CH_2), 26.31 (CH_2), 22.87 (CH_2), 14.11 (Me-1), 14.09 (Me-19). +ve TOF MS *m/z* (rel. int.): 284 $[M]^+$ ($C_{19}H_{40}O$), (4.4), 269 (11.6), 239 (8.5).

II.5. Isodocosanol (2)

Elution of the column with petroleum ether-chloroform (1:1) yielded pale yellow crystals of 2, recrystallized from chloroform-methanol (1:1), 130 mg (0.062 %yield). R_f : 0.65 (petroleum ether-$CHCl_3$-MeOH (1:9:1). m. p.: 110-11^{0}C; IR υ_{max} (KBr): 3427, 2917, 1437, 1317, 1021, 953, 709 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.66 (1H, m, $w_{1/2}$=16.5 Hz, H-9α), 2.02 (2H, m, CH_2), 1.25 (36H, brs, 18 x CH_2), 0.88 (3H, t, J = 6.7 Hz, Me-1), 0.85 (3H, d, J = 6.1 Hz, Me-22); ^{13}C NMR ($CDCl_3$): 68.13 (C-9), 31.93 (CH_2), 29.69 (15 × CH_2), 29.37 (2 × CH_2), 22.69 (CH_2), 14.10 (Me-1), 14.08 (Me-22). +ve TOF MS *m/z* (rel. int.): 326 $[M]^+$ ($C_{22}H_{46}O$) (1.5), 213 (23.6).

II.6. *n*-Octacosanyl alcohol (3)

Further elution of the column with petroleum ether-chloroform (1:1) yielded colourless crystals of 3, recrystallized from chloroform-methanol (9:1), 105 mg (0.050 % yield). R_f: 0.71 (petroleum ether: chloroform: methanol; 2:7:1).m.p. 70-71^{o}C. IR υ_{max} (KBr): 3433, 2916, 2849, 1462, 1317, 1021, 953, 719 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 3.66 (2H, t, J = 6.6 Hz, H_2-1), 2.01 (2H, m, CH_2), 1.87 (2H, m, CH_2), 1.75 (2H, m, CH_2), 1.69 (2H, m, CH_2), 1.56 (2H, m, CH_2), 1.53 (2H, m, CH_2), 1.25 (40H, brs, 20 × CH_2), 0.88 (3H, t, J = 6.5 Hz, Me-28). ^{13}C NMR ($CDCl_3$): δ 62.89 (C-1), 53.21 (CH_2), 44.35 (CH_2), 37.19 (CH_2), 36.03 (CH_2), 32.80 (CH_2), 31.92 (CH_2), 29.68 (18 × CH_2), 25.73 (CH_2), 22.68 (CH_2), 14.09 (Me-28). +ve TOF MS *m/z* (rel. int.) : 410 $[M]^+$ ($C_{28}H_{58}O$) (6.9).

II.7. *n*-Heptadecanyl linoleate (4)

Elution of the column with petroleum ether-chloroform (1:4) produced colourless crystals of 4, recrystallized from chloroform-methanol (1:1), 124 mg (0.059 % yield), R_f: 0.62 (petroleum ether: chloroform; 3:7)., m.p. 64-65^{o}C. IR υ_{max} (KBr): 2917, 2849, 1737, 1623, 1463, 1378, 1169, 1020, 720 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.34 (2H, m, H-10, H-12), 5.09 (1H, m, H-9), 4.98 (1H, m, H-13), 4.06 (2H, t, J = 7.2 Hz, H_2-1′), 2.28 (2H, m, H_2-2), 2.02 (2H, m, H_2-11), 1.93 (2H, m, H_2-8), 1.81(2H, m, H_2-14), 1.56 (2H, m, CH_2), 1.52 (2H, m, CH_2), 1.47 (2H, m, CH_2), 1.25 (40H, brs, 20 × CH_2), 0.87 (3H, t, J = 6.5 Hz, Me-18), 0.80 (3H, t, J = 6.3 Hz, Me-17′). ^{13}C NMR ($CDCl_3$): δ 173.06 (C-1), 139.87 (C-10), 131.09 (C-9), 122.56 (C-12), 121.01 (C-13), 66.25 (C-1′), 50.86 (CH_2), 42.30 (CH_2), 37.34 (CH_2), 33.77 (CH_2), 32.75 (CH_2), 31.88 (CH_2), 29.65 (18× CH_2), 25.71 (CH_2), 22.64 (CH_2), 14.06 (Me-18), 11.99 (Me-17′);). +ve TOF MS *m/z* (rel. int.): 518 $[M]^+$ ($C_{35}H_{66}O_2$), (1.5), 263 (11.2), 255 (10.7).

