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PHYTOCHEMICAL INVESTIGATIONS OF THE STEM BARK OF *ACACIA NILOTICA* (L.) DELILE, FRUITS OF *CARISSA CARANDAS* L. AND SEEDS OF *WITHANIA SOMNIFERA* (L.) DUNAL

Shahnaz Sultana[1,2], Mohammed Ali[1]* and Showkat Rassol Mir[1]

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

[2]College of Pharmacy, Jazan University, Jazan, Saudi Arabia.

***Corresponding Author: Dr. Mohammed Ali**

Phytochemistry Research Laboratory, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi - 110 062, India.

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ABSTRACT

Acacia nilotica (L.) Delile (Leguminosae) is distributed in tropical Africa and Asia. Its bark is used to treat eye and skin diseases, coughs, cystitis, diarrhoea, dysentery, fever, bleeding gums, impotence, intestinal worms, leucorrhea, piles, sclerosis, scurvy, smallpox, syphilis, tuberculosis, mouth ulcers, vaginitis and wounds. *Carissa carandas* L. (Apocynaceae) is found in southern Asia and its fruits are used to treat acidity, biliousness, diabetes, indigestion, skin diseases, urinary disorders, wounds, to improve appetite and to strengthen the cardiac muscles. *Withania somnifera* (L.) Dunal (Solanaceae) is distributed in India, Sri Lanka, Afghanistan, Baluchistan, Sind and the Mediterranean regions. The seeds are regarded as a diuretic and hypnotic and used for coagulating milk. Our study was planned to isolate chemical constituents from the stem bark of *A. nilotica*, fruits of *C. carandas* and seeds of *W. somnifera* and to characterize their structures on the basis of spectral data analysis and chemical reactions. Phytochemical investigation of the methanol extract of the stem bark of *A. nilotica* afforded four phytoconstituents identified as *n*-tridecanyl dotriacontanoate (*n*-tridecanyl lacceroate, **1**), *n*-hexanyl O-β-D- glucuronopyranoside (*n*-hexanyl β-D-glucuronoside, **2**), β-D-arabinopyranosyl-(4→1')-O-β-D-glucuronopyranoside (β-D-arabinoglucuronoside, **3**) and β-D-glucuronopyranosyl-(4→1')-O-β-D-glucuronopyranoside (β-D-diglucuronoside, **4**). The fruits of *C. carandas* gave a new tetracyclic triterpenic acid characterized as lanost-5-en-3α-ol 21-oic acid (3-epi-lanostenol 21-oic acid, **5**). The seeds of *W. somnifera* afforded glyceryl-1-linoleio-2-arachidyl-3-docos-9‴,12‴-dienoate **(6).**

KEYWORDS: *Acacia nilotica*, *Carissa carandas*, *Withania somnifera*, phytoconstituents, isolation, characterization.

INTRODUCTION

Acacia nilotica (L.) Delile, syn. *A. arabica* (Lam.) Willd., *A. subalata* Vatke, *Mimosa nilotica* L. (Leguminosae), known as gum Arabic tree, babul, kikar, black piquant, Egyptian thorn and prickly acacia, is widespread in tropical Africa and Asia, and occurs in Australia, Egypt, India, Burma, Sri Lanka, Saudi Arabia, Egypt and South Africa. It is a moderate sized tree with a dense spreading crown, bark black and fissured.[1] Its tender growing tops and leaves are used to treat diabetes, diarrhoea, dysentery, dropsy, gonorrhea, itches, leucorrhea, spermatorrhoea, bleeding ulcers and wounds. The leaves and the gum are utilized for gargling for relaxing sore throat and spongy gums.[2,3] The bark is effective as an antiscorbutic, antiseptic, aphrodisiac, lactagogue, nerve stimulant and to cure cancerous and syphilitic affections, conjunctivitis, coughs, cystitis, diarrhoea, dysentery, eczema, fever, bleeding gums, impotence, intestinal worms, leprosy, leucorrhea, indurations of the liver and spleen, ophthalmia, piles, sclerosis, scurvy, smallpox, syphilis, tuberculosis, mouth ulcers, vaginitis, prolapse of the uterus and wounds.[2,3] The plant is given in veterinary medicine as a molluscicide. The tender twig is used as a toothbrush. The resin is mixed with an infusion of the orange flower and ingested to reduce typhoid fever. The wood is effective to relieve smallpox. A decoction of the pods is given to prevent excessive bleeding during menstruation. Babool gum powder is consumed to alleviate arthritis.[2,4,5] The flowers are regarded as an antidiarrhoeal, anti-dysentery, febrifuge, tonic and to calm down earache.[6]

