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PHYTOCHEMICAL STUDIES OF *Calotropis procera* STEM BARK

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Calotropis procera is a medicinal plant usually growing 1.8–2.4 m high; it belongs to the family Asclepidaceae and is distributed more or less throughout India (common name Madar). *C. procera* is used as a laxative and anthelmintic, and also cures ulcers [1]. The latex of the plant contains uscharin, lupeol, taraxasteryl acetate, β-amyrin, β-calotropenol, and triterpene 3-epimoretenol. The leaves contain proteolytic enzymes and a thiol-activated proteinase. Fresh flowers contain α- and β-amyrin, stigmasterol, β-sitosterol, multiflorenol, cyclosadol, procerasterol, and calotropenyl acetate. The plant also contains the cardenolide proceragenin [2]. The stem bark of *C. procera* has shown significant anti-inflammatory and gastromucosal protective effect [3]. In continuation of our studies, during the phytochemical investigations of *Calotropis procera* stem bark, five known compounds have been isolated for the first time.

The stem bark was collected, shade-dried, and powdered mechanically. About 2000 g of the stem bark powder was subjected to successive extraction with 4000 mL of chloroform and 4000 mL of aqueousalcohol (ethanol–water 60:40) by maceration at room temperature for 48 h using a mechanical shaker. The extract was dried at 40°C under vacuum by using a rotary evaporator (BUCHI Rotavapor R-215). The chloroform extract was fractionated with *n*-hexane (NF1), 1-butanol (BF1), ethyl acetate (EF1), and chloroform (CF1) by using the solid-liquid partition technique. The aqueousalcoholic extract (HE) of the stem bark was fractionated by using the liquid-liquid partition technique with *n*-hexane (NF2), 1-butanol (BF2), ethyl acetate (EF2), chloroform (CF2), and water (WF2).

Bioactivity-guided fractionation led to the isolation of triterpenoids from the stem bark of *C. procera*. When the chloroform extract of *C. procera* was developed on silica gel TLC, pink and purple spots appeared after spraying with 10% H_2SO_4 solution and heating, indicating the presence of triterpenoids in the extract.

NF1 (30 g) was chromatographed on a silica gel column, eluting with benzene–ethyl acetate (9:1, 8.5:1.5, 7.5:2.5), to give three fractions (A1–A3) after concentration at reduced pressure. Fraction A2 was applied to a silica gel column, eluting with benzene–ethyl acetate (10:1–1:10), to yield 600 mg of lupeol (**1**) [4, 5], 300 mg of calotropenol (**2**), and 450 mg of oleanolic acid (**3**) [6].

BF2 (15 g) was chromatographed on a silica gel column, eluting with *n*-hexane–acetone. The chromatographically identical fractions were combined to give two fractions, F1 (5.10 g, *n*-hexane–acetone, 70:30) and F2 (3.75 g, *n*-hexane–acetone, 60:40), after concentration at reduced pressure. Fraction F1 was applied to a silica gel column, eluting with *n*-hexane–acetone, 70:30, to yield luteolin (**4**) (600 mg), and F2 after eluting with *n*-hexane–acetone, 60:40, yielded epicatechin (**5**) [7, 8] (450 mg).

Lupeol (1). White needles, mp 208–210°C. IR (KBr, ν_{max}, cm^{-1}): 3443 (OH), 3032, 1051 and 839 (terminal methylene group). EI-MS *m/z* 413 [M^+]. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.68 and 4.54 (each 1H, m, H-29), 3.21 (1H, dd, H-3), 1.64 (3H, s, H-30), 1.04 (3H, s, H-26), 0.98 (3H, s, H-23), 0.91 (3H, s, H-27), 0.86, 0.76, and 0.65 (each 3H, s, H-25, 28, 24). ^{13}C NMR (100 MHz, DMSO-d_6): 38.6 (C-1), 27.4 (C-2), 78.9 (C-3), 38.4 (C-4), 55.2 (C-5), 18.2 (C-6), 34.2 (C-7), 40.7 (C-8), 50.3 (C-9), 37.1 (C-10), 20.9 (C-11), 25.0 (C-12), 38.0 (C-13), 42.7 (C-14), 27.4 (C-15), 35.5 (C-16), 42.9 (C-17), 48.2 (C-18), 47.9 (C-19), 150.8 (C-20), 29.8 (C-21), 39.9 (C-22), 28.0 (C-23), 15.4 (C-24), 16.1 (C-25), 15.9 (C-26), 14.5 (C-27), 17.9 (C-28), 109.3 (C-29), 19.2 (C-30).

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Calotropenol (2). Colorless needles, mp 166–168°C. IR (KBr, ν_{max}, cm^{-1}): 3343 (OH), 3065, 1636, 880. EI-MS *m/z* 426 [M^+], 411, 410, 393, 271, 257, 218 and 207. ^1H NMR (300 MHz, DMSO-d_6, δ, ppm): 4.65 and 4.70 (each 1H, s, H-29), 3.23 (1H, dd, H-3), 1.12 (3H, s, H-27), 1.03 (3H, s, H-23), 0.99 (3H, s, H-26), 0.93 (3H, d, H-30), 0.84 (3H, s, H-25), 0.82 and 0.80 (each 3H, s, H-24, 28). ^{13}C NMR (100 MHz, DMSO-d_6): 38.5 (C-1), 27.0 (C-2), 79.0 (C-3), 38.8 (C-4), 55.5 (C-5), 18.4 (C-6), 34.0 (C-7), 40.9 (C-8), 48.8 (C-9), 37.4 (C-10), 21.6 (C-11), 25.6 (C-12), 39.3 (C-13), 42.2 (C-14), 26.0 (C-15), 28.0 (C-16), 37.6 (C-17), 59.7 (C-18), 154.4 (C-19), 39.4 (C-20), 31.0 (C-21), 37.9 (C-22), 28.0 (C-23), 16.3 (C-24), 15.7 (C-25), 16.7 (C-26), 25.0 (C-27), 28.0 (C-28), 107.7 (C-29), 19.0 (C-30).

Oleanolic Acid (3). Colorless needles, mp 303–305°C.

Luteolin (4). Pale yellow powder, mp 340–342°C.

Epicatechin (5). White amorphous powder, mp 248–249°C.

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