II.8. *n*-Octadecanyl linoleate (5)

Further elution of the column with petroleum ether-chloroform (1:4) afforded pale yellow mass of 5, recrystallized from methanol, 125 mg (0.064 %yield). R_f :0.62 (petroleum ether- chloroform-methanol, 2:7:1).m. p. 87-88^0 C. IR υ_{max}(KBr): 2919, 2850, 1740, 1641, 1463, 1377, 1019, 953, 719 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 5.76 (1H, m, H-10), 5.39 (1H, m, H-12), 5.01 (1H, m, H-9), 4.94 (1H, m, H-13), 4.12 (1H, t, J = 7.2 Hz, H_2-1), 2.71 (2H, m, H_2-11), 2.28 (1H, d, J = 7.2 Hz, H_2-2), 2.02 (2H, m, CH_2), 1.99 (2H, m, CH_2), 1.70 (2H, m, CH_2), 1.68 (2H, m, CH_2), 1.55 (4H, m, 2 × CH_2), 1.33 (44H, brs, 22 × CH_2), 0.85 (3H, t, J = 6.8 Hz, Me-18), 0.82 (3H, t, J = 6.5 Hz, Me-18′). ^{13}C NMR ($CDCl_3$): δ 169.83 (C-1), 139.24 (C-10), 130.56 (C-12), 123.97(C-9), 114.04 (C-13), 65.02 (C-1′), 39.34 (CH_2), 37.09 (CH_2), 33.80 (CH_2), 32.78 (CH_2), 31.92 (CH_2), 31.42 (CH_2), 29.68 (21× CH_2), 26.74 (CH_2), 22.67

(CH_2), 14.23 (Me-18), 14.09 (Me-18′); +ve TOF MS *m/z* (rel. int.): 532 $[M]^+$ ($C_{36}H_{68}O_2$) (2.8), 269 (12.6), 263 (13.7).

II.9. *n*-Nonacosanyl linoleate (6)

Elution of the column with chloroform afforded light brown crystals of 6, recrystallized from chloroform-methanol (1:1), 110 mg (0.052 % yield). R_f: 0.32 (petroleum ether-chloroform; 3:7); m. p. 92-93^{0}C. IR υ_{max} (KBr): 2919, 2850, 1739, 1641, 1463, 1316, 1186, 1023, 954, 718 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 5.74 (1H, m, H-10), 5.26 (1H, m, H-12), 5.11 (1H, m, H-9), 5.06 (1H, m, H-13), 4.08 (1H, t, J = 6.8 Hz, H_2-1′), 2.71 (2H, m, H_2-11), 2.21 (2H, m, H_2-2), 1.95 (2H, m, H_2-8), 1.92 (2H, m, H_2-14), 1.61 (2H, m, CH_2), 1.53 (2H, m, CH_2), 1.51 (2H, m, CH_2), 1.45 (2H, m, CH_2), 1.25 (66H, brs, 33 × CH_2), 0.80 (3H, t, J = 6.3 Hz, Me-18), 0.76 (3H, t, J = 6.7 Hz, Me-29′). ^{13}C NMR ($CDCl_3$): δ 169.83 (C-1), 139.81 (C-10), 124.40 (C-12), 119.09 (C-9), 114.03 (C-13), 64.06 (C-1′), 37.40 (CH_2), 34.81 (CH_2), 33.80 (CH_2), 32.76 (CH_2), 31.91 (CH_2), 31.39 (CH_2), 29.68 (25× CH_2), 29.36 (3 × CH_2), 27.98 (CH_2), 27.07 (CH_2), 25.75 (CH_2), 24.96 (CH_2), 22.67 (CH_2), 19.69 (Me-18), 14.07 (Me-29′). +ve TOF MS *m/z* (rel. int.): 686 $[M]^+$ ($C_{47}H_{90}O_2$), (100), 423 (5.1), 263 (8.2).