The plant contained phenolic acids, tryptamines, β-carbolines, mesculine, bufoteinine, nicotine, L-arabinose, catechol, galactan, galactoaraban, galactose, N-acetyl djenkolic acid, pentosan, tannins, flavonoids, proanthocyanidins, chlorogenic acid, androstene, D-

pinitol, calycanthidine, catechine, pipecolic acid, erythritol and malic, linoleic and stearic acids. [7 -9] The bark yielded phenolics, tannin, phlobatannin, gallic and protocatechuic acids, (-) epicatechin, (+) dicatechin, quercetin, (+) leucocyanidin gallate, α-amyrin, β-sitosterol, sucrose and (+) - catechin- 5-gallate.[10,11] An essential oil of the stem bark was composed mainly of menthol and limonene.[12] The roots afforded polygalloyl tannin.[13] The flowers contained kaempferol-3-glucoside, iso-quercitrin, leucocyanidin and stearic acid.[11]

Carissa carandas L., syn. *C. salicina* Lam., *Arduina carandas* (L.) Baill., *Echites spinosus* Burm.f., *Jasminonerium carandas* (L.) Kuntze (Apocynaceae), known as karonda, kali maina, Bengal currant and Christ's thorn, is found in India, Nepal, Afghanistan, Myanmar, Philippines, Bangladesh and Sri Lanka at elevations between 30 - 1,800 m. It is a straggly, woody, 3- m tall, climbing shrub, rich in white, gummy latex, with numerous, spreading, sharp thorny branches; evergreen, opposite, oval or elliptic, dark-green, leathery leaves; fragrant and tubular flowers in terminal clusters; oblong, dark-purple fruits, black when ripe; 2 to 8 small, flat, brown seeds.[14] The fruits are used as an antiscorbutic, refrigerant and to treat acidity, biliousness, diabetic, indigestion, skin diseases, urinary disorders, wounds, to improve appetite and to strengthen the cardiac muscles. The unripe fruits are considered as an anthelmintic, appetizer, astringent, antidiarrheal, aphrodisiac and thermogenic. The roots are useful as a bitter stomachic, fly repellent, vermifuge and to relieve acidity, flatulence, indigestion, difficulty in micturition, ulcers, urinary disorders and wounds. The stem bark is utilized to subside skin diseases. The leaves are effective to cure diarrhea, earache, fevers, soreness of the mouth and throat and syphilitic pains.[15] The fruits possessed an essential oil composed of 2-phenyl ethanol, linalool, β-caryophyllene, isoamyl alcohol and benzyl acetate, carissol, myo-inositol, α- and β-amyrins, their acetates, 1-pentatriacontanol, ursolic acid, carinol, ascorbic acid, lupeol, β-sitosterol and oderoside H glycoside.[14,16-18] The roots yielded 2-acetyl phenol, a lignan carbinol, carissone, carindone, lupeol, β-sitosterol, its glycoside, 16β-hydroxybetulinic acid, α-amyrin, des-N-methylnoracronycine, ursolic acid, lupa-12,20(29)-dien-3β, 28-diol and urs-12-ene- 3β, 22β-diol.[19] The leaves contained triterpenoids, carissin, carandinol, tannins, oleanolic acid, ursolic acid, stigmasterol and β-sitosterol.[20-22]

Withania somnifera (L.) Dunal (Solanaceae), known as ashwagandha, Indian ginseng, poison gooseberry and winter cherry, is distributed in India, Sri Lanka, Afghanistan, Baluchistan, Sind and the Mediterranean regions. It is an erect, evergreen, branching, tomentose shrub, up to 150 cm in height, with simple, petiolate, elliptic-ovate, entire leaves and pale green monoceous flowers.[23] Its roots possess abortifacient, adaptogen, alterative, anti-stress, aphrodisiac, deobstruent, diuretic, immune stimulant, narcotic, rejuvenate and tonic properties; used to treat arthritis, asthma, backache, bronchitis, constipation, cough, epilepsy, hiccups, insomnia, leucoderma, liver diseases, memory loss, menstrual problems, paralysis, rheumatism, muscle and senile debilities, dropsy, emaciation of children, hiccup, insomnia, leucoderma, nervous exhaustion, spermatorrhoea and ulcers.[23-27] The leaves are prescribed to cure boils, carbuncles, fever, inflammation, swellings, ophthalmia, skin lesions, tumors and ulcers. The seeds are regarded as a diuretic and hypnotic, used for coagulating milk.[23-27] The roots contained alkaloids (isopellertierine, anaferine, withanine, somniferine, somnine, somniferinine, withananine, pseudo-withanine, tropine, pseudo-tropine, 3-a-gloyloxytropane, choline, cuscohygrine, isopelletierine, anaferine andanahydrine like anahygrine), steroidal lactones (withanolides and withaferins), sitoindosides, scoopoletin and steroidal lactones.[28-34] The leaves yielded β-sitosterol and chlorogenic acid. The fruits possessed cysteine.[35]

