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ALIPHATIC CONSTITUENTS OF *TRIBULUS TERRESTRIS* L. FRUITS AND *DURANTA ERECTA* L. LEAVES AND AN AROMATIC ESTER FROM *PEDALIUM MUREX* L. FRUITS

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ABSTRACT

Tribulus terrestris L. (Family: Zygophyllaceae) is used to treat abdominal, liver, urethra and urinary tract disorders, calculus affections, cystitis, dysuria, edema, gonorrhea, gout, impotency, influenza, kidney stones, leucorrhea, mastitis, micturition, piles, rheumatism, spermatorrhoea, venereal diseases and vitiligo. *Duranta erecta L. (Family Verbenaceae) is* utilized to cure asthma, bronchitis, fevers, infertility, intestinal worms, malaria, menstrual and neuralgic disorders, pneumonia and skin diseases. The leaves and fruits of *Pedalium murex L. (Family Pedaliaceae)* are prescribed to relieve amenorrhea, asthma, colds, cough, dropsy, fevers, flatulence, gonorrhea, inflammation, impotency, leucorrhoea, puerperal diseases, rheumatism, skin diseases, spasmodic affections, spermatorrhoea, urethral stones, venereal diseases and wounds. A petroleum ether fraction of a methanolic extract of the fruits of *T. terrestris* on GC-MS analysis showed the presence mainly of fifteen aliphatic alcohols (91.8%) including *n*- tetracosan-4-ol (26.3 %), *n*-dotriacont-3-en-29-ol (17.3 %), *n*- pentacosan-4-ol (14.8 %), *n*-octacos-3,7-dien-25-ol (5.1%), *n*-tetradec-2,4-dien-7-ol, *n*- pentadecan-5,6-diol, heptan-2,5-diol, *n*-henetriacontan-5,21,26-triacontan-5,21,26-triol, *n*-tritriacont-19,22,27-trien-3-ol and isopentanol. The methanolic extract of the fruits on concentration furnished β-sitosterol 3-O-β-D-glucopyranoside (**1**). The methanolic extracts of the leaves of *Duranta erecta* and fruits of *Pedalium murex* on subjection to silica gel column afforded *n*-dotriacontan-12α, 21α-diol (**2**) and 5′β-hydroxytridecanyl benzoate (**3**), respectively, as the new phytoconstituents.

KEYWORDS: *Tribulus terrestris*, *Duranta erecta*, *Pedalium murex*, Chemical constituents.

INTRODUCTION

Tribulus terrestris L., syn. *T. lanuginosus* L. (Family: Zygophyllaceae), known as gokharu, nanagokharu, nerinjil, khar-e-khusak khurd and puncture vine, is found in Mediterranean, subtropical, and desert climate regions around the world, viz. India, China, southern USA, Mexico, Spain, and Bulgaria. It is considered a toxic weed to livestock causing a distressing irreversible asymmetric locomotor disorder with symptoms similar to those of Parkinson's disease in humans.[1] It is an annual, drought-tolerant, small prostrate, 10-60 cm high, hirsute or silky hairy shrub; roots slender, branched, fibrous, cylindrical, with small rootlets; leaves opposite, unequal, paripinnate, pinnae 5 - 8 pairs, elliptical or oblong lanceolate; flowers yellow; fruits stellate shaped, compressed, five angled, covered with prickles; seeds in each crocus with transverse partitions between them, oily. The plant and fruits are regarded as an analgesic, antihypertensive, aphrodisiac, astringent, blood purifier, diuretic, mild laxative, lithotriptic, palliative, stomachic, nervine tonic and urinary disinfectant; used to treat abdominal distension, albuminuria, asthma, bladder and urethra disorders, Bright's disease, calculus affections, common colds, conjunctivitis, coughs, cystitis, debility, diarrhoea, dysuria, edema, emission, eye and throat infections, fecundity, flatulence, genitourinary tract disorders, gleet, gonorrhea, gout, headache, impotency, influenza, kidney stones, leucorrhea, depressed liver, mastitis, micturition, phosphaturia, piles, rheumatism, scorpion sting, sexual dysfunction, sexual debility, spermatorrhoea, urinary tract troubles, burning urination, uterine disorders, venereal diseases and vitiligo. The roots and fruits are useful in rheumatism, piles, renal and vesicle calculi, menorrhagia, impotency and premature ejaculation. A leaf paste is applied on the bald head for hair growth. A stem infusion is taken to cure gonorrhea. The plant in combination with *Hyoscyamous niger* and opium is given to reduce inflammation of the urinary passage.[2-4]