II.10. *cis*-Cos-10-enoic acid (7)

Elution of the column with chloroform-methanol (49:1) furnished colourless crystals of 7, recrystallized from chloroform-methanol (1:4), 108 mg (0.051 % yield), R_f: 0.74 (chloroform). m.p.: 90-91 ^{0}C; IR υ_{max} (KBr): 3435, 2921, 2845, 1690, 1643, 1436, 1317, 1021, 953, 710 cm^{-1}.^{1}H NMR ($CDCl_3$): δ 5.16 (2H, m, $w_{1/2}$ = 9.2 Hz, H-10, H-11), 2.50 (2H, m, CH_2), 2.30 (2H, t, J = 7.2 Hz, H_2-2), 2.01 (2H, m, CH_2), 1.79 (2H, m, CH_2), 1.57 (2H, m, CH_2), 1.46 (2H, m, CH_2), 1.25 (22H, brs, 11 × CH_2), 0.86 (3H, t, J = 6.3 Hz, Me-20). ^{13}C NMR ($CDCl_3$) : δ 181.03 (C-1), 122.17 (C-10), 120.27 (C-11), 51.46 CH_2), 40.68 (CH_2), 34.88 (CH_2), 31.53 (CH_2), 29.62 (9 × CH_2), 29.57 (CH_2), 22.62 (CH_2), 14.05 (CH_3 -20). +ve TOF MS *m/z* (rel. int.): 310 $[M]^+$ ($C_{20}H_{38}O_2$) (11.6), 153 (31.3).

II.11. *cis*- Cos-9-enoic acid (8)

Further elution of the column with chloroform-methanol (49:1) provided colourless crystals of 8, recrystallized from methanol, 125 mg (0.060 %yield). R_f : 0.69 (petroleum ether- chloroform-methanol, 2:7:1). m.p. 90-91^{0}C. IR υ_{max} (KBr): 3317, 2917, 2849, 1690, 1645, 1462, 1317, 1022, 953, 718 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 5.35 (1H, m, $w_{1/2}$ = 9.2 Hz, H-9), 5.01 (1H, m, $w_{1/2}$ = 8.9 Hz, H-10), 2.30 (2H, t, J = 7.2 Hz, H_2-2), 2.02 (4H, m, H_2-8, H_2-11), 1.44 (4H, m, 2 × CH_2), 1.25 (22H, brs, 11 × CH_2), 0.87 (3H, t, J = 6.5 Hz, Me-20). ^{13}C NMR ($CDCl_3$): δ 183.41 (C-1), 133.16 (C-9), 120.87 (C-10), 44.73 (CH_2), 34.55 (CH_2), 34.19 (CH_2), 31.97 (CH_2), 31.93 (CH_2), 29.69 (9× CH_2), 27.82 (CH_2), 22.68 (CH_2), 14.09 (Me-20). +ve TOF MS *m/z* (rel. int.): 310 $[M]^+$ ($C_{20}H_{38}O_2$) (21.6).

II .12. *n*-Heptadecanyl glucoside (9)

Elution of the column with chloroform-methanol (49:1) gave light brown crystals of 9, recrystallized from chloroform-methanol (1:1), 110 mg (0.057 %yield). R_f: 0.43 (petroleum ether-$CHCl_3$-MeOH (2:7:1). m.p. 110-11^{0}C. IR υ_{max} (KBr): 3425, 3350, 2918, 2849, 1437, 1318, 1022, 953, 709 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 5.01 (1H, d, J = 7.1 Hz, H-1′), 4.90 (1H, m, H-5′), 4.83 (2H, m, CH_2), 4.05 (1H, m, H-5′), 3.87 (1H, m, H-4), 3.65 (2H,m, H-1), 3.31 (2H, m, H_2-6′), 2.29 (2H, m, CH_2), 2.02 (2H, m, CH_2), 1.59 (2H, m, CH_2), 1.25 (36H, brs, 12 × CH_2), 0.87 (3H, t, J = 6.5 Hz, Me-17). ^{13}C NMR ($CDCl_3$): δ 101.25 (C-1′), 78.13 (C-5′), 73.58 (C-2′), 68.47 (C-3′), 65.89 (C-4′), 63.15 (C-1), 60.83 (C-6′), 56.83 (C-2), 39.34 (CH_2), 31.93 (CH_2), 29.70 (10 × CH_2), 29.65 (CH_2), 22.69 (CH_2), 14.10 (CH_3 -17). +ve TOF MS *m/z* (rel. int.): 418 $[M]^+$ ($C_{23}H_{46}O_6$) (2.5), 239 (21.3), 179 (26.8).

