ejpmr, 2020,7(10), 482-487

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

CHEMICAL CONSTITUENTS OF THE AERIAL PARTS OF *DIDYMOCARPUS PEDICELLATA* R. BR.

Irshad Ahmad Lone[1,3], Mohammad Ali[2]*, M. Sarwar Alam[1], Showkat Rasool Mir[2] and Shahnaz Sultana[2,4]

[1]Department of Chemistry, School of Chemical and Life Sciences, Jamia Hamdard (Hamdard University), New Delhi – 110 062, India.

[2]Phytochemistry Research Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard (Hamdard University), New Delhi - 110 062, India.

[3]Present Address: Department of Chemistry, Faculty of Science, Govt. Degree College, Baramulla-Kupwara National Highway, Handwara-193002, Jammu & Kashmir, India.

[4]Present Address: College of Pharmacy, Jazan University, Jazan, Saudi Arabia.

***Corresponding Author: Mohammad Ali**
Phytochemistry Research Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard (Hamdard University), New Delhi - 110 062, India.

Article Received on 26/07/2020 **Article Revised on 16/08/2020** **Article Accepted on 06/09/2020**

ABSTRACT

Didymocarpus pedicellatus R.Br. (family Gesneriaceae) is a small stemless herb used to treat chest pain, cough, cuts, diabetes, diarrhoea, epilepsy, heart and renal diseases, bladder and kidney stones, splenitis, stomach ache, syphilis, ulcers and wound. The present study was designed to isolate and characterize chemical constituents of the aerial parts of *D. pedicellata*. Phytochemical investigations of a chloroform fraction of the alcoholic extract of aerial parts resulted in the isolation of 4-(hept-7-enyl) – phenol (p-heptenyl phenol, **1**) and four aliphatic acids identified (all Z)- n-hexacosa-9, 19, 21-trienoic acid (**2**), n-hexacosa-8 (Z)- en-l-oic acid (**3**), n-hexacosa-10 (Z)-enl-oic acid (**4**) and (all Z)- n-octacosa-5, 15,19, 22-tetraen-l-oic acid (**5**). GC-MS analysis of a petroleum ether fraction of the alcoholic extract of the aerial parts showed high amounts of aliphatic alcohols (49.3 %) and aliphatic esters. The predominant components were *n*-heneicosan-1,6-diol (44.5 %), glyceryl oleate (22.7 %) and glyceryl linoleate (6.8%). The other aliphatic alcohols detected in small amounts included *n*-tetradec-5,7,9-trien-1-ol, *n*-nonadec-9,11,13-trien-1-ol, *n*-nonadec-6,10,13-trien-9-ol and 1-phenyl-*n*-docos-11-en-2,3,6-triol.

KEYWORDS: *Didymocarpus pedicellatus*, aerial parts, ethanolic extract, phytoconstituents, isolation, characterization.

INTRODUCTION

Didymocarpus pedicellatus R.Br., syn. *D. platypus* C.B. Clarke (family Gesneriaceae), known as stone flower, shila pushpa, shanta pushpi , pasanbheda , charela and patharphori, is distributed in the Himalayas in north-eastern India, Nepal and Bhutan. It is a small stemless herb, leaves 2-3 pairs, opposite, glandular, round ovate, glabrous, glandular-punctate; flowers purple, funnel shaped calyx.[1] The leaves are antiseptic and diuretic, effective against chest pain, cough, cuts, diabetes, diarrhoea, epilepsy, heart and renal diseases, bladder and kidney stones, splenitis, stomach ache, syphilis, ulcers and wound. It is a major ingredient in polyherbal formulations prescribed to treat kidney and bladder stones.[2-4]

The leaves of *D. pedicellata* contained chalcones,[5] flavones, flavanones, [6-9] pedicellic acid[10], phenolic phenolic components[6], *n*-heneicosanyl linolenate and aromatic monoterpenic esters.[11,12] The aerial parts afforded 21-hydroxy-β-sitosteryl n-octadec-9′,12′,15 ′-trienoate, 21-hydroxy- β-stigmasteryl n-octadec-9′,12′,15′-trienoate, stigmasterol 3-O-β-D-glucopyranoside[13] and β-carotene.[14] The plant essential oil was composed of α-humulene (62.15%), cedroxyde (5.69%), (-)-α- panasinsanene (3.70%), longifolol (2.80%), 1-octen-3-one (2.32 %), E-caryophyllene (1.96 %) and α-cadina-4, 9-diene (1.77%).[14] Keeping in view the various therapeutic values of the plants and the development of ecofriendly, biodegradable and safer herbal preparations, aerial parts of *D. pedicellata* were screened for the isolation and characterization of their chemical constituents.

