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ISOLATION AND CHARACTERIZATION OF GLYCOSIDES FROM *CONVOLVULUS PROSTRATUS, FICUS VIRENS, PHOENIX DACTIFERA, SPONDIAS MANGIFERA* AND *TERMINALIA BELERICA*

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ABSTRACT

Glycosides occur in plants as pigments and are used in medication. Our study was planned to isolate the glycosides from five medicinal plants, viz. *Convolvulus prostratus, Ficus virens, Phoenix dactifera, Spondias pinnata.* and *Terminalia belerica*. *Convolvulus prostratus* Forssk. (Convolvulaceae), used to cure mental disorders, contained geranilan-3-ol-1- carboxylate -1-O-β-D-xylopyranosyl-(2′→1″)-O-β-D-xylopyranoside.(**1**). *Ficus virens* Aiton (Moraceae) is used to treat apoplexy, blood diseases, bone fracture, delirium, diabetes, leucorrhoea, pain, rheumatism, skin ulcers and vertigo. Phytochemical investigation of a methanolic extract of the stem bark afforded α-D-glucopyranosyl-(6→1′)-O-α-D-glucopyranosyl-(6′→1″)-O-α-D-glucopyranosyl- (6″→1‴)-O-α-D-glucopyranoside (6-α-D- tetraglucoside, **2**). *Phoenix dactylifera* L. (Arecaceae) fruits are taken as an essential healthy food in the human diet and showed anticancer, antihyperlipidemic, anti-inflammatory, anti-oxidant, antiseptic and hepatoprotective properties. Column chromatography of a methanolic extract of the fruits gave a new α-D-pentaglucoside derivative characterized as α-D-glucopyranosyl-(6a→1b)-O-α-D-glucopyranosyl-(6b→1c)-O-α-D-glucopyranosyl-(6c→1d)-O-α-D-glucopyranosyl-(6d→1e)-O-α-D-glucopyranoside (**3**). The fruits of *Spondias pinnata* (L. f.) Kurz. (Anacardiaceae) are used as an antiscorbutic, aphrodisiac, astringent, blood purifier, tonic and to cures bilious dyspepsia, dysentery, burning sensation and earache. Subjection of a methanolic extract of the fruits of *S. pinnata* to silica gel column chromatography furnished propane-1,2-dioic acid-3-carboxyl O-β-D-glucopyranosyl- (6′→1″)-O-β-D-glucofuranoside, (tricarballylic acid 6-O-β-D- diglucoside, **4**). The fruits of *Terminalia bellerica* (Gaertn.) Roxb. (Combretaceae) are used to alleviate asthma, bronchitis, cardiac disorders, colds, cough, diabetes, dropsy, dyspepsia, eye and skin diseases, jaundice, leprosy, ulcer, urinary disorders and vomiting. Geranilan-10-oxy-10-O-β-D-xylopyranosyl 2′-benzoate (**5**) was isolated from the fruits of *T. bellerica*. The structures of these glycosides have been established by analysis of spectral data and chemical reactions.

KEYWORDS: Medicinal Plants, Glycosides, Isolation, Characterization.

INTRODUCTION

Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides which can be activated by enzymatic hydrolysis. Many glycosides occur in plants, often in flowers and fruits as pigments and are used as medication. Various medicines, condiments and dyes from plants occur as glycosides. Several antibiotics are glycosides (*e.g.*, streptomycin). Nucleosides derived from the partial breakdown of nucleic acids are also glycosides. Cardiac glycosides are steroidal constituents which relieve symptoms due to a congestive heart failure and reduce some atrial arrhythmias. Digoxin improves the cardiac function and treats supra-ventricular arrhythmias, particularly atrial fibrillation. Steviol glycosides are preferred as natural sweeteners for diabetic patients. Saponins are glycosides that lower the surface tension of water and used as cleansing agents and as an expectorant. Phenolic glycosides possess antiseptic properties that are useful for the health of the urinary tract. Flavonoid glycosides include naringin, hesperidin, quercitrin and rutin. These glycosides have antioxidant properties and improve capillary debility. Apterin, a coumarin glycoside, expands the coronary arteries and functions as a calcium channel blocker. Salicin is an alcoholic glycoside found in the genus Salix. Our body transforms salicin into salicylic acid that is intimately associated with aspirin and possesses analgesic, febrifuge and anti-inflammatory properties. Anthraquinone glycosides are antineoplastic. The cyanogenic glycosides from *Prunus* species (cherry), particularly amygdalin and prunasin, have anticarcinogenic activity.[1-3] Five

medicinal plants, *viz. Convolvulus prostratus* aerial parts, *Ficus virens* bark, *Phoenix dactifera* fruits, *Spondias mangifera* fruits and *Terminalia belerica* fruits were selected for isolation of the glycosides and their structural formulae were established on the basis of spectral data analysis and chemical reactions.

Convolvulus prostratus Forssk., syn. *C. pluricaulis* Choisy (Convolvulaceae), known as shankapushpi, shankhuli and speedwheel, grows in India, Myanmar and Sri Lanka. It is a small, hairy, procumbent, diffuse, perennial herb with prostrate branches, small elliptic to oblong, lanceolate, obtuse, mucronate leaves and light blue, solitary flowers.[4] It is useful to enhance brain power and to treat anorexia, attention deficit hyperactivity disorder (ADHD), mild convulsions, dementia, depression, emotional stress, epilepsy, headache due to stress, mental debility, memory loss, mental hypersensitivity, schizophrenia, sleeplessness, stress disorders, and vertigo. It is added in some formulations with other drugs used for aggressive behavior disorders, depression, epilepsy and schizophrenia.[5] Shankhpushpi contained D-glucose, rhamnose, maltose, sucrose, starch, proteins, amino acids, alkaloids convolvine, convolamine, confoline, phyllabine, convolidine, convoline, convosine, subhirsine and convolvidine, wax, scopoletin, glacial acetic acid, β-sitosterol, kaempferol, linoleic and palmitic acids, hextriacontane, 20-oxodotriacontanol, ceryl alcohol, octacosanol, tetracosane, tetratriacontanoic acid, 29- oxodotriacontanol, di-hydroxycinnamic acid, β-sitosterol glucoside, microphyllic acid, pentyl hexacosanoate, tetracyclohexanyl caproate, dicyclohexyl cyclo-octyl acetic acid, cyclo-octadecanyl methanol, 7-hydroxyheptadecanyl-1,7, 17-tricarboxylic acid and 1-pentyl-2-tridecanyl cyclopentyl cyclohexane carboxylate.[6-9]