III . RESULT AND DISCUSSION

Compound **1**, named isononadecanol, showed characteristic IR absorption bands for hydroxyl group (3433 cm^{-1}) and long aliphatic chain (709 cm^{-1}). On the basis of mass spectrum, the molecular ion peak of **1** was established at *m/z* 284 consistent with the molecular formula of a saturated aliphatic alcohol $C_{19}H_{40}O$. The formation of an ion peak at *m/z* 269 $[CH_3\ (CH_2)_{16}CH\ (OH),\ M\text{-}Me]^+$ and 239 $[CH_3\ (CH_2)_{16}]^+$ indicated the location of the hydroxyl group at C-2. The ^1H NMR spectrum of **1** displayed a one-proton multiplet at δ 3.66 with half-width of 15.3 Hz assigned to α-oriented carbinol H-2 proton. Three two-proton multiplets at δ 2.30, 2.03 and 1.60 and a broad singlet at δ 1.26 (26H) were attributed to the methylene protons. A three-proton doublet at δ 0.87 (J = 6.3 Hz) and a three-proton triplet at δ 0.85 (J = 6.1 Hz, Me-19) were ascribed to secondary C-1 and primary C-19 methyl protons, respectively. The ^{13}C NMR spectrum of **1** displayed signals for carbinol carbon at δ 64.16 (C-2), methylene carbons between δ 37.85-22.87 and methyl carbons at δ 14.11 (C-1) and 14.09 (C-19). The absence of any signal beyond δ 3.66 in the ^1H NMR spectrum and δ 64.16 in the ^{13}C NMR spectrum supported saturated nature of the molecule. Based on these evidences structure of **1** has been established as *n*-nonadecan-2β-ol.

19 **2** **1**
$CH_3\ (CH_2)_{16}CH\ (OH)\ CH_3$ (1)

Compound **2**, designated as isodocosanol, showed IR absorption bands for hydroxyl group (3427cm-^{1}) and long aliphatic chain (709 cm^{-1}). It had a molecular peak at *m/z* 326 in the mass spectrum corresponding to the molecular formula of an aliphatic alcohol $C_{22}H_{46}O$. The generation of an ion peak at *m/z* 213 $[C_8\text{-}C_9$ fission, $CH_3\ (CH_2)_{12}\ CHOH]^+$ indicated the location of the hydroxyl group at C-9. The ^1H NMR spectrum of **2** exhibited a one–proton multiplet at δ 3.66 ($w_{1/2}$=16.5 Hz) assigned to carbinol H-9α proton. A two-proton multiplet at δ 2.02 and a broad singlet at δ 1.25 (36H) were associated with the methylene protons. Two three-proton triplets at δ at δ 0.88 (J = 6.7 Hz) and δ 0.85 (J = 6.1 Hz) were attributed to primary C-1 and C-22 methyl protons, respectively. The ^{13}C NMR spectrum of **2** displayed signals for carbinol carbon at δ 68.13 (C-9), methylene carbons between δ 31.93 - 22.69 and methyl carbons at δ 14.10 (Me-1), 14.08 (Me-22). The absence of any signal beyond δ 3.66 in the ^1H NMR spectrum and δ 68.13 in the ^{13}C NMR spectrum suggested saturated nature of the molecule. On the basis of foregoing discussion, the structure of **2** has been formulated as *n*-docosan-9β-ol.

22 9 1
$CH_3\ (CH_2)_{12}CH\ (OH)(CH_2)_7CH_3$ (**2**)

Compound **3**, an aliphatic alcohol, exhibited IR absorption bands for hydroxyl group (3430 cm^{-1}) and long aliphatic chain (719 cm^{-1}). On the basis of mass spectrum, the molecular ion peak of **3** has been established at *m/z* 410 corresponding to a long chain aliphatic alcohol, $C_{28}H_{58}O$. The ^1H NMR spectrum of **3** displayed a two-proton triplet at δ 3.66 (J=6.6 Hz) assigned to hydroxyl methylene H_2-1 protons. Six two-proton multiplets between δ 2.01- 1.53 and a broad singlet at δ 1.25 (40H) were associated with the remaining methylene protons. A three-proton triplet at δ 0.88 (J=6.5 Hz) was accounted to C-28 primary methyl protons. The ^{13}C NMR spectrum of **3** exhibited signals for hydroxyl methylene carbon at δ 62.89 (C-1), methylene carbon between δ 53.21-22.68 and methyl carbons at δ 14.09 (C-28). The absence of any signal beyond δ 3.66 in the ^1H NMR spectrum and δ 62.89 in the ^{13}C NMR spectrum supported saturated nature of the molecule. On the basis of foregoing account, the structure of **3** has been formulated as 1-octacosanol.