MATERIALS AND METHODS

General procedures

The melting points were measured by means of a thermoelectrically operated Perfit apparatus and are uncorrected. UV spectra were recorded on Beckman DU-6 spectrophotometer in either methanol or chloroform. FT-IR spectra were recorded on Jasco FT/ IR-55000 spectrophotometer using KBr pellet; υmax values are given in cm^{-1}. NMR spectra were recorded on Bruker Spectrospin 400-MHz instrument with $CDCl_3$ as solvent

and tetramethyl silane as the 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_{254} (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 materials

The aerial parts of *D. pedicellata* were purchased from Khari Baoli, New Delhi, India 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 (No. NISCAIR/RHMD/Consult/-2007-08/865/49) has been preserved in the herbarium of NISCAIR, New Delhi.

Extraction and fractionation

The powdered shade-dried aerial parts of the plant (2.0 Kg) were Soxhlet-extracted exhaustively with ethanol (95%). The alcoholic extract was concentrated to dryness under reduced pressure at 40 ^{0}C in a rotary evaporator to yield light brown mass (628.0 g). A portion of this extract (550.0 g) was suspended in water and fractionated successively with petroleum ether, chloroform and *n*-butanol. These fractions were dried under reduced pressure to yield petroleum ether (45 g), chloroform (131 g) and *n*-butanol (272 g) soluble extracts. All these extracts were stored at 4 ^{0}C until use.

Isolation of chemical constituents from the chloroform fraction

The chloroform extract residue (130.0 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_f values were combined and crystallized with solvents. The isolated compounds were recrystallized to get pure compounds.

***p*-Heptenyl phenol (1)**

Elution of the column with petroleum ether - chloroform (9:1) gave pale yellow crystals of **1,** (93.6 mg), R_f 0.34 (toluene – acetone - formaldehyde, 5:3:1), m. p. 156-158 °C; UV λmax 253, 276 nm (log ε 3.6, 5.3); IR υ_{max} (KBr): 3390, 2922, 2845, 1640,1525,1470, 1385, 1195, 1035 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.82 (2H, m, H-3, H-5), 7.73 (2H, m, H-2, H-6), 6.94 (1H, d, J = 11.2 Hz, H-7), 6.90 (1H, brm, $w_{1/2}$ = 12.6 Hz, H-8), 2.68 (2H, m, H_2-9), 2.27 (2H, m, H_2-10), 1.42 (2H, m, H_2-11), 1.35 (2H, m, H_2-12), 0.64 (3H, t, J = 7.6 Hz, H_3-13); ^{13}C NMR ($CDCl_3$): δ 148.68 (C-1), 120.79 (C-2), 140.15 (C-3), 159.62 (C-4), 136.86 (C-5), 120.73 (C-6), 118.78 (C-7), 112.13 (C-8), 43.85 (C-9), 32.51 (C-10), 29.63 (C-11), 29.62 (C-12), 14.73 (C-13); +ve FAB MS *m/z* (rel. int.): 190 $[M]^+$ ($C_{13}H_{18}O$) (29.8), 175 (15.1), 173 (14.9), 119 (33.2), 97 (100), 93 (72.1).

(All *Z*)-*n*-Hexacosa-9,19,21-trienoic acid (2)

Elution of the column with petroleum ether - chloroform (3:1) afforded yellow crystals of **2,** 68.8 mg, R_f 0.76 (acetone-toluene, 1:1) , m. p. 97 - 99 ^{o}C; UV λmax 224 nm (log ε 5.3); IR υ_{max} (KBr): 3415, 2920, 2840, 1702, 1645, 1415, 1375, 1187, 939, 722 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.33 (1H, m, $w_{1/2}$ = 4.3 Hz, H-9), 5.31 (1H, m, $w_{1/2}$ = 4.1 Hz, H-10), 5.29 (2H, m, $w_{1/2}$ = 4.3 Hz, H-19, H-20), 5.27 (1H, m, $w_{1/2}$ = 4.1 Hz, H-21), 5.23 (1H, m, $w_{1/2}$ = 3.8 Hz, H-22), 2.28 (2H, t, J = 7.2 Hz, H_2-2), 2.05 (2H, m, H_2-8), 2.01 (2H, m, H_2-11), 1.92 (2H, m, H_2-18), 1.89 (2H, m, H_2-23), 1.60 (2H, m, H_2-3), 1.51 (2 H, m, H_2-4), 1.24 (4H, m, H_2-5, H_2-6), 1.18 (18 H, brs, 9 × CH_2), 0.86 (3H, t, J = 6.4 Hz, Me-26); ^{13}C NMR ($CDCl_3$): δ 179.20 (C-1), 33.05 (C-2), 28.94 (C-3), 28.75 (C-4), 28.69 (C-5), 28.66 (C-6), 28.42 (C-7), 30.89 (C-8), 129.19 (C-9), 129.03 (C-10), 30.51 (C-11), 28.33 (C-12), 26.95 (C-13), 26.42 (C-14), 26.18 (C-15), 26.16 (C-16), 24.60 (C-17), 23.64 (C-18), 129.01 (C-19), 128.70 (C-20), 127.03 (C-21), 126.67 (C-22), 21.68 (C-23), 21.65 (C-24), 21.57 (C-25), 13.10 (C-26); +ve FAB MS *m/z* (rel. int.): 390 $[M]^+$ ($C_{26}H_{46}O_2$) (13.8), 281 (56.1), 247 (8.9), 221 (82.5), 169 (11.3), 143 (23.5), 109 (63.2), 83 (38.6), 57 (100).