Ficus virens Aiton, syn. *F. ampla* Kunth et Bouche, *F. infectoria* Roxb., *F. wightiana* Wall. (Moraceae), known as pilkhan, pakhad, spotted white fig, is a medium to large-sized deciduous tree without aerial roots, frequently epiphytic with greenish-grey, smooth bark, leaves oblong- elliptic to ovate or lanceolate, apex acuminate, base truncate to cordate, glabrous; fruits pinkish brown to white figs, borne amongst leaves, usually paired. It grows in India, Sri Lanka, the Solomon Islands, China, Japan, through tropical Asia to New Guinea, Australia, Malaysia and New Guinea between 300–2,700 m altitude.[10]

Its bark, latex, leaves and fruits are used to treat apoplexy, blood diseases, bone fracture, delirium, diabetes, leucorrhoea, pain, rheumatism, skin ulcers, vertigo and as a gargle in salivation.[11-13]

The leaves yielded quercetin, kaempferol, their glycosides and vogelin J.[14] The bark possessed phenolics, *n*-octadecanyl-O-α-D-glucopyranosyl(6′ → 1″)-O-α-D-glucopyranoside, proanthocyanidins and flavonoids.[15-19]

Phoenix dactylifera L., syn. *Palma dactylifera* (L.) Mill., *Phoenix chevalieri* D. Rivera, S. Ríos et Obón (Arecaceae), known as date, is a native to the Egypt's Nile valley, Morocco, Algeria and Tunisia; grown in Libya, Israel, Iraq, Iran, Mexico and the United States.[20] Dates have medicinal uses including anticancer, antihyperlipidemic, anti-inflammatory, anti-oxidant, antiseptic, hepatoprotective properties and thereby serving as an essential healthy food in the human diet.[21,22] Dates contain as major carotenoids lutein, β-carotene, neoxanthin, violaxanthin and antheraxanthin[23], phytosterols[24], isoflavones, lignans, seco-isolariciresinol, coumestrol, phenolic acids[25-28], cinnamic acid derivatives and flavones.[29,30]

Spondias pinnata (L. f.) Kurz., syn. *S. mangifera* (Linn. F.) Kurz., *S. paniculata* Roxb. ex Wight et Arn., *Mangifera pinnata* L. f. (Anacardiaceae), known as amara, Andaman mombin, Indian mombin, bing lang qing, Indian hog-plum and wild mango, is distributed in southern China, India, Nepal, Myanmar, Thailand, Cambodia, Laos, Vietnam, Malaysia, Indonesia, Philippines and the Solomon Islands up to 1200 m. It is a medium-sized deciduous tree, 10–20 m tall, with large, imparipinnate leaves, crowded at the end of branchlets, leaflet blade ovate-oblong to elliptic-oblong, papery, glabrous, margins serrate or entire, apex acuminate; flowers sessile, small, white and glabrous; fruit a drupe, ellipsoid, yellowish orange at maturity; seeds two or three.[31,32] The bark is antiemetic, aromatic, astringent, rubefacient and tonic; used to treat arthritic, diarrhoea, dysentery, biliousness, gonorrhea, rheumatism, mental disorders, tuberculosis and vomiting. A bark paste is applied to relieve joint and stomach pains, ringworm and skin diseases. The roots are given to regulate menstruation. The leaves are appetizing and astringent; leaf juice is dropped into the ear to cure earache. The unripe fruit is good for rheumatism and sore throat. The fruit is antiscorbutic, aphrodisiac, astringent, blood purifier and tonic; cures bilious dyspepsia, dysentery, burning sensation and earache.[31-34] The fruits contained β-amyrin, oleanolic acid, amino acids and polysaccharides. The aerial parts gave lignoceric acid, 24-methylenecycloartanone, stigmast- 4-en-3-one, β-sitosterol, its glucoside, daucosterol, 24-methylene cycloartanone, ellagitannins, galloylgeranin and β – carotein.[31,35-37] The bark possessed rutin and tannic acid.[38]

Terminalia bellerica (Gaertn.) Roxb. (Combretaceae), known as bahera and belliric myrobalan, is a large, fast-growing deciduous tree up to 50 m in height with a large, globose crown; leaves simple, alternate, spiral, clustered at the twig ends; inflorescence axillary spikes; flowers sessile, creamy white; drupes globose or ovoid, 1-seeded. It is found in deciduous forests of India, China, Indonesia, Sri Lanka, Nepal, Pakistan and Malaysia. The

