

Analysis of Spectral Data of the Chemical Constituents from the Leaves of *Jasminum grandiflorum* L., *Achyranthes aspera* L. and *Tinospora cordifolia* (Willd.) Miers

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ABSTRACT

Jasminum grandiflorum L. (Oleaceae) is used to relieve coughs, depression, dizziness, eye diseases, facial paralysis, general debility, fevers, headache, psoriasis, sciatica, skin diseases and vertigo. *Achyranthes aspera* L. (Amaranthaceae) is utilized to treat anorexia, ascites, respiratory problems, kidney, brain and skin diseases, cholera, convulsions, diabetes, fistula, hysteria, insect bites, malaria, night blindness, obesity, piles, snake bites, stomach disorders, swellings, tooth aches, tumors and wounds. *Tinospora cordifolia* (Willd.) Miers (Menispermaceae) is effective to alleviate anemia, debility, diabetes, diarrhea, dysentery, dyspepsia, fevers, jaundice, rheumatism, urinary and skin diseases, scorpion stings and snake bites. The air-dried plant leaves were exhaustively extracted with methanol individually in a Soxhlet apparatus. The concentrated methanol extracts were adsorbed on silica gel for column and chromatographed over silica gel column separately. The columns were eluted with petroleum ether, chloroform and methanol successively to isolate the phytoconstituents. Phytochemical investigation of the leaves of *J. grandiflorum* afforded glyceryl behenate (2,3-dihydroxypropyl docosanoate, **1**), glycerol cerotate (2,3-dihydroxypropyl 1-hexacosanoate, **2**), cerotyl O-β-D-diarabinoside (*n*-hexacosanoyl-O-β-D-arabinopyranosyl-(2'→1'')-O-β-D-arabinopyranoside / cerotyl O-β-D-arabinopyranosyl-(2'→1'')-O-β-D-arabinopyranoside, **3**), stearyl-O-α-D-triglucoside (stearyl glucopyranosyl-(6'→1'')-O-α-D-glucopyranosyl-(6''→1''')-O-α-D-glucopyranoside, **4**) and behenyl-O-α-D-glucopyranosyl-(6'→1'')-O-α-D-glucopyranosyl-(6''→1''')-O-α-D-glucopyranoside, **5**). The leaves of *A. aspera* and *T. cordifolia* furnished a new diterpenoid ester aromadendr-10(14)-en-15-olyl (*E*)-ferulate **6**) and an aromatic ester phenyl ethyl behenate **7**), respectively. The structures of these phytoconstituents have been established on the basis of spectral data analysis and glycosidic and phenolic chemical reactions.

Keywords: *Jasminum grandiflorum* L., *Achyranthes aspera* L., *Tinospora cordifolia* (Willd.) Miers, spectral data, phytoconstituents

INTRODUCTION

Jasminum grandiflorum L., syn. *J. officinale* L. (Oleaceae), known as mogra, chameli, common jasmine, poet's jasmine and royal jasmine, is a native to the sino-Himalayan region, south Asia, the Arabian peninsula, north-east Africa and the Yunnan and Sichuan regions of China. It is an evergreen, scandent, 2 - 3 m high long shrub [1,2]. Jasmine essential oil has analgesic, anodyne, anthelmintic, anti-depressant, anti-inflammatory, antiseptic, aphrodisiac,

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astringent, dentifrice, deobstruent, depurative, diuretic, emmenagogue, emollient, neurasthenia, stimulant, vermifuge, thermogenic and tonic properties. It is effective to alleviate depression, nervous exhaustion, psoriasis, stress and during labor in child birth. The flowers and roots are used to relieve coughs, dizziness, eye diseases, facial paralysis, general debility, fevers, headache, sciatica, skin diseases and vertigo. A flower paste is applied on the breasts to increase milk production. The leaf juice is put to cure corns, gingivitis, dental caries, otorrhoea, mouth ulcers, skin diseases and wounds. The plant leaves and flowers are recommended to treat erectile dysfunction, eye diseases, gingivitis, headache, painful periods, respiratory problems, skin diseases, mouth ulcers and wounds [1-4]. A root paste is utilized as a face pack to improve the complexion and to ameliorate headache and paralysis [1-4]. The plant possessed anti-acne, anti-inflammatory, antimicrobial, antioxidant, antiulcer, angiotensin converting enzyme inhibitor, chemoprotective, cytoprotective, spasmolytic and wound healing activities [5-7]. The major chemical constituents of Jasmine essential oil are benzoic acid, benzaldehyde, benzyl acetate, benzyl alcohol, indole, benzyl benzoate, methyl jasmonate, *cis*-3-hexenyl benzoate, *cis*-jasmonone, ceosol, eugenol, farnesol, geraniol, linalool, phenyl acetic acid, methyl anthranilate, *p*-cresol, nerol, γ -terpineol, nerolidol, isohytol and phytol [8,9]. The flowers contained kaempferol-3-O-glycosides, 7-ketologanin, oleoside-11-methyl ester, 7-glucosyl-11-methyl oleoside, ligstroside, oleuropein and hederagenin glycosides, secoiridoids viz., jasgranoside, jaspolyoside, 8-epi-kingiside, 10-hydroxy-oleuropein, 10-hydroxy-ligstroside and oleoside-7, 11-dimethyl ester [10-12]. The plant afforded 2"-epifraxamoside, demethyl-2"-epifraxamoside, jasminanhydride, phenolics and a triterpene [13].

