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CHEMICAL CONSTITUENTS FROM THE FRUITS OF *MOMORDICA BALSAMINA* L.

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ABSTRACT

Momordica balsamina L. (family Cucurbitaceae) is a tendril-bearing annual vine with ovoid-ellipsoid, orange-red, warty fruits. Its fruits are anthelmintic, antiseptic, edible, purgative and vermifuge, used to treat burns, chapped hands, diabetes, headache, intestinal and stomach complaints, malaria, piles, sores and wounds. The present study was designed to isolate and characterize chemical constituents from the fruits of *M. balsamina*. Phytochemical investigations of a defatted ethanolic extract of the fruits resulted in the isolation of a fatty acid characterized as *n*-docosanoic acid (behenic acid, **1**), a new acyl galactoside identified as (*Z*)-13-docos-13-enoyl O-β-D-galactopyranoside (erucicyl O-β-D-galactopyranoside, **3**) and three tetracyclic triterpenoids recognized as cucurbit-5,7-dien-3β-ol (**2**), cucurbit-5-en-3β-ol 3-O-β-D-glucopyranoside (**4**) and cucurbit-5-en-3β-olyl 3-O-β-D-glucopyranosyl-(4′→1″)-O-β-D-glucopyranosidic 4″-linolenate (**5**). All these chemical constituents are reported for the first time from this plant.

KEYWORDS: *Momordica balsamina*, fruits, extraction, phytoconstituents, isolation, spectral data, characterization.

INTRODUCTION

International survey of drugs utilization suggests that 80% of world populations prescribe traditional herbal medicines obtained from over 20,000 plant species. Currently in the United States nearly 25% of pharmaceutical prescriptions contain at least one herbal ingredient. Majority of the dietary products obtained from plants show antioxidant property and are effective to reduce attack of several diseases. Various chemical constituents present in the foods like essential amino acids, vitamins, antioxidants and fatty acids play a vital role in preventing and delaying the premature onset of chronic diseases in life. Consumption of the fruits and vegetables minimizes the risk of cancer, cardiovascular diseases, cerebrovascular disorders and mortality by 15-30 %. Phytoconstituents are natural and bioactive plant products which provide health for human beings, protection to plants cells from disease, damage, stress, drought, light exposure and pathogenic attack and contribute their colour, aroma and flavour. Their dietary intake as nutraceuticals is remarkable. There are 4,000 characterized phytochemicals, about 150 chemical compounds have been studied in detail. These phytoconstituents are isolated from various plant parts including fruits, leaves, legumes, whole grains, nuts, seeds, fungi, herbs and spices. They are biosynthesized in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds. The pigment molecules are often accumulated in the outer layers of the various plant tissues. These compounds are known as secondary plant metabolites and have biological properties such as antioxidant, antimicrobial, enzyme detoxification, immune system stimulation, decrease of platelet aggregation, hormone metabolism modulation and anticancer properties. Phytoconstituents are essential nutrients required by the human body for sustaining life and they prevent common diseases.[1,2]

Momordica balsamina L., syn. *M. involucrata* E. Meyer ex Sonder, *M. Schinzii* Cogn. ex Schinz (family Cucurbitaceae), known as Balsam pear, Balsam apple, African cucumber, Bad kareliya, is a native to the tropical regions of Africa and introduced in Asia, Australia, and Central America. In India, it occurs naturally in forest in the rainy season.[3] It is a tendril-bearing annual vine with unbranched, hairless tendrils, rootstock tuberous, leaves round, cordate, alternate, waxy, margin dentate, stalked; flower pale yellow with a dark spot, deeply veined, round, solitary; fruits ovoid-ellipsoid, beaked, narrowed at ends, orange-red when ripped, warty; seeds numerous, ovate, covered with a scarlet, sticky coating, compressed. The plant is anthelmintic, emetic, galactagogue, laxative, purgative, stomachic, tonic, tranquillizer and vermifuge, used to treat burns, chest pains, diabetes, fever, mental illness, scabies, stomach complaints and yaws.[4] The plant is added as an ingredient in Strophanthus arrow poison. The twigs are taken orally to relieve liver diseases.[5] The