The fruits of *T. terrestris* contained steroidal saponins,[5-12] kaempferol-3-gentiobioside, isorhamnetin-3-gentiobioside,[10] **f**urostanol saponins,[6,7,13-19] terrestribisamide, 25R-spirost-4-en 3, 12-dione, tribulusterine, N-p-coumaroyltyramine, terrestriamide, hecogenin, aurantiamide acetate, xanthosine, fatty acid ester, ferulic acid, vanillin, p-hydroxybenzoic acid and β-sitosterol,[20] tribulusamides A and B (lignanamides), *N-trans*-feruloyltyramine, terrestriamide, *N-trans*-coumaroyltyramine,[21] terresoxazine,[22] neohecogenin glycosides,[23] tribulusimide C, N-p- coumaroyltyramine, terrestriamide, N-trans-caffeoyltyramine,[24] glycerol monopalmitate, butanedioic acid, vanillic acid, 3-hydroxy-stigmast-5-en-7-one, 4-ketopinoresinol, terrestriamide, and N-trans-caffeoyltyramine,[25] feruloyl amide,[26] pregnane and steroidal glycosides,[27] alkaloids harmane and norharmane.[28] The aerial parts possessed tribol, spirostanol, steroidal saponins, sitosterol glucoside,[14,29,30] protodioscin, 5,6-dihydroprotodioscin, neoprotodioscin and their respective sulfates,[31] sodium salts of methyl prototribestin and prototribestin,[32] fatty acid and esters,[33] oligosaccharides, di-*p*-coumaroylquinic acid,[34] flavonoid glycosides,[35,36] α-amyrin and linoleic, linolenic and stearic acids.[37] The leaves yielded kaempferol, kaempferol-3-glucoside, kaempferol-3-rutinoside and tribuloside (kaempferol3-β-d-(6″-p-coumaroyl) glucoside.[38,39]

Duranta erecta L., syn. *D. angustifolia* Salisb., *D. dentata* Pers., *D. ellisiae* Jacq., *D. inermis* L., *D. racemosa* Mill., *D. repens* L., *D. spinosa* Mill. (Family Verbenaceae), known as golden dewdrop, pigeon berry and sky flower, is distributed from Mexico to South America and the Caribbean. It is cultivated as an ornamental plant in fences and naturalized in southern USA, Philippines, India, China and several Pacific islands.[40] It is a sprawling, upright, smooth shrub of a small tree, up to 6 m tall; branches drooping spiny, bark light gray, rough, fissured when old, spines along the stems; leaves small, light green, elliptic to ovate, entire, opposite; flowers light-blue or lavender, in clusters located on terminal and axillary stems; fruits small, fleshy, globose, yellow or orange berries, in hanging clusters; seeds several. The leaves and unripened berries are toxic.[41] The whole plant is used as an antidote to toxins, insect repellent, larvicide and stimulant and to treat asthma, bronchitis, fevers, infertility, malaria, neuralgic disorder, pneumonia and skin itches.[42,43] The fruits are considered as an antifungal, diuretic, febrifuge, insecticide, larvicidal, slightly toxic and vermifuge, taken to treat abscesses, intestinal worms, malaria and parasitism. The fruit induces derangement in man. The flowers are stimulant. The leaves are diuretic, used to cure abscesses, cataracts and malaria. Fruit powder mixed with *Ferula asafetida* is given internally against sores and ulcers. The leaves mixed with *Styrax benzoin*, infused in coconut milk and given orally and applied externally to children to relieve cough with vomiting, fevers, malaria and measles. A leaf decoction is administered orally to comfort menstrual disorders. A leaf paste is applied to heal wounds. The bark is administered internally to relieve malaria.[4,42,44-46]