Achyranthes aspera L., syn. *A. acuminata* E. Mey. ex Cooke et Wright, *A. indica* (L.) Mill. (Amaranthaceae), known as amarga, prickly chaff flower, devil's horsewhip and chirchita, is distributed throughout India and the tropical world including Baluchistan, Australia, Sri Lanka, Kenya, Tanzania and Uganda [14]. The plant has alexiteric, alterative, anthelmintic, anti-inflammatory, anti-phlegmatic, antiseptic, antispasmodic, appetizer, astringent, deobstruent, diuretic, expectorant, purgative and stomachic properties, used for induction of labor pain, cessation of postpartum bleeding and to treat acne, anorexia, ascites, asthma, bladder and kidney stones, brain diseases, high cholesterol, cholera, colds, colic pain, cough, convulsions, diarrhoea, diabetes, fistula, hiccups, hysteria, insect bites, itching, malaria, night blindness, obesity, piles, pneumonia, skin diseases, snake bites, stomach aches, swellings, tooth aches, tumors, uterine retention and wounds [14,15]. The roots are useful to cure cancer and bladder stones [14, 15]. The plant possesses analgesic, anti-allergic, anti-arthritis, antiparasitic, anticarcinogenic, anti-depressant, antidiabetic, anti-inflammatory, antimicrobial, antioxidant, antiparasitic, antipyretic, antiviral, bronchoprotective, cardiovascular, diuretic, hepatoprotective, hypoglycaemic, hypolipidemic, immunomodulatory, immunostimulates, nephroprotective, spermicidal and wound healing properties [16-18]. The plant contained aliphatic constituents, 27-cyclohexyl heptacosan-7-ol, phytosterols, oleanolic acid, bisdesmosidic triterpenoid saponins, tannins, ecdysterone and 20-hydroxyecdysone [19 - 25]. A volatile oil from the leaves was mainly composed of *p*-benzoquinone, hydroquinone, spathulenol, nerol, α -ionone, asarone and eugenol [26]. The stem furnished 3-acetoxy-6-benzoyloxyapangamide [27]. The seeds yielded cyclic chain aliphatic fatty acid, saponins, saponin and oleanolic acid [28, 29].

Tinospora cordifolia (Willd.) Miers, syn. *Menispermum cordifolium* Willd. (Menispermaceae), known as amrita, guduchi, giloe and heart-leaved moonseed, is a glabrous, deciduous, climbing shrub distributed throughout tropical India, China, Sri Lanka, Bangladesh and Myanmar. Its stem is used to treat anemia, debility, diabetes, diarrhea, dysentery, dyspepsia, fevers, jaundice, rheumatism, urinary and skin diseases, scorpion stings and snake bites [30-32]. A stem paste is applied to fix bone fractures and taken orally to expel brain toxins [30 - 32]. A leaf decoction is drunk to relieve gout [30 - 32]. The stem showed ameliorative, analgesic, antibacterial, anti-cancer/anti-tumour, antidiabetic, antidiarrhoeal, antidyslipidemic, antifeedant, anti-inflammatory, antioxidant, anti-stress, anti-ulcer, aphrodisiac, cardioprotective, cytoprotective, digestive, gastroprotective, hepatoprotective, hyperglycaemic, hypolipidaemic, immunobiological, neuroprotective, nootropic and radioprotective properties [33 - 40].

The plant contained clerodane derived diterpenes [41, 42], giloin glycoside, giloinin, gilosterol, columbin, chasmanthin, palmarin, tinosporon, tinosporic acid, tinosporol, phenolic lignans, phenolic propane glycosides, phytoecdysones, tinosporafuranol, tinosporafurandiol, β -sitosterol, alkaloids berberine, tinosporin, palmitine, tembetarine, choline, isocolumbin, and tetrahydropalmatine; octacosanol, heptacosanol, nonacosan-15-one, hydroxyecdysone, makisterone, giloinsterol, 18-nonderodane glycoside, furanoid diterpene glycosides, tinocordifoliside, tinocordiside, cordiside, cordifoliside, plamatosides and syringing [43-46]. The stem possessed cetyl alcohol, *trans*-cinnamoyl amides, geranil arabinoside, 2-naphthol-2-O- α -L-arabinosides and tinolabdenyl flavanone [47]. Keeping in view the high reputation and wide application of leaves of *J. grandiflorum*, *A. aspera* and *T. cordifolia* in many indigenous medicinal systems, it has been aimed to analyze the spectral data to establish structures of the phytoconstituents isolated from the leaves of these plants procured from Delhi.

EXPERIMENTAL

General Procedures

Melting points were determined on a Perfit melting point apparatus and are uncorrected. UV spectra were determined on Shimadzu-120 double beam spectrophotometer with methanol as a solvent. IR spectra were recorded in KBr pellet on a Shimadzu FTIR-8400 spectrophotometer. The ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were scanned on a Bruker DRX instruments using TMS as an 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 direct inlet prob system. The m/z values of the more intense peaks are mentioned and the figures in bracket attached to each m/z values indicated relative intensities with respect to the base peak. Column chromatography was performed on silica gel (60-120 mesh; Qualigen, Mumbai, India). TLC was run on silica gel G 60 F₂₅₄ precoated TLC plates (Merck, Mumbai, India). Spots were visualised by exposing to iodine vapours and UV radiations (254 and 366 nm) and spraying with ceric sulphate solution.

Plant Material

The leaves of *J. grandiflorum*, *A. aspera* and *T. cordifolia*, were collected locally from Delhi and authenticated by Prof. M. P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard, New Delhi. The voucher specimens of these plant parts are preserved in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

Extraction and Isolation

The leaves of each plant (1 kg) were coarsely powdered and extracted exhaustively with methanol individually in a Soxhlet apparatus. The extracts were concentrated under reduced pressure to get dark brown masses, 110.3 g, 129.4 g and 119.7 g, respectively. The dried residue (100 g each) was dissolved in a minimum amount of methanol and adsorbed on silica gel column grade (60-120 mesh) individually to obtain a slurry. Each slurry was air-dried and chromatographed over silica gel columns loaded in petroleum ether (b. p. 60 - 80°C) separately. The columns were 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*) mixtures. Various fractions were collected singly 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 the following pure compounds:

Isolation of phytoconstituents from the leaves of *Jasminum grandiflorum*

Glyceryl behenate (1)