leaves are anthelmintic, anti-emetic and edible, utilized to relieve diabetes, fever, gastroenteritis, hepatitis, malaria, menstrual pain, rheumatism, skin disorders, syphilis and uterine bleeding. The leaf sap is effective as a metal cleaner. The leaves and stems serve as a cattle fodder.[6] The fruits are anthelmintic, antiseptic, purgative and vermifuge, useful to cure burns, chapped hands, diabetes, headache, intestinal and stomach complaints, malaria, piles, sores and wounds. The bitter young fruits are edible. The ripe fruits are toxic and cause vomiting and diarrhoea.[3,7,8] The seeds are anthelmintic. The pounded seeds are soaked in water and inserted into the neck of womb to procure abortion. The seeds, fruits and roots are used as a remedy for urethral discharges, piles and as an abortifacient.[3] The extract of various parts of this plant showed analgesic, anti-diarrhoeal, anti-HIV, anti-inflammatory, antimicrobial, antioxidant, antiplasmodial, antiseptic, antiviral, insecticidal, nutritional, shigellocidal and wound healing properties.[3,9-20]

The leaves contained vitamin C, alkaloids, saponins, tannins and amino acids. [21, 22] The plant yielded a bitter principle momordicin and pimarane diterpenes.[23] The fruits produced gallic acid, quercetin, tannins, fatty acid, carbohydrates, lupeol, ursolic acid, β-amyrin, β-sitosterol, cucurbit-5,7-dien-3β-ol, cucurbita-5-en-3β-ol-3-*O*-β-D-glucopyranoside and cucurbit-5-en-3β-ol-3-*O*-β-D-glucopyranosyl-(4′→1′′)-*O*-β-D-glucopyranoside.[18,24,25] The seeds afforded a protein, viz. balsamin, lipids, carbohydrates, alkaloids, flavonoids, tannins, phenols and saponins.[26–28] Keeping in view the various therapeutic values of the plant and the development of ecofriendly, biodegradable and safer herbal preparations, the fruits of *M. balsamina* collected from Moga, Punjab were extracted with ethanol. The concentrated ethanolic extract was used for the isolation of chemical constituents. Structures of the isolated phytoconstituents were established using detailed spectral studies.

MATERIALS AND METHODS

The protocols of all methodologies (procedures, experimental designs and analysis assays) were adopted from the earlier published work.[29, 30]

General procedures

The melting points were measured by means of a thermoelectrically operated Perfit apparatus and are uncorrected. UV spectra were recorded on Lambda Bio 20 spectrophotometer (Perkin Elmer, Schwerzenbach, Switzerland) in methanol. The IR spectra were taken on a Jasco FT/IR-5000 Spectrometer (FTS 135, Hong Kong) using KBr pellet. The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were scanned on Bruker DRX Spectrometer (Rheinstetten, 2 Germany) using $CDCl_3$ or DMSO-d_6 as a solvent and TMS (Fluka analytical, Sigma-Aldrich, Netherland) as an internal standard. Mass spectrometric detections were carried out on (Q-TOF-ESI) (Waters Corp., UK) instrument with a +ve ESI mode technique. Column chromatography was performed on silica gel (Qualigens, Mumbai, India) with 60-120 mesh particle size and petroleum ether, chloroform, methanol and other solvents used were purchased from Merck Specialties (E. Merck Pvt. Ltd., New Delhi, India). The purity of the isolated compounds was checked on precoated TLC plates with silica gel 60 F_{254} (Merck, 0.25 mm) and the spots were visualized by exposure to iodine vapours or under UV radiations and spraying with ceric sulphate solution.

Collection of plant material

The fresh fruits of *M. balsamina* were procured from a local vegetable market of Moga, Punjab and authenticated by Dr. H.B. Singh, Taxonomist, National Institute of Science Communication and Information Resources (CSIR), New Delhi. A voucher specimen is preserved in the herbarium of the Phytochemistry Research Laboratory, Jamia Hamdard, New Delhi.

