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CHARACTERIZATION OF CHEMICAL CONSTITUENTS OF *Calotropis procera*

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Calotropis procera belongs to the family Asclepiadaceae with 180 genera and 2200 species distributed mainly in the tropical and subtropical regions of the world.

Two species, *Caloropis procera* and *Calotropis gigantea*, are of economic importance. These two species closely resemble each other in structure and find similar medicinal uses. Both plants yield latex, which is present in all parts of this plant.

The milky juice of *Calotropis procera* is used as purgative, while the flowers are used as a digestive, stomachic, and tonic, and in the treatment of cough, asthma, and loss of appetite. The root bark promotes secretion and is useful in treating skin diseases, enlargement of abdominal viscera, intestinal worms, ascites, and anasarca. The alcoholic root extract also has analgesic, anticonvulsant, and sedative properties [1–3].

The plant *Calotropis procera* is locally name madar. The root extract of *Calotropis procera* showed strong cytotoxic effects against KB cell lines *in vitro*.The root extract has potential antipyretic activity against both yeast-induced and typhoid vaccine induced fever, but these studies are not enough for identifying and characterizing the bioactive compounds in this plant. The purpose of this study is to identify and characterize the bioactive principles from the root of *Calotropis procera* [3, 4].

The root of *Calotropis procera* was collected during the summer of 2007 from Pousara, Katni (M.P.), India. The plant was identified by Dr. K. P. Sahu, Department of Botany, Govt. Autonomous Model Science College, Jabalpur (M.P.), India, and a voucher Specimen No. 26 collected in 30 July 2007 was kept in the Department of Botany, Autonomous Model Science College, Jabalpur (M.P.), India. The dried root powder (7 kg) was subjected to hot extraction with MeOH by a Soxhlet extractor, and after solvent evaporation on a rotary evaporator, 10 g crude extract was found. A small portion of the MeOH extract dissolved in petroleum ether was spotted on TLC plates. Then TLC plates were run through various solvent systems and viewed individually under UV light. Through several classical chromatographic separations, the compounds of the MeOH extract were separated by *n*-hexane–ethyl acetate (8:2), *n*-hexane–ethyl acetate (1:1), and finally with ethyl acetate. Thirteen fractions were found to be homogeneous on TLC plates using *n*-hexane–ethyl acetate (8:2), petroleum ether–ethyl acetate (8:2), and petroleum ether–methanol (8:2) solvent systems. These fractions were crystallized and named CP-1–CP-13.

The mp of CP-1–CP-13 were 176, 133, 167, 255.5, 211–213, 167–168, 254–255, 210–211, 198, 167, 189, 133–135, and 97°C, respectively, and all were positive for alcohols and steroids. The DIMS of CP-1–CP-13 showed a parent M^+ peak at *m/z* 412, 414, 374, 878, 391, 426, 374, 404, 468, 428, 426, 440, 412, respectively, which correspond to the molecular formulas $C_{29}H_{48}O$, $C_{29}H_{50}O$, $C_{23}H_{34}O_4$, $C_{41}H_{64}O_{13}$, $C_{23}H_{34}O_5$, $C_{30}H_{50}O$, $C_{23}H_{34}O_4$, $C_{29}H_{40}O_{10}$, $C_{32}H_{52}O_2$, $C_{29}H_{48}O_2$, $C_{30}H_{50}O$, $C_{31}H_{52}O$, and $C_{29}H_{48}O$. The ^1H NMR and ^{13}C NMR data of the above compounds are summarized in the data on CP-1– CP-13.

The mp, co-TLC, co-IR, and spectroscopic data prove that the compounds were stigmasterol (**1**), *β*-sitosterol (**2**), digitoxigenin (**3**), digitoxin (**4**), digoxigenin (**5**), calotropenol (**6**), proceragenin (**7**), calotoxin (**8**), calotropenyl acetate (**9**), procesterol (**10**), multiflorenol (**11**), cyclosadol (**12**), and *β*-sitostenone (**13**).

Calotropenol (6). Yield 10.00 mg, mp 167–168°C, colorless, shining needles. IR ($CHCl_3$, ν, cm^{-1}): 3420, 3075, 1645, 890. EI-MS *m/z* (rel. int. %): $[M]^+$ 426 ($C_{30}H_{50}O$) (30), $[M - Me]^+$ 411 (10), $[M - H_2O]^+$ 408 (16), $[M - Me - H_2O]^+$ 393 (8), 272 (10), 257 (20), 218 (6), 207 (100), $[207 - H_2O]^+$ 189 (62).