plant is used to treat asthma, ophthalmia, diabetes, diarrhoea, dropsy, dysentery, fevers, liver complaints, hypertension, piles, skin diseases and wounds. The bark is diuretic and taken to relieve anaemia, chest pain, heart diseases and leucoderma. The fruits are antiemetic, anthelmintic, febrifuge, anti-inflammatory, expectorant, flatulence, laxative, ophthalmic and tonic, used to alleviate asthma, blood pressure, bronchitis, cardiac disorders, colds, cough, diabetes, dropsy, dyspepsia, jaundice, eye and skin diseases, leprosy, scorpion stings, thirst, ulcer, urinary disorders and vomiting. The ripe fruits are taken along with *T. chebulia* and *Phyllanthus emblica* as a Triphala drug. The seed oil is applied to cure inflammation, rheumatism, alopecia, skin disorders and premature graying of hair.[39-41] The bark contains β-sitosterol, tannins, belleric, ellagic and gallic acids, catechol, arjungenin, its glycosides and belericosides. The fruits possessed gallic, chebulagic, elllagic, quinic, shikimic and dehydroshikimic acids, bellericosides, hexahydroxydiphenic acid ester, ethyl gallate, β-sitosterol, galloyl glucose, mannitol, glucose, galactose, rhamnose, tannins, lignans, trigalloyl and pentagalloyl glucogallin, corilagin, chebulin, terchebulin, chebupentol, arjugenin, arjunolic acid and sennoside A.[42-50] The leaves gave 4-hydroxy-(2-methylbutanol) benzoic acid, tannins, gallic and ellagic acids, methyl gallate, luteolin, quercetin, kaempferol and flavonol glycosides.[50,51] The seed oil was composed of palmitic, oleic and linoleic acids as the major fatty acids.[52]

MATERIAL AND METHODS

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 ^{1}H and ^{13}C NMR spectra were scanned on a Bruker DRX (300 MHz) instrument 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 F_{254} 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 aerial parts of *Convolvulus prostratus,* stem bark of *Ficus virens*, and fruits of *Phoenix dactifera, Spondias mangifera* and *Terminalia belerica* were collected locally from Delhi and authenticated by Prof. M.P. Sharma, Taxonomist, Department of Botany, Jamia Hamdard, New Delhi. Their voucher specimens were preserved in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

Extraction and isolation

Each 1 kg of the aerial parts of *C. prostratus,* stem bark of *F. virens*, and fruits of *P. dactifera, S. mangifera* and *T. belerica* were coarsely powdered and extracted exhaustively separately with methanol in a Soxhlet apparatus. The extracts were concentrated under reduced pressure to get dark brown masses, 121.3 g, 127.4 g, 118.6 g, 224.8 g, 185.1 g and 127.6 g, respectively. The dried residue (100 g each) was dissolved in minimum amount of methanol and adsorbed on silica gel column grade (60-120 mesh) separately to obtain slurries. Each slurry was air-dried and chromatographed over silica gel columns loaded in chloroform individually. Each column was eluted with chloroform and mixtures of 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. The isolated compounds were recrystallized to get pure compounds. The following compounds were isolated :

Isolation of a glycoside from *Convolvulus prostrates* aerial parts

Geranilan-3*α*-ol-1-carboxylate 1-O-β--D-dixyloside (1): Elution of the column with chloroform - methanol (19:1) provided a colourless semisolid mass of **1**, yield 214 mg, UV λ_{max} (MeOH), 210 nm (log ε 3.4); IR γ_{max} (KBr): 3398, 3260, 3130, 2925, 2854, 1740, 1636, 1403, 1381, 1237, 1166, 1099, 756 cm^{-1}; ^{1}H NMR (MeOD): δ 5.35 (1H, d, J = 7.2 Hz, H-1′), 4.59 (1H, m, H-2′), 4.12 (2H, m, H_2-5′), 4.12 (1H, m, H-3′), 3.65 (1H, m, H_2-4′), 5.16 (1H, d, J = 7.3 Hz, H-1″), 4.43 (1H, m, H-2″), 4.23 (2H, m, H_2-5″), 3.95 (1H, m, H-3″), 3.62 (1H, m, H_2-4″), 2.36 (1H, s, H_2-2a), 2.29 (1H, s, H_2-2b), 2.08 (2H, m, H_2-4), 1.72 (2H, m, H_2-5), 1.68 (2H, m, H_2-6), 1.60 (1H, m, H-7), 1.25 (3H, s, Me-10), 0.88 (3H, d, J = 6.6 Hz, Me-8), 0.83 (1H, d, J = 6.4 Hz, Me-9); ^{13}C NMR (MeOD): δ 169.23 (C-1), 46.26 (C-2), 74.32 (C-3), 36.67 (C-4), 30.29 (C-5), 34.72 (C-6), 42.24 (C-7), 18.19 (C-8), 17.21 (C-9), 21.62 (C-10), 98.34 (C-1′), 75.21 (C-2′), 70..67 (C-3′), 67.36 (C-4′), 64.52 (C-5′), 96.73 (C-1″), 71.18 (C-2″), 68.87 (C-3″), 66.71 (C-4″), 6394 (C-5″), ESI MS *m/z* (rel. int.): 452 $[M]^+$ ($C_{20}H_{36}O_{11}$) (1.4), 281 (10.8), 187 (9.4), 149 (6.1), 133 (21.2).

Isolation of a glycoside from *Ficus virens* stem bark 6-*α*-D- Tetraglucoside (2)

Elution of the column with chloroform -methanol (13:7) yielded a colourless sticky mass of **2**, recrystallized from chloroform - methanol (1:1 *v/v*), yield 295 mg, R_f 0.64 (chloroform - methanol, 4 : 1 *v/v*); UV λ_{max} (MeOH) 285 nm; IR γ_{max} (KBr): 3505, 3429, 3365, 3021, 2927, 2891, 1636, 1408, 1215, 1114, 926 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.18 (1H, d, J = 4.4 Hz, H-1), 4.22 (1H, m, H-5), 3.89 (1H, m, H-2), 3.74 (1H, m, H-3), 3.51 (1H, m, H-4), 3.34 (2H, d, J = 13.2 Hz, H_2-6), 5.05 (1H, d, J = 4.1 Hz, H-1′),