Extraction and isolation

The powdered shade-dried fruits of the plant (2.0 kg) were defatted with petroleum ether and then extracted exhaustively with ethanol (95%) in a Soxhlet apparatus. The ethanolic extract was concentrated to dryness under reduced pressure to yield a brown mass (131.2 g). A portion of this extract (120.0 g) was dissolved in a minimum quantity of methanol and adsorbed on silica gel (60-120 mesh) for preparation of a slurry. It was dried in air and chromatographed over silica gel for column packed in chloroform. The column was eluted with chloroform and chloroform - methanol (99:1, 49:1, 19:5, 9:1, 17:3, 4:1 7:3, 1:1, v/v) mixtures successively. 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.

Behenic acid (1)

Elution of the column with chloroform gave colorless crystals of **1**, recrystallized from acetone-methanol (1:1), 170 g, R_f 0.47 (chloroform-methanol, 1:1), m. p. 78 – 80 ^{0}C; UV λmax (MeOH): 211 nm; IR υ_{max} (KBr): 3280, 2917, 2849, 1707, 1463, 1410, 1298, 1176, 1193, 938, 719 cm^{-1}; ^{1}H NMR ($CDCl_3$) : δ 2.49 (2H, J = 7.3 Hz, H_2-2), 1.62 (2H, m, H_2-3), 1.55 (2H, m, H_2-4), 1.41 (2H, m, H_2-5), 1.28 (32 H, brs, 16 x CH_2), 0.85 (3 H, t, J = 6.6 Hz, Me-22); ^{13}C NMR ($CDCl_3$): δ 178.90 (C-1), 50.89 (C-2), 33.95 (C-3), 31.95 (C-4), 30.98 (C-5), 29.73 (C-6), 29.62 (C-7), 29.56 (C-8), 29.47 (9 x CH_2), 27.81 (C-18), 26.27 (C-19), 24.73 (C-20), 22.72 (C-21), 14.16 (C-22); +ve ESI MS *m/z* (rel. int.): 340 $[M]^{+}$ ($C_{22}H_{44}O_2$) (14.8).

Cucurbit-5, 7-dien-3β-ol (2)

Further elution of the column with chloroform yielded a pale yellow waxy compound **2**, purified by preparative TLC using chloroform; 125 mg, R_f 0.58 (benzene–chloroform–methanol, 5:4:1); IR υ_{max} (KBr): 3438, 2928,

2854, 1641, 1445, 1382, 1215, 1072, 1019, 928, 932, 758 cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.13 (1H, d, J = 5.6 Hz, H-6), 5.86 (1H, d, J = 5.6 Hz, H-7), 3.81 (1H, dd, J = 5.8, 8.9 Hz, H-3α), 1.28 (3H, brs, Me-28), 1.25 (3H, brs, Me-19), 1.16 (3H, d, J = 6.1 Hz, Me-21), 1.04 (3H, brs, Me-29), 0.82 (3H, d, J = 6.6 Hz, Me-26), 0.79 (3H, d, J = 6.3 Hz, Me-27), 0.75 (3H, brs, Me-18), 0.70 (3H, brs, Me-30), 2.71 – 1.39 (22H, m, 9 x CH_2, 4 x CH); ^{13}C NMR ($CDCl_3$): δ 25.29 (C-1), 34.21 (C-2), 76.30 (C-3), 42.24 (C-4), 143.72 (C-5), 118.89 (C-6), 135.01 (C-7), 136.92 (C-8), 46.17 (C-9), 42.41 (C-10), 33.69 (C-11), 50.75 (C-12), 52.82 (C-13), 48.18 (C-14), 44.33 (C-15), 33.51 (C-16), 56.96 (C-17), 16.39 (C-18), 18.65 (C-19), 36.23 (C-20), 26.12 (C-21), 27.26 (C-22), 34.06 (C-23), 38.39 (C-24), 29.94 (C-25), 29.48 (C-26), 27.49 (C-27), 23.61 (C-28), 32.33 (C-29), 17.59 (C-30); ESI-MS *m/z* (rel. int.): 426 $[M]^+$ $C_{30}H_{50}O$ (2.7).