28 1
$CH_3\ (CH_2)_{26}CH_2OH$ (**3**)

Compound **4,** named *n*-heptadecanyl linoleate, showed IR absorption bands for ester group (1737 cm^{-1}), unsaturation (1623 cm^{-1}) and long aliphatic chain (720 cm^{-1}). On the basis of mass spectrum, the molecular ion peak of **4** was determined at *m/z* 518 corresponding to the molecular

formula of a fatty acid ester, $C_{35}H_{66}O_2$. The generation of the ion peaks at *m/z* 263 [$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7CO]^+$ and 255 [M-263; $CH_3(CH_2)_{16}O]^+$ indicated that linoleic acid was esterified with a C_{17} alcohol. The ^{1}H NMR spectrum of **4** displayed a two-proton multiplet at δ 5.34 assigned to vinylic H-10 and H-12 and two one-proton multiplets at δ 5.09 and 4.98 ascribed to the vinylic H-9 and H-13 protons, respectively. A two-proton triplet at δ 4.06 (J=7.2 Hz) was attributed to oxymethylene H_2-1′ proton. A two–proton multiplet at δ 2.28 was accounted to methylene H_2-2 protons adjacent to the ester group. The other methylene protons appeared between δ 2.02 – 1.25. Two three-proton triplets at δ 0.87 (J= 6.5 Hz) and 0.80 (J= 6.3 Hz) were due to C-18 and C-17' primary methyl protons, respectively. The ^{13}C NMR spectrum of **4** showed signals for ester carbon at δ 173.06 (C-1), vinylic carbons between δ 139.87 - 121.01, oxymethylene carbon at δ 66.25 (C-1′), methylene carbons in the range of δ 50.86- 22.64 and methyl carbons at δ 14.06 (C-18) and 11.99 (C-17'). On the basis of spectral data analysis, the structure of **4** has been identified as *n*-heptadecanyl *n*-octadec-9,12-dienoate.

18 13 12 11 10 9 1 1′ 17′

$CH_3(CH_2)_4CH{=}CH\ CH_2CH{=}CH\ (CH_2)_7COOCH_2\ (CH_2)_{15}CH_3$ (**4**)

Compound **5**, named *n*-octadecanyl linoleate, decolorized bromine water indicating unsaturated nature of the molecule. Its IR spectrum showed distinctive absorption bands for ester group (1740 cm^{-1}), unsaturation (1641 cm^{-1}) and long aliphatic chain (719 cm^{-1}).

On the basis of mass spectrum, the molecular ion peak of **5** was determined at *m/z* 532 corresponding to a fatty acid ester $C_{36}H_{68}O_2$. The generation of the ion peaks at *m/z* 263 [$CH_3(CH_2)_4CH{=}CH\text{-}CH_2CH{=}CH(CH_2)_7CO]^+$ and 269 [M-263; $CH_3\text{-}(CH_2)_{17}O]^+$ suggested that linoleic acid was esterified with a C_{18} alcohol. The ^{1}H NMR spectrum of **5** displayed four one-proton multiplets at δ 5.76, 5.39, 5.01 and 4.94 assigned correspondingly to vinylic H-10, H-12, H-9 and H-13 protons, respectively. A two-proton triplet at δ 4.12 (J=7.2 Hz) was attributed to oxymethylene H_2-1′ protons. A two-proton multiplet at δ 2.71 was ascribed to methylene H_2-11. A two-proton triplet at δ 2.28 (J=7.2 Hz) was due to methylene H_2-2 protons adjacent to ester group. The other methylene protons appeared as multiplets between δ 2.02 – 1.55 and as a broad singlet at δ 1.33 (44H). Two three-proton triplets at δ 0.85 (J=6.8 Hz) and 0.82 (J= 6.5 Hz) were accounted to C-18 and C-18′ primary methyl protons, respectively. The ^{13}C NMR spectrum of **5** showed signals for ester carbon at δ 169.83 (C-1), vinylic carbons between δ 139.24 - 114.04, oxymethylene carbon at δ 65.02 (C-1′), methylene carbons from δ 39.34 to δ 22.67 and methyl carbons at δ l4.23 (C-18) and 14.09 (C-18′). Based on these evidences the structure of **5** has been established as *n*-octadecanyl *n*-octadec-9,12,-dienoate.