***n*-Hexacosa-8 (Z)-en-1-oic acid (3)**

Elution of the column with petroleum ether-chloroform (3:2) yielded pale yellow sticky mass of **3**, recrystallized from chloroform-methanol (1:1), 63.6 mg, R_f 0.55 (acetone-toluene, 1:1), UV λmax 227 nm (log ε 4.8); IR υ_{max} (KBr): 3435, 2916, 2848, 2676, 1701, 1635, 1463, 1191, 851, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.31 (1H, m, $w_{1/2}$ = 3.8 Hz, H-8), 5.26 (1H, m, $w_{1/2}$ = 4.1 Hz, H-9), 2.25 (2H, t, J = 7.6 Hz, H_2-2), 2.01 (2H, m, H_2-7), 1.98 (2H, m, H_2-10), 1.56 (2H, m, H_2-3), 1.24 (4H, m, H_2-6, H_2-11), 1.21 (8H, m, 4 x CH_2), 1.18 (24 H, brs, 12 × CH_2), 0.79 (3H, t, J = 6.8 Hz, Me-26); ^{13}C NMR ($CDCl_3$): δ 179.91 (C-1), 39.38 (C-2), 31.91 (C-3), 29.96 (C-4), 29.61 (C-5), 29.50 (C-6), 34.87 (C-7), 130.21 (C-8), 128.08 (C-9), 34.41(C-10), 29.45 (C-11), 29.43 (C-12), 29.42 (C-13), 29.42 (C-14), 29.35 (C-15), 29.32 (C-16), 29.22 (C-17), 29.04 (C-18), 27.98 (C-19), 27.22 (C-20), 27.16 (C-21), 26.33 (C-22), 25.76 (C-23), 24.98 (C-24), 22.67 (C-25), 14.04 (C-26); +ve FAB MS *m/z* (rel. int.): 394 $[M]^+$ ($C_{26}H_{50}O_2$) (43.1), 265 (21.7), 239 (24.1), 155 (13.9), 129 (18.6), 111 (21.5), 85 (31.0).

***n*-Hexacosa-10 (Z)-en-1-oic acid (4)**

Elution of the column with petroleum ether-chloroform (1:1) furnished pale yellow crystals of **4**, recrystallized from acetone, 58.3 mg, R_f 0.60 (acetone-toluene, 1:1), UV λmax 217 nm (log ε 4.3); IR υ_{max} (KBr): 3441, 2917, 2850, 1703, 1640, 1470, 1360, 1262, 793 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.36 (1H, m, $w_{1/2}$ = 3.6 Hz, H-10), 5.33 (1H, m, $w_{1/2}$ = 3.2 Hz, H-11), 2.36 (2H, t, J = 7.5 Hz, H_2-2), 2.04 (2H, m, H_2-9), 1.99 (2H, m, H_2-12), 1.55 (2H, m, H_2-3), 1.32 (2H, m, H_2-8), 1.30 (2H, m, H_2-13), 1.28 (8H, brs, 4 x CH_2), 1.20 (20 H, brs, 10 × CH_2), 0.87 (3H, t, J = 6.6 Hz, Me-26); ^{13}C NMR ($CDCl_3$): δ 180.01 (C-1), 39.07 (C-2), 31.93 (C-3), 29.98 (C-4), 29.68 (C-5), 29.53 (C-6), 29.57 (C-7), 29.98 (C-8), 34.04 (C-9), 130.02 (C-10), 129.73 (C-11), 31.93 (C-12), 29.52 (C-13), 29.44 (C-14), 29.42 (C-15), 29.36 (C-16), 29.33 (C-17), 29.24 C-18), 27.98 (C-19), 27.22 (C-20), 27.16 (C-21), 26.33 (C-22), 25.76 (C-23), 24.98 (C-24), 22.67 (C-25), 14.04 (C-26); +ve FAB MS *m/z* (rel. int.): 394 $[M]^+$ ($C_{26}H_{50}O_2$) (19.8), 379 (8.3), 350 (10.6), 237 (21.2), 211 (12.8), 183 (13.1), 157 (25.6), 139 (21.8), 113 (19.5).