Erucicyl O-β-D-galactoside (3)

Elution of the column with chloroform-methanol (19:1) furnished a buff coloured powder of **3**, recrystallized from chloroform-methanol (1:1), 210 mg, m. p. 80 – 82 ^{o} C, UV λmax (MeOH): 213 nm; IR υ_{max} (KBr): 3410, 3366, 3281, 2931, 2851, 1722, 1647, 1415, 1322, 1239, 1104, 1051, 1023, 771 cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.35 (1H, m, $w_{1/2}$ = 5.6 Hz, H-13), 5.32 (1H, m,, $w_{1/2}$ = 5.9 Hz, H-14), 2.23 (2H, t, J = 7.3 Hz, H_2-2), 2.15 (2H, m, H_2-12), 2.05 (2H, m, H_2-15), 1.65 (2H, m, H_2-3), 1.53 (2H, m, H_2-11), 1.39 (4H, m, H_2-10, H_2-9), 1.28 (22H, brs, 11 x CH_2), 0.86 (3H, t, J = 6.6 Hz, Me-22), 5.21 (1H, d, J = 7.2 Hz, H-1'), 4.36 (1H, m, H-5'), 4.21 (1H, m, H-2'), 3.60 (1H, m, H-3'), 3.52 (1H, m, H-4'), 3.08 (2H, d, J = 8.8 Hz, H_2-6'); ^{13}C NMR (DMSO-d_6): δ 169.21 (C-1), 52.06 (C-2), 37.23 (C-3), 29.40 (C-4), 28.73 (C-5), 28.68 (C-6), 28.65 (C-7), 28.63 (C-8), 28.60 (C-9), 29.36 (C-10), 32.26 (C-11), 44.18 (C-12), 122.63 (C-13), 118.40 (C-14), 42.38 (C-15), 29.48 (C-16), 28.70 (C-17), 28.55 (C-18), 28.51 (C-19), 25.17 (C-20), 22.68 (C-21), 14.21 (C-22), 108.38 (C-1'), 70.19 (C-2'), 68.27 (C-3'), 65.83 (C-4'), 74.11 (C-5'), 61.21 (C-6'); ESI-MS *m/z* (rel. int.): 500 $[M]^+$ ($C_{28}H_{52}O_7$) (1.8), 337 (39.2), 321 (10.2), 179 (6.7), 163 (10.6).

Cucurbit-5-en-3β-ol 3-O- β-D-glucoside (**4**)

Elution of the column with chloroform–methanol (9:1) afforded a yellow amorphous powder of **4**, recrystallized from chloroform–methanol (9:1), yield 148 mg, m. p. 187 – 188 ^{0} C, R_f 0.45 (chloroform–methanol, 9:1); UV λ_{max} (MeOH): 211 nm (log ε 2.6, 5.8); IR υ_{max} (KBr): 3311, 3250, 2918, 2847, 1733, 1468, 1392, 1178, 1104, 1047, 991, 943, 721 cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.22 (1H, m, H-6), 5.18 (1H, d, J = 7.1Hz, H- 1'), 4.85 (1H, m, H-5'), 4.85 (1H, m, H- 5'), 4.40 (1H, dd, J = 7.1, 8.0 Hz, H-2'), 3.66 (1H, dd, J = 5.5, 8.9 Hz, H-3), 3.56 (1H, m, H- 3'), 3.43 (1H, m, H-4'), 3.12 (1H, d, J = 10.5 Hz, H_2-6'a), 3.09 (1H, d, J = 10.3 Hz, H_2-6'b), 1.28 (3H, brs, Me-28), 1.20 (3H, brs, Me-19), 0.98 (3H, d, J = 6.6 Hz, Me-21), 0.95 (3H, brs, Me-29), 0.81 (3H, d, J = 6.5 Hz, Me-26), 0.78 (3H, d, J = 6.4 Hz, Me-27), 0.74 (3H, brs, Me-18), 0.51 (3H, brs, Me-30), 2.32 – 1.31 (25H, m, 10 x CH_2, 5 x CH); ^{13}C NMR (DMSO-d_6): δ 22.81 (C-1), 32.15 (C-2), 76.27 (C-3), 42.19 (C-4), 148.23 (C-5), 118.42 (C-6), 24.14 (C-7), 51.39 (C-8), 46.25 (C-9), 43.17 (C-10), 28.18 (C-11), 49.23 (C-12), 50.16 (C-13), 48.27 (C-14), 42.21 (C-15), 30.62 (C-16), 57.36 (C-17), 15.61 (C-18), 22.52 (C-19), 34.33 (C-20), 23.73 (C-21), 24.19 (C-22), 31.87 (C-23), 39.28 (C-24), 29.17 (C-25), 25.71 (C-26), 26.03 (C-27), 21.77 (C-28), 29.48 (C-29), 18.22 (C-30), 103.61 (C-1'), 74.51 (C-2'), 68.76 (C-3'), 65.03 (C-4'), 76.17 (C-5'), 60.85 (C-6'); ESI-MS *m/z* (rel. int.): 590 $[M]^+$ $C_{36}H_{62}O_6$ (1.8).