The *D. erecta* plant contained 5,6-dimethoxyflavone glycosides and derivatives of di- and tri-methoxyflavones,[47] coumarinolignoids durantins A-C, cleomiscosins A, 6,7,8-trimethoxycoumarin and 5,4′- dihydroxy-3,6,7-trimethoxyflavone, [48] tetrahydroxyflavone,[49] isoprenylated flavonoids, isoprenylated acetophenone derivative, 5-hydroxy-3,6,7,4'-tetramethoxyflavone, rosenonolactone, 6,7-dimethoxycoumarin, 5α,8α-epidioxyergosta-6,22-dien-3β-ol and 5α,8α-epidioxyergosta-6,9(11),22-trien-3beta-ol,[50] iridoid glycosides duranterectoside A, lamiidoside, durantosides II and III, deacetyl asperulosidic acid methyl ester and 6′-O-sinapoylgeniposide,[51] steroid,[52] phenylethanoid glycoside acteoside, lamiide and pseudo-ginsenoside-RT1[53] and durantol.[54] The leaves yielded scutellarein, pectolinarigenin,[55] iridoids durantosides I, II, IV and V, oleanolic, ursolic, (E)-cinnamic and p-methoxycinnamic acids, β-sitosterol 3-O-β-D-glucoside, kusaginin, glucose,[56] triterpenoid saponins durantanins IV and V, oleanolic acid, phenylethanoids campenoside I, cistanoside E, acteoside, flavonoids acacetin, diosmetin, apigenin, luteolin, quercetin,[57] iridoid glucosides, duranterectosides A, B, C and D, lamiide, lamiidoside, verbascoside,[58] C-alkylated flavonoids and the *trans*-clerodane diterpenoids.[59] The seeds furnished a fixed oil composed of fatty acids, catechin, saponin, rutin, linamarin and tannins. The leaves possessed a fixed oil, kaempferol, rutin and saponins.[60] The stem contained duranterectoside A, durantosides I, II and III, lamiidoside,[58] β-amyrin and 12-oleanene 3ß, 21ß-diol.[61] The fruits afforded repennoside (iridiod glucoside) and trans-cinnamic acid.[62,63] The flowers produced oleanolic acid, α- and β-amyrins, phytyl fatty acid esters and triacylglycerols.[64]

Pedalium murex L., synonyms *P. microcarpum* Decne., *P. muricatum* Salisb., *Rogeria microcarpa* Klotzsch (Family Pedaliaceae), known as bara gokhru, gokhru-big and large caltrops, is distributed from India to Indonesia, in Sri Lanka, Mexico and tropical Africa. It is a foetid-smelling, annual, up to 70 cm tall herb with slightly succulent, spreading, glandular, much-branched stems; roots turmeric-like in colour, leaves simple, opposite, oblong-obovate, truncate or obtuse, crenate-serrate, glabrous above, minutely scaly below; flowers yellow, axillary, solitary, scaly outside, persistent; fruits indehiscent, 4-angled with stout, sharp, conical horizontal spines, narrowed at the base; seeds 1 - 2 per locule, oblong, cylindrical, black. The leaves are antibilious, anti-inflammatory, antispasmodic, aphrodisiac, appetizer, blood purifier, carminative, demulcent, digestive, diuretic, emmenagogue, lithontriptic, mucilaginous, nutrient, oxytocic, refrigerant, rejuvenating, stomachic, sweet and tonic; useful to treat amenorrhea, aphthae, ardor urinae, asthma, bladder stone, burning micturition, calculi, colds, cough, general debility, dropsy, dysmenorrhea, dyspepsia,

dysuria, ephemeral fever, flatulence, gleet, gonorrhea, inflammation, impotency, leucorrhoea, lumbago, nocturnal emissions, puerperal diseases, renal and vesical calculi, rheumatism, skin diseases, spasmodic affections, spermatorrhoea, splenomegaly, strangury, ulcers, urethral stones, urinary incontinence, venereal diseases and wounds. The cooked leaves are eaten as a vegetable.[3,65] The leaves with *Sida acuta* leaves are given to induce ovulation. Mucilaginous water of the leaves is effective against babesiosis parasitic disease.[4] The fruits are antispasmodic, aphrodisiac, demulcent, diuretic and used to treat general debility, fever, genitourinary disorders, impotence, kidney stones, menstrual irregularities, nocturnal emissions, puerperal disorders, rheumatism, spermatorrhoea, ulcers, urinary disorders, and wounds. The root is considered antibilious.[3] A fruit decoction with roots of *Abrus precatorius* is taken orally to prevent syphilis. A root decoction is beneficial in gonorrhea.[4] The plant milky mucilage is beneficial to cure gonorrhea.[65] The flowers with sesame seeds are given to comfort gonorrhea.[4]