MATERIALS AND METHODS

General procedures

Melting points were recorded using one end open capillary tubes on a thermoelectrically heated Melting Point M-560 apparatus (Perfit, India) without correction. UV spectra were determined with Lambda Bio 20 Spectrophotometer (Perkin Elmer, Schwerzenbach, Switzerland) in methanol. IR spectra were recorded by using KBr pellets, with Jasco FT/IR-5000 Spectrometer (FTS 135, Hong Kong). The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on Bruker DRX-Spectrometer (Rheinstetten, 2 Germany), using $CDCl_3$ and DMSO-d_6 and TMS (Fluka analytical, Sigma-Aldrich, Netherland) as an internal standard. Mass-spectrometric detection was carried out on (Q-TOF-ESI) (Waters Corp., UK) with a +ve ESI technique. Column chromatography was performed on silica gel (Qualigens, Mumbai, India), 60–120 mesh and 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 $60F_{254}$ (Merck, 0.25 mm) and the spots were visualized by exposure to iodine vapors or under UV radiations and spraying with ceric sulfate solution.

Plant material

The stem bark of *A. nilotica*, fruits of *C. carandas* and seeds of *W. somnifera* were purchased from a local market of Delhi, Khari Baboli and identified by Prof. M. Sharma, Department of Botany, Jamia Hamdard, New Delhi. The voucher specimens of the samples were deposited in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

Extraction and isolation

The stem bark of *A. nilotica*, fruits of *C. carandas* and seeds of *W. somnifera* (1 kg each) were coarsely powdered and extracted separately and exhaustively with

methanol in a Soxhlet apparatus. The extracts were concentrated under reduced pressure to get dark brown masses, 112.5 g, 129.3 g and 117.6 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) individually to obtain slurries. Each slurry was air-dried and chromatographed over silica gel columns loaded in petroleum ether (b. p. 60 - 80°C) one by one. Each 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.

Isolation of phytoconstituents from the stem bark of *Acacia nilotica*

***n*-Tridecanyl lacceroate (1):** Elution of the column with petroleum ether gave colourless crystals of **1**, yield 281 mg, m. p. 59 - 60 °C, UV λ_{max} (MeOH): 204 nm (log ε 4.1); IR γ_{max} (KBr): 2924, 2854, 1726, 1605, 1462, 1373, 1218, 1114, 1031, 729 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.06 (2H, t, J = 6.8 Hz, H_2-1′), 2.24 (2H, m, H_2-2), 2.17 (2H, m, CH_2), 1.58 (2H, m, CH_2), 1.28 (40H, brs, 20 x CH_2), 1.25 (36H, brs, 18 x CH_2), 0.89 (3H, t, J = 6.6 Hz, Me-32), 0.86 (3H, t, J = 6.6 Hz, Me-13′); ^{13}C NMR ($CDCl_3$): δ 173.16 (C-1), 67.14 (C-1′), 56.94 (C-2), 35.04 (CH_2), 33.22 (CH_2), 32.29 (CH_2), 30.89 (30 x CH_2), 30.61 (CH_2), 30.57 (CH_2), 30.36 (CH_2), 30.32 (CH_2), 26.31 (CH_2), 25.42 (CH_2), 23.82 (CH_2), 14.57 (Me-32), 14.53 (Me-13′); ESI MS *m/*z (rel. int.): 662 $[M]^+$ ($C_{45}H_{90}O_2$) (100), 479 (3.3), 463 (5.9), 199 (4.3).

***n*-Hexanyl β-D-glucuronoside (2):** Elution of the column with chloroform - methanol (19 : 1) yielded colourless crystals of **2**, yield 147 mg, m. p. 239 – 241 °C; UV λ_{max} (MeOH): 209 nm (log ε 2.4); IR γ_{max} (KBr): 3512, 3415, 3342, 2936, 2849, 1695, 1625, 1442, 1349, 1220, 1019, 731 cm^{-1}; ^1H NMR (MeOD): δ 4.61 (1H, d, J = 7.1 Hz, H-1'), 4.02 (1H, m, H-5'), 3.70 (1H, m, H-2'), 3.62 (1H, m, H-3'), 3.59 (1H, m, H-4'), 3.40 (2H, t, J = 6.8 Hz, H_2-1), 1.52 (2H, m, H_2-2), 1.28 (6H, brs, 3 × CH_2), 0.89 (3H, t, J = 6.5 Hz, Me-6); ^{13}C NMR (MeOD): δ 60.05 (C-1), 52.03 (C-2), 33.26 (C-3), 29.63 (C-4), 22.58 (C-5), 14.36 (C-6), 110.59 (C-1'), 78.11 (C-2'), 69.89 (C-3'), 65.71 (C-4'), 82.03 (C- 5'), 187.61 (C-6'); ESI MS *m/*z (rel. int.): 278 $[M]^+$ ($C_{12}H_{22}O_7$) (100), 193 (6.1).

