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CHEMICAL CONSTITUENTS FROM THE ROOTS OF *CALOTROPIS PROCERA* (AIT) R. BR.

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

[1]Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi 110 062, India.
[2]Present address: College of Pharmacy, Jazan University, Jazan, Saudi Arabia.

***Corresponding Author: Mohammed Ali**
Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi 110 062, India.

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ABSTRACT
Calotropis procera (Ait) R. Br. (Asclepiadaceae) is a small, pubescent, evergreen and erect shrub which grows wild in south eastern Asia. Its roots are anthelmintic, depurative, diaphoretic, emetic, expectorant, febrifuge and purgative; used to treat anasarca, asthma, ascites, bronchitis, cough, skin diseases, intestinal worms, leprosy and eczema. An air-dried root powder of *C. procera* was extracted with methanol to obtain a dark brown viscous mass. It was dissolved in small amount of methanol and adsorbed on silica gel (60-120 mesh) for preparation of a slurry. The dried slurry was subjected to chromatography over silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, chloroform and methanol to isolate four new phytoconstituents identified as (all *cis*)-*n*-triacont-3,7,11,15,19,25-hexaene-1-ol (**3**), (*cis*)-*n*-tetratriacont-18-en-1-oic acid (**4**), urs-12, 20(30)-dien-3β-olyl laurate (**5**) and 1′-β-sitosteryl-2′-*n*-hexanoyl-glycerol (**6**) together with the known compounds β-sitosteryl linoleate (**1**) and nervonic acid (**2**). The structures of all the isolated phytoconstituents have been established on the basis of spectral data analyses and chemical reactions.

KEYWORDS: *Calotropis procera*, roots, polyalkene, triterpenic ester, fatty acids, steroids.

INTRODUCTION
Calotropis procera (Ait) R. Br. (Asclepiadaceae), known as Apple of Sodom, Milkweed or Swallow-wort, is a small, hardy, pubescent, evergreen, erect and compact shrub, up to 4.5 m high, covered with cottony tomentose. It exudates a copious milky sap when cut. It grows wild in south eastern Asia including India, Pakistan, Afghanistan, tropical Africa, Indochina, Morocco and Senegal mainly in drier and warm regions up to 1,050 m altitude on course, sandy and alkaline soils. Its growth is luxuriant on rubbish heaps, waste or fallow lands, along roadsides, sea shores and river banks.[1] The root is cylindrical, branched, curved, light, woody and grayish white. It resembles with the root of *Cephaelis ipecacuanha* (Rubiaceae) in action and is substituted for it. The roots are alterative, anthelmintic, depurative, diaphoretic, emetic, expectorant, febrifuge and purgative; prescribed to treat anasarca, asthma, ascites, bronchitis, cough, skin diseases, intestinal worms, leprosy and eczema.[2, 3] The root powder promotes gastric secretion; fresh root is used as a tooth brush to cure toothache.[1] A root paste mixed with the leaves of *Ocimum sanctum* is taken orally to relieve menorrhoea.[4] The phytoconstituents cardenolides,[5,6] flavone glycoside,[7] pentacyclic triterpenoids,[8-14] sterols,[7,15] monoterpenic, diterpenic and phenolic glycosides,[16-18] fatty acids[5] and a norditerpenyl ester[12] have been reported from the roots. This manuscript describes the isolation and characterization of four new chemical constituents along with β-sitosteryl linoleate and nervonic acid from the roots of *C. procera* collected from a local region.

EXPERIMENTAL
General
Melting points were measured on a thermoelectrically operated Perfit 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 Shimadzu FTIR-8400 spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were scanned by Bruker spectrospin NMR instrument using TMS as internal standard. FAB mass spectra were recorded on a Jeol D-300 spectrometer. For column chromatography, silica gel (60-120 mesh, Merck, Mumbai, India) was used and thin-layer chromatography was performed on silica gel G 60 F_{254} precoated TLC plates (Merck, Mumbai, India). The spots were visualized by exposure to iodine vapors and UV radiations (254 and 366 nm).