Elution of the column with ethyl acetate-methanol (19 : 1) yielded light yellow mass of **1**, recrystallized from methanol, yield 423 mg, UV λ_{max} (MeOH): 211 nm, m. p. 75 - 77 °C; IR γ_{max} (KBr): 3448, 3361, 2937, 2843, 1723, 1629, 1451, 1382, 1257, 1051, 935, 718 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 4.36 (2H, m, H₂-1), 4.07 (1H, m, H-2), 3.39 (2H, m, H₂-3), 2.23 (2H, m, H₂-2'), 1.91 (2H, m, CH₂), 1.85 (2H, m, CH₂), 1.55 (2H, m, CH₂), 1.18 (32H, brs, 16 × CH₂), 0.82 (3H, t, J = 6.6 Hz, Me-22'); ^{13}C NMR (DMSO- d_6): δ 172.81 (C-1'), 71.32 (C-2), 69.58 (C-1), 63.64 (C-3), 48.21 (CH₂), 34.36 (CH₂), 31.53 (CH₂), 28.99 (7 × CH₂), 28.86 (CH₂), 28.83 (CH₂), 28.78 (CH₂), 28.70 (CH₂), 28.62 (CH₂), 28.53 (CH₂), 27.31 (CH₂), 25.46 (CH₂), 24.33 (CH₂), 22.70 (CH₂), 14.13 (Me-22'); ESI MS m/z (rel. int.): 414 [M]⁺ (C₂₅H₅₀O₄) (1.6), 383 (26.3), 353 (22.8), 339 (11.), 323 (8.7), 295 (12.5).

Glycerol cerotate (2)

Further elution of the column with ethyl acetate - methanol (19 : 1) gave a light yellow mass of **2**, recrystallized from methanol, yield 133 mg, UV λ_{max} (MeOH): 209 nm, m. p. 78 - 80 °C; IR γ_{max} (KBr): 3415, 3355, 2926, 2850, 1721, 1611, 1460, 1383, 1281, 1194, 1085, 1020, 873, 720 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 4.43 (2H, m, H₂-1), 4.06 (1H, m, H-2), 3.44 (2H, d, J = 5.6 Hz, H₂-3), 2.51 (2H, m, H₂-2'), 1.99 (2H, m, CH₂), 1.67 (2H, m, CH₂), 1.57 (2H, m, CH₂), 1.21 (40H, brs, 20 × CH₂), 0.82 (3H, t, J = 6.5 Hz, Me-26'); ^{13}C NMR (DMSO- d_6): δ 170.11 (C-1'), 71.23 (C-2), 69.60 (C-1), 63.62 (C-3), 36.25 (C-2'), 34.03 (C-3'), 31.48 (C-4'), 29.97 (C-5' to C-12'), 29.85 (C-13'), 29.73 (C-14'), 29.68 (C-15'), 29.61 (C-16'), 29.57 (C-17'), 29.53 (C-18'), 29.51 (C-19'), 29.46 (C-20'), 29.33 (C-21'), 29.26 (C-22'), 27.61 (C-23'), 25.27 (C-24'), 22.69 (C-25'), 14.21 (Me-26'); ESI MS m/z (rel. int.): 470 [M]⁺ (C₂₉H₅₈O₄) (1.1), 379 (24.8).

Cerotyl O-β-D-diarabinoside (3)

Elution of the column with ethyl acetate - methanol (9 : 1) afforded a light yellow mass of **3**, recrystallized from methanol, yield 151 mg, R_f : 0.21 (toluene - ethyl acetate - formic acid, 5 : 4 : 1.8), UV λ_{max} (MeOH): 211 nm, m. p. 86 - 87 °C; IR γ_{max} (KBr): 3510, 3429, 3357, 2927, 2841, 1722, 1647, 1404, 1384, 1260, 1083, 725 cm^{-1} ; 1H NMR (DMSO- d_6): δ 5.28 (1H, d, J = 7.3 Hz, H-1'), 4.47 (1H, m, H-2'), 4.24 (1H, m, H-4'), 4.05 (1H, m, H-3'), 3.57 (2H, d, J = 9.6 Hz, H₂-5'), 4.92 (1H, d, J = 7.5 Hz, H-1''), 4.34 (1H, m, H-2''), 4.13 (1H, m, H-4''), 3.79 (1H, m, H-3''), 3.48 (2H, d, J = 8.8 Hz, H₂-5''), 2.24 (2H, m, H₂-2), 2.01 (2H, m, CH₂), 1.69 (2H, m, CH₂), 1.67 (2H, m, CH₂), 1.57 (4H, m, 2 × CH₂), 1.50 (2H, m, CH₂), 1.37 (2H, m, CH₂), 1.29 (32H, brs, 16 × CH₂), 0.98 (3H, t, J = 6.5 Hz, Me-26); ^{13}C NMR (DMSO- d_6): δ 168.96 (C-1), 56.13 (C-2), 33.48 (C-3), 31.27 (C-4), 28.99 (C-5 to C-15), 28.91 (C-16), 28.88 (C-17), 28.79 (C-18), 28.68 (C-19), 28.65 (C-20), 28.63 (C-21), 28.59 (C-22), 27.71 (C-23), 25.43 (C-24), 22.69 (C-25), 15.37 (Me-26), 103.57 (C-1'), 77.48 (C-2'), 71.24 (C-3'), 73.02 (C-4'), 63.69 (C-5'), 93.28 (C-1''), 76.07 (C-2''), 70.13 (C-3''), 69.62 (C-4''), 62.18 (C-5''); ESI MS m/z (rel. int.): 660 [M]⁺ (C₃₆H₆₈O₁₀) (1.1), 379 (14.2), 265 (5.1), 149 (11.9), 133 (10.3).