β-D-Arabinoglucuronoside (3): Elution of the column with chloroform - methanol (9:1) afforded colourless crystals of **3**, yield 318 mg, m. p. 159 - 161 °C; UV λ_{max} (MeOH): 221 nm (log ε 4.2); IR γ_{max} (KBr): 3509, 3369, 3255, 3210, 2945, 2832, 1683, 1448, 1219, 1114, 1029 cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.01 (1H, d, J = 7.2 Hz, H-1), 3.81 (1H, m, H-5), 3.57 (1H, m, H-4), 3.35 (1H, m, H-2), 3.30 (1H, m, H-3), 4.57 (1H, d, J = 7.1 Hz, H-1'), 3.37 (1H, m, H- 2'), 3.33 (1H, m, H-3′), 3.28 (1H, m, H-4'), 3.13 (2H, m, H_2-5'); ^{13}C NMR (DMSO-d_6): δ 104.32 (C-1), 70.28 (C-2), 71.25 (C-3), 73.21 (C-4), 62.54 (C-5), 101.63 (C-1'), 72.18 (C-2'), 67.69 (C-3'), 65.82 (C-4'), 77.15 (C- 5'), 180.04 (C-6'); ESI MS *m/*z (rel. int.): 326 $[M]^+$ ($C_{11}H_{18}O_{11}$) (16.8), 193 (5.7), 133 (3.8).

β-D-Diglucuronoside (4): Further elution of the column with chloroform - methanol (9:1) furnished colourless crystals of **4**, yield 176 mg, m. p. 209 - 210 °C; IR γ_{max} (KBr): 3410, 3355, 3255, 3217, 2946, 2834, 1685, 1449, 1115, 1027 cm^{-1}; ^1H NMR (MeOD): δ 4.88 (1H, d, J = 7.3 Hz, H-1), 4.57 (1H, m, H-5), 3.81 (1H, m, H-4), 3.34 (1H, m, H-2), 3.11 (1H, m, H-3), 4.85 (1H, d, J = 7.2 Hz, H-1'), 4.55 (1H, m, H-5'), 3.57 (1H, m, H-4′), 3.31 (1H, m, H-2'), 3.13 (1H, m, H-3'); ^{13}C NMR (DMSO d-$_6$): δ 102.13 (C-1), 71.50 (C-2), 68.72 (C-3), 67.18 (C-4), 78.33 (C-5), 181.21 (C-6), 100.76 (C-1'), 72.21 (C-2'), 67.42 (C-3'), 66.37 (C-4'), 77.61 (C- 5'), 179.26 (C-6'); ESI MS *m/*z (rel. int.): 370 $[M]^+$ ($C_{12}H_{18}O_{13}$) (23.8), 193 (10.5), 177 (2.9).

Isolation of a triterpenic acid from the fruits of *Carissa carandas*

3-Epi-lanostenol 21-oic acid (5): Elution of the column with chloroform – methanol (19 : 1) afforded a colourless amorphous powder of **5**, yield 314 mg, m. p. 176 - 178 ^{o} C; UV $_{\lambda\ max}$ (MeOH): 209 nm (log ε 3.7); IR υ_{max} (KBr): 3450, 3260, 2924, 2845, 1701, 1637, 1432, 1216, 1031, 929 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.28 (1H, d, J = 9.9 Hz, H-6), 3.72 (1H, dd, J = 5.1, 5.3 Hz, H-3β), 2.19 (1H, m, H-20), 1.29 (3H, brs, M- 29), 1.08 (3H, brs, Me-19), 1.03 (3H, brs, Me-30), 0.95 (3H, d, J = 6.8 Hz, Me-26), 0.90 (3H, d, J = 6.6 Hz, Me- 27), 0.88 (3H, brs, Me-28), 0.78 (3H, brs, Me-18), 2.08 - 1.32 (24H, 10 x CH_2, 4 x CH); ^{13}C NMR ($CDCI_3$): δ 37.73 (C-1), 27.68 (C-2), 66.17 (C-3), 38.45 (C-4), 138.01 (C-5), 123.16 (C-6), 29.17 (C-7), 41.88 (C-8), 48.28 (C-9), 38.34 (C-10), 21.60 (C-11), 35.85 (C-12), 44.23 (C-13), 54.96 (C-14), 34.27 (C-15), 30.11 (C-16), 50.72 (C-17), 18.02 (C-18), 21.03 (C-19), 40.42 (C-20), 181.15 (C-21), 36.15 (C-22), 22.68 (C-23), 45.16 (C-24), 33.52 (C-25), 22.15 (C-26), 22.08 (C-27), 22.21 (C-28), 22.03 (C-29), 16.33 (C-30); ESI MS *m/*z (rel. int.): 458 $[M]^+$ ($C_{30}H_{50}O_3$) (18.2), 440 (100), 413 (20.1), 412 (32.7), 315 (5.8), 247 (21.2), 289 (13.5), 206 (6.8), 192 (11.3), 143 (8.2).