18 13 12 11 10 9 1 1′ 18′

$CH_3(CH_2)_4CH{=}CH\ CH_2CH{=}CH\ (CH_2)_7CO\text{-}OCH_2\ (CH_2)_{16}\ CH_3$ (**5**)

Compound **6**, designated as *n*-nonacosanyl linoleate, showed IR bands for ester group (1739 cm^{-1}), unsaturation (1641 cm^{-1}) and long aliphatic chain (718 cm^{-1}). Its mass spectrum displayed a molecular ion peak at *m/z* 686 corresponding to a fatty acid ester, $C_{47}H_{90}O_2$. The formation of ion peaks at *m/z* 263 [$CH_3(CH_2)_4\,CH{=}CH\ CH_2\ CH{=}CH(CH_2)_7CO]^+$ and 423 [M – 263]$^+$ suggested that linoleic acid was esterified with a C_{29} alcohol. The ^{1}H NMR spectrum of **6** exhibited four one–proton multiplets between δ 5.74 – 5.06 assigned to vinylic protons H-10, H-12, H-9 and H-13, respectively. A two -proton triplet at δ 4.08 (J=6.8 Hz) was attributed to oxymethylene H_2-1′ protons. The remaining methylene protons resonated in the range of δ 2.71- 1.25. Two three-proton triplets at δ 0.80 (J= 6.3 Hz) and δ 0.76 (J= 6.7 Hz) were accounted to C-18 and C-29′ primary methyl protons, respectively. The ^{13}C NMR spectrum of **6** showed signals for ester carbon at δ 169.83 (C-1), vinylic carbons from δ 139.24 to 114.03, oxymethylene carbon at δ 64.06 (C-1′), methylene carbons between δ 37.40 - 22.67 and methyl carbons at δ l9.69 (C-18) and 14.07 (C-29′). On the basis of spectral data analysis, the structure of **6** has been characterised as *n*-nonacosanyl *n*-octadec-9, 12-dienoate.

18 13 12 11 10 9 1 1′ 29′

$CH_3(CH_2)_4CH{=}CH\ CH_2CH{=}CH\ (CH_2)_7CO\text{-}OCH_2\ (CH_2)_{27}CH_3$ (**6**)

Compound **7**, an unsaturated fatty acid, decolorized bromine water and produced effervescence with sodium bicarbonate indicating fatty acid with vinylic linkage. Its IR spectrum displayed distinctive absorption bands for carboxylic group (3435, 1690 cm^{-1}), unsaturation (1643 cm^{-1}) and long aliphatic chain (710 cm^{-1}). On the basis of mass spectrum, its molecular weight was established at *m/z* 310 relating to the molecular formula of a fatty acid, $C_{20}H_{38}O_2$. It had two degree of unsaturation; one each of them was adjusted in the vinylic linkage and carboxylic function. The formation of an ion peak at *m/z* 153 $[CH_3\ (CH_2)_8CH{=}CH]^+$ supported the existence of the vinylic linkage at C-10. The ^{1}H NMR spectrum of **7** displayed a two–proton multiplet at δ 5.16 ($w_{1/2}$ = 9.2 Hz) assigned to *cis*-oriented vinylic H-10 and H-11 protons. A two-proton triplet at δ 2.30 (J=7.2 Hz) was ascribed to methylene H_2-2 proton adjacent to the carboxylic group. The other methylene protons appeared as two-proton multiplets at δ 2.50, 2.01, 1.79, 1.57 and 1.46 and a broad singlet at δ 1.25 (22H). A three-proton triplet at δ 0.86 (J= 6.3 Hz) was ascribed to C-20 primary methyl protons. The ^{13}C NMR spectrum of **7** exhibited signals for carboxylic carbon at δ 181.03 (C-1), vinylic carbons at δ 122.17 (C-10) and 120.27(C-11), methylene carbons between δ 51.46 – 22.62 and methyl carbon at δ 14.05 (C-20). On the basis of spectral data analysis and chemical reactions, the structure of **7** has been characterised as *n*-cos-(Z)-10-enoic acid.

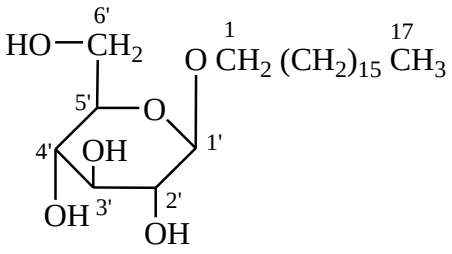
20 11 10 1
$CH_3\,(CH_2)_8CH{=}CH\,(CH_2)_8COOH$ (**7**)