Plant Material
The roots of *C. procera* were collected locally and identified by Prof. M. P. Sharma, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi. A

voucher specimen (NO. PRL/ JH / 08 / 32) is deposited in the herbarium of the Faculty of Pharmacy, Jamia Hamdard, New Delhi.

Extraction and Isolation

The air-dried roots (2 kg) of *C. procera* were coarsely powdered and extracted exhaustively in a Soxhlet apparatus with methanol for 72 hr. The methanolic extract was concentrated under reduced pressure to obtain a dark brown viscous mass. Small portion of the extract was analyzed chemically to determine the presence of different chemical constituents. The viscous dark brown mass was adsorbed on silica gel (60-120 mesh) for column chromatography, after being dissolved in a small quantity of methanol for preparation of a slurry. The slurry (200 g) was air- dried and subjected to chromatography over silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, mixture of petroleum ether and chloroform (9:1, 3:1, 1:1 and 1:3), chloroform and the mixture of chloroform and methanol (99:1, 97:3, 95:5, 92:8, 9:1, 3:1, 1:1, 1:3). 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 the following pure compounds:

β-Sitosteryl linoleate (1)

Elution of the column with petroleum ether – chloroform (7 : 3) afforded a pale yellow semisolid mass of **1**, 127 mg; R_f 0.63 (petroleum ether-chloroform, 1:3), m. p.: 117-118°C; IR ν_{max} (KBr): 2928, 2850, 1723, 1645, 1465, 1371, 1283, 1168, 1048, 955, 798, 717 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.31 (1H, m, H-6), 5.19 (1H, m, H-9′), 5.11 (1H, m, H-10′), 5.04 (1H, m, H-12′), 5.01 (1H, m, H-13′), 4.12 (1H, brm, $w_{1/2}$ = 18.2 Hz, H-3α), 2.34 (2H, t, J = 7.3 Hz, H_2-2′), 1.04 (3H, brs, Me-19), 0.94 (3H, d, J = 6.3 Hz, Me-21), 0.89 (3H, t, J = 6.1 Hz, Me-18′), 0.85 (3H, d, J = 6.5 Hz, Me-26), 0.81 (3H, d, J = 6.1 Hz, Me-27), 0.77 (3H, t, J = 6.6 Hz, Me-29), 0.69 (3H, brs, Me-18), 2.17 – 1.10 (51H, m, 22 x CH_2, 7 x CH); ^{13}C NMR ($CDCl_3$): δ 37.21 (C-1), 31.55 (C-2), 71.83 (C-3), 39.81 (C-4), 140.76 (C-5), 121.62 (C-6), 31.91 (C-7), 33.96 (C-8), 50.17 (C-9), 36.34 (C-10), 21.19 (C-11), 39.81 (C-12), 42.13 (C-13), 56.79 (C-14), 24.31 (C-15), 28.27 (C-16), 56.11 (C-17), 11.81 (C-18), 19.24 (C-19), 36.18 (C-20), 18.77 (C-21), 33.98 (C-22), 23.18 (C-23), 45.81 (C-24), 29.67 (C-25), 19.37 (C-26), 19.13 (C-27), 23.19 (C-28), 11.28 (C-29), 173.31 (C-1′), 37.61 (C-2′), 29.69 (C-3′), 29.67 (C-4′), 29.65 (C-5′), 29.61 (C-6′), 29.50 (C-7′), 29.27 (C-8′), 128.06 (C-9′), 130.50 (C-10′), 34.78 (C-11′), 139.25 (C-12′), 116.03 (C-13′), 35.51 (C-14′), 28.86 (C-15′), 25.10 (C-16′), 22.69 (C-17′), 14.08 (C-18′); +ve FAB MS *m/z* (rel. int.): 677 $[M+1]^+$ ($C_{47}H_{81}O_2$) (2.3), 413 (22.1), 397 (51.8), 280 (15.3).