Cucurbit-5-en-3β-ol diglucosidic linolenate (5)

Further elution of the column with chloroform–methanol (9:1) produced yellow crystals of **5**; R_f 0.62 (benzene–chloroform–methanol, 5:4:1); m. p. 237 – 239 ^{0} C, UV λ_{max} (MeOH): 212 nm; IR υ_{max} (KBr): 3528, 3438, 3315, 2918, 2849, 1732, 1640, 1445, 1381, 1197, 1072, 1019, 987, 932, 720 cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.29 (1H, m, H-6), 3.98 (1H, dd, J = 5.5, 9.3 Hz, H-3α), 1.34 (3H, brs, Me-28), 1.31 (3H, brs, Me-19), 0.97 (3H, d, J = 6.3 Hz, Me-21), 0.92 (3H, brs, Me-29), 0.84 (3H, d, J = 6.2 Hz, Me-26), 0.78 (3H, d, J = 6.3 Hz, Me-27), 0.72 (3H, brs, Me-18), 0.63 (3H, brs, Me-30), 5.78 (1H, d, J = 7.3 Hz, H-1'), 4.81 (1H, m, H- 5'), 4.52 (1H, m, H-4'), 4.49 (1H, m, H-2'), 4.38 (1H, m, H-3'), 3.20 (2H, d, J = 8.2 Hz, H_2-6'), 5.73 (1H, d, J = 7.1 Hz, H-1"), 4.76 (1H, m, H-5"), 4.60 (1H, m, H-4"), 4.46 (1H, m, H-2"), 4.33 (1H, m, H-3"), 3.18 (2H, d, J = 9.1 Hz, H_2-6"), 6.25 (2H, m, H-10"', H-12"'), 5.31 (1H, m, H-13"'), 5.27 (1H, m, H-9"'), 5.24 (1H, m, H-16"'), 5.21 (1H, m, H-17"'), 2.52 (2H, t, J = 7.2 Hz, H_2-2"'), 0.88 (3H, t, J = 6.5 Hz, Me-18"'), 2.31 – 1.19 (45H, m, 20 x CH_2, 5 x CH); ^{13}C NMR (DMSO-d_6): δ 22.93 (C-1), 32.88 (C-2), 76.86 (C-3), 42.49 (C-4), 138.72 (C-5), 120.46 (C-6), 25.56 (C-7), 51.50 (C-8), 46.51 (C-9), 45.16 (C-10), 27.14 (C-11), 47.85 (C-12), 50.32 (C-13), 48.22 (C-14), 41.86 (C-15), 30.11 (C-16), 50.51 (C-17), 16.10 (C-18), 20.58 (C-19), 35.17 (C-20), 23.20 (C-21), 21.01 (C-22), 31.85 (C-23), 38.21 (C-24), 28.96 (C-25), 24.39 (C-26), 25.14 (C-27), 21.35 (C-28), 29.30 (C-29), 18.13 (C-30), 103.25 (C-1'), 69.04 (C-2'), 68.57 (C-3'), 73.52 (C-4'), 75.20 (C-5'), 60.35 (C-6'), 91.64 (C-1"), 71.70 (C-2"), 66.45 (C-3"), 70.08 (C-4"), 74.06 (C-5"), 61.03 (C-6"), 171.24 (C-1"'), 53.90 (C-2"'), 33.72 (C-3"'), 29.81 (C-4"'), 29.57 (C-5"'), 29.23 (C-6"'), 29.20 (C-7"'), 25.61 (C-8"'), 131.82 (C-9"'), 130.04 (C-10"'), 57.23 (C-11"'), 129.03 (C-12"'), 127.93 (C-13"'), 56.73 (C-14"'), 125.75 (C-15"'), 115.98 (C-16"'), 22.63 (C-17"'), 14.70 (C-18"'); ESI-MS *m/z* (rel. int.): 1012 $[M]^+$ ($C_{60}H_{100}O_{12}$) (1.8), 585 (9.3), 570 (8.1), 427 (11.2), 410 (12.1), 308 (21.1), 277 (6.9), 163 (12.4).