Isolation of a glyceride from the seeds of *Withania somnifera*

Glyceryl-1-linoleio-2-arachidyl-3-docos-9′′′, 12′′′-dienoate (6): Elution of the column with petroleum ether furnished a yellow semisolid mass of **6**, purified by preparative TLC using petroleum ether - chloroform (1:1), UV λ_{max} (MeOH): 212 nm (log ε 2.4); IR γ_{max} (KBr): 2927, 2855, 1737, 1721, 1645, 1463, 1377, 1272, 1244, 1176, 1115, 1059, 985, 724 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.45 (2H, m, H-10′, H-12′), 5.37 (2H, m, H-10′′′, H-12′′′), 5.34 (2H, m, H-9′′′, H-13′′′), 5.32 (1H, m, H-11′), 5.29 (1H, m, H-13′), 4.21 (1H, m, H-2), 4.16 (2H, m, H_2-1), 4.13 (2H, m, H_2-3), 2.80 (2H, m, H_2-11′),

2.76 (2H, m, H_2-11‴), 2.36 (2H, t, J = 7.2 Hz, H_2-2′), 2.32 (2H, t, J = 7.5 Hz, H_2-2″), 2.29 (2H, t, J = 6.9 Hz, H_2-2‴), 2.05 (6H, m, H_2-8′, H_2-14′, H_2-8‴), 2.01 (2H, m, H_2-14‴), 1.60 (8H, brs, 4 x CH_2), 1.36 (6H, m, 3 x CH_2), 1.28 (14H, brs, 7 x CH_2), 1.25 (16H, brs, 8 x CH_2), 1.22 (30H, brs, 15 x CH_2), 0.86 (3H, t, J = 6.3 Hz, Me-18′), 0.84 (3H, t, J = 6.5 Hz, Me-20″), 0.82 (3H, t, J = 6.6 Hz, Me-22‴); ^{13}C NMR ($CDCl_3$): δ 172.8 (C-1′), 169.23 (C-1″), 168.71 (C-1‴), 132.81 (C-9′), 131.96 (C-9′), 130.23 (C-13′), 130.01 (C-9‴), 128.30 (C-9‴), 128.08 (C-10′), 127.90 (C-12′), 127.76 (C-11‴), 127.11 (C-12‴), 70.29 (C-2), 66.16 (C-1), 62.35 (C-3), 41.35 (C-2′) 36.07 - 29.83 (8 x CH_2), 29.69 (7 x CH_2), 29.65 – 29.05 (10 x CH_2), 28.99 (CH_2), 28.20 (CH_2), 27.66 (CH_2), 27.19 (2 x CH_2), 26.91 (2 x CH_2), 25.83 – 25.28 (4 x CH_2), 24.89 (CH_2), 24.72 (CH_2), 22.80 – 22.56 (3 x CH_2), 20.68 (4 x CH_2), 14.25 (Me-18′), 14.09 (Me-20″), 11.40 (Me-22‴); ESI MS *m/*z (rel. int.): 968 $[M]^+$ ($C_{63}H_{116}O_6$) (1.8), 335 (27.6), 311 (70.1) 295 (8.3), 279 (18.2).

RESULTS AND DISCUSSION

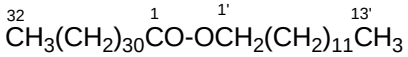
Compound **1**, named *n*-tridecanyl lacceroate, showed characteristic IR absorption bands for ester group (1726 cm^{-1}) and a long aliphatic chain (729 cm^{-1}). Its mass spectrum exhibited a molecular ion peak at *m/*z 662 corresponding to a molecular formula of a fatty acid ester, $C_{45}H_{90}O_2$. The ion fragments arising at *m/*z 463 $[CH_3(CH_2)_{30}CO]^+$, 479 $[CH_3(CH_2)_{30}COO]^+$ and 199 $[M – 463]^+$ suggested that lacceroic acid was esterified with a C_{13} alcohol. The ^{1}H NMR spectrum of **1** displayed a two-proton triplet at δ 4.06 (J = 6.8 Hz) assigned to oxymethylene H_2-1′. Three two-proton multiplets at δ 2.24, 2.17 and 1.58 and two broad singlets at δ 1.28 (40H) and 1.25 (36H) were ascribed to the methylene protons. Two three-proton triplets at δ 0.89 (J = 6.6 Hz) and 0.86 (J = 6.6 Hz) were attributed to terminal C-32 and C-13′ primary methyl protons, respectively. The ^{13}C NMR spectrum of **1** exhibited signals for the ester carbon at δ 173.16 (C-1), oxymethylene carbon at δ 67.14 (C-1′), other methylene carbons from δ 56.94 to 23.82 and methyl carbons at δ 14.57 (C-32) and 14.53 (C-13′). The absence of any signal beyond δ 4.06 in the ^{1}H NMR spectrum and between δ 173.16 - 67.14 in the ^{13}C NMR spectrum suggested saturated nature of the molecule. On the basis of these evidences the structure of **1** has been characterized as *n*-tridecanyl dotriacontanoate (Fig 1).