Nervonic acid (2)

Elution of the column with petroleum ether - chloroform (1:1) gave colorless mass of **2**, 68 mg, m. p. 42 - 43°C, UV λmax (MeOH): 213 nm (log ε 4.2); IR γ max (KBr): 3335, 2926, 2850, 1701, 1705, 1645, 1429, 1379, 1268, 1028, 932, 807, 721 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.13 (1H, m, H-15), 5.10 (1H, m, H-16), 2.10 (2H, t, J = 7.3 Hz, H_2-2), 1.79 (2H, m, H_2-14), 1.70 (2H, m, H_2-17), 1.61 (2H, m, CH_2), 1.52 (2H, m, CH_2), 1.28 (8H, m, 4 x CH_2), 1.23 (22H, brs, 11 x CH_2), 0.63 (3H, t, J = 5.7 Hz, Me-34); +ve FAB MS *m/z* (rel.int.): 367 $[M+1]^+$ ($C_{24}H_{47}O_2$) (23.6), 253 (21.6), 227 (18.9), 113 (8.6).

***n*-Triaconthexene-1-ol (3)**

Elution of the column with chloroform produced colorless crystalline product **3**; 118 mg, m. p. 85-87°C; IR γ max (KBr): 3414, 2937, 2841, 1645, 1424, 1387, 1268, 1150, 1087, 725 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.23 (2H, m, $w_{1/2}$ = 8.2 Hz, H-3, H-4), 5.18 (2H, m, $w_{1/2}$ = 7.8 Hz, H-7, H-8), 5.07 (2H, m, $w_{1/2}$ = 7.6 Hz, H-11, H-12), 5.02 (2H, m, $w_{1/2}$ = 8.8 Hz, H-15, H-16), 4.99 (2H, m, $w_{1/2}$ = 8.9 Hz, H-19, H-20), 4.95 (2H, m, $w_{1/2}$ = 7.7 Hz, H-25, H-26), 3.21 (2 H, t, J = 7.3 Hz, H_2-1), 2.18 (4 H, m, 2 x CH_2), 2.13 (6H, m, 3 x CH_2), 2.06 (6H, m, 3 x CH_2), 1.83 (4H, m, 2 x CH_2), 1.65 (4H, m, 2 x CH_2), 1.55 (2H, m, CH_2), 1.24 (6H, brs, 3 x CH_2), 0.86 (3H, t, J = 6.3 Hz, Me-30); +ve FAB MS *m/z* (rel. int.): 427 $[M+1]^+$ ($C_{30}H_{51}O$) (6.7), 381 (12.8), 355 (15.6), 301 (18.7), 247 (14.8), 193 (12.1), 139 (17.9), 111 (23.4), 83 (33.6), 57 (100).

***n*-Tetratriacont-18-enoic acid (4)**

Elution of the column with chloroform gave colorless crystalline product **4**; 96 mg, m. p. 88-90°C; IR γmax (KBr): 3228, 2925, 2845, 1702, 1642, 1438, 1365, 1259, 1142, 1031, 722 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.35 (1H, m, $w_{1/2}$ = 8.6 Hz, H-18), 5.32 (1H, m, $w_{1/2}$ = 8.2 Hz, H-19), 2.31 (2 H, t, J = 7.3 Hz, H_2-2), 2.18 (2 H, m, H_2-17), 2.11 (2H, m, H_2-20), 1.63 (4H, m, 2 x CH_2), 1.55 (2H, m, CH_2), 1.34 (2H, m, CH_2), 1.29 (16H, brs, 8 x CH_2), 1.25 (30H, m, 15 x CH_2), 0.89 (3H, t, J = 6.9 Hz, Me-34); ^{13}C NMR ($CDCl_3$): δ 178.08 (C-1), 130.78 (C-18), 120.08 (C-19), 56.71 (C-2), 35.18 (C-17), 33.12 (C-20), 30.71 (CH_2), 31.64 (CH_2), 29.61 (18x CH_2), 28. 21 (6 x CH_2), 22.61 (CH_2), 14.43 (C-34); +ve FAB MS *m/z* (rel. int.): 507 $[M+1]^+$ ($C_{34}H_{67}O_2$) (10.7), 462 (13.8), 269 (36.9), 211 (8.7).