The fruits of *P. murex* contained 2',4',5'-trihydroxy-5,7-dimethoxyflavone, triacontanyl dotriacontanoate, luteolin, rubusic acid, diosgenin, nonacosane, tritriacontane, triacontanoic acid, tritriacontanoic acid and β-sitosterol 3O-D-glucoside,[66] alkaloid isatin, amino acids,[67] aliphatic constituents, vanillin, caffeic, ferulic and protocatechuic acids.[68] The leaves and flowers yielded flavonoids dinatin, its 7-glucoronide, diosmetin, its 7-glucuronide, quercetin, luteolin, pedaltin and pedalin,[69] rubusic acid, lupeol acetate and ursolic acid,[70] quercetin and kaempferol.[71] The root contained 2-(5,6-dimethyl pyrazinyl) methyl phenol.[72]

MATERIALS AND METHODS

General Procedures

Melting points were measured using one end open capillary tubes on a thermoelectrically heated melting point apparatus (Perfit, India) without correction. UV spectra were determined with Lambda Bio 20 spectrophotometer (Perkin Elmer, Schwerzenbach, Switzerland) in methanol. The IR spectra were obtained 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 as solvents. TMS (Fluka analytical, Sigma-Aldrich, Netherland) was taken as an internal standard and the 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 (Qualigens, Mumbai, India) with 60-120 mesh particle size. The purity of the isolated compounds was checked on precoated TLC plates with silica gel 60 F_{254} (0.25 mm, Merck, Mumbai, India). The spots were visualized by exposure to iodine vapors and under UV radiations at 254 and 366 nm and spraying with ceric sulphate solution.

Plant materials

The fruits of *Tribulus terrestris* and *Pedalium murex* and leaves of *Duranta erecta* were collected from Delhi and identified by Prof. M. P. Sharma, Department of Botany, School of Chemical and Life Sciences, Jamia Hamdard University, New Delhi. The voucher specimens of the samples are preserved in the herbarium of the Department.

Isolation of phytoconstituents from the fruits of *Tribulus terrestris*

The air-dried coarsely powder of the fruits (500 g) was exhaustively extracted with methanol in a Soxhlet apparatus for 16 h. The extract was concentrated on a steam-bath and dried under reduced pressure to get 53 g of dark brown mass. The residue was dissolved in petroleum ether (b. p. 60 – 80 °C), concentrated and stored at 4°C in the dark for subsequent experiments. The remaining residue was re-dissolved in chloroform – methanol (1:1) and kept at 4 °C for crystallization.

β-Sitosterol glucoside (1)

The methanolic extract of the fruits after removal of the petroleum ether soluble fraction was dissolved in chloroform – methanol (1 : 1) and crystallized to get an amorphous powder of **1**, recrystallized from chloroform-methanol (1:3), yield 56 mg, Rf 0.4 (chloroform-methanol, 5:2). m.p. 275-277 °C; UV λmax (MeOH): 208 nm (log ε 2.9); IR νmax (KBr): 3441, 3376, 2927, 2851, 1642, 1451, 1392, 1262, 1086, 824 cm^{-1}; ^{1}H NMR ($DMSOd_6$): δ 5.33 (1H, m, H-6), 5.14 (1H, d, *J* = 7.3 Hz, H-1′), 4.81 (1H, m, H-5′), 3.76 (1H, m, H-2′), 3.54 (1H, m, H-3′), 3.42 (1H, m, H-4′), 3.53 (1H, brs, $w_{1/2}$ = 18.3 Hz, H-3), 3.17 (2H, d, J = 8.2 Hz, H_2- 6′), 0.91 (3H, brs, Me-19), 0.84 (3H, d, J = 6.3 Hz, Me-21), 0.81 (3H, d, J = 6.5 Hz, Me-26), 0.78 (3H, d, *J* = 6.5 Hz, Me-27), 0.76 (3H, t, J = 6.8 Hz, Me-29), 0.61 (3H, brs, Me-18), 2.76 - 1.03 (29H, m, 11 × CH_2, 7 × CH); ^{13}C NMR ($CDCl_3$): δ 38.36 (C-1), 31.86 (C-2), 73.61 (C-3), 42.23 (C-4), 139.41 (C-5), 123.19 (C-6), 33.09 (C-7), 31.83 (C-8), 51.07 (C-9), 37.13 (C-10), 25.59 (C-11), 39.16 (C-12), 43.21 (C-13), 56.68 (C-14), 24.32 (C-15), 28.34 (C-16), 55.69 (C-17), 12.48 (C-18), 19.47 (C-19), 36.68 (C-20), 20.89 (C-21), 31.04 (C-22), 28.61 (C-23), 48.71 (C-24), 29.69 (C-25), 19.94 (C-26), 20.22 (C-27), 23.07 (C-28), 12.89 (C-29), 101.69 (C-1'), 76.21 (C-2'), 73.58 (C-3'), 69.85 (C-4'), 78.18 (C-5'), 61.72 (C- 6'); ESI-MS *m/z* (rel. int.): 576 $[M]^+$ ($C_{35}H_{60}O_6$) (8.1), 413 (22.1), 381 (10.8), 273 (12.1), 179 (15.3).