(All *Z*)- *n*-Octacosa-5, 15,19, 22-tetraen-l-oic acid (5)

Elution of the column with petroleum ether - chloroform (2 : 3) gave light greenish crystals of **5**, 101.9 mg, R_f 0.12 (petroleum ether – chloroform, 1 :1), m. p. 101-103 °C , UV λmax 206 nm (log ε 4.3) , IR υ_{max} (KBr): 3410, 2916, 2849, 1702, 1642, 1471, 1370, 1021, 794 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.34 (1H, m, $w_{1/2}$ = 3.7 Hz, H-5), 5.32 (1H, m, $w_{1/2}$ = 4.1 Hz, H-6), 5.30 (1H, m, $w_{1/2}$ = 4.1 Hz, H-15), 5.28 (1H, m, $w_{1/2}$ = 4.9 Hz, H-16), 5.25 (1H, m, $w_{1/2}$ = 4.3 Hz, H-19), 5.23 (1H, m, $w_{1/2}$ = 5.2 Hz, H-20), 5.05 (1H, m, $w_{1/2}$ = 4.9 Hz, H-22), 5.03 (1H, m, $w_{1/2}$ = 4.6 Hz, H-23), 2.75 (2H, t, J = 6.4 Hz, H_2-2), 2.35 (2H, dd, J = 7.6, 7.2 Hz, H_2-21), 2.25 (2H, m, H_2-4), 2.09 (2H, m, H_2-7), 1.98 (2H, m, H_2-14), 1.93 (2H, m, H_2-17), 1.59 (2H, m, H_2-18), 1.55 (2H, m, H_2-24), 1.22 (4H, m, H_2-3, H_2-8), 1.18 (12 H, brs, H_2-9 to H_2-13, H_2-25), 1.08 (4H, m, H_2-26, H_2-27), 0.81 (3H, t, J = 6.4 Hz, Me-28); ^{13}C NMR ($CDCl_3$): δ 178.65 (C-1), 32.97 (C-2), 30.51 (C-3), 29.15 (C-4), 123.99 (C-5), 126.80 (C-6), 28.93 (C-7), 29.68 (C-8), 28.95 (C-9), 26.95 (C-10), 26.41 (C-11), 26.12 (C-12), 25.37 (C-13), 24.61 (C-14), 127.01 (C-15), 128.73 (C-16), 31.17 (C-17), 30.91 C-18), 129.03 (C-19), 134.18 (C-20), 38.04 (C-21), 129.19 (C-22), 129.01 (C-23), 23.68 (C-24), 22.41 (C-25), 21.64 (C-26), 21.67 (C-27), 14.38 (C-28); +ve FAB MS *m/z* (rel. int.): 416 $[M]^+$ ($C_{28}H_{48}0_2$) (21.3), 329 (17.8), 305 (11.5), 303 (5.3), 279 (23.6), 251 (12.8), 191 (22.7), 165 (24.2), 137 (58.3), 113 (12.7), 111 (25.6), 97 (61.1), 87 (14.6).