4.16 (1H, m, H-5′), 3.87 (1H, m, H-2′), 3.70 (1H, m, H-3′), 3.47 (1H, m, H-4′), 3.27 (2H, d, J = 12.0 Hz, H_2-6′), 4.89 (1H, d, J = 4.6 Hz, H-1″), 4.11 (2H, m, H-5″), 3.80 (1H, m, H-2″), 3.64 (1H, m, H-3″), 3.44 (1H, m, H-4″), 3.20 (2H, d, J = 8.8 Hz, H_2-6″), 4.85 (1H, d, J = 5.1 Hz, H-1‴), 4.01 (1H, m, H-5‴), 3.78 (1H, m, H-2‴), 3.55 (1H, m, H-3‴), 3.39 (1H, m, H-4‴), 3.09 (2H, d, J = 8.3 Hz, H_2-6‴); ^{13}C NMR (DMSO-d_6): δ 104.06 (C-1), 75.82 (C-2), 72.88 (C-3), 71.66 (C-4), 82.56 (C-5), 63.05 (C-6), 101.99 (C-1′), 75.24 (C-2′), 72.78 (C-3′), 69.89 (C-4′), 79.01 (C-5′), 62.17 (C-6′), 97.31 (C-1″), 74.35 (C-2″), 72.61 (C-3″), 68.82 (C-4″), 77.16 (C-5″), 61.22 (C-6″), 91.78 (C-1‴), 73.68 (C-2‴), 71.89 (C-3‴), 66.79 (C-4‴), 76.74 (C-5‴), 60.52 (C-6‴); ESI MS *m/z* (rel. int.): 666 $[M]^+$ ($C_{24}H_{42}O_{21}$) (12.5), 503 (3.9), 487 (7.8), 342 (17.5), 179 (7.2), 163 (4.6).

Isolation of a glycoside from *Phoenix dactylifera* fruits 6-α-D-Pentaglucoside (3)

Elution of the column with chloroform - methanol (1:1) furnished colourless crystals of **3**, yield 361 mg, m. p. 182-185 °C; IR γ_{max} (KBr): 3421, 3350, 3261, 2924, 2853, 1618, 1445, 1384, 1285, 1109, 1048 cm^{-1}; ^{1}H NMR (DMSO d-$_6$): δ 5.17 (1H, d, J = 4.1 Hz, H-1a), 4.27 (1H, m, H-5a), 3.78 (1H, m, H-2a), 3.58 (1H, m, H-3a), 3.36 (1H, m, H-4a), 3.20 (2H, d, J = 8.0 Hz, H_2-6a), 5.12 (1H, d, J = 3.8 Hz, H-1b), 4.25 (1H, m, H-5b), 3.75 (1H, m, H-2b), 3.52 (1H, m, H-3b), 3.34 (1H, m, H-4b), 3.17 (2H, d, J = 9.5 Hz, H_2-6b), 4.83 (1H, d, J = 4.3 Hz, H-1c), 3.97 (1H, m, H-5c), 3.73 (1H, m, H-2c), 3.48 (1H, m, H-3c), 3.31 (1H, m, H-4c), 3.15 (2H, d, J = 8.1 Hz, H_2-6c), 4.54 (1H, d, J = 3.2 Hz, H-1d), 3.87 (1H, m, H-5d), 3.70 (1H, m, H-2d), 3.45 (1H, m, H-3d), 3.27 (1H, m, H-4d), 3.12 (2H, d, J = 8.3 Hz, H_2-6d), 4.47 (1H, d, J = 3.9 Hz, H-1e), 3.84 (1H, m, H-5e), 3.65 (1H, m, H-2e), 3.42 (1H, m, H-3e), 3.24 (1H, m, H-4e), 3.06 (2H, d, J = 12.0 Hz, H_2-6e); ^{13}C NMR (DMSO d-$_6$): δ 104.16 (C-1a), 75.94 (C-2a), 73.23 (C-3a), 71.79 (C-4a), 82.63 (C-5a), 63.19 (C-6a), 102.11 (C-1b), 75.45 (C-2b), 73.03 (C-3b), 71.79 (C-4b), 81.97 (C-5b), 63.10 (C-6b), 98.21 (C-1c), 75.45 (C-2c), 72.95 (C-3c), 70.72 (C-4c), 77.27 (C-5c), 62.31 (C-6c), 97.01 (C-1d), 74.97 (C-2d), 72.49 (C-3d), 70.44 (C-4d), 77.02 (C-5d), 61.35 (C-6d), 91.92 (C-1e), 74.46 (C-2e), 72.06 (C-3e), 69.98 (C-4e), 76.83 (C-5e), 60.64 (C-6e); ESI MS *m/z* (*rel.int.*): 828 $[M]^+$ ($C_{30}H_{52}O_{26}$) (2.1), 503 (4.1), 487 (3.1), 341 (10.2), 325 (7.1), 179 (3.6), 163 (5.6).

Isolation of a glycoside from *Spondias pinnata* fruits Tricarballylic acid 6-O-β-D- diglucoside (4)