GC-MS analysis of the extract

GC-MS analysis of the petroleum ether soluble portion was carried out on a Shimadzu Gas Chromatograph instrument fitted with a capillary column TR-5MS (30 m x 0.25 mm), film thickness 0.25 µm. The carrier gas He,

flow rate 1.2 ml/min. The initial temperature was 70 °C and then heated at a rate of 15 °C per minute to 290 °C and held for 16 minutes. The chromatograph was coupled to Shimadzu QP2010 Ultra MS detector 70 eV.

Identification of chemical constituents

The most constituents were identified by GC-MS by comparing their retention indices with those of authentic standards available in the laboratory or with the retention indices. Further identification was achieved by mass fragmentation patterns of the mass spectra and compared with those stored in the spectrometer data base using the NIST08 and Wiley 9 built libraries.

Isolation of an aliphatic dihydroxy alcohol from the leaves of *Duranta erecta*

The dried and coarsely pulverized leaves (500 g) of *D. erecta* were extracted exhaustively with methanol in a Soxhlet apparatus. The extract was concentrated under reduced pressure to get a dark brown mass (61.8 g). A small portion of the extract was analyzed chemically to determine the presence of different chemical constituents. The dried extract (50 g) was dissolved in a minimum quantity of methanol and adsorbed on silica gel (60-120 mesh) for the preparation of a slurry. It was dried in air and chromatographed over silica gel columns (1.6 m x 16 mm x 2 mm) packed in petroleum ether. The column was eluted successively in increasing order of polarity in various combinations with petroleum ether alone and petroleum ether - chloroform mixtures (9:1, 3:1, 1:1, 1:3, *v/v*). The 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 compound were recrystallized to get the following pure compound:

***n*-Dotriacontan-12α, 21α-diol (2)**

Elution of the column with petroleum ether - chloroform (9:1, *v/v*) furnished a colourless amorphous powder of **2**, yield 241 mg, m. p. 85-87 °C, UV λ max (MeOH): 210 nm; IR υmax (KBr): 3445, 3395, 2926, 2747, 1481, 1384, 830, 722 cm^{-1}; ^1H NMR ($CDCl_3$) : δ 3.82 (1H, m, J = 7.2 Hz, H-12β), 3.77 (1H, m, $w_{1/2}$ = 6.6 Hz, H-21β), 1.63 (2H, m, H_2 -11), 1.55 (4H, m, H_2 -13, H_2 -20), 1.34 (2 H, m, H_2 -22), 1.29 (16 H, brs, 8 x CH_2), 1.25 (32 H, brs, 16 x CH_2), 1.21 (3H, t, J = 7.8 Hz, Me-1), 1.16 (3H, t, J = 6.6 Hz, Me-32); ^{13}C NMR ($CDCl_3$): δ 67.58 (C-12), 66.93 (C-21), 35.46 (C-11), 32 .72 (C-13), 30.81 (C-20), 29.85 (C-22), 29.71 (CH_2), 29.67 (CH_2), 29.63 (CH_2), 29.58 (CH_2), 29.54 (CH_2), 29.45 (CH_2), 29.39 (3 x CH_2), 25.76 (8 x CH_2), 22.69 (7 x CH_2), 14.16 (Me-1), 14.02 (Me-32); +ve FAB MS *m/*z (rel. int.) : 482 $[M]^+$ ($C_{32}H_{66}O_2$) (21.3), 327 (5.2), 155 (65.7).

Isolation of an aromatic ester from the fruits of *Pedalium murex*

The fruit powder (500 g) of *P. murex* was extracted exhaustively with methanol in a Soxhlet apparatus and the extract was concentrated to obtain a yellow mass (48.6 g). The dried extract (40 g) was dissolved in a minimum quantity of methanol and adsorbed on silica gel (60-120 mesh) for the preparation of a slurry. The dried slurry was loaded over a silica gel column and processed similarly to that of *D. erecta*.