Urs-12,20(30)-dienyl laurate (5)

Elution of the column with chloroform yielded pale yellow crystals of **5**, recrystallized from chloroform–methanol (1:1), 64 mg; m. p. 197 - 198°C; IR γmax (KBr): 2929, 2853, 1733, 1645, 1458, 1382, 1295, 1187, 1085, 989, 876 cm^1; ^1H NMR ($CDCl_3$): δ 5.17 (1H, m, H-12), 4.68 (2H, brs, H_2-30), 4.35 (1H, dd, J = 5.3, 9.3 Hz, H-3β), 2.33 (2H, t, J = 7.2 Hz, H_2-2′), 2.01 (1H, d, J = 4.5 Hz, H-18β), 1.04 (3H, s, Me-23), 0.99 (3H, s, Me-24), 0.97 (3H, s, Me-25), 0.85 (3H, t, J = 6.5 Hz, Me-12′), 0.83 (3H, d, J = 6.3 Hz, Me-29), 0.80 (3H, s, Me-28), 0.77 (3H, s, Me-27), 0.75 (3H, s, Me-26), 1.25 (12H, brs, 6 x CH_2), 1.07 (4H, brs, 2 x CH_2), 2.23- 1.13 (23 H, m, 10 x CH_2 , 3 x CH); ^{13}C NMR ($CDCl_3$): δ 42.12 (C-1), 25.42 (C-2), 78.96 (C-3), 38.79 (C-4), 55.14 (C-5), 18.37 (C-6), 32.96 (C-7), 39.19 (C-8), 47.63 (C-9),

38.26 (C-10), 23.53 (C-11), 124.35 (C-12), 139.58 (C-13), 50.45 (C-14), 27.99 (C-15), 26.23 (C-16), 32.53 (C-17), 59.01 (C-18), 41.51 (C-19), 154.53 (C-20), 34.06 (C-21), 38.53 (C-22), 28.08 (C-23), 21.37 (C-24), 16.81 (C-25), 17.45 (C-26), 15.39 (C-27), 16.25 (C-28), 31.29 (C-29), 107.19 (C-30), 172.12 (C-1′), 55.32 (C-2′), 47.96 (C-3′), 34.79 (C-4′), 30.19 (C-5′), 29.67 (C-6′), 29.56 (C-7′), 29.34 (C-8′), 29.11 (C-9′), 28.72 (C-10′), 22.65 (C-11′), 15.62 (C-12′); +ve FAB MS *m/z* 607 $[M+1]^+$ ($C_{42}H_{71}O_2$) (22.6), 591 (12.1), 576 (10.2), 423 (70.5), 406 (63.3), 217 (100), 206 (59.5), 200 (41.5), 189 (63.4), 187 (58.5), 183 (42.6), 176 (68.2).

1′-β-Sitosteryl-2′-caproyl glycerol (**6**)