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 µm 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 µL of sample extract was injected in a split less mode. The injection port was set at 320 ^{o} C and temperature of oven was initially set at 70^o C for 5 minutes. The oven temperature was subsequently ramped to 205 ^{o} C at a rate of 5^o C/min for 5 minutes, 280^0 C at a rate of 5^o C/min for 5 minutes and finally to 300^o C at a rate of 5^o C/min for 5 minutes. The maximum oven temperature was fixed at 320^{o}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^{o}C and 350^{o}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 of phenols, had UV absorption maxima at 253, 276 nm for aromatic compounds and characteristic IR absorption bands for a hydroxyl group (3390 cm^{-1}), unsaturation (1640 cm^{-1}) and aromatic ring (1525, 1035 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, its molecular ion peak was established at *m/z* 190 consistent with a molecular formula of an alkylated phenol, $C_{13}H_{18}O$. The ion peaks arising at *m/z* 175 $[M - Me]^+$, 173 $[M - OH]^+$, 119 $[M – (CH_2)_4\text{-}CH_3]^+$ and 93 $[M – CH{=}CH(CH_2)_4\text{-}CH_3]^+$ suggested the presence of a heptenyl unit linkage to the phenolic compound. The ^1H NMR spectrum of **1** exhibited downfield signals for aromatic protons as two two-proton multiplets at δ 7.82 and 7.73 assigned correspondingly to H-3, H-5 and H-2, H- 6 protons. A one-proton doublet at δ 6.94 (J = 11.2 Hz) and a one-proton multiplet at δ 6.90 with half-width of 12.6 Hz were ascribed to *cis*-oriented vinylic H-7 and H- 8 protons, respectively. The remaining methylene protons resonated between 8 2.68 - 1.35. A three-proton triplet at δ 0.64 (J = 7.6 Hz) was accounted to primary C-13 methyl protons. In its ^{13}C NMR there were thirteen carbons including aromatic and vinylic carbons between δ 159.62 – 112.13, methylene carbons in the range of δ 43.85 – 29.62 and the terminal primary methyl C-13 carbon in the upfield region at δ 14.73. The DEPT spectrum supported the presence of one methyl, four methylene, six methine and two quaternary carbon signals. The HMBC spectrum of **1** showed correlations of H-2 and H-5 with C-4; and H-2, H-6 and H-7 with C-1; H- 7 and H_2 -9 with C-8; and H_2-12 with C-13. On the basis of above spectral data analysis and chemical reactions, the structure of **1** was established as 4-(hept-7-enyl) – phenol, a new alkylated phenol (Fig 1).

Compound **2** produced effervescences with sodium bicarbonate solution and decolourized bromine water suggesting unsaturated nature of the fatty acid. Its IR spectrum showed characteristic absorption bands for a carboxylic group (3415, 1702 cm^{-1}), unsaturation (1645 cm^{-1}) and long aliphatic chain (722 cm^{-1}). Its molecular weight was established at *m/z* 390 on the basis of mass spectrum consistent with a molecular formula of an unsaturated fatty acid with three vinylic linkages, $C_{30}H_{56}O_2$. The ion peaks arising at *m/z* 143 [C_8-C_9 fission, $(CH_2)_7$ -COOH, $C_8H_{15}O_2]^+$, 169 [C_{10}-C_{11} fission, CH=CH-$(CH_2)_7$ -COOH, $C_{10}H_{17}O_2]^+$, 247 [M – 143]$^+$, and 221 [M – 169]$^+$ suggested the presence of one of the vinylic linkage at C-9 carbon atom. The ion fragments produced at *m/z* 109 [C_{18} – C_{19} fission, $C_8H_{13}]^+$, 83 [C_{20} – C_{21} fission, $C_6H_{11}]^+$, 57 [C_{22} – C_{23} fission]$^+$ and 281 [M – 109]$^+$ supported the existence of another vinylic linkages at C-19 and C-21 carbon atoms.

The ^{1}H NMR spectrum of **2** showed four one-proton multiplets at δ 5.33, 5.31, 5.27 and 5.23 and a two-proton multiplet at δ 5.29 with half-width between 3.8 – 4.3 Hz assigned to cis-oriented vinylic H-9, H-10, H-21, H-22, and H-19, H-20 protons, respectively. A two-proton triplet at δ 2.28 (J = 7.2 Hz) was ascribed to methylene H_2-2 protons adjacent to the carboxylic function. The other methylene protons appeared as multiplets from δ 2.05 to 1.24 and as a singlet at δ 1.18 (18H). A three-proton triplet at δ 0.86 (J = 6.4 Hz) was accounted to C-26 primary methyl protons. The ^{13}C NMR spectrum of **2** exhibited signals for the carboxylic carbon at δ 179.20 (C-1), vinylic carbons in the range of δ 129.19 - 126.67, methylene carbons between δ 33.05 – 21.57 and methyl carbon at δ 13.10 (C-26). The DEPT spectrum of compound **2** displayed the presence of one methyl, eighteen methylene and six methine carbon signals of a long chain aliphatic structure with a primary methyl at one end and a carboxylic group at the other end. The ^{1}H-^{1}H COSY spectrum of **2** showed correlations of H_2-8, H-9 and H_2-11 with H-10; H_2-18, H-19, H-20 and H-22 with H-21; and H_2-24 and H_2-25 with H_3-26. The HMBC spectrum of **2** exhibited interactions of H_2-3 and H_2-2 with C-1; H_2-8 and H-9 with C-10; H_2-18, H-19, H-22 and H-21 with C-20; H_2-25 with C-26. On the basis of spectral data analysis and chemical reactions, the structure of **2** has been elucidated as (all Z)- *n*-hexacosa-9, 19, 21-trienoic acid, a new fatty acid (Fig 1).