5′β-Hydroxytridecanyl benzoate (3)

Elution of the column with petroleum ether – chloroform (3:1) gave pale yellow gummy mass of **3**, yield 173 mg, UV λ max (MeOH): 276 nm; IR υmax (KBr): 3415, 2928, 2841, 1725, 1637, 1527, 1465, 1341, 1261, 1056, 990, 825, 723 cm^{-1}; ^1H NMR ($CDCl_3$) : δ 7.72 (2H, m, H-2, H-6), 7.54 (2H, m, H-3, H-5), 7.29 (1H, m, H-4), 4.09 (2H, t, J = 6.1 Hz, H_2-1′), 3.76 (1H, brm, $w_{1/2}$ = 18.8 Hz, H-5′α), 2.21 (2H, m, H_2-4′), 2.02 (2H, m, H_2-6′), 1.68 (2H, m, H_2 -2′), 1.44 (4H, m, H_2 -3′, H_2 -7′), 1.29 (4 H, m, H_2 -8′, H_2 -9′), 1.25 (6 H, m, H_2 -10′, H_2 -11′, H_2 -12′), 0.99 (3H, t, J = 6.5 Hz, Me-13′); ^{13}C NMR ($CDCl_3$): δ 132.48 (C-1), 128.86 (C-2), 130.95 (C-3), 132.36 (C-4), 130.79 (C-5), 128.72 (C-6), 167.73 (C-7), 65.88 (C-1′), 29.38 (C-2′), 29.34 (C-3′), 27.72 (C-4′), 71.81 (C-5′), 38.05 (C-6′), 31.94 (C-7′), 30.57 (C-8′), 28.24 (C-9′), 25.54 (C-10′), 22.72 (C-11′), 20.18 (C-12′), 14.02 (Me-13′); +ve FAB MS *m/*z (rel. int.) : 320 $[M]^+$ ($C_{20}H_{32}O_3$) (14.5), 215 (2.7), 207 (10.3), 199 (3.4), 177 (3.6), 143 (45.1), 121 (2.8), 113 (59.2), 105 (73.5).

RESULTS AND DISCUSSION

The chemical compositions of the petroleum ether extract of the fruits of *Tribulus terrestris* are tabulated in Table 1. The extract was consisted mainly of fifteen aliphatic alcohols (91.8%), two sesquiterpenes (5.1 %) and one each of fatty acid (0.5 %) and aliphatic ketone (2.6 %).

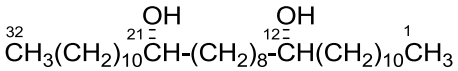
The predominant aliphatic alcohols were *n*- tetracosan-4-ol (26.3 %), *n*-dotriacont-3-en-29-ol (17.3 %) and *n*-pentacosan-4-ol (14.8 %) followed by *n*-octacos-3,7-dien-25-ol (5.1%), *n*-tetradec-2,4-dien-7-ol (4.4%), *n*-pentadecan-5,6-diol (3.8 %), heptan-2,5-diol (3.5 %), *n*-henetriacontan-5,21,26-triacontan-5,21,26-triol (3.1 %), *n*-tritriacont-19,22,27-trien-3-ol (2.6 %) and isopentanol (2.2 %). Heptan-2,5-diol (3.5 %) and *n*- pentadecan-5,6-diol (3.8 %) were detected as dihydroxyaliphatic alcohols and *n*-henetriacontan-5,21,26-triacontan-5,21,26-triol (3.1 %) was identified as a trihydroxy aliphatic alcohol. The sesqiterpenes detected in the fruit petroleum extract included epibicyclosesquiphellandrene (1.9 %) and caryophyllenol (3.2 %). The chemical constituents *n*-eicosan-9-one (2.6 %) and palmitoleic acid (0.5 %) were characterized as an aliphatic ketone and fatty acid, respectively. One of the aliphatic alcohol *n*- docosan-5-ol and the fatty acid palmitoleic acid occurred in trace amount. The fruit petroleum ether was devoid of mono-, di- and triterpenes, steroidal constituents and aromatic compounds.

Table 1: Chemical constituents of the petroleum ether extract of *Tribulus terestris* fruits.