Stearyl-O-α-D-triglucoside (4)

Elution of the column with ethyl acetate - methanol (4:1) afforded a light yellow mass of **4**, recrystallized from methanol, yield 318 mg, R_f : 0.14 (toluene - ethyl acetate - formic acid, 5:4:1.8), UV λ_{max} (MeOH): 211 nm, m. p. 68 - 70 °C; IR γ_{max} (KBr): 3510, 3465, 3395, 3251, 2927, 2842, 1721, 1634, 1440, 1384, 1269, 1147, 1051, 772 cm^{-1} . 1H NMR (DMSO- d_6): δ 5.28 (1H, d, J = 4.3 Hz, H-1'), 4.79 (1H, m, H-5'), 4.53 (1H, m, H-2'), 4.16 (1H, m, H-3'), 3.75 (1H, m, H-4'), 3.36 (2H, d, J = 6.1 Hz, H₂-6'), 5.23 (1H, d, J = 4.9 Hz, H-1''), 4.73 (1H, m, H-5''), 4.33 (1H, m, H-2''), 3.98 (1H, m, H-3''), 3.69 (1H, m, H-4''), 3.29 (2H, d, J = 6.4 Hz, H₂-6''), 5.08 (1H, d, J = 3.6 Hz, H-1'''), 4.61 (1H, m, H-5'''), 4.27 (1H, m, H-2'''), 3.85 (1H, m, H-3'''), 3.67 (1H, m, H-4'''), 3.11 (2H, d, J = 11.2 Hz, H₂-6'''), 2.41 (2H, m, H₂-2), 2.05 (2H, m, CH₂), 1.62 (2H, m, CH₂), 1.50 (2H, m, CH₂), 1.20 (8H, brs, 4 × CH₂), 1.07 (16H, brs, 8 × CH₂), 0.86 (3H, t, J = 6.5 Hz, Me-18); ^{13}C NMR (DMSO- d_6): δ 172.31 (C-1), 56.14 (C-2), 34.26 (C-3), 32.11 (C-4), 29.73 (C-5), 29.61 (C-6), 29.57 (C-7), 29.51 (C-8), 29.47 (C-9), 29.38 (C-10), 29.31 (C-11), 29.47 (C-12), 29.47 (C-13), 29.47 (C-14), 27.89 (C-15), 24.51 (C-16), 22.68 (C-17), 18.28 (C-18), 101.37 (C-1'), 73.12 (C-2'), 71.25 (C-3'), 69.91 (C-4'), 76.57 (C-5'), 64.89 (C-6'), 96.74 (C-1''), 70.20 (C-2''), 70.72 (C-3''), 70.13 (C-4''), 76.38 (C-5''), 63.65 (C-6''), 92.12 (C-1'''), 71.52 (C-2'''), 70.42 (C-3'''), 69.64 (C-4'''), 74.63 (C-5'''), 61.21 (C-6'''); ESI MS m/z (rel. int.): 770 [M]⁺ (C₃₆H₆₆O₁₇) (12.8), 341 (24.2), 283 (4.3), 179 (38.6).

Behenyl-O-α-D-triglucoside (5)

Elution of the column with ethyl acetate : methanol (3:1) yielded a pale yellow mass of **5**, recrystallized from methanol, yield 468 mg, R_f : 0.17 (toluene - ethyl acetate - formic acid, 5:4:1.8), UV λ_{max} (MeOH): 210 nm, m. p. 73 - 75 °C; IR γ_{max} (KBr): 3521, 3463, 3342, 3270, 2926, 2855, 1721, 1637, 1461, 1384, 1268, 1076, 771 cm^{-1} ; 1H NMR (DMSO- d_6): δ 5.22 (1H, d, J = 4.8 Hz, H-1'), 4.31 (1H, m, H-5'), 3.92 (1H, m, H-2'), 3.78 (1H, m, H-3'), 3.65 (1H, m, H-4'), 3.31 (2H, d, J = 11.2 Hz, H₂-6'), 5.06 (1H, d, J = 4.0 Hz, H-1''), 4.16 (1H, m, H-5''), 3.86 (1H, m, H-2''), 3.75 (1H, m, H-3''), 3.56 (1H, m, H-4''), 3.26 (2H, d, J = 9.2 Hz, H₂-6''), 4.96 (1H, d, J = 2.8 Hz, H-1'''), 4.02 (1H, m, H-5'''), 3.82 (1H, m, H-2'''), 3.68 (1H, m, H-3'''), 3.50 (1H, m, H-4'''), 3.11 (2H, d, J = 7.6 Hz, H₂-6'''), 2.35 (2H, m, H₂-2), 2.02 (2H, m, CH₂), 1.67 (2H, m, CH₂), 1.55 (2H, m, CH₂), 1.37 (2H, m, CH₂), 1.33 (2H, m, CH₂), 1.28 (28H, brs, 14 × CH₂), 0.88 (3H, t, J = 6.7 Hz, Me-26); ^{13}C NMR (DMSO- d_6): δ 172.01 (C-1), 48.25 (C-2), 37.26 (C-3), 33.43 (C-4), 31.79 (C-5 to C-7), 29.61 (C-8 to C-11), 29.55 (C-12), 29.47 (C-13), 29.42 (C-14), 29.37 (C-15), 29.25 (C-16), 29.16 (C-17), 27.33 (C-18), 25.82 (C-19), 24.51 (C-20), 22.68 (C-21), 14.18 (Me-22), 103.73 (C-1'), 73.99 (C-2'), 71.76 (C-3'), 70.37 (C-4'), 76.50 (C-5'), 63.59 (C-6'), 96.70 (C-1''), 73.09 (C-2''), 71.30 (C-3''), 70.14 (C-4''), 74.58 (C-5''), 62.56 (C-6''), 92.01 (C-1'''), 72.14 (C-2'''), 71.20 (C-3'''), 69.59 (C-4'''), 76.34 (C-5'''), 61.13 (C-6'''); ESI MS m/z (rel. int.): 826 [M]⁺ (C₄₀H₇₄O₁₇) (1.1), 501 (3.1), 485 (11.7), 342 (35.8), 339 (26.3), 323 (2.5), 179 (39.5).