Elution of the column with chloroform-methanol (9 : 1) afforded a pale yellow semi-solid mass of **4**, yield 178 mg, R_f 0.62 (chloroform - methanol, 9 :1), UV (MeOH): λ_{max} 219 (log ε 2.8); IR γ_{max} (KBr): 3511, 3425, 3360, 3255, 2925, 2845, 1752, 1701, 1628, 1486, 1360, 1221, 1087, 772 cm^{-1}; ^{1}H NMR (MeOD): δ 2.98 (2H, d, J = 4.5 Hz, H_2-3), 2.77 (1H, m, H-2), 2.71 (1H, d, J = 4.2 Hz, H_2-1a), 2.65 (1H, d, J = 4.5 Hz, H_2-1b), 5.01 (1H, d, J = 7.8 Hz, H-1′), 4.36 (1H, m, H-5′), 3.88 (1H, m, H-2′), 3.79 (1H, m, H-3′), 3.35 (1H, m, H-4′), 3.28 (2H, d, J = 8.5 Hz, H_2-6′), 5.38 (1H, d, J = 8.5 Hz, H-1″), 3.76 (1H, m, H-2″), 3.72 (1H, m, H-3″), 3.40 (2H, s, H_2-5″), 3.20 (2H, s, H_2-6″), ^{13}C NMR (MeOD): δ 41.08 (C-1), 42.91 (C-2), 53.36 (C-3), 176.01 (C-4), 175.21 (C-5), 173.81 (C-6), 101.16 (C-1′), 76.17 (C-2′), 70.26 (C-3′), 74.13 (C-4′), 78.66 (C-5′), 64.07 (C-6′), 105.98 (C-1″), 86.35 (C-2″), 68.70 (C-3″), 84.31 (C-4″), 65.34 (C-5″), 62.12 (C-6″); ESI MS *m/z* (rel. int., %): 500 $[M]^+$ ($C_{18}H_{28}O_{16}$) (3.2), 325 (12.8), 179 (18.4), 175 (21.2), 163 (9.4).

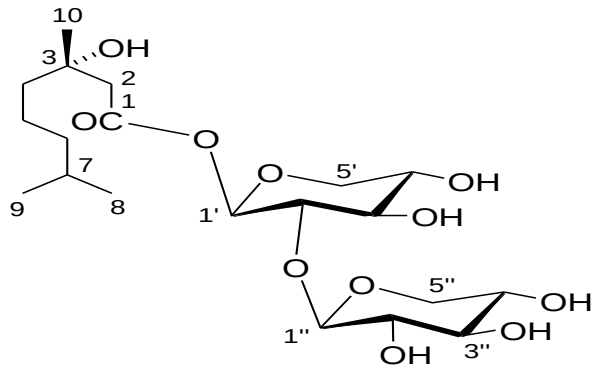
Isolation of a glycoside from *Terminalia belerica* fruits Geranilan-10-oxy-10-O-β-D-xylopyranosyl 2′-benzoate (5)

Elution of the column with chloroform - methanol (4:1) provided a pale yellow powder of **5**, yield 214 mg, R_f 0.42 (chloroform - methanol, 4:1), m. p. 108 -110 ^{0}C, UV λ_{max} (MeOH), 243 nm (log ε 3.6); IR γ_{max} (KBr): 3401, 3315, 2926, 2864, 1725, 1646, 1525, 1435, 1384, 1215, 1156, 1033, 758 cm^{-1}; ^{1}H NMR (MeOD): δ 7.55 (1H, m, H-2″), 7.49 (1H, m, H-6″), 7.08 (1H, m, H-3″), 7.05 (1H, m, H-5″), 6.98 (1H, m, H-4″), 4.69 (1H, d, J = 7.2 Hz, H-1′), 4.39 (1H, m, H-2′), 4.07 (1H, m, H-4′), 3.76 (1H, m, H-3′), 3.56 (2H, d, J = 5.7 Hz, H_2-5′), 3.34 (2H, d, J = 6.3 Hz, H_2-10), 2.92 (1H, m, H-3), 2.59 (1H, m, H-7), 2.55 (2H, m, H_2-5), 2.49 (2H, m, H_2-4), 1.35 (2H, m, H_2-6), 1.29 (2H, m, H_2-2), 1.21 (3H, d, J = 6.1 Hz, Me-8), 1.18 (3H, d, J = 6.3 Hz, Me-9), 0.85 (1H, t, J = 6.7 Hz, Me-1); ^{13}C NMR ($CDCl_3$): δ 13.19 (C-1), 22.06 (C-2), 46.38 (C-3), 30.65 (C-4), 36.28 (C-5), 38.92 (C-6), 42.19 (C-7), 15.09 (C-8), 15.11 (C-9), 62.67 (C-10), 90.33 (C-1′), 75.08 (C-2′), 69.87 (C-3′), 74.17 (C-4′), 64.53 (C-5′), 146.45 (C-1″), 139.63 (C-2″), 123.47 (C-3″), 110.49 (C-4″), 118.36 (C-5″), 122.10 (C-6″), 170.48 (C-7″); ESI MS *m/z* (rel. int.): 394 $[M]^+$ ($C_{22}H_{34}O_6$) (1.4), 253 (9.7), 237 (13.9), 141 (4.6), 121 (12.5).

RESULTS AND DISCUSSION

Compound **1**, named geranilan-3α-ol-1-carboxylate 1-O-β--D-dixyloside, gave positive tests for glycosides and showed IR absorption bands for hydroxyl groups (3398, 3260, 3130 cm^{-1}) and ester function (1740 cm^{-1}). Its molecular ion peak was determined at *m/z* 452 on the basis of mass and ^{13}C NMR spectral data consistent with the molecular formula of a monoterpenoate dipentosidic ester, $C_{20}H_{36}O_{11}$. The ion peaks generating at *m/z* 187 [O - $C_{1'}$ fission, $C_{10}H_{19}O_3]^+$, 133 $[C_5H_9O_4]^+$, 149 $[C_5H_9O_5]^+$ and 281 $[C_5H_9O_4$- $C_5H_8O_5]^+$ indicated that a hydroxymonoterpenoate unit was linked with a dipentoside moiety. The ^{1}H NMR spectrum of **1** exhibited two one – proton deshielded doublets at δ 5.35 (J = 7.2 Hz) and 5.16 (J = 7.3 Hz) ascribed to anomeric H-1′ and H-1″ protons, respectively, other sugar protons between δ 4.59 – 3.63, two one - proton singlets at δ 2.36 and 2.29 due to methylene protons adjacent to the ester function, a three-proton singlet at δ 1.25 accounted to C-10 methyl protons located on a C-3 tertiary oxycarbon, two three-proton doublets at δ 0.88 (J = 6.6 Hz) and 0.83 (J = 6.4 Hz) attributed correspondingly to secondary C-8 and C-9 methyl protons and the remaining methine and