Further elution of the column with chloroform furnished colorless crystals of **6**, 69 mg, m. p.: 124 - 126° C; UV λmax (MeOH): 214 nm (log ε 3.8); IR γ max (KBr): 3461, 2920, 2851, 1737, 1640, 1470, 1367, 1248, 1165, 1051, 990 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.36 (1H, m, H-6), 4.30 (1H, m, H-2′), 3.65 (1H, brm, $w_{1/2}$ = 18.2 Hz, H-3α), 3.48 (2H, d, J = 6.4 Hz, H_2-1′), 3.28 (2H, d, J = 5.6 Hz, H_2-3′), 2.28 (2H, t, J = 7.2 Hz, H_2-2″), 1.25 (4H, brs, H_2-3″, H_2-4″,), 1.07 (3H, brs, Me-19), 0.97 (3H, d, J = 6.7 Hz Me-21), 0.88 (3H, t, J = 6.3 Hz Me-6″), 0.86 (3H, d, J = 6.3 Hz Me-26), 0.83 (3H, d, J = 6.0 Hz, Me-27), 0.80 (3H, t, J = 6.3 Hz Me-29), 0.67 (3H, brs, Me-18), 2.76 – 1.10 (31H, m, 12 x CH_2, 7 x CH); ^{13}C NMR ($CDCl_3$): δ 37.28 (C-1), 31.65 (C-2), 71.79 (C-3), 40.73 (C-4), 140.77 (C-5), 121.66 (C-6), 31.93 (C-7), 33.92 (C-8), 50.15 (C-9), 36.42 (C-10), 21.09 (C-11), 39.79 (C-12), 42.32 (C-13), 56.74 (C-14), 24.37 (C-15), 28.25 (C-16), 56.09 (C-17), 11.88 (C-18), 19.84 (C-19), 36.17 (C-20), 18.79 (C-21), 33.97 (C-22), 23.08 (C-23), 45.81 (C-24), 29.63 (C-25), 19.33 (C-26), 19.15 (C-27), 23.09 (C-28), 11.31 (C-29), 66.79 (C-1′), 68.61 (C-2′), 62. 05 (C-3′), 173.36 (C-1″), 38.83 (C-2″), 27..18 (C-3″), 25.62 (C-4″), 24.85 (C-5″), 14.09 (C-6″); +ve FAB MS *m/z* (rel. int.): 587 $[M+H]^+$ ($C_{38}H_{66}O_4$) (11.3), 413 (42.2), 398 (39.5), 396 (91.2), 383 (49.7), 271 (12.2), 255 (17.1), 240 (13.3), 213 (22.5), 198 (20.5), 189 (17.6), 173 (16.5).

RESULTS AND DISCUSSION

Compounds **1** and **2** were the known phytoconstituents characterized as β-sitosteryl linoleate[19,20] and nervonic acid.[21]

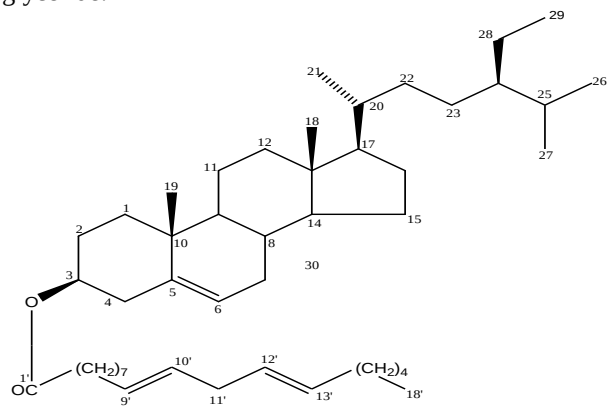
Compound **3** showed characteristic IR absorption bands for hydroxyl group (3414 cm^{-1}), unsaturation (1645 cm^{-1}) and long aliphatic chain (725 cm^{-1}). The mass spectrum of **3** exhibited a molecular ion peak at *m/z* 427 $[M+1]^+$ corresponding to a molecular formula of an unsaturated aliphatic alcohol $C_{30}H_{51}O$. It indicated six degrees of unsaturation which were adjusted in the six vinylic linkages. The ion fragments arising at *m/z* 57 $[CH_3(CH_2)_3]^+$, 139 $[CH_3(CH_2)_3CH{=}CH(CH_2)_4]^+$, 193 $[CH_3(CH_2)_3CH{=}CH(CH_2)_4CH{=}CH(CH_2)_2]^+$, 381 $[M - CH_2CH_2OH]^+$, 355 $[M - CH{=}CHCH_2CH_2OH]^+$, 301 $[M - CH{=}CH(CH_2)_2CH{=}CH\text{-}CH_2CH_2OH]^+$, 247 $[CH_3(CH_2)_5C_{12}H_{18}]^+$, 111 $[CH_3(CH_2)_3CH{=}CH\text{-}CH_2CH_2]^+$ and 83 $[CH_3(CH_2)_3CH{=}CH]^+$ indicated the existence of the vinylic linkages at C-3, C-7, C-11, C-15, C-19 and C-25 positions. The ^1H NMR spectrum of **3** displayed four two-proton multiplets at δ 5.23, 5.18, 5.07 and 4.95 and a four-proton signal at δ 5.02 with half-width between 8.9 – 7.3 Hz assigned to *cis*-oriented vinylic protons. A two-proton triplet at δ 3.21 (J = 7.3 Hz) was ascribed to hydroxymethylene H_2-1 protons. The methylene protons appeared as four-proton multiplets at δ 2.18, 1.83 and 1.64, as six-proton singlets at δ 2.13, 2.06 and 1.24 and a two-proton multiplet at δ 1.55. A three-proton triplet at δ 0.86 (J= 6.5 Hz) was accounted to C-30 primary methyl protons. On the basis of these evidences the structure of **3** has been elucidated as (all *cis*)- *n*-triacont-3,7,11,15,19,25-hexaene-1-ol, a new aliphatic alcohol.