Compound **2**, designated as *n*-hexanyl β-D-glucuronoside, responded for glycoside tests positively and exhibited distinctive IR absorption bands for hydroxyl groups (3512, 3415, 3342 cm^{-1}) and carboxylic function (1695 cm^{-1}). Its molecular ion peak was established at *m/*z 278 on the basis of mass and ^{13}C NMR spectra corresponding to a molecular formula of an alkyl glurunoside, $C_{12}H_{22}O_7$. An ion peak arising at *m/*z 193 $[C_6H_9O_7]^+$ indicated that a hexanose acid was linked with a hexane unit. The ^{1}H NMR spectrum of **2** displayed a one - proton doublet at δ 4.61 (J = 7.1 Hz) assigned to anomeric H-1′ proton. The other sugar protons appeared between δ 4.02 - 3.59. A two-proton triplet at δ 3.40 (J = 7.2 Hz) was ascribed to oxymethylene H_2-1. The remaining methylene protons resonated as a two-proton multiplet at δ 1.52 and as a six – proton singlet at δ 1.28. A three - proton triplet at δ 0.89 (J = 6.5 Hz) was attributed to terminal C-6 primary methyl protons. The ^{13}C NMR spectrum of **2** displayed signals for carboxylic carbon at δ 187.61 (C-6′), anomeric carbon at δ 110.59 (C-1'), other sugar carbons from δ 82.03 to 65.71, methyl carbon at δ 14.21 (C-6) and methylene carbons between δ 52.03 - 22.58. On the basis of foregoing discussion, the structure of compound **2** has been characterized as *n*-hexanyl O-β-D-glucuronopyranoside, a new alkyl glucuronoside (Fig 1).

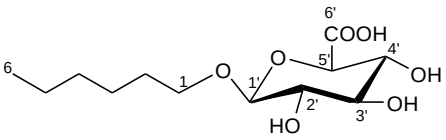
Compound **3**, named β-D-arabinoglucuronoside, gave positive tests for glycosides and showed IR absorption bands for hydroxyl groups (3515, 3312, 3260 cm^{-1}) and carboxylic function (3210, 1683 cm^{-1}). On the basis of mass and ^{13}C-NMR spectra, the molecular ions peak of **3** was determined at *m/*z 326 consistent with a molecular formula of a disaccharide, $C_{11}H_{18}O_{11}$. The ion peaks arising at *m/*z 193 $[C_6H_9O_7]^+$ and 133 $[C_5H_9O_4]^+$ indicated that a pentose sugar unit was linked with a hexose acid. The ^{1}H NMR spectrum of **3** exhibited two one-proton doublets at δ 5.01 (J = 7.2 Hz) and 4.57 (J = 7.1 Hz) assigned correspondingly to anomeric H-1 and H-1′. The other sugar protons resonated between δ 3.81 - 3.13. The ^{13}C NMR spectrum of compound **3** displayed signals for anomeric carbons at δ 104.32 (C-1) and 101.63 (C-1'), carboxylic carbon at δ 180.04 (C-6') and the remaining sugar carbons from δ 77.15 to 62.54. The presence of the sugar H-4 signal in the deshielded region at δ 3.57 in the ^{1}H NMR spectrum and C-4 carbon signal at δ 73.21 in the ^{13}C NMR spectrum suggested (4→1′) linkage of the sugar units. Acid hydrolysis of **3** yielded D-glucuronic acid, R_f 0.26 (*n*-butanol- acetic acid – water, 4 : 1 : 5) and β-arabinose, R_f 0.42 (*n*-butanol-pyridine - water, 3 : 1 : 1). On the basis of these evidences the structure of **3** has been formulated as β-D-arabinopyranosyl-(4→1')-O-β-D-glucuronopyranoside, a new disaccharide (Fig 1).