Compound **8**, an unsaturated fatty acid, decolorized bromine water and produced effervescence with sodium bicarbonate indicating unsaturated nature of the fatty acid. The IR spectrum of **8** showed characteristic absorption bands for carboxylic group (3317, 1690 cm^{-1}), unsaturation (1645 cm^{-1}) and long aliphatic chain (718 cm^{-1}). It mass spectrum displayed a molecular ion peak at *m/z* 310 corresponding to a molecular formula of a fatty acid $C_{20}H_{38}O_2$. A prominent ion peak arising at *m/z* 167 $[CH_3(CH_2)_9CH{=}CH]^+$ suggested the presence of the vinylic linkage at C_9 carbon. The ^{1}H NMR spectrum of **8** exhibited two one-proton multiplets at δ 5.35 ($w_{11/2}$=9.2 Hz) and 5.01 ($w_{1/2}$=8.9 Hz) assigned to cis-oriented vinylic H-9 and H-10 protons, respectively. A two –proton triplet at δ 2.30 (J=7.2 Hz) was ascribed to methylene H_2-2 protons adjacent to the carboxylic group. The other methylene protons appeared as four–proton multiplets at δ 2.02 and 1.44 and as a broad singlet at δ 1.25 (22H). A three-proton triplet at δ 0.87 (J= 6.5 Hz) was accounted to C-20 primary methyl protons. The ^{13}C NMR spectrum of **8** displayed signals for carboxylic carbon at δ 183.41 (C-1), vinylic carbons at δ 133.16 (C-9) and 120.87(C-10), methylene carbons between δ 44.73-22.68 and methyl carbon at δ 14.09 (C-20). On the basis of spectral data analysis and chemical reactions, the structure of **8** has been formulated as *n*-cos-(Z)-9-enoic.acid.

20 10 9 1
$CH_3\,(CH_2)_9CH{=}CH\,(CH_2)_7COOH$ (**8**)

Compound **9**, named *n*-heptadecanyl glucoside, gave positive tests for glycosides. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3425, 3350 cm^{-1}) and long aliphatic chain (709 cm^{-1}). On the basis of mass spectrum, the molecular ion peak of **9** has been determined at *m/z* 418 consistent with the molecular formula of an alcoholic glycoside $C_{23}H_{46}O_6$. The formation of the prominent ion peaks at *m/z* 179 $[C_6H_{11}O_6]^+$ and 239 [M-179, $CH_3\,(CH_2)_{15}\,CH_2]^+$ suggested that a C_{17} alcohol was linked with a hexose unit. The ^{1}H NMR spectrum of **9** displayed a one–proton doublet at δ 5.01 (J=7.1 Hz) assigned to anomeric H-1′ proton. Four one-proton multiplets between δ 4.90- δ 3.87 were ascribed to the sugar carbinol protons. Two two-proton multiplets at δ 3.65 and δ 3.31 were accounted to oxymethylene H_2-1 and H_2-6′ protons, respectively. Three two-proton multiplets at δ 2.29, 2.02 and 1.59 and a broad singlet at δ 1.25 (24 H) were associated with the methylene protons of the carbon chain. A three-proton triplet at δ 0.87 (J = 6.5 Hz) was due to C-17 primary methyl protons. The ^{13}C NMR spectrum of **9** displayed signals for anomeric carbon at δ 101.25 (C-1′), other sugar carbons between δ 78.13 - 65.89, oxymethylene carbon at δ 63.15 (C-1) and hydroxymethylene carbon at δ 60.83 (C-6′), methylene carbons in the range of δ 56.83 - 22.69 and methyl carbon at δ 14.10 (C-18). Acid hydrolysis of **9** yielded D-glucose, co-TLC comparable. On the basis spectral data analysis and chemical reactions, the chemical

structure of **9** has been formulated as *n*-heptadecanyl-ß-D-glucopyronoside. This is a new aliphatic alcoholic glucoside.



9

IV. CONCLUSION

The presence of *iso*-alkanes and their functionalized derivatives have been demonstrated in several plants. The plant alkanes and their derivatives can be considered good chemotaxonomic markers at the familial and, sub-familial levels. The investigation yielded long-chain alkane, aliphatic ester, alcohols and fatty acid products which possibly serve as chemotaxonomic marker for this *Artemisia* species. Some of the compounds may be products of induced biogenesis through plant-environment interaction in response to self-defense mechanism of the plant and working as plant defensins and phytoalexins.

V. References

[1] Klayman, D.L.; Lin, A.J.; Acton, N. Isolation of artemisinin (qinghaosu) from *Artemisia annua* growing in the United States J Nat Prod., 47(4)) (1984) 715-717.