Compound **4** showed IR absorption bands for carboxylic group (3228, 1702 cm^{-1}), unsaturation (1642 cm^{-1}) and long aliphatic chain (722 cm^{-1}). Its mass spectrum displayed a molecular ion peak at *m/z* 507 $[M+1]^+$ corresponding to a molecular formula of a fatty acid, $C_{34}H_{67}O_2$. The ion peaks arising at *m/z* 269 [C_{17}-C_{18} fission, $(CH_2)_{16}COOH]^+$, 211 [C_{19}-C_{20} fission, $CH_3(CH_2)_{14}]^+$ and 462 $[M - COOH]^+$ supported the presence of vinylic linkage at C_{18}. The ^1H NMR spectrum of **4** displayed two one-proton multiplets at δ 5.35 ($w_{1/2}$ = 8.6 Hz) and 5.32 ($w_{1/2}$ = 8.2 Hz) assigned to *cis*-oriented vinylic protons H-18 and H-19, respectively. A two-proton triplet at δ 2.31 (*J*=7.3 Hz) was ascribed to H_2-2 methylene protons adjacent to the carboxylic group. Five multiplets between δ 2.18 – 1.34 and two broad signals at δ 1.29 (16H) and 1,25 (30H) were associated with the other methylene protons. A three-proton triplet at δ 0.89 (J = 6.9 Hz) was accounted to primary C-34 methyl protons. The ^{13}C NMR spectrum of **4** exhibited important signals for carboxylic carbon at δ 178.08 (C-1), vinylic carbons at δ 130.78 (C-18) and 120.08 (C-19), methylene carbons from δ 56.71 to 22.61 and primary methyl carbon at δ 14.43 (CH_3-34). On the basis of above discussion the structure of **4** was characterized as (*cis*)-*n*-tetratriacont-18-en-1-oic acid.

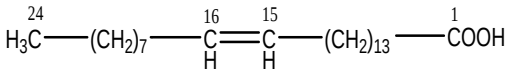
Compound **5**, named urs-12, 20(30)-dienyl laurate**,** responded positively to Liebermann-Burchardt test for triterpenoids and showed IR absorption bands for ester group (1733 cm^{-1}) and unsaturation (1645 cm^{-1}). Its molecular ion peak was determined at *m/z* 607 $[M+1]^+$ on the basis of mass and ^{13}C NMR spectra corresponding to a molecular formula of a pentacyclic triterpenic ester, $C_{42}H_{71}O_2$. The characteristic ion fragments arising at *m/z* 591 $[M - Me]^+$, 576 $[591 - Me]^+$, 183 [O – $C_{1'}$ fission, $OC\text{-}(CH_2)_{10}\text{-}CH_3]^+$, 423 $[M - 183]^+$, 200 [C_3 – O fission, $OOC\text{-}(CH_2)_{10}\text{-}CH_3]^+$ and 406 $[M - 200]^+$ indicated that a lauryl group was attached to the triterpenic unit. The ion peaks generating at *m/z* 206 and 217 from the mass unit at *m/z* 423 due to retro-Diels-Alder fragmentation suggested the existence of one of the vinylic linkage in ring C at C_{12} and another olefinic bond in ring E.[22] The ^1H NMR spectrum of **5** displayed a downfield one-proton multiplet at δ 5.17 and a two-