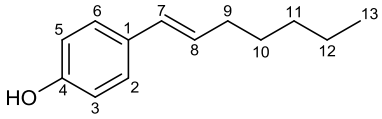
Compound **3**, [M] $^+$ at *m/z* 394 ($C_{26}H_{50}O_2$), gave positive tests for carboxylic acids and unsaturation and showed IR typical absorption bands for a carboxylic function (3435, 1701 cm^{-1}), unsaturation (1635 cm^{-1}) and long aliphatic chain (725 cm^{-1}). The ion peaks generated at *m/z* 129 [C_7 - C_8 fission, $(CH_2)_6$ -COOH, $C_7H_{13}O_2]^+$, 155 [C_9 -C_{10} fission, CH=CH-$(CH_2)_6$ -COOH, $C_9H_{15}O_2]^+$, 265 [M – 129, $C_{19}H_{37}]^+$ and 239 [M – 155, $C_{17}H_{35}]^+$ suggested existence of the vinylic linkage at C-8 carbon atom. The ^{1}H NMR spectrum of **3** displayed two one-proton multiplets at δ 5.31 ($w_{1/2}$ = 3.8 Hz) and 5.26 ($w_{1/2}$ = 4.1 Hz) assigned to cis-oriented vinylic H-8 and H-9 protons, respectively. A two-proton triplet at δ 2.25 (J = 7.5 Hz) was ascribed to methylene H_2-2 protons nearby to the carboxylic function. The other methylene protons resonated as multiplets from δ 2.01 to 1.21 and as a singlet at δ 1.18 (24H). A three-proton triplet at δ 0.79 (J = 6.8 Hz) was accounted to C-26 primary methyl protons. The ^{13}C NMR spectrum of **3** exhibited signals for carboxylic carbon at δ 179.91 (C-1), vinylic carbons at δ 130.21 (C-8) and 128.08 (C-9), methylene carbons between δ 39.38 – 22.67 and methyl carbon at δ 14.01 (C-26). The DEPT spectrum of **3** showed the presence of one methyl, twenty two methylene, two methine and one quaternary carbon signals. The ^{1}H-^{1}H COSY spectrum of **3** exhibited correlations of H_2-7, H-8 and H_2-10 with H-9; and H_2-25 with H_3-26. The HMBC spectrum of **3** showed that H_2-2 and H_2-3 interacted with C-1; H_2-7, H-8 and H_2-10 interacted with C-9; and H_2-25 interacted with C-26. On the basis of these evidences the structure of **3** was formulated as *n*-hexacosa-8 (*Z*)- en-l-oic acid, a fatty acid reported for the first time from this plant (Fig 1).

Compound **4**, $[M]^+$ at *m/z* 394 ($C_{26}H_{50}O_2$), was a position isomer of **3**. Its mass spectrum showed ion peaks at *m/z* 379 [394 – Me]$^+$ and 350 [394 – CO_2]$^+$ indicating the presence of a carboxylic group. The ion peaks generated at *m/z* 157 [C_9 – C_{10} fission, $(CH_2)_8$ -COOH, $C_9H_{17}O_2]^+$, 211 [C_{11} -C_{12} fission, CH_3-$(CH_2)_{14}$, $C_{15}H_{31}]^+$, 237 [M – 157, $C_{17}H_{33}]^+$, and 183 [M – 211, $C_{11}H_{19}O_2]^+$ supported the existence of the vinylic linkage at C-10 carbon atom. The ^{1}H NMR spectrum values of **4** were similar to those of **3** indicating the cis-oriented vinylic H-10 and H-11 protons. The analysis of ^{13}C NMR, DEPT and 2D spectral data of **4** led to establish its structure as *n*-hexacosa-10 (*Z*)-en-l-oic acid, an unknown fatty acid (Fig 1).

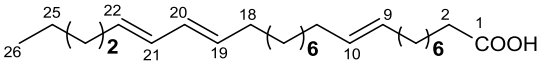
Compound **5**, $[M]^+$ at *m/z* 416 ($C_{28}H_{48}0_2$), gave positive tests for unsaturation and a carboxylic function in the molecule and showed IR absorption bands for the carboxylic group (3410, 1702 cm^{-1}), unsaturation (1642 cm^{-1}) and long aliphatic chain (794 cm^{-1}). The ion fragments arising at *m/z* 97 [C_{21}- C_{22} fission, $CH_3(CH_2)_4CH=CH]^+$, 111 [C_{20} - C_{21} fission, C_5H_{11}-CH=CH-CH_2], 137 [C_{18} – C_{19} fission, C_5H_{11}-CH=CH-CH_2-CH=CH]$^+$, 305 [M – 111]$^+$ and 279 [M – 137]$^+$ indicated the existence of the vinylic linkage at C-22 and C-19 carbons. The ion peaks generated at *m/z* 165 [C_{16} – C_{17} fission, C_5H_{11}-$(CH=CH-CH_2)_2CH_2]^+$, 191 [C_{14} – C_{15} fission, $C_{12}H_{19}$-CH_2-CH=CH]$^+$ and 251 [M – 165]$^+$ supported another vinylic linkage at C-15 carbon. The ion peaks formed at *m/z* 87 [C_4 – C_5 fission, HOOC-$(CH_2)_3]^+$, 113 [C_6 – C_7 fission, HOOC-$(CH_2)_3$-CH=CH]$^+$, and 303 [M – 113]$^+$ suggested the presence of one of the vinylic linkage at C-6 carbon with terminal carboxylic group.