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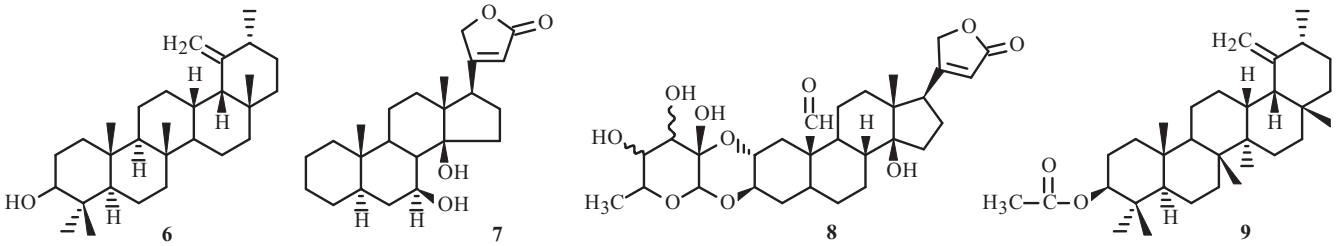

6 **7** **8** **9**

^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.82 (s, H_3-24, H_3-28), 0.84 (s, H_3-25), 0.93 (d, J = 6.2, H_3-30), 0.99 (s, H_3-26), 1.03 (s, H_3-26), 1.12 (s, H_3-27), 3.23 (dd, $J_{ax,\,ax}$ = 9.2, $J_{ax,\,eq}$ = 4.3, Hα-3), 4.65–4.70 (br.s, H_2-29). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 38.49 (t, C-1), 27.00 (t, C-2), 79.05 (d, C-3), 38.80 (s, C-4), 55.47 (d, C-5), 18.42 (t, C-6), 33.95 (t, C-7), 40.95 (s, C-8), 48.76 (d, C-9), 37.40 (s, C-10), 21.58 (t, C-11), 25.55 (t, C-12), 39.26 (d, C-13), 42.20 (s, C-14), 25.95 (t, C-15), 28.01 (t, C-16), 37.62 (s, C-17), 59.71 (d, C-18), 154.37 (s, C-19), 39.40 (d, C-20), 30.99 (t, C-21), 37.85 (t, C-22), 28.00 (q, C-23), 16.38 (q, C-24), 15.69 (q, C-25), 16.73 (q, C-26), 24.94 (q, C-27), 28.01 (q, C-28), 107.72 (t, C-29), 19.00 (q, C-30) [5, 6].

Proceragenin (7). Yield 5.00 mg, mp 254–255°C, colorless needles. IR (KBr, ν, cm^{-1}): 3550, 3420, 1775, 1730 and 1625. EI-MS *m/z* (rel. int. %): $[M]^+$ 374 (5), $[M - H_2O]^+$ 356 (12), $[M - Me - H_2O]^+$ 341 (4), $[M - 2H_2O]^+$ 338 (6), $[ion\ a]^+$ 264 (4), $[ion\ a - H_2O]^+$ 246 (6), $[ion\ a - 2H_2O]^+$ 228 (3), 203 (100), 111 (58).

^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.79 (s, H_3-19), 0.85 (s, H_3-18), 3.57 (m, H-3), 4.82–4.92 (dd, J = 17.90, 1.8, H_2-21), 5.85 (dd, J = 1.82, 1.17, H-22) [7]. ^{13}C NMR (75 MHz, $CDCl_3$, δ): 37.95 (t, C-1), 24.39 (t, C-2), 28.48 (t, C-3), 30.60 (t, C-4), 42.00 (d, C-5), 37.12 (t, C-6), 71.17 (d, C-7), 44.41 (d, C-8), 49.80 (d, C-9), 35.12 (s, C-10), 21.21 (t, C-11), 39.00 (t, C-12), 49.80 (s, C-13), 85.00 (s, C-14), 33.13(t, C-15), 26.88 (t, C-16), 50.88 (d, C-17), 15.76 (q, C-18), 12.22 (q, C-19), 175.30 (t, C-20), 73.44 (t, C-21), 116.70 (d, C-22), 174.10 (s, C-23).

Calotoxin (8). Yield 6.00 mg, mp 210–211°C, colorless needles. IR (KBr, ν, cm^{-1}): 3410, 1775, 1730, 1710 and 1625. UV (EtOH, λ_{max}, nm): 215 (log ε 4.5). EI-MS *m/z* (rel. int. %): $[M]^+$ 404 (6), $[M - H_2O]^+$ 386 (36), $[M - H_2O - CHO]^+$ 357 (13), $[M - C_9H_{11}O_4]^+$ 233 (79), $[M - C_9H_{11}O_4 - H_2O]^+$ 215 (46), $[M - C2_9H_{40}O_{10} - M - C_{23}H_{32}O_6]^+$ 128 (20).

^{1}H NMR (500 MHz, C_5D_5N, δ, ppm, J/Hz): 0.89 (s, H_3-18), 1.65 (d, J = 6.2, H_3-6), 4.53–4.97 (dd, J = 17 and 1.16, H_2-21), 4.95 (s, C-1), 5.46 (s, H-22), 10.0 (s, H-19). ^{13}C NMR (100 MHz, C_5D_5N, δ): 36.57 (t, C-1), 69.58 (d, C-2), 70.16 (d, C-3), 32.51 (t, C-4), 43.48 (d, C-5), 27.98 (t, C-6), 27.93 (t, C-7), 42.63 (d, C-8), 48.70 (d, C-9), 53.00 (s, C-10), 22.23 (t, C-11), 39.24 (t, C-12), 49.85 (s, C-13), 85.00 (s, C-14), 33.99 (t, C-15), 27.15 (t, C-16), 50.19 (d, C-17), 15.87 (q, C-18), 207.99 (s, C-19), 175.52 (s, C-20), 73.66 (t, C-21), 117.79 (d, C-22), 174.38 (s, C-23), 95.26 (C-1), 93.73 (s, C-2), 75.29 (d, C-3), 75.22 (d, C-4), 72.28 (d, C-5), 18.75 (q, C-6) [6, 8].