proton signal at δ 4.68 assigned to vinylic H-12 and exocyclic methylene H_2-30 protons, respectively. A one-proton double-doublet at δ 4.35 with coupling interactions of 5.3 and 9.3 Hz was ascribed to oxymethine H-3α proton. A one-proton triplet at δ 2.33 (J = 7.2 Hz) was due to methylene H_2-2′ proton adjacent to the ester group. The other methylene and methine protons resonated as broad signals at δ 1.25 (12H) and 1.07 (4H) and as multiplets between δ 2.23- 1.13. Six three-proton broad singlets at δ 1.04 (Me-23), 0.99 (Me-24), 0.97 (Me-25), 0.80 (Me-28), 0.77 (Me-27) and 0.75 (Me-26), a three-proton doublet at δ 0.83 (*J* = 6.3 Hz, Me-29) and a three-proton triplet at δ 0.85 (J = 6.5 Hz, Me-12′) were attributed to methyl protons of a ursene-type triterpene and to the primary C-12′ methyl protons, all attached to the saturated carbons. The ^{13}C NMR spectrum of **5** displayed signals for forty two carbons and important signals were appeared due to vinylic carbons at δ 124.35 (C-12), 139.58 (C-13), 154.53 (C-20) and 107.19 (C-30), ester carbon at δ 172.12 (C-1′), oxymethine carbon at δ 78.96 (C-3) and methyl carbons from δ 28.08 to 15.62. The assignments of the carbon chemical shifts were made by comparison with δ values of corresponding carbon atom of urs-12-enes.[22-24] On the basis of above discussion the structure of **5** was elucidated as urs-12, 20(30)-dien-3β-olyl laurate. This is a new pentacyclic triterpene ester.

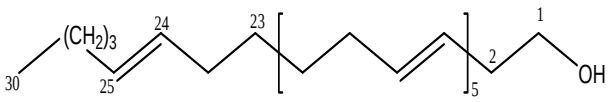
Compound **6**, named 1′-β-sitosteryl-2′-caproyl glycerol, exhibited characteristic IR absorption bands for hydroxyl function (3461 cm^{-1}), ester group (1737 cm^{-1}) and unsaturation (1640 cm^{-1}). Its molecular ion peak was determined at *m/z* 587 $[M+H]^+$ on the basis of mass and ^{13}C NMR spectra consistent with the molecular formula of a steroidal acyl glycerol, $C_{38}H_{66}O_4$. The ion peaks arising at *m/z* 413 [O – $C_{1'}$ fission, $C_{29}H_{49}O]^+$, 398 [413 – Me]$^+$, 396 [413 - OH]$^+$, 383 [398 – Me]$^+$, 271 [413 – side chain]$^+$, 255 [271 – Me]$^+$, 240 [255 – Me]$^+$. 213 [255 – ring D fission]$^+$, 198 [213 – Me]$^+$, 189 [OCH_2-$CHOCO(CH)_4CH_3$-$CH_2OH]^+$ and 173 [CH_2-$CHOCO(CH)_4CH_3$-$CH_2OH]^+$ suggested that caproyl glycerol was linked to β-sitosterol. The ^{1}H NMR spectrum of **6** displayed a one-proton multiplet at δ 5.36 assigned to vinylic H-6 proton, two one-proton multiplets at δ 4.30 and 3.65 ($w_{1/2}$ = 18.2 Hz) ascribed to oxymethine H-2′ and H-3α protons, respectively, two doublets at δ 3.48 (2H, J = 6.4 Hz) and 3.28 (2H, J = 5.6 Hz) attributed correspondingly oxymethylene H_2-1′ and H_2-3′ and a two-proton triplets at δ 2.28 (J= 7.2 Hz) accounted to methylene H_2-2″ protons adjacent to the ester group. Two three-proton broad singlets at δ 1.07 and 0.67, three three-protons doublets at δ 0.97 (J = 6.7 Hz), 0.86 (J = 6.3 Hz) and 0.83 (J = 6.0 Hz) and two three-proton triplets at δ 0.88 (J = 6.3 Hz) and 0.80 (J = 6.3 Hz) were associated with tertiary C-19 and C-18, secondary C-21, C-26 and C-27 and primary C-6″ and C-29 methyl protons, respectively. The remaining methylene and methine protons resonated as a four-proton singlet δ 1.29 and as multiplets from δ 2.76 – 1.10. The ^{13}C NMR spectrum of **6** displayed signals for ester carbon at δ 173.36 (C-1″), vinylic carbons at δ 140.77 (C-5) and 121.66 (C-6), oxymethine carbons at δ 71.79 (C-3) and 68.61 (C-2′), oxymethylene carbons at δ 66.79 (C-1′) and 62.05 (C-3′) and methyl carbons between δ 19.84 – 11.88. The ^{1}H and ^{13}C NMR spectral data of steroidal nucleus were compared with the reported spectral values of steroids.[25-27] On the basis these evidences the structure of **6** has been elucidated as 1′-β-sitosteryl-2′- *n*- hexanoyl-glycerol, a new steroidal glyceride.