Isolation of a phytoconstituent from the leaves of *Achyranthes aspera*

Aromadendrene-15-oyl ferulate (6)

Elution of the column with chloroform - methanol (49 : 1) mixture furnished a pale yellow mass of **6**; recrystallized from chloroform-methanol (1 : 1); yield 233 mg; m. p. 127-129 °C; UV λ_{max} (MeOH): 213, 274 nm (log ϵ 2.7, 2.3); IR γ_{max} (KBr): 3248, 2935, 2846, 1727, 1641, 1525, 1431, 1369, 1116, 1063, 895 cm^{-1} ; 1H NMR (DMSO- d_6): δ 6.84 (1H, d, J = 2.5 Hz, H-2'), 6.80 (1H, dd, J = 2.5, 9.0 Hz, H-6'), 6.77 (1H, d, J = 9.0 Hz, H-5'), 6.05 (1H, d, J = 18.3 Hz, H-7'), 5.82 (1H, d, J = 18.3 Hz, H-8'), 3.34 (3H, s, OMe), 4.91 (2H, brs, H₂-14), 4.07 (2H, d, J = 8.2 Hz, H₂-15), 2.72 (1H, s, H-1), 2.50 (2H, m, H₂-9), 2.31 (2H, m, H₂-2), 2.03 (2H, m, H₂-3), 2.15 (1H, m, $w_{1/2}$ = 14.9 Hz, H-4 α), 1.98 (2H, m, H₂-8), 1.36 (1H, m, H-5), 1.23 (3H, brs, Me-12), 0.84 (3H, brs, Me-13), 0.19 (1H, m, H-6), 0.10 (1H, m, H-7); ^{13}C NMR (DMSO- d_6): δ 55.96 (C-1), 30.36 (C-2), 29.72 (C-3), 48.41 (C-4), 50.28 (C-5), 31.82 (C-6), 21.90 (C-7), 22.58 (C-8), 34.08 (C-9), 138.66 (C-10), 17.32 (C-11), 27.07 (C-12), 29.21 (C-13), 113.07 (C-14), 62.82 (C-15), 145.24 (C-1'), 130.81 (C-2'),

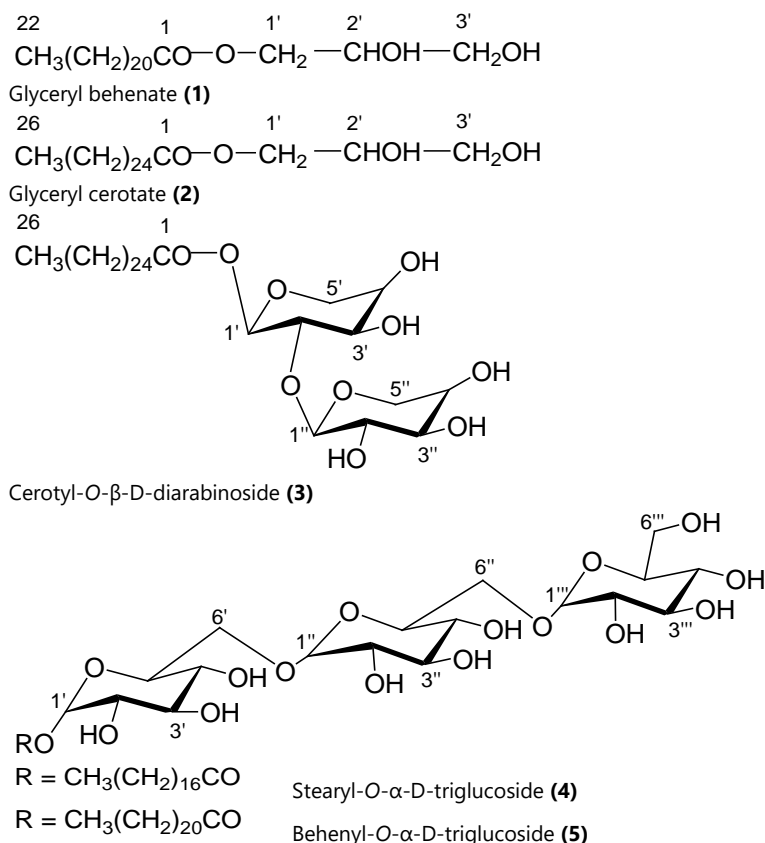


Figure 1. Chemical constituents **1 - 5** isolated from the leaves of *Jasminum grandiflorum*

147.93 (C-3'), 152.16 (C-4'), 120.94 (C-5'), 123.07 (C-6'), 115.82 (C-7'), 115.63 (C-8'), 169.86 (C-9'), 51.31 (OMe); ESI MS m/z (rel. int.): 396 [M]⁺ (C₂₅H₃₂O₄) (12.6), 203 (8.2), 193 (12.1).

Isolation of a phytoconstituent from the leaves of *Tinospora cordifolia*

Phenylethyl behenate (**7**)

Elution of the column with petroleum ether - chloroform (1:1) gave a pale yellow gummy mass of **7**, yield 212 mg, UV λ_{max} (MeOH): 211, 276 nm (log ϵ 2.8, 1.9); IR γ_{max} (KBr): 2926, 2845, 1724, 1645, 1525, 1452, 1387, 1256, 1163, 1031, 725 cm⁻¹; ¹H NMR (CDCl₃): δ 7.19 (1H, d, J = 7.2 Hz, H-2'), 7.13 (1H, d, J = 7.2 Hz, H-6'), 7.10 (1H, m, H-5'), 7.07 (1H, m, H-4'), 7.03 (1H, m, H-3'), 4.05 (2H, t, J = 7.2 Hz, H₂-8'), 2.27 (2H, t, J = 7.5 Hz, H₂-2), 2.21 (2H, t, J = 7.3 Hz, H₂-7'), 1.54 (2H, m, CH₂-3), 1.32 (2H, m, CH₂), 1.18 (34H, brs, 17 × CH₂), 0.82 (3H, t, J = 6.3 Hz, Me-22); ¹³C NMR (CDCl₃): δ 173.93 (C-1), 137.86 (C-1'), 129.05 (C-2', C-6'), 128.24 (C-3', C-5'), 125.32 (C-4'), 60.15 (C-8'), 34.42 (C-7'), 32.80 (CH₂), 31.98 (CH₂), 31.64 (CH₂), 30.21 (CH₂), 30.09 (CH₂), 29.75 (5 × CH₂), 29.71 (3 × CH₂), 29.51 (CH₂), 29.42 (CH₂), 29.32 (CH₂), 29.20 (CH₂), 26.81 (CH₂), 22.73 (CH₂), 21.46 (CH₂), 14.13 (Me-22); ESI MS m/z (rel. int.): 444 [M]⁺ (C₃₀H₅₂O₂) (9.3), 339 (6.7), 323 (3.6), 121 (5.6).