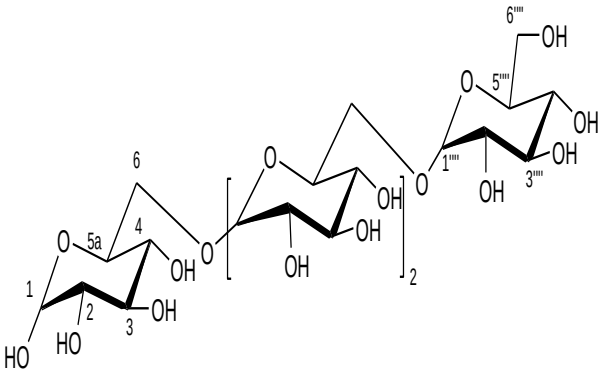
methylene protons between δ 2.08 – 1.60. The ^{13}C NMR spectrum of **1** displayed signals for ester carbon at δ 169.23 (C-1), anomeric carbons at δ 98.34 (C-1′) and 96.73 (C-1″), other sugar carbons between δ 75.21 - 63.94, methyl protons at δ 18.19 (C-8), 17.21 (C-9) and 21.62 (C-10), oxygenated tertiary carbon at δ 74.32 (C-3) and the remaining methylene and methine carbons from δ 46.26 to 30.29. The presence of the sugar H-2′ proton signal in the downfield region as a one – proton multiplet at δ 4.59 and its respective carbon signal at δ 75.21 (C-2′) suggested the (2′→1″) linkage of the sugar units. Acid hydrolysis of **1** yielded D- xylose, R_f 0.2 (*n*-butanol: acetic acid: water; 4:1:5, top). On the basis of above discussion the structure of **1** was elucidated as geranilan-3-ol-1- carboxylate -1-O-β-D-xylopyranosyl-(2′→1″)-O-β-D-xylopyranoside (**1**), a new monoterpenic dixyloside (Fig 1).



Geranilan-3α-ol-1-carboxylate-1-O-β-D-dixyloside (1)

Fig. 1. Compound 1 isolated from the aerial parts of *Convolvulus prostrates.*

Compound **2,** named as 6-α-D-tetraglucoside, showed IR diagnostic absorption bands for hydroxyl groups (3505, 3429, 3365, 3021 cm^{-1}). It responded positively to general test for glycosides. On the basis of mass and ^{13}C NMR spectral data the molecular ion peak of **2** was determined at *m/z* 666 consistent to a molecular formula of a tetraglycoside, $C_{24}H_{42}O_{21}$. The ion peaks generating at *m/z* 163 $[C_6H_{11}O_5]^+$, 179 $[C_6H_{11}O_6]^+$, 342 $[C_6H_{11}O_6\text{-}C_6H_{11}O_5]^+$ and 503 $[M\text{-}163]^+$ suggested that hexose units were present in the compound **2.** The ^1H NMR spectrum of **2** showed four one-proton doublets at δ 5.18 (J = 4.4 Hz), 5.05 (J = 4.1 Hz), 4.89 (J = 4.6 Hz) and 4.85 (J = 5.1 Hz) ascribed as α-oriented anomeric H-1′, H-1″, H-1‴ and H-1 protons, respectively. The sugar units present in **2** were identified as α-D-glycopyranose by analysis of the coupling constants of the anomeric signals of sugar protons as one proton doublets from δ 5.18 to 4.85 having coupling constants between 5.1 – 4.1 Hz. Four one-proton multiplets appeared between δ 4.22 - 4.01 which were assigned to oxymethine H-5, H-5′, H-5″ and H-5‴ protons, respectively. The remaining hydroxyl methine protons resonated from δ 3.89 to 3.39. Four two - proton doublets at δ 3.34 (J = 13.2 Hz), 3.27 (J = 12.0 Hz), 3.20 (J = 8.8 Hz) and 3.09 (J = 8.3 Hz) were attributed to oxymethylene H_2-6, H_2-6′, H_2-6″ and H_2-6‴ protons, respectively. The ^{13}C NMR of **2** exhibited important signals for anomeric carbons at δ 104.01 (C-1), 101.99 (C-1′), 97.31 (C-1″) and 91.78 (C-1‴). The other sugar carbons appeared between δ 82.56 - 60.521. The presence of the oxymethylene H_2-6, H_2-6′ and H_2-6″ proton signals in the ^1H NMR spectrum in the deshielded region at δ 3.34, 3.27 and 3.20, respectively, and the corresponding carbon signals of C-6, C-6′ and C-6″ in the ^{13}C NMR spectrum at δ 63.05, 62.17 and 61.22 suggested (6→1) linkages of the sugar units. Acid hydrolysis of **2** yielded D-glucose, R_f 0.26 (*n*-butanol-acetic acid – water, 4 : 1 : 5). On the basis of above discussion the structure of **2** was elucidated as α-D-glucopyranosyl-(6→1′)-O-α-D-glucopyranosyl-(6′→1″)-O-α-D-glucopyranosyl- (6″→1‴)-O-α-D-glucopyranoside (Fig 2).