RESULTS AND DISCUSSION

Compound **1** was a known long chain saturated fatty acid identified as *n*-docosanoic acid (behenic acid). [31] Compound **2** was a recognized cucurbitane-type triterpenoid identified as cucurbit-5,7-dien-3β-ol (Fig.1)[18]

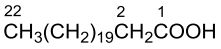
Compound **3**, designated as erucicyl O-β-D-galactoside, $[M]^+$ at *m/z* 500 ($C_{28}H_{52}O_7$), gave positive tests for glycosides and displayed characteristic IR absorption bands for hydroxyl groups (3410, 3366, 3281 cm^{-1}), ester function (1722 cm^{-1}), unsaturation (1647 cm^{-1}) and long chain aliphatic hydrocarbon (771 cm^{-1}). The mass ion fragments generated at *m/z* 163 [$C_{1'}$ - O fission, $C_6H_{11}O_5]^+$, 179 [C_1 – O fission, $C_6H_{11}O_6]^+$, 337 [M – 163]$^+$ and 321 [M – 179]$^+$ indicated that a hexose unit was linked with a C-22 unsaturated fatty acid. The ion peaks arising at *m/z* 361 [$C_{12} – C_{13}$ fission, $(CH)_{11}$-COO-$C_6H_{11}O_5]^+$, and 387 [$C_{14} – C_{15}$ fission, CH=CH-$(CH)_{11}$-COO-$C_6H_{11}O_5]^+$, suggested the presence of the vinylic linkage between C_{13} and C_{14} carbons supporting erucic acid. The ^{1}H NMR spectrum of **3** exhibited two one-proton multiplets at δ 5.35 and 5.32 with half-widths of 5.6 and 5.9 Hz assigned to cis-oriented vinylic H-9 and H-10 protons, respectively, methylene protons between δ 2.23 – 1.28, a three-proton triplet at δ 0.86 (J = 6.6 Hz) ascribed to primary C-22 methyl protons, a one-proton doublet at δ 5.21 (J = 7.2 Hz) attributed to anomeric H-1′ proton, other sugar protons as one-proton multiplets from δ 4.36 to 3.52 accounted to carbinol H-2′ to H-4′ protons and as a two-proton doublet at δ 3.08 (J = 8.8 Hz) associated with hydroxymethylene H_2-6′ protons. The ^{13}C NMR spectrum of **3** displayed signals for the ester carbon at δ 169.21 (C-1), anomeric carbons at δ 108.51 (C-1′), other sugar carbons in the range from δ 74.11 to 61.21, methylene carbons between δ 52.06 - 22.68 and methyl carbon at δ 14.21 (C- 22). The ^{1}H-^{1}H COSY spectrum of **3** exhibited correlations of H_2-12, H-13, H_2-15 with H-14; H_2-20 and H_2-21 with H_3-22; H-2', H-5' and H_2-2 with H-1'; and H_2-6', H-3' and H-4' with H-5'. Acid hydrolysis of **3** yielded erucic acid, m. p. 31-33 ^{o} C, and D-galactose, m. p. 168 – 170 ^{0} C, $[\alpha]^{20}_D$ +78° to +81.5° (2 % water), R_f : 0.58 (*n*-butanol-acetic acid-water, 4:1:1.6). On the basis of these evidences the structure of **3** has been elucidated as (*Z*)-13-docos-13-enoyl O-β-D-galactopyranoside (erucicyl O-β-D-galactopyranoside), a new acyl galactoside (Fig. 1).