S. No.	Retention time	Component	% Area	Molecular formula / Molecular weight
1	32.46	Isopentanol	2.2	$C_5H_{12}O$, MW 88
2	34.19	Heptan-2,5-diol	3.5	$C_7H_{16}O_2$, MW 132
3	37.06	*n*- Nona-4-ol	1.5	$C_9H_{20}O$, MW 144
4	38.66	Dodec-3,6-dien-8-ol	1.1	$C_{12}H_{22}O$, MW 182
5	45.13	Epibicyclosesquiphellandrene	1.9	$C_{15}H_{24}$, MW 204
6	47.16	*n*-Tetradec-2,4-dien-7-ol	4.4	$C_{14}H_{26}O$, MW 210
7	49.04	*n*-Pentadecan-5-ol	1.2	$C_{15}H_{32}O$, MW 228
8	54.41	Caryophyllenol	3.2	$C_{15}H_{24}O$, MW 220
9	55.10	*n*-Hexadecan-5-ol	1.8	$C_{16}H_{34}O$, MW 242
10	57.94	*n*-Eicosan-9-one	2.6	$C_{20}H_{40}O$, MW 296
11	61.23	*n*- Pentadecan-5,6-diol	3.8	$C_{15}H_{32}O_2$, MW 244
12	62.91	*n*- Docosan-5-ol	0.5	$C_{22}H_{46}O$, MW 326
13	64.13	*n*- Tetracosan-4-ol	26.3	$C_{24}H_{50}O$, MW 354
14	67.20	*n*- Pentacosan-4-ol	14.8	$C_{25}H_{52}O$, MW 368
15	68.08	*n*-Octacos-3,7-dien-25-ol	5.1	$C_{28}H_{54}O$, MW 406
16	70.73	Palmitoleic acid	0.5	$C_{16}H_{30}O_2$, MW 254
17	72.9	*n*-Dotriacont-3-en-29-ol	17.3	$C_{32}H_{64}O$, MW 464
18	74.12	*n*-Triitriacont-19,22,27-trien-3-ol	2.6	$C_{33}H_{62}O$, MW 474
19	77.59	*n*-Henetriacontan-5,21,26-triacontan-5,21,26-triol	3.1	$C_{31}H_{64}O_3$, MW 484

The compound **1** was identified as β-sitosterol 3-O-β-D-glucopyranoside on the basis of spectral data analysis and chemical reactions.[73,74]

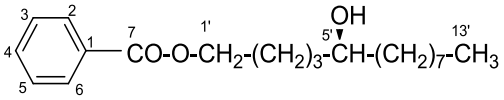
The IR spectrum of compound **2** showed IR absorption bands for hydroxyl groups (3445, 3395 cm^{-1}) and long aliphatic chain (722 cm^{-1}). Its mass spectrum exhibited a molecular ion peak at *m/*z 482 corresponding to a saturated aliphatic diol, $C_{32}H_{66}O_2$. The ion fragments arising at *m/*z 155 [C_{21} – C_{22} fission, $CH_3(CH_2)_{10}]^+$ and 327 [C_{11} – C_{12} fission, $CH_3(CH_2)_{10}$ – CH(OH)-$(CH_2)_8$-CH(OH)]$^+$ indicated the presence of one of the hydroxyl group at C-12 and another one at C-21 positions. The ^{1}H NMR spectrum of **2** displayed two one-proton multiplets at δ 3.82 ($w_{1/2}$ = 7.2 Hz) and 3.77 ($w_{1/2}$ = 6.6 Hz) assigned to β-oriented secondary H-12 and H-21 carbinol protons, respectively. Three multiplets at δ 1.63 (2H), 1.55 (4H) and 1.32 (2H) and two broad singlets at δ 1.29 (16 H) and 1.25 (32 H) were ascribed to the methylene protons. Two three- proton triplets at δ 1.21 (J = 7.8 Hz) and 1.16 (J = 6.6 Hz) were accounted to terminal C-1 and C-32 primary methyl protons. The ^{13}C NMR spectrum of **2** exhibited signals for carbinol carbons at δ 67.58 (C-12) and 66.93 (C-21), methylene carbons from δ 35.46 to 22.69 and methyl carbons at δ 14.16 (C-1) and 14.02 (C-32). The absence of any signal beyond δ 3.82 in the ^{1}H NMR spectrum and carbon signal after δ 67.58 in the ^{13}C NMR spectrum ruled out the existence of any vinylic linkage in the molecule. On the basis of these spectral data analysis, the structure of **2** has been elucidated as *n*-dotriacontan-12α, 21α-diol, a new aliphatic dihydroxy alcohol.