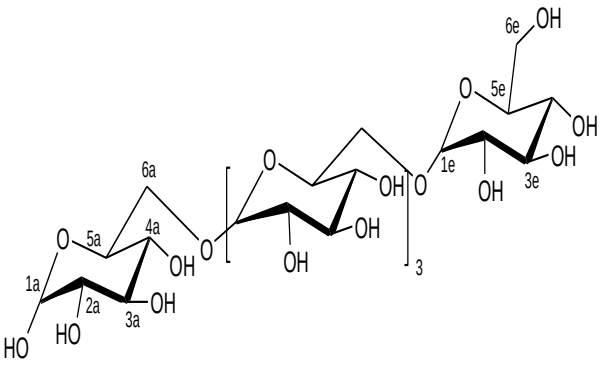


6-α-D-Tetraglucoside (2)

Fig. 2. Compound 2 isolated from the bark of *Ficus virens.*

Compound **3,** named 6-α-D-pentaglucoside**,** $[M]^+$ at *m/z* 828 ($C_{30}H_{52}O_{26}$), gave positive tests for glycosides and showed IR absorption bands for hydroxyl groups (3421, 3350, 3261 cm^{-1}). The ion peaks arising at *m/z* 163 $[C_6H_{11}O_5]^+$, 179 $[C_6H_{11}O_6]^+$, 325 $[C_6H_{11}O_5\text{-}C_6H_{10}O_5]^+$, 503 $[M - 325]^+$, 487 $[C_6H_{11}O_5\text{-}C_6H_{10}O_5\text{-}\ C_6H_{10}O_5]^+$ and 341 $[M - 487]^+$ indicated that hexose units were linked in the pentasaccharide unit. The ^1H NMR spectrum of compound **3** exhibited five one – proton signals for anomeric protons as doublets at δ 5.17 (J = 4.1 Hz), 5.12 (J = 3.8 Hz), 4.83 (J = 4.3 Hz), 4.54 (J = 3.2 Hz) and 4.47 (J = 3.9 Hz) assigned to α-oriented anomeric H-1a to H-1e protons. The other sugar protons resonated as multiplets between δ 4.27 - 3.24 due to oxymethine protons and as two-proton doublets at δ 3.20 (J = 8.0 Hz), 3.17 (J = 9.5 Hz), 3.15 (J = 8.1 Hz), 3.12 (J = 8.3 Hz) and 3.06 (J = 12.0 Hz) associated with the hydroxymethylene H_2-1a to H_2-1e protons, respectively. The ^{13}C NMR spectrum of compound **3** displayed signals for anomeric carbons from δ 104.16 to 91.92 and other sugar carbons between 82.63 - 60.64. The presence of the sugar protons from H_2-6a to H_2-6d in the deshielded region from δ 3.20 to 3.12 in the ^1H NMR spectrum and carbon signals C-6a to C-6d between δ 63.19 – 61.35 in the ^{13}C NMR spectrum suggested (6→1) linkages of the sugar units. Acid hydrolysis of **3** yielded D-glucose, R_f 0.26 (*n*-butanol- acetic acid – water, 4 : 1 : 5). On the

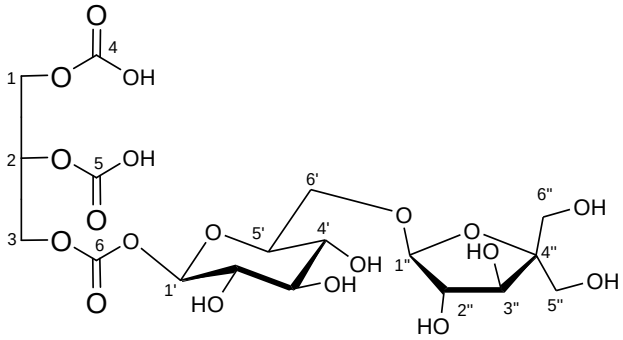
basis of the foregoing discussion the structure of **3** has been established as α-D-glucopyranosyl-(6a→1b)-O-α-D-glucopyranosyl-(6b→1c)-O-α-D-glucopyranosyl-(6c→1d)-O-α-D-glucopyranosyl-(6d→1e)-O-α-D-glucopyranoside, a new α-D-pentaglucoside derivative (Fig 3).



6-α-D-Pentaglucoside (3)

Fig. 3. Compound 3 isolated from fruits of *Phoenix dactylifera.*

Compound **4**, named tricarballylic acid 6-O-β-D-diglucoside, gave effervescence with sodium bicarbonate solution and responded positively to glycoside tests. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3511, 3425, 3360 cm^{-1}), ester group (1752 cm^{-1}) and carboxylic functions (3255, 1701 cm^{-1}). On the basis of mass and ^{13}C NMR spectra the molecular ion peak of **4** was determined at *m/z* 500 $[M]^+$ consistent with a molecular formula of propane tricarboxylic acid diglycoside, $C_{18}H_{28}O_{16}$. The ion peaks arising at *m/z* 163 $[C_6H_7O_5]^+$, 179 $[C_6H_{11}O_5]^+$,325 $[C_6H_{11}O_5\text{-}C_6H_{10}O_5]^+$, 337 $[M - 163]^+$ and 175 $[M - 325, C_6H_{11}O_6]^+$ indicated that propane tricarboxylic acid was linked to a dihexose sugar unit. The ^1H NMR spectrum of **4** displayed two one-proton doublets at δ 5.01 (J = 7.8 Hz) and 5.38 (J = 7.6 Hz) ascribed to anomeric H-1′ and H-1″protons, respectively. The other sugar protons appeared in the range of δ 4.36 - 3.20. The deshielded resonance of the sugar H_2-6′ protons as a doublet at δ 3.28 (J = 8.5 Hz) indicated the attachment of other sugar unit at C-6′ by (6′→1″) linkage. A two-proton doublet at δ 2.98 (J = 4.5 Hz) and two one-proton doublets at δ 2.71 (J = 4.2 Hz) and 2.65 (J = 4.5 Hz) were associated correspondingly with methylene H_2-3 adjacent to the ester group and with methylene H_2-1a and H_2-1b protons near by the carboxylic groups. A one-proton multiplet at δ 2.77 was accounted to methine H-2 proton. The ^{13}C NMR spectrum of **4** showed important signals for carboxylic carbons at δ 176.01 (C-4) and 175.21 (C-5), ester carbon at δ 173.81 (C-6), anomeric carbons at δ 101.16 (C-1′) and 105.98 (C-1″), other sugar carbons between δ 86.35 - 62.12, methylene carbons at δ 41.08 (C-1) and 53.36 (C-3) and methine carbon at δ 42.91 (C-2). The appearance of the sugar carbons in the downfield region at δ 86.35 (C-2″) and 84.31 (C-4″) supported furanose ring system of one of the sugar unit. The existence of the oxymethylene C-6′ carbon in the downfield region at δ 64.07 (C-6′) supported the location of the second sugar unit at C-6′. Acid hydrolysis of **4** yielded tricarballylic acid, m. p. 156 – 159 °C and D-glucose, R_f 0.26 (*n*-butanol- acetic acid – water, 4 : 1 : 5). On the basis of spectral data analysis and chemical reactions, the structure of **4** has been characterized as propane-1,2-dioic acid-3-carboxyl O-β-D-glucopyranosyl- (6′→1″)-O-β-D-glucofuranoside, a new tricarballylic acid diglucoside (Fig 4).