RESULTS AND DISCUSSION

Compound **1** was a known glycerol ester identified as glycerol behenate (2,3-dihydroxy-propyl docosanoate) [48] (**Figure 1**).

Compound **2**, designated as glycerol cerotate, showed IR absorption bands for hydroxyl groups (3415, 3355 cm⁻¹), ester group (1721 cm⁻¹) and long aliphatic chain (720 cm⁻¹). On the basis of mass and ¹³C NMR spectra its molecular ion peak was determined at m/z 470 corresponding to a molecular formula of an acyl glycerol C₂₉H₅₈O₄. An ion peak arising at m/z 379 [C₁' - O fission, CH₃-(CH₂)₂₄-CO]⁺ indicated that cerotic acid was linked with glycerol. The ¹H NMR spectrum of **2** exhibited a multiplet at δ 4.43 and a doublet at δ 3.44 (J = 5.6 Hz) integrating for two protons each assigned to oxymethylene H₂-1 and H₂-3 protons, respectively, a one-proton multiplet at δ 4.06 ascribed to hydroxymethine H-2 proton, a three-proton triplet at δ 0.82 (J = 6.5 Hz) attributed to terminal C-26' primary methyl protons and the methylene protons in the range from δ 2.51 to 1.21. The ¹³C NMR spectrum of **2**

displayed signals for ester carbon at δ 170.11 (C-1'), oxymethylene carbons at δ 69.60 (C-1) and 63.62 (C-3), hydroxymethine carbon at δ 71.23 (C-2), methylene carbons between δ 36.25 - 22.69 and methyl carbon at δ 14.21 (C-26'). The presence of ^1H NMR signals for H₂-1 in deshielded region at δ 4.43 and ^{13}C NMR signals for C-1 at δ 69.60 suggested that the attachment of cerotic acid at C-1. The absence of any signal beyond δ 4.43 in the ^1H NMR spectrum and between δ 170.11 - 71.23 in the ^{13}C NMR spectrum supported saturated nature of the molecule. On the basis of these evidences the structure of **2** has been elucidated 2,3-dihydroxypropyl 1-hexacosanoate (**Figure 1**).

Compound **3**, specified as cerotyl O- β -D-diarabinoside, responded for glycoside tests positively and exhibited distinctive IR absorption bands for hydroxyl groups (3510, 3429, 3357 cm^{-1}), ester function (1722 cm^{-1}) and long aliphatic chain (725 cm^{-1}). Its molecular ion peak was established at m/z 660 on the basis of mass and ^{13}C NMR spectra corresponding to a molecular formula of an acyl diglycoside, $\text{C}_{36}\text{H}_{68}\text{O}_{10}$. The ion peaks arising at m/z 133 [$\text{C}_5\text{H}_9\text{O}_4$]⁺, 149 [$\text{C}_5\text{H}_9\text{O}_5$]⁺, 265 [$\text{C}_5\text{H}_9\text{O}_4$ - $\text{C}_5\text{H}_8\text{O}_4$]⁺ and 379 [C_1 - O fission, CH_3 -(CH_2)₂₄-CO]⁺ indicated that cerotic acid was esterified with a dipentoside unit. The ^1H NMR spectrum of **3** displayed two one-proton doublets at δ 5.28 (J = 7.3 Hz) and 4.92 (J = 7.5 Hz) assigned to anomeric H-1' and H-1'' protons, respectively. The other sugar protons appeared as one - proton multiplets between δ 4.47 - 3.79 ascribed to oxymethine protons and as two-proton doublets at δ 3.57 (J = 9.6 Hz) and 3.48 (J = 8.8 Hz) attributed to oxymethylene H₂-5' and H₂-5'', respectively. Seven multiplets between δ 2.24 - 1.37 and as a singlet at δ 1.29 (32H) were ascribed to methylene protons. A three - proton triplet at δ 0.98 (J = 6.5 Hz) was accounted to terminal C-26 primary methyl protons. The ^{13}C NMR spectrum of **3** displayed signals for ester carbon at δ 168.96 (C-1), anomeric carbons at δ 103.57 (C-1') and 93.28 (C-1''), other sugar carbons from δ 77.48 to 62.18, methyl carbon at δ 15.37 (C-26) and methylene carbons between δ 56.13 - 22.69. The presence of H-2' signal in the deshielded region at δ 4.47 in the ^1H NMR spectrum and C-2' signal at δ 77.48 in the ^{13}C NMR spectrum suggested (2'→1'') linkages of the sugar units. Acid hydrolysis of **3** yielded cerotic acid, m. p. 87 °C, and D-arabinose, R_f 0.70 (*n*-butanol- acetic acid - water, 4 : 1 : 1.6). On the basis of foregoing discussion, the structure of **3** has been characterized as *n*-hexacosanoyl-O- β -D- arabinopyranosyl-(2'→1'')-O- β -D-arabinopyranoside / cerotyl O- β -D-arabinopyranosyl-(2'→1'')-O- β -D-arabinopyranoside, a new acyl diarabinoside (**Figure 1**).

