

Isolation and Characterization of Chemical Constituents from the Roots of *Carissa carandas*

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In the present study, three triterpenoids were isolated and characterized from petroleum ether extract of the roots of *Carissa carandas*. The compounds ursolic acid, lupa-12,20(29)-dien-3 β ,28-diol and urs-12-ene-3 β ,22 β -diol were reported for the first time in this plant.

Key Words: *Carissa carandas*, Roots, Triterpenoids.

INTRODUCTION

Carissa carandas Linn (Apocynaceae) is a dichotomously branched evergreen shrub with short stem and strong thorns in pairs, probably native and common throughout in India, Sri Lanka, Java, Malaysia, Myanmar and Pakistan. The plant is an anthelmintic, astringent, appetizer, antipyretic, used in biliousness, stomach disorder, rheumatism and disease of the brain¹. Earlier studies have shown that the extract of the plant possesses cardiogenic, antipyretic and antiviral activity²⁻⁴. Various cardiac glycosides like odoroside H, odoroside G, evomoside, rhamnoside, rhamnoglycoside and a triterpenoidal constituent carissone and β -sitosterol were reported from the root extract of the plant^{2,5}. In the present study, roots of the plant have been chemically characterized.

EXPERIMENTAL

All the melting points were determined in a Toshniwal melting point apparatus and were uncorrected. The IR spectra of the compounds were recorded using KBr pellet method on Rx-1 Perkin-Elmer FTIR. ¹H NMR and ¹³C NMR spectra were run on Bruker Avance II 400 spectrophotometer using CDCl₃ as a solvent. Mass spectra (FAB-MS) were obtained on a Jeol SX 102/DA-6000 mass spectrometer.

Extraction and isolation: The roots of *C. carandas* were collected from Udipi, Karnataka, during April 2006. It was authenticated by Dr. Gopalakrishna Bhat, Department of Botany, Poorna Prajna College, Udipi, Karnataka, India. The dried root bark (1 Kg) were powdered and soaked in 95 % ethyl alcohol and extracted in cold for 4 d with occasional shaking. After decanting the solvent, the process was

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repeated for 4 times. The solvent from the total extract was filtered and the concentrate was evaporated on a water bath to syrupy consistency and then evaporated to dryness under reduced pressure to give ethanol extract (16 % w/w yield). The ethanol extract (125 g) was suspended in distilled water and then successive extraction with petroleum ether (60-80 °C, 8 × 500 mL). All fractions were then washed with distilled water, dried over anhydrous sodium sulphate and freed of solvent by distillation to give petroleum ether soluble fraction (40 g). Petroleum ether fraction (25 g) was saponified by refluxing for 6 h in 500 mL 5% methanolic KOH and then allowed to stand at room temperature for 20 h. The unsaponifiable portion was then extracted with ether (5 × 300 mL). All the ethereal fractions were combined and washed with distilled water. The solvent was evaporated and dried over anhydrous sodium sulphate to afford a yellowish residue (10 g)⁶.

The residue (8 g) was dissolved in chloroform (10 mL) and adsorbed on to silica gel (60-120 mesh, 20 g). After evaporation of the solvent it was subjected to column chromatography over silica gel (150 g) prepared in petroleum ether (60-80 °C). The elution was carried out successfully with 100 mL portions each of petroleum ether (60-80 °C) alone, petroleum ether-chloroform graded mixtures (95:5, 90:10 up to 100 % chloroform) and chloroform-methanol similar graded mixtures. The fractions were combined based on their TLC pattern and individual compounds were further purified by preparative thin layer chromatography on silica gel G; afforded three compounds, compound **I** (120 mg), **II** (55 mg) and **III** (46 mg).

Compound I (ursolic acid): Pale yellow powder; R_f 0.53 in petroleum ether-chloroform (8:2); m.p. 271-274 °C; IR ν_{max} (KBr): 3454, 2940, 2854, 1643, 1461, 1374, 1054 cm^{-1} ; 1H NMR ($CDCl_3$, 400 MHz): δ 1.02 to 1.99 (m, 22H), 3.45 (1H, dd, $J = 10.1, 5.8$ Hz, H-3), 2.27 (1H, d, $J = 4.46$ Hz, H-18), 1.04 (s, 3H, Me-23), 0.91 (s, 3H, Me-24), 0.76 (s, 3H, Me-25), 0.93 (s, 3H, Me-26), 0.94 (s, 3H, Me-27), 0.86 (s, 3H, Me-29), 0.83 (s, 3H, Me-30), 2.27 (m, 1H, OH), 5.35 (s, 1H, H-12); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 36.80 (C-1), 28.02 (C-2), 78.59 (C-3), 39.97 (C-4), 41.89 (C-5), 22.45 (C-6), 29.30 (C-7), 39.09 (C-8), 51.99 (C-9), 30.59 (C-10), 22.32 (C-11), 125.74 (C-12), 137.39 (C-13), 39.53 (C-14), 29.18 (C-15), 26.40 (C-16), 55.93 (C-17), 38.21 (C-18), 32.99 (C-19), 37.01 (C-20), 27.24 (C-21), 38.09 (C-22), 14.99 (C-23), 14.78 (C-24), 18.17 (C-25), 16.53 (C-26), 23.54 (C-27), 177.56 (C-28), 20.65 (C-29), 16.13 (C-30); FAB-MS m/z (rel.int): 456 [M^+] ($C_{30}H_{48}O_3$) (74), 428 (55), 289 (52.5), 277 (41), 248 (100), 149 (37), 85 (9.5), 83 (39).

Compound II (lupa-12, 20(29)-dien-3 β ,28-diol): White amorphous powder; R_f 0.59 in petroleum ether-chloroform (8:2); m.p. 265-270 °C; IR ν_{max} (KBr): 3448, 2961, 2925, 2858, 1659, 1374, 1343, 880 cm^{-1} ; 1H NMR ($CDCl_3$, 400 MHz): δ 0.84 (s, 3H), 0.86 (s, 3H), 0.88 (s, 3H), 0.92 (s, 3H), 1.01 