[2] Mueller, M.S.; Karhagomba, I.B.; Hirt, H.M.; Wemakor, E. The potential of *Artemisia annua* L. as a locally produced remedy for malaria in the tropics: agricultural, chemical and clinical aspects. J Ethnopharmacol 73(3) (2000) 487-493.

[3] Abdin, M.Z.; Israr, M.; Rehmann, R.U.; Jain, S.K. Artemisinin, a novel antimalarial drug. Biochemical and molecular approaches for enhanced production. Planta Med 69 (2003) 289-299.

[4] Zheng, G.C. Cytotoxic terpenoids and flavonoids from *Artemisia annua.* Planta Med 60 (1) (1994) 54-57.

[5] Mohamed, A.E.H.; El-Sayed, M.A.; Hegazy, M.E.; Helaly, S.E.; Esmail, A.M.; Mohamed, N.S. Chemical constituents and biological activities of *Artemisia herba- alba*. Rec Nat Prod 4 (2010) 1–25.

[6] Ferreira, J.F.S.; Luthria, D.L.; Sasaki, T.; Heyerick, A. Flavonoids from *Artemisia annua* L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. Molecules 15 (5) (2010) 3135–3170.

[7] Lai, J.-P.; Lim, Y.H.; Su, J.; Shen, H.-M.; Ong, C.N. Identification and characterization of major flavonoids and caffeoylquinic acids in three Compositae plants by LC/DAD-APCI/MS J Chrom B 2007, 848, 215–225.

[8] Brown, G.D. Two new compounds from *Artemisia annua*. J Nat Prod 55 (1992)1756–1760.

[9] Bhakuni, R.S.; Jain, D.C.; Sharma, R.P.; Kumar, S. Secondary metabolites of *Artemisia annua* and their biological activity. Curr Sci 80 (2001) 35-48.

[10] Elford, B.C.; Roberts, M.F.; Phillipson, J.D.; Wilson, R.J. Potentiation of the antimalarial activity of qinghaosu by methoxylated flavones. Trans R Soc Trop Med Hyg 81 (1987) 434–436.

[11] Rice-Evans, C.A.; Miller, N.J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Rad Biol Med 20 (1996) 933– 956.

[12] Zhao, Y.W.; Ni, F.Y.; Song, Y.L.; Wang, S.Y.; Huang, W.Z.; Wang, Z.Z.; Xiao, W. Chemical constituents from Artemisia annua. Zhongguo Zhong Yao Za Zhi 39 (24) (2014) 4816-4821.

[13] Anonymous. The Wealth of India, a dictionary of Indian raw materials and industrial products, National Institute of Science Communication and Information Resources, CSIR, New Delhi, First supplement, 1 (2006) 91-92.

[14] Carbonara, T.; Pascale, R.; Argentieri , M.P.; Papadia, P.; Fanizzi, F.P.; Villanova, L.; Avato , P. Phytochemical analysis of a herbal tea from *Artemisia annua* L. J Pharma Biomed Analysis 62 (2012) 79–86.

[15] Cavar, S.; Maksimovic, M.; Vidic, D.; Paric A. Chemical composition and antioxidant and antimicrobial activity of essential oil of *Artemisia annua* L. from Bosnia. Industrial Crops and Products. 37 (1) (2012) 479 – 485.

[16] Bilia, A.R.; Santomauro, F.; Sacco, C.; Bergonzi, M.C.; Donato, R. Essential oil of *Artemisia annua* L.: an extraordinary component with numerous antimicrobial properties. Evidence-based Complementary and Alternative Medicine. 2014, Article ID 159819, 7 pages.
[17] Goel, D.; Goel, R.; Singh, V.; Ali M.; Mallavarapu, G.R.; Kumar, S. Composition of the essential oil from the roots of *Artemisia annua*. J Nat Med 61 ((2007) 458-461.
[18] Goel, D.; Singh, V.; Ali, M.; Mallavarapu, G.R.; Kumar, S. Essential oils of petal, leaf and stem of the antimalarial plant *Artemisia annua*. J Nat Med 61 (2007) 187-191.

Please cite this Article as:

Vijender Singh, Mohammed Ali, Archana Malik and Shahnaz Sultana, New aliphatic constituents from the aerial parts of *Artemisia annua* L., ***Algerian J. Nat. Products,* 5:2 (2017) 515-523**

www.univ-bejaia.dz/ajnp
Online ISSN: 2353-0391
Editor in chief: Prof. Kamel BELHAMEL

Access this article online	
Website: www.univ-bejaia.dz/ajnp	**Quick Response Code**
DOI: https://doi.org/10.5281/zenodo.1117151	