Compound **4**, named β-D-diglucuronoside, $[M]^+$ at *m/*z 370 ($C_{12}H_{18}O_{13}$), responded positively to glycoside tests and displayed IR absorption bands for hydroxyl groups (3410, 3355, 3255 cm^{-1}) and carboxylic functions (3217, 1685 cm^{-1}). The ion peaks arising at *m/*z 193 $[C_6H_9O_7]^+$ and 177 $[C_6H_9O_6]^+$ indicated that two hexose acid units were linked with each other. The ^{1}H NMR spectrum of **4** showed two one-proton doublets at δ 4.88 (J = 7.3 Hz) and 4.85 (J = 7.2 Hz) assigned to anomeric H-1 and H-1′, respectively. The other sugar protons resonated between δ 4.57 - 3.11. The ^{13}C NMR spectrum of compound **4** exhibited signals for anomeric carbons at δ 102.13 (C-1) and 100.76 (C-1'), carboxylic carbons at δ 181.21 (C-6) and 179.26 (C-6') and the remaining sugar carbons from δ 78.33 to 66.37. The presence of the sugar H-4 signal in the deshielded region at δ 3.81 in the ^{1}H NMR spectrum and C-4 carbon signal at δ 67.18 in the ^{13}C NMR spectrum suggested (4→1′) linkage of the sugar units.

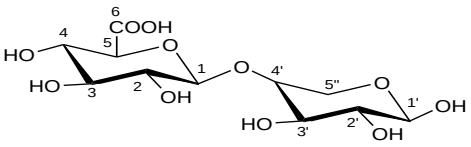
Acid hydrolysis of **4** yielded D-glucuronic acid, R_f 0.26 (*n*-butanol- acetic acid – water, 4 : 1 : 5). On the basis of these evidences the structure of **4** has been formulated as β-D-glucuronopyranosyl- (4→1')-O-β- D-glucuronopyranoside, a new disaccharide (Fig 1).



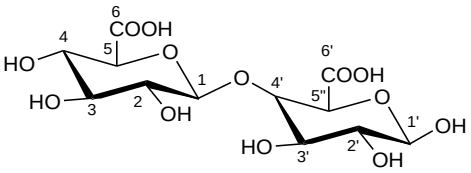
***n*-Tridecanyl lacceroate (1)**



***n*-Hexanyl β-D-glucuronoside (2)**



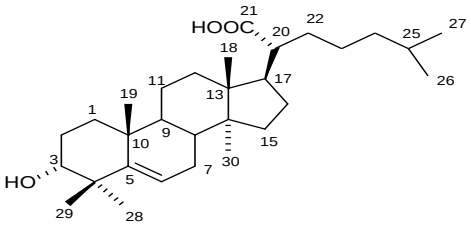
β-D-Arabinoglucuronoside (3)



β-D-Diglucuronoside (4)

Fig. 1: Compounds 1 – 4 isolated from the stem bark of *Acacia nilotica.*

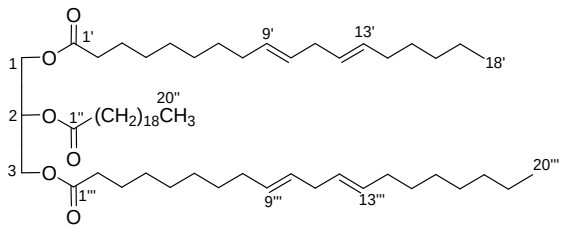
Compound **5**, named 3-epi-lanostenol 21-oic acid, yielded effervescence with sodium bicarbonate solution and showed IR absorption bands for a hydroxyl group (3450 cm^{-1}), carboxylic function (3260, 1701 cm^{-1}) and unsaturation (1637 cm^{-1}). Its molecular ion peak was determined at *m/*z 458 on the basis of mass and ^{13}C NMR spectra relating to a triterpenic acid, $C_{30}H_{50}O_3$. The ion fragments generating at *m/*z 440 $[M - H_2O]^+$, 413 $[M - COOH]^+$ and 412 $[M - HCOOH]^+$ suggested the presence of one each of the hydroxyl and carboxyl groups in the molecule. The ion peaks produced at *m/*z 192 $[C_{8,14} - C_{9,11}$ fission$]^+$, 206 $[C_{8,14} - C_{11,12}$ fission$]^+$ and 247 $[C_{12,13} - C_{13,14} - C_{14,15}$ fission$]^+$ indicated the existence of the vinylic linkage at C_5 and saturated nature of the ring C. The ion fragments arising at *m/*z 143 $[C_{17} - C_{20}$ fission, side chain $C_8H_{15}O_2]^+$ and 315 $[M - COOH]^+$ supported the location of the carboxylic group in the saturated side chain. The ^1H NMR spectrum of **5** exhibited a one-proton doublet at δ 5.28 (J = 9.9 Hz) assigned to vinylic H-6 proton, a one-proton double doublet at δ 3.72 (J = 5.1, 5.3 Hz, H- 3α) ascribed to β-oriented oxymethine H-3 proton, five three-proton broad singlets at δ 1.29, 1.08, 1.03, 0.88 and 0.78 due to tertiary C-29, C-19, C-30, C-28 and C-18 methyl protons and as two three-proton doublets at δ 0.95 (J = 6.8 Hz) and 0.90 (J = 6.6 Hz) associated with secondary C-26 and C-27 methyl protons. The remaining methylene and methine protons appeared between δ 2.19 – 1.32. The ^{13}C NMR spectrum of **5** displayed signals for vinylic carbons at δ 138.01 (C-5) and 123.16 (C-6), carbinol carbon at δ 66.17 (C-3), carboxylic carbon at δ 181.15 (C-21) and methyl carbons from δ 22.15 to 16.33. The ^1H and ^{13}C NMR spectral data of the triterpenic unit of **5** were compared with the reported spectral data of lanostene-type triterpenoids.[36 - 38] On the basis of these evidences the structure of **5** was established as lanost-5-en-3α-ol 26-oic acid, a new lanostenoic acid (Fig 2).