The ^{1}H NMR spectrum **5** displayed eight one-proton downfield multiplets from δ 5.34 to 5.03 assignable to H-5, H-6 , H-15, H-16, H-19, H-20, H-22 and H-23 vinylic

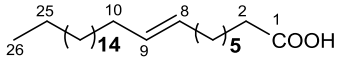
protons. A two-proton triplet at δ 2.75 (J = 6.4 Hz, each) was ascribed to methylene H_2 -2 protons adjacent to the carboxylic group. The other methylene protons resonated between δ 2.35 -1.08. A three-proton triplet at δ 0.81 (J = 6.4 Hz) was attributed to primary C-28 methyl protons. The ^{13}C NMR spectrum exhibited signals for carboxylic carbon at δ 178.65 (C-1), vinylic carbons between δ 134.18 - 123.99 and methyl carbon at δ 14.38 (C-28). The DEPT spectrum of **5** showed the presence of one methyl, eighteen methylene, eight methine and one quaternary carbon signals. The ^1H-^1H COSY spectrum of **5** showed correlations of H_2-4, H-5 and H_2-7 with H-6; H_2 -14, H-15 and H_2-17 with H-16; H_2 -21, H-19, H-23 and H-22 with H-20; and H_2 -27 with H_3-28. The HMQC spectrum of **5** showed that H_2 -2 interacted with C-1; H_2-4, H-5 and H_2-7 interacted with C-6; H_2 -14, H-15 and H_2-17 interacted with C-16; H_2 -21, H-19, H-23 and H-22 interacted with C-20; and H_2 -27 interacted with C-28. On the basis of above discussion the structure of **5** was established as (all *Z*)- *n*-octacosa-5, 15,19, 22-tetraen-l-oic acid, a new unsaturated higher fatty acid (Fig 1).



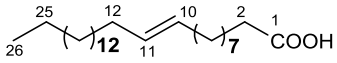
***p*-Heptenyl phenol (1)**



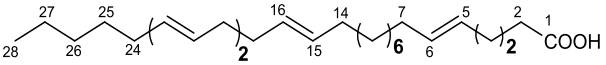
(All *Z*)-*n*-Hexacosa-9,19,21-trienoic acid (2)



***n*-Hexacosa-8-enoic acid (3)**



***n*-Hexacosa-10-enoic acid (4)**



(All *Z*)-*n*-Octacosa-5,15,19,22-tetraenoic acid (5)

Fig. 1: Chemical constituents 1 - 5 isolated from the aerial parts of *Didymocarpus pedicellata.*

Chemical composition of the petroleum ether fraction

The chemical composition of the petroleum ether fraction of the ethanolic extract of the aerial parts of *D. pedicellata* is tabulated in Table 1. The components are arranged in order of GC elution on DB-1 column. The extract was characterized by high amounts of aliphatic alcohols (49.3 %) and aliphatic esters. Among eight aliphatic constituents, the predominant component was *n*-heneicosan-1,6-diol (44.5 %) followed by glyceryl mono-oleate (22.7 %) and glyceryl linoleate (6.8%). The other aliphatic alcohols detected in small amounts included *n*-tetradec-5,7,9-trien-1-ol (1.1 %), *n*-nonadec-9,11,13-trien-1-ol (0.9 %) and *n*-nonadec-6,10,13-trien-9-ol (1.2 %). 1-Phenyl *n*-docos-11-en-2,3,6-triol (1.6 %) was the only aromatic constituent linked with a C_{22} aliphatic triol unit. Glyceryl linolenate (0.9 %) was a fatty ester present in small amount. The aerial parts were devoid of fatty acids, saponins and di- and triterpenoids. This is the first report of chemical composition of petroleum ether fraction of the aerial parts of *D. pedicellata*.