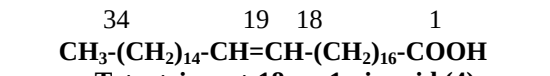
β-Sitosteryl linoleate (1)



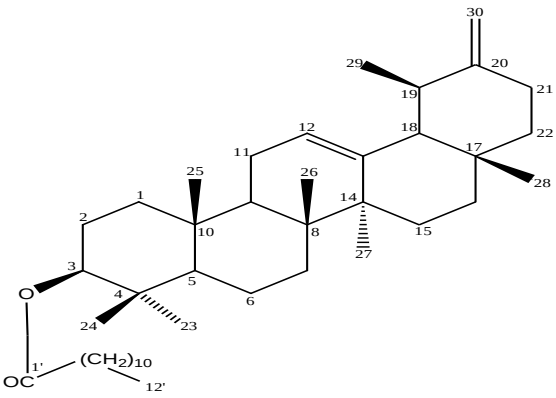
Nervonic acid (2)



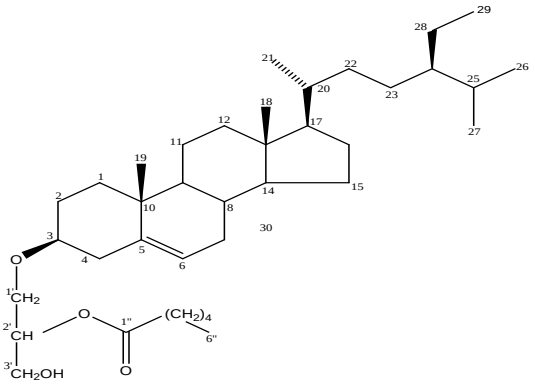
***n*-Triaconthexene-1-ol (3)**



CH_3-$(CH_2)_{14}$-CH=CH-$(CH_2)_{16}$-COOH
***n*-Tetratriacont-18-en-1-oic acid (4)**



Urs-12,20(30)-dienyl laurate (5)



1′-β-Sitosteryl-2′-caproyl glycerol (6)

CONCLUSION

Phytochemical investigation of a methanolic extract of the roots of *C. procera* resulted in the isolation of two steroidal derivatives, two fatty acids and one each of polyalkene alcohol and urs-12, 20(30)-dienyl laurate. This work has enhanced understanding about the phytoconstituents of the plant. These compounds may be used as chromatographic markers for standardization of the roots.

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