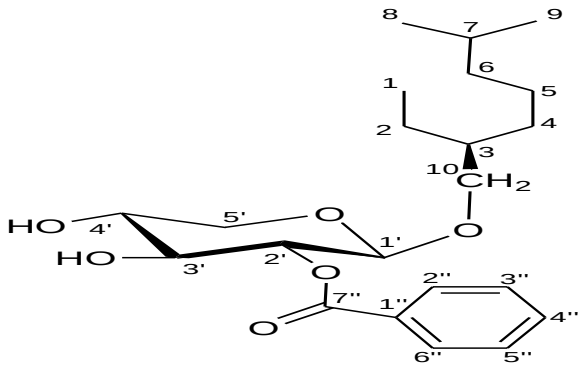


Tricarballylic acid-6-O-β-D-diglucoside (4)

Fig. 4. Compound 4 isolated from *Spondias mangifera* fruits.

Compound **5**, named geranilan-10-oxy-10-O-β-D-xylopyranosyl 2′-benzoate, responded glycosidic tests positively and showed IR absorption bands for hydroxyl groups (3401, 3315, cm^{-1}), ester function (1725 cm^{-1}) and aromatic ring (1525, 1033 cm^{-1}). On the basis of mass and ^{13}C NMR spectral data, the molecular ion peak of **5** was established at *m/z* 394 consistent with the molecular formula of a monoterpenic glycosidic benzoate, $C_{22}H_{34}O_6$. The ion peaks generating at *m/z* 141 [O - C_{10} fission, $C_{10}H_{21}]^+$, 253 $[M - 141]^+$, 237 $[C_{1'}$ - O fission, $C_5H_8O_4\text{-}CO\text{-}C_6H_5]^+$ and 121 $[OOC\text{-}C_6H_5]^+$ indicated that a monoterpenic glycoside was linked with benzoyl unit. The ^1H NMR spectrum of **5** displayed five one – proton deshielded multiplets between δ 7.55 – 7.98 assigned to aromatic H-2″ to H-6″. A one – proton doublet at δ 4.69 (J = 7.2 Hz) was ascribed to anomeric H-1′ proton. The other sugar protons appeared as one – proton multiplets at δ 4.39 (H-2′), 4.07 (H-4′) and 3.76 (H-3′) and as a two – proton doublet at δ 3.56 (J= 5.7 Hz, H_2-5′). A two - proton doublet at δ 3.34 (J = 6.3 Hz) was attributed to oxymethylene H_2-10 protons. Two three-proton doublets at δ 1.21 (J = 6.1 Hz) and 1.18 (J = 6.3 Hz) and a three – proton triplet at δ 0.85 (J = 6.7 Hz) were accounted to secondary C-8 and C-9 and primary C-1 methyl protons, respectively. The other methine and methylene protons resonated from δ 2.92 to 1.29. The ^{13}C NMR spectrum of **5** displayed signals for aromatic carbons between δ 146.45 to 110.49, ester carbon at δ 170.48 (C-7″), anomeric carbon at δ 90.33 (C-1′), other sugar carbons between δ 75.08 - 64.53, methyl protons at δ 13.19 (C-1), 15.09 (C-8) and 15.11 (C-9), oxymethylene carbon at δ 62.67 (C-10) and the remaining methylene and methine carbons from δ 46.38 to 17.39. The presence of proton H-2′ signal in the downfield region as a one – proton multiplet at δ 4.39 and its respective carbon signal at δ

75.08 (C-2′) suggested the (2′→7″) linkage of the benzoyl unit with the sugar moiety. Acid hydrolysis of **5** yielded D- xylose, R_f : 0.2 (*n*-butanol: acetic acid: water; 4:1:5, top). On the basis of above discussion the structure of **5** was elucidated as geranilan-10-oxy-10-O-β-D-xylopyranosyl 2′-benzoate, a new monoterpenic xylosidic benzoate (Fig 5).



Geranyl-10-oxy-10-O-β-D-xylopyranoside 2′-benzoate (5)

Fig. 5. Compound 5 isolated from *Terminalia belerica* fruits.

CONCLUSION

Phytochemical investigation of a methanolic extract of the aerial parts of *Convolvulus prostratus* gave geranilan-3-ol-1- carboxylate -1-O-β-D-xylopyranosyl-(2′→1″)-O-β-D-xylopyranoside. The stem bark of *Ficus virens* afforded a 6-α-D- tetraglucoside. From the fruits of *Phoenix dactylifera* L. a new α-D-pentaglucoside derivative was isolated. The fruits of *Spondias pinnata* furnished tricarballylic acid 6-O-β-D-diglucoside, The fruits of *Terminalia bellerica* yielded a monoterpenic xylosidic ester geranilan-10-oxy-10-O-β-D-xylopyranosyl 2′-benzoate. This work has enhanced understanding about the phytoconstituents of these plants. These secondary metabolites can be used as analytical markers for quality control of these herbal drugs.

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