Compound **4** was a known cucurbitane-type triterpenoid glucoside identified as cucurbit-5-en-3β-ol 3-O- β-D-glucopyranoside (Fig. 1).[18]

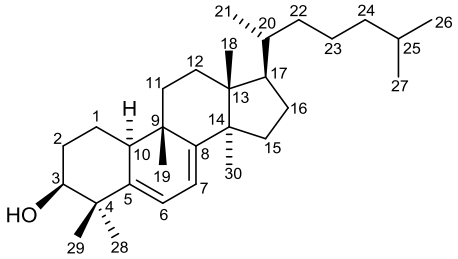
Compound **5,** named cucurbit-5-en-3β-ol diglucosidic linolenate, gave positive tests for glycosides and showed characteristics IR absorption bands for hydroxyl groups (3528, 3438, 3315 cm^{-1}), ester function (1732 cm^{-1}), unsaturation (1640 cm^{-1}) and long aliphatic chain (720 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of **5** was determined at *m/z* 1012 corresponding to the molecular formula of a tetracyclic triterpenic diglycosidic ester, $C_{60}H_{100}O_{12}$. The ion fragments arising at *m/z* 427 [$C_{1'}$ – O fission, $C_{30}H_{51}O]^+$ and 410 [C_3 – O fission, $C_{30}H_{51}]^+$ indicated the presence of tetracyclic triterpenic unit. The ion peaks generated at *m/z* 585 [M – 427]$^+$, 570 [585 - Me]$^+$, 277 [$C_{4''}$ – O fission, $C_{18}H_{29}O_2]^+$, and 308 [585 – 277]$^+$ suggested the existence of two hexose units linked with the triperpenic unit and a triple unsaturated C-18 fatty acid (linolenic acid) attached to the disaccharide unit. The ^{1}H NMR spectrum of **5** showed signals for vinylic protons as a two-proton multiplet at δ 6.25 assigned to H-10''' and H-12''' protons, and five one-proton multiplets between δ 5.31 – 5.21 due to triterpenic vinylic H-6 and fatty acid vinylic H-13''', H-9''', H-16''' and H-17''' protons. A one-proton double doublet at δ 3.98 (J = 5.5, 9.3 Hz, H-3α) was due to oxymethine H-3α proton. Five three-proton singlets at δ 1.34, 1.31, 0.92, 0.72 and 0.63, three three-proton doublets at δ 0.97 (J = 6.3 Hz), 0.84 (J = 6.2 Hz) and 0.78 (J = 6.3 Hz) and one three-proton triplet at 0.88 (J = 6.5 Hz) were ascribed correspondingly to tertiary C-28, C-19, C-29, C-18 and C-30, secondary C-21, C-26 and C-27 and primary C-18''' methyl protons. A two-proton triplet at δ 2.52 (J = 7.2 Hz) were attributed to methylene H_2-2''' protons adjacent to the ester function. The signals between δ 2.31 – 1.19 were assigned to the remaining methylene and methine protons. Two one-proton doublets at δ 5.78 (J = 7.3 Hz) and 5.73 (J = 7.1 Hz, H-1") were accounted to β-oriented anomeric H-1′ and H-1" protons, respectively. Two one-proton doublets at δ 3.20 (J = 8.2 Hz) and 3.18 (J = 9.1 Hz) were due to hydroxymethylene H_2-6' and H_2-6" protons. The other sugar oxymethine protons resonated as a one-proton multiplets in the range of δ 4.81 – 4.33. The ^{13}C NMR spectrum of **5** showed important signals for oxymethine carbon at δ 76.86 (C-3), ester carbon at δ 171.24 (C-1'"), vinylic carbons from δ 138.72 to 115.98, methyl carbons between δ 29.30 – 14.70, anomeric carbons at δ 103.25 (C-1′) and 91.64 (C-1"), other sugar carbons from δ 75.20 to 61.03 and the remaining methylene and methine carbons between δ 53.90 - 22.63. The ^{1}H NMR and ^{13}C NMR spectral data of the triterpenic nucleus were compared with other cucurbitene–type molecules.[18, 32, 33] The presence of ^{1}H NMR signal of H-4' in the deshielded region at δ 4.52 and ^{13}C NMR signal for C-4' at δ 73.52 suggested the attachment of the second sugar unit at C-4' carbon. The existence of ^{1}H NMR signal of H-4" in the downfield region at δ 4.60 and C-4" carbon signal at δ 70.08 supported the location of the linolenyl group at C-4" carbon.