n-Dotriacontan-12α,21α-diol (**2**)

Compound **3** showed UV absorption maximum at 276 nm for aromatic ring and IR absorption bands for a hydroxyl group (3415 cm^{-1}), ester function (1725 cm^{-1}), aromatic ring (1637, 1527 cm^{-1}) and long aliphatic chain (723 cm^{-1}). On the basis of mass and ^{13}C NMR spectra its molecular ion peak was determined at *m/*z 320 consistent with a molecular formula of a phenyl tridecandiol ester, $C_{20}H_{32}O_3$. The ion peaks arising at *m/*z 105 [C_7 - O fission, C_6H_5-CO]$^+$, 215 [M – 105, O-$(CH_2)_4$-CH(OH)-$(CH_2)_7$-$CH_3]^{+\cdot}$ 121 [$C_{1'}$ - O fission, C_6H_5-COO]$^+$, 199 [M – 121]$^{+\cdot}$ 177 [$C_{4'}$ - $C_{5'}$ fission, C_6H_5-COO-$(CH_2)_4]^+$, 143 [M – 177, CH(OH)-$(CH_2)_7$-$CH_3]^{+\cdot}$ 207 [$C_{5'}$ - $C_{6'}$ fission, C_6H_5-COO-$(CH_2)_4$CH(OH)]$^+$ and 143 [M – 207, $(CH_2)_7$-$CH_3]^+$ indicated that tridecandiol was esterified with benzoic acid and the hydroxy groups were present at C-1′ and C-5′ positions. The ^{1}H NMR spectrum of **3** displayed two two-proton multiplets at δ 7.72 and 7.54 and a one-proton multiplet at δ 7.29 assigned to aromatic H-2 and H-6, H-3, H-5 and H-4 protons, respectively, a two-proton triplet at δ 4.09 (J = 6.1 Hz) ascribed to oxymethylene H_2-1′ protons, a one-proton multiplet at δ 3.76 with half-width of 18.8 Hz accounted to α-oriented carbinol H-5′ proton, six multiplets at δ 2.02 (2H), 1.68 (2H), 1.44 (4H), 1.29 (4H) and 1.25 (6 H) associated with the methylene protons and a three-proton triplet δ 0.99 (J = 6.5 Hz) attributed to terminal C-13′ primary methyl protons. The absence of any signal between δ 7.29 – 4.09 in the ^{1}H NMR spectrum ruled out the existence of any vinylic proton in the molecule. The ^{13}C NMR spectrum of **3** exhibited signals for ester carbon at δ 167.73 (C-7), aromatic carbons between δ 132.48 – 128.72, carbinol carbon at δ 71.81 (C-5′), oxymethylene carbon at δ 65.88 (C-1′), other methylene carbons from δ 38.05 to 20.18 and methyl carbon at δ 14.02 (C-13′). Acid hydrolysis of 1 yielded benzoic acid, m. p. 121 -122

°C, R_f 0.75 [ethanol (96 %) – aqueous ammonia (25%) – water, 5: 3: 1], and *n*-tridecan-1,5-diol, $[M]^+$ at m/z 206 ($C_{13}H_{28}O_2$). On the basis of the aforementioned spectral data analysis and chemical reactions, the structure of **3** has been elucidated as 5′β-hydroxytridecanyl benzoate, a new aromatic ester.



5′β-Hydroxytridecanyl benzoate (**3**)

CONCLUSION

A petroleum ether fraction of a methanolic extract of the fruits of *Tribulus terrestris* on GC-MS analysis showed the presence mainly of fifteen aliphatic alcohols (91.8%) including *n*- tetracosan-4-ol, *n*-dotriacont-3-en-29-ol, *n*-pentacosan-4-ol, *n*-octacos-3,7-dien-25-ol and *n*-tetradec-2,4-dien-7-ol as the major constituents. The methanolic extract of the fruits furnished β-sitosterol 3-O-β-D-glucopyranoside (**1**). The methanolic extracts of the leaves of *Duranta erecta* and fruits of *Pedalium murex* contained *n*-dotriacontan-12α, 21α-diol (**2**) and 5′β-hydroxytridecanyl benzoate (**3**), respectively, as the new phytoconstituents.

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