Table 1: Chemical composition of the petroleum ether extract of the aerial parts of *D. pedicellata.*

S.No.	Components	RT	% Area
1.	*n*-Tetradec-5,7,9-trien-1-ol	3.12	1.1
2.	*n*-Nonadec-9,11,13-trien-1-ol	16.80	0.9
3.	*n*-Nonadec-6,10,13-trien-9-ol	17.16	1.2
4.	1-Phenyl-n-docos-11-en-2,3,6-triol	17.95	1.6
5.	*n*-Heneicosan-1,6-diol	19.27	44.5
6.	Glyceryl mono-oleate	22.50	22.7
7.	Glyceryl linoleate	23.45	6.8
8.	Glyceryl linolenate	24.90	0.9

RT = Retention time

CONCLUSION

Phytochemical investigation of a chloroform fraction of the ethanolic extract of the aerial parts of *Didymocarpus pedicellata* led to the isolation of 4-(hept-7-enyl) – phenol (**1**) and four unsaturated aliphatic acids (**2 - 5**). GC-MS analysis of the petroleum ether fraction of the aerial parts showed high amounts of aliphatic alcohols (49.3 %) and aliphatic esters. *n*-Heneicosan-1,6-diol (44.5 %), glyceryl oleate (22.7 %) and glyceryl linoleate (6.8%) were the predominant 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

The authors are thankful to the Heads, Advanced Instrumentation Research Facility, Jawaharlal Nehru University, New Delhi and Saphosticated Instrumentation Analytical Facility, Central Drug Research Institute, Lucknow for recording spectral data of the compounds.

REFERENCES

1. Shah CS, Shah N, Mody KD. Pharmacognostic study of pashanbhed: I – III: *Bergenia ciliata* and *Didymocarpus pedicellata*. Quarterly Journal of Crude Drug Research, 1972; 12(1): 182 – 193.
2. Handa SS, Kaul MK. Supplement to Cultivation and Utilization of Medicinal Plants. Jammu Tawi: Regional Research Laboratory (Council of Scientific and Industrial Research), 1997; 93-94.
3. Singh AP. *Didymocarpus pedicellata*: The Lithontriptic Ethnomedicine. Ethnobotanical Leaflets, 2007; 11: 73-75.
4. Quattrocchi U. CRC World Dictionary of Medicinal and Poisonous Plants: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology, CRC Press, Boca Raton, Florida, 2012; 1399.
5. Bose PC, Adityachaudhary N. Isodidymocarpin, a new chalcone from *Didymocarpus pedicellata*, Journal of Indian Chemical Society, 1978; 55: 1198-1200.
6. Rathore JS, Garg SK, Nagar A, Sharma ND, Gupta SR. New phenolic components of *Didymocarpus pedicellata*. Planta Medica, 1981; 43: 86-88.
7. Guha PK, Bhattacharya A. 5, 8-Dihydroxyflavone from the immature leaves of *Didymocarpus pedicellata*. Phytochemistry, 1992; 31(5): 1833-1834.
8. Bose PC, Adityachaudhary N. Didymocarpin, a new flavanone from *Didymocarpus pedicellata*. Phytochemistry, 1978; 17: 587-588.
9. Bhattacharyya A, Choudhuri A, Adityachaudhary N. Didymocarpin-A, a new flavanone from *Didymocarpus pedicellata.* Chemistry and Industry (London), 1979; 10: 348-349.
10. Rao KV, Seshadri TR, Sood MS. Isolation and constitution of pedicellic acid: a new dicarboxylic acid from the leaves of *Didymocarpus pedicellata*. Tetrahedron, 1966; 22(4): 1495-1498.
11. Ahmad W, Ali A, Ali M, Mir SR, Zaidi SMA, Ahmad S. New fatty acid and aromatic monoterpenic esters from the leaves of *Didymocarpus pedicellata* R. Br. Indian Drugs, 2017; 54(11): 28 – 32.
12. Goyal PK, Verma SK, Sharma I, Sharma A, Sharma AK. Ethnobotanical and phytopharmacological updates on *Didymocarpus pedicellata*. Indo American Journal of Pharm Research, 2015; 5(08): 2747 – 2755.
13. Lone IA, Ali M, Alam MS, Lone SA. Isolation, characterization and antioxidant activities of steroidal constituents from the aerial parts of *Didymocarpus pedicellata* R. Br. Journal of Pharmacognosy and Phytochemistry, 2016; 5(3): 36-42.
14. Prasad K, Chandra D. Antioxidant activity, phytochemical and nutrients of *Didymocarpus pedicellata* R. Br. from Pithoragarh, Uttarakhand Himalayas, India. Journal of Pharmacology & Clinical Research, 2017; 4(3): 555640; DOI: 10.19080/JPCR.2017.04.555640.