3-*Epi*-lanostenol-21-oic acid (5)

Fig. 2: Compound 5 isolated from the fruits of *Carrisa crandas.*

Compound **6** gave positive tests for glycerides and showed IR absorption bands for ester groups (1737, 1721 cm^{-1}), unsaturation (1645 cm^{-1}) and long aliphatic chain (724 cm^{-1}). On the basis of its mass and ^{13}C NMR spectra, its molecular ion peak was determined at *m/*z 968 consistent with a molecular formula of a mixed glyceride, $C_{63}H_{116}O_6$. The generation of a predominant ion peak at *m/*z 311 $[CH_3\text{-}(CH_2)_{18}COO]^+$ and another ion peak at *m/*z 295 $[CH_3\text{-}(CH_2)_{18}CO]^+$ suggested that arachidyl group was linked at the secondary C-2 carbon of glycerol. The ion fragments arising at *m/*z 279 $[CH_3\text{-}(CH_2)_4\text{-}CH{=}CH{-}CH_2{-}CH{=}CH{-}(CH_2)_7{-}COO]^+$ and 335 $[CH_3\text{-}(CH_2)_8\text{-}CH{=}CH{-}CH_2{-}CH{=}CH{-}(CH_2)_7{-}COO]^+$ indicated the location of the linoleiyl and docos-9,12-dienoyl units at the terminal carbons of glycerol. The ^1H NMR spectrum of **6** exhibited five deshielded multiplets from δ 5.45 to 5.29 assigned to vinylic protons. A one-proton multiplet at δ 4.21 and two two-proton multiplets 4.16 and 4.13 were ascribed to oxymethine H-2 and oxymethylene H_2-1 and H_2-3 protons, respectively. Three triplets at δ 0.86 (J = 6.3 Hz), 0.84 (J = 6.5 Hz) and 0.82 (J = 6.6 Hz) integrating for three protons each were attributed correspondingly to primary C-18′, C-20″ and C-22‴ methyl protons. The remaining methylene protons resonated from δ 2.80 to 1.22 . The ^{13}C NMR spectrum of **6** showed important signals for ester carbons at δ 172.8 (C-1′), 169.23 (C-1″) and 168.71 (C-1‴), vinylic carbons from δ 132.81 to 127.11, oxymethine carbon at δ 70.29 (C-2), oxymethylene carbons at δ 66.16 (C-1) and 62.35 (C-3) and other methylene carbons between δ 41.35 – 20.68. The remaining primary methyl carbons appeared at δ 14.25 (C-18′), 14.09 (C-20″) and 11.40 (C-22‴). On the basis of these findings, the structure of **6** has been established as glyceryl 1-linoleiyl-2-arachidyl - 3-docos-9‴, 12‴-dienoate, a new mixed glyceride (Fig 3).



Glyceryl-1-linoleio-2-arachidyl-3-docos-9‴,12‴-dienoate (6)

Fig. 3: Compound 6 isolated from the seeds of *Withania somnifera.*

CONCLUSION

Phytochemical investigation of a methanolic extract of the stem bark of *Acacia nilotica* gave *n*-tridecanyl lacceroate, *n*-hexanyl β-D-glucuronoside and diglycosides. The fruits of *Carrisa crandas* afforded 3-*epi*-lanostenol-21-oic acid. The seeds of *Withania somnifera* furnished a lipid component characterized as glyceryl-1- linoleio-2-arachidyl-3-docos-9‴,12‴-dienoate. 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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