Compound **4**, named stearyl-O- α -D-triglucoside, [M]⁺ at m/z 770 ($\text{C}_{36}\text{H}_{66}\text{O}_{17}$), showed IR absorption bands for hydroxyl groups (3510, 3465, 3395 cm^{-1}), ester function (1721 cm^{-1}) and long aliphatic chain (772 cm^{-1}). The ion fragments generating at m/z 283 [C_1 - O fission, $\text{CH}_3(\text{CH}_2)_{16}\text{COO}$]⁺, 179 [$\text{C}_6\text{H}_{11}\text{O}_6$]⁺ and 341 [$\text{C}_6\text{H}_{11}\text{O}_6$ - $\text{C}_6\text{H}_{10}\text{O}_4$]⁺ suggested that a trihexoside chain was linked to stearic acid. The ^1H NMR spectrum of **4** displayed three one-proton doublets at δ 5.28 (J = 4.3 Hz), 5.23 (J = 4.9 Hz) and 5.08 (J = 3.6 Hz) assigned to α -oriented anomeric H-1', H-1'' and H-1''' protons, respectively. The other sugar protons appeared as one - proton multiplets from δ 4.79 to 3.67 ascribed to oxymethine protons and as two-proton doublets at δ 3.36 (J = 6.1 Hz), 3.29 (J = 6.4 Hz) and 3.11 (J = 11.2 Hz) associated correspondingly with oxymethylene H₂-6', H₂-6'' and H₂-6''' protons. The signals from δ 2.41 to 1.07 were due to the methylene protons. A three-proton triplet at δ 0.86 (J = 6.5 Hz) was accounted to terminal C-18 primary methyl protons. The ^{13}C NMR spectrum of **4** showed signals for ester carbon at δ 172.31 (C-1), anomeric carbons at δ 101.37 (C-1'), 96.74 (C-1'') and 92.12 (C-1'''), other sugar carbons from δ 76.57 to 61.21, methylene carbons between δ 56.14 - 22.68 and methyl carbon at δ 18.28 (C-18). The existence of the oxymethylene ^1H NMR signals H₂-6' at δ 3.36 and H₂-6'' at δ 3.29 and carbon signals at δ 64.89 (C-6') and 63.65 (C-6'') in the downfield region in the ^{13}C NMR spectrum suggested (6'→1'') and (6''→1''') linkages of the sugar units. Acid hydrolysis of **4** yielded stearic acid, m. p. 68 - 69 °C and D-glucose, R_f 0.55 (*n*-butanol-acetic acid- water, 2:1:1). On the bases of the foregoing accounts, the structure of this triglucosidic ester has been elucidated as stearyl glucopyranosyl-(6'→1'')-O- α -D-glucopyranosyl-(6''→1''')-O- α -D- glucopyranoside, a new acyl tri-glucoside (**Figure 1**).

Compound **5**, named behenyl-O- α -D-triglucoside, [M]⁺ at m/z 826 ($\text{C}_{40}\text{H}_{74}\text{O}_{17}$), exhibited IR absorption bands for hydroxyl groups (3521, 3463, 3342, 3270 cm^{-1}), ester group (1721 cm^{-1}) and long aliphatic chain (771 cm^{-1}). The important mass fragment peaks arising at m/z 323 [CH_3 -(CH_2)₂₀-CO, $\text{C}_{22}\text{H}_{43}\text{O}$]⁺, 339 [CH_3 -(CH_2)₂₀-COO, $\text{C}_{22}\text{H}_{43}\text{O}_2$]⁺, 485 [CH_3 -(CH_2)₂₀-COO- $\text{C}_6\text{H}_{10}\text{O}_4$]⁺, 501 [CH_3 -(CH_2)₂₀-COO- $\text{C}_6\text{H}_{10}\text{O}_5$]⁺, 179 [$\text{C}_6\text{H}_{11}\text{O}_6$]⁺ and 342 [M - 485, $\text{C}_6\text{H}_{11}\text{O}_6$ - $\text{C}_6\text{H}_{10}\text{O}_5$]⁺ indicated that behenic acid was linked with a trihexose unit. The ^1H NMR spectrum of **5** showed three one-proton doublets at δ 5.22 (J = 4.8 Hz), 5.06 (J = 4.0 Hz) and 4.96 (J = 2.8 Hz) assigned to α -oriented anomeric H-1', H-1'' and H-1''' proton, respectively, other sugar protons appeared as one-proton multiplets between δ 4.31 - 3.50 ascribed to oxymethine protons and as two-proton doublets at δ 3.31 (J = 11.2 Hz), 3.26 (J = 9.2 Hz,) and 3.11 (J = 7.6 Hz) accounted correspondingly to oxymethylene H₂-6', H₂-6'' and H₂-6''' protons. A three-proton triplet at δ 0.88 (J = 6.7 Hz) was attributed to C-26 primary methyl protons. The remaining methylene protons resonated from δ 2.35 to 1.28. The ^{13}C NMR spectrum of **5** exhibited signals for ester carbon at δ 172.01 (C-1), anomeric carbons at δ 103.73 (C-1'), 96.70 (C-1'') and 92.01 (C-1'''), other sugar carbons in the range of δ 76.50 - 61.13, methylene carbons between δ 48.25 - 22.68 and methyl carbon at δ 14.18 (C-22). The presence of ^1H NMR signals for oxymethylene protons in deshielded region at δ 3.31 (H₂-6') and 3.26 (H₂-6'') and their respective carbon signals in the ^{13}C NMR spectrum at δ 63.59 (C-6') and 62.56 (C-6'') suggested (6'→1'') and (6''→1''') linkages of sugar units, respectively. Acid hydrolysis of **5** yielded behenic acid, m. p. 79 - 80 °C and D-glucose, R_f 0.55 (*n*-butanol-acetic acid- water, 2:1:1). On the basis of spectral analysis and chemical reactions, the structure of **5** has been elucidated as *n*-docosanoyl-

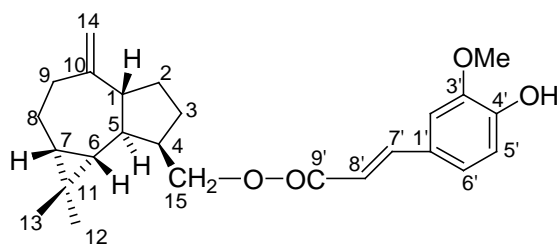
Aromadendrene-15-olyl ferulate (**6**)

Figure 2. Chemical constituent **6** isolated from the leaves of *Achyranthes aspera*

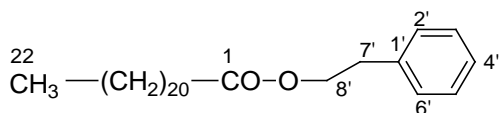
Phenyl ethyl behenoate (**7**)

Figure 3. Chemical constituent **7** isolated from the leaves of *Tinospora cordifolia*

O- α -D-glucopyranosyl-(6'→1'')-O- α -D-glucopyranosyl-(6''→1''')-O- α -D-glucopyranoside / behenyl-O- α -D-glucopyranosyl-(6'→1'')-O- α -D-glucopyranosyl-(6''→1''')-O- α -D-glucopyranoside, a new acyl triglucoside (**Figure 1**).