Calotropenyl Acetate (9). Yield 4.00 mg, mp 198°C. IR (KBr, ν, cm^{-1}): 1725, 1350 (ester carbonyl), 3090, 1640 and 880 (C=CH_2). MS *m/z* (rel. int. %): 468 $[C_{32}H_{52}O_2, M]^+$ (45), $[C_{32}H_{52}O_2 - CH_3]^+$ (7), 408 $[C_{32}H_{52}O_2 - AcOH]^+$ (10), 393 $[C_{32}H_{52}O_2 - CH_3 - AcOH]^+$ (8), 272 (5), 257 $[C]^+$ (4), 249 $[a]^+$ (17), 218 (51) and 189 $[a - AcOH]^+$ (100) [6, 8].

^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.82 (6H, s, H-24 and H-28), 0.83 (3H, s, H-25), 0.91 (3H, d, J = 6.5, H-30), 1.00 (3H, s, H-26), 1.01 (3H, s, H-23), 1.03 (3H, s, H-27), 2.08 (3H, s, $OCOCH_3$), 4.52 (1H, dd, $J_{ax,ax}$ = 9.8, $J_{ax,eq}$ = 4.7, H-3α), 4.60–4.68 (2H, br.s, H-29). ^{13}C NMR (75.4 MHz, $CDCl_3$, δ): 38.54 (C-1), 23.76 (C-2), 80.95 (C-3), 37.85 (C-4), 55.54 (C-5), 18.26 (C-6), 34.10 (C-7), 41.01 (C-8), 48.78 (C-9), 37.57 (C-10), 21.54 (C-11), 25.68 (C-12), 39.26 (C-13), 42.11 (C-14), 26.22 (C-15), 28.70 (C-16), 37.57 (C-17), 59.40 (C-18), 154.57 (C-19), 39.44 (C-20), 31.22 (C-21), 38.97 (C-22), 28.00 (C-23), 18.54 (C-24), 15.96 (C-25), 16.37 (C-26), 25.54 (C-27), 28.10 (C-28), 107.24 (C-29), 19.53 (C-30), 170.80 ($OCOCH_3$), 21.20 ($OCOCH_3$).

Procesterol (10). Yield 5 mg, mp 167°C. IR ($CHCl_3$, ν, cm^{-1}): 3575 (OH), 1675 (conjugated ketone) 3050, 1635 and 815 (C=CH). MS *m/z* (rel. int. %): 428 $[C_{29}H_{48}O, M]^+$ (15), 413 $[C_{29}H_{48}O_2 - CH_3]^+$ (23), 410 $[C_{29}H_{48}O_2 - H_2O]^+$ (18), 287 $[C_{29}H_{48}O_2 - C_{10}H_{21}$(entire substituent at C-17)$]^+$ (38), 269 $[C_{29}H_{48}O_2 - C_{10}H_{21} - H_2O]^+$ (25), 245 $[C_{29}H_{48}O_2 - C_{10}H_{21} - 42]^+$ (43), 227 $[C_{29}H_{48}O_2 - C_{10}H_{21} - 42 - H_2O]^+$ (45), and 152 $[C_{29}H_{48}O_2 - C_{10}H_{21} - 42 - 93]^+$ (100).

^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.760 (3H, s, H-18), 0.803 (3H, d, J = 6.7, H-26), 0.827 (3H, d, J = 6.7, H-27), 0.847 (3H, t, J = 7.3, H-29), 0.905 (3H, d, J = 6.3, H-21), 1.379 (3H, s, H-19), 4.450 (1H, oct, $J_{7\alpha,6\beta}$ = 12.1, $J_{7\beta,6\beta}$ = 4.7, $J_{6\beta,\,4}$ = 1.8, H-6), 5.780 (1H, d, J = 1.8, H-4). ^{13}C NMR (75.4 MHz, $CDCl_3$, δ): 38.66 (C-1), 34.31 (C-2), 200.30 (C-3), 126.59 (C-4), 168.43 (C-5), 73.36 (C-6), 37.17 (C-7), 45.93 (C-8), 53.72 (C-9), 38.08 (C-10), 21.04 (C-11), 39.68 (C-12), 42.59 (C-13), 56.79 (C-14), 24.32 (C-15), 28.22 (C-16), 56.16 (C-17), 11.92 (C-18), 19.84 (C-19), 36.28 (C-20), 18.78 (C-21), 33.99 (C-22), 26.43 (C-23), 46.11(C-24), 29.01(C-25), 19.09(C-26), 19.59 (C-27), 23.16 (C-28), 12.30 (C-29) [6, 9].

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