The DEPT spectrum of **5** showed the presence of nine methyl, twenty two methylene, twenty three methine and six quaternary carbons. The ^{1}H-^{1}H COSY spectrum of **5** exhibited correlations of H_2-1, H_2-2 and H-1' with H-3; H_2-7, H-8 and H-9 with H-6; H- H-3', H-5' and H-1" with H-4'; H-3", H-5" and H_2-4'" with H-4"; and H_2-8'", H-9'", H-10'", H-12'" and H-13'" with H_2-11'". The HMBC spectrum of **5** displayed interactions of H_2-2, H-3, H-6 and H-9 with C-4; H-3, H-2' and H-5' with C-1'; H-4', H-2", and H-5" with C-1"; H-4" and H_2-2'" with C-1'"; and H-9'", H-10'", H_2-11'", H-13'" with C-12'". Acid hydrolysis of **5** yielded cucurbit-5-en-3β-ol, linolenic acid and β-D-glucose, R_f 0.26 (*n*-butanol- acetic acid – water, 4 : 1 : 5), specific rotation, $[\alpha]^D_{25^o}$ + 52^o (water). On the basis of spectral data analysis and chemical reactions, the structure of **5** has been characterized as cucurbit-5-en-3β-olyl 3-O-β-D-glucopyranosyl-(4′→1″)-

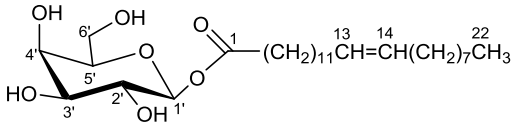
O-β-D-glucopyranosidic 4″- linolenate, a new cucurbit-5-ene diglucosidic ester (Fig. 1).



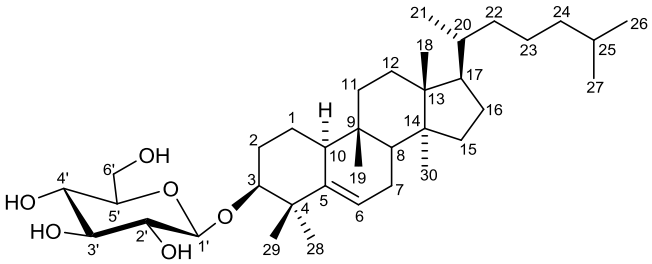
Behenic acid (1)



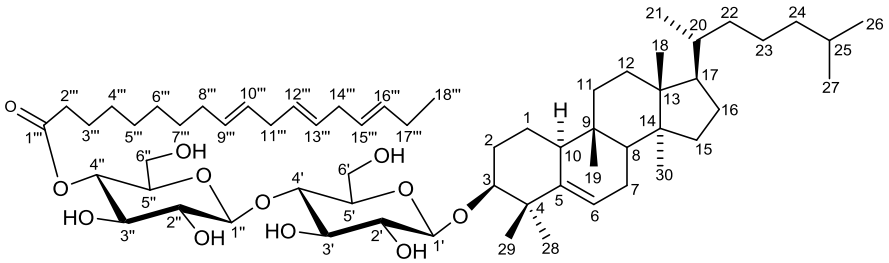
Cucurbit-5,7-dien-3β-ol (2)



Erucicyl-*O*-β-D-galactoside (3)



Cucurbit-5-en-3β-ol 3-*O*-D-glucoside (4)



Cucurbit-5-en-3β-ol diglucosidic linolenate (5)

Fig. 1: Chemical constituents 1 – 5 isolated from the fruits of *Momordica balsamina* L.

CONCLUSION

Phytochemical investigations of a defatted ethanolic extract of the fruits of *Momordica balsamina* L. afforded a fatty acid characterized as *n*-docosanoic acid (behenic acid, **1**), a new acyl galactoside identified as erucicyl O-β-D- galactopyranoside (**3**), and three tetracyclic triterpenoids recognized as cucurbit-5, 7-dien-3β-ol (**2**), cucurbit-5-en-3β-ol 3-O- β-D-glucopyranoside (**4**) and cucurbit-5-en-3β-olyl 3-O-β-D-glucopyranosyl-(4′→1″)-O-β-D-glucopyranosidic 4″- linolenate (**5**). This work has enhanced understanding about the phytoconstituents of the undertaken plant. All these chemical constituents are reported for the first time from this plant and can be used for quality control of the plant.

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