Compound **6**, named aromadendrene-15-olyl ferulate, responded to phenolic tests positively and showed UV absorption maxima at 274 nm for aromatic ring and IR absorption bands for hydroxyl group (3248 cm^{-1}), ester function (1727 cm^{-1}) and aromatic ring and unsaturation (1641, 1525, 1063 cm^{-1}). Its molecular ion peak was established at m/z 396 on the basis of mass and ^{13}C NMR spectra corresponding to a molecular formula of a sesquiterpenoid ester $\text{C}_{25}\text{H}_{32}\text{O}_4$. The ions peaks arising at m/z 193 [$\text{C}_{15} - \text{O}$ fission, $\text{C}_{10}\text{H}_9\text{O}_4$] $^+$ and m/z 203 [$\text{M} - 193$] $^+$ indicated that ferulic acid was esterified with the sesquiterpenoid unit. The ^1H NMR spectrum of **6** displayed aromatic signals as one-proton doublets at δ 6.84 ($J = 2.5$ Hz) and 6.77 ($J = 9.0$ Hz) and as a one - proton double doublet at δ 6.80 ($J = 2.5, 9.0$ Hz) assigned to *meta*-coupled H-2', *ortho*-coupled H-5' and *meta*-, *ortho*-coupled H-6' protons, respectively, a three - proton singlet at δ 3.34 due to methoxy protons, two one-proton doublets at δ 6.05 ($J = 18.3$ Hz), 5.82 ($J = 18.3$ Hz) attributed correspondingly to (*E*)- or *trans*-oriented vinylic H-7' and H-8' protons, a two-proton singlet at δ 4.91 accounted to exocyclic methylene H_2 -14 protons, a two-proton doublet at δ 4.07 ($J = 8.2$ Hz) ascribed to oxymethylene H_2 -15 protons, two three-proton singlets at δ 1.23 and 0.84 due to tertiary C-12 and C-13 methyl protons, respectively, two one-proton multiplets in upfield region at δ 0.19 (H-6) and 0.10 (H-7) due to attachment of a tricyclic ring to a heptacyclic ring indicating aromadendrene-type skeleton of the molecule and other methine and methylene protons in the range of δ 2.72 - 1.36. The ^{13}C NMR spectrum of **6** demonstrated the presence of aromatic and vinylic carbon signals between δ 152.16 - 113.07, oxymethylene carbon at δ 62.82 (C-15), ester carbon at δ 169.86 (C-9'), methoxy carbon at δ 51.31 and methyl carbons at δ 27.07 (C-12) and 29.21 (C-13). Acid hydrolysis of **6** yielded ferulic acid, m. p. 168 - 170° C, R_f 0.70 (chloroform : methanol: formic acid, 85 : 15 : 1). Analysis of the spectral data and chemical reaction led to elucidate the structure of **6** as aromadendr-10(14)-en-15-olyl (*E*)-ferulate, a new diterpenoid ester (**Figure 2**).

Compound **7**, [M] $^+$ at m/z 534 ($\text{C}_{36}\text{H}_{70}\text{O}_2$), showed IR absorption bands for ester group (1724 cm^{-1}), aromatic ring (1645, 1525, 1031 cm^{-1}) and long aliphatic chain (725 cm^{-1}). It had UV absorption maxima at 276 nm indicating the presence of an aromatic ring in the molecule. The generation of the ion peaks at m/z 323 [$\text{CH}_3(\text{CH}_2)_{20}\text{CO}$] $^+$, 339 [$\text{CH}_3(\text{CH}_2)_{20}\text{COO}$] $^+$ and 121 [$\text{M} - 323, \text{C}_6\text{H}_5(\text{CH}_2)_2\text{O}$] $^+$ indicated that phenyl ethyl alcohol was esterified with behenic acid. The ^1H NMR spectrum of **7** exhibited two one - proton doublets at δ 7.19 ($J = 7.2$ Hz) and 7.13 ($J = 7.2$ Hz) and as multiplets at δ 7.10, 7.07 and 7.03 assigned to aromatic protons, a two - proton triplet at δ 4.05 ($J = 7.2$ Hz) ascribed to oxymethylene H_2 -8', other methylene protons from δ 2.27 to 1.18 and a three-proton triplet at δ 0.82 ($J = 6.3$ Hz) accounted to terminal C-22 primary methyl protons. The ^{13}C NMR spectrum of **7** displayed signals for ester carbon at δ 173.93 (C-1), oxymethylene carbon at 60.15 (C-8'), other methylene carbons between δ 34.42 - 21.46, methyl carbon at δ 14.13 (C-22) and aromatic carbons in the range of δ 137.86 - 125.32. On the basis of the foregoing description, the structure of **7** was formulated as phenyl ethyl behenate, a new fatty ester (**Figure 3**).

CONCLUSION

Phytochemical investigation of the aerial parts of *Jasminum grandiflorum* gave two known glycerol esters (**1**, **2**), a cerotyl-O- β -D-diarabinoside (**3**) and two acyl O- α -D-triglucosides (**4**, **5**) as the new phytoconstituents. The leaves of *A. aspera* and *T. cordifolia* afforded new compounds aromadendr-10(14)-en-15-olyl (*E*)-ferulate (**6**) and phenyl ethyl behenate (**7**), respectively. This work has enhanced understanding about the phytoconstituents of the plant.

These secondary metabolites can be used as analytical markers for quality control of these plant. Further research is recommended to screen bioactivities of the isolated phytoconstituents with a view for supplementing conventional drug development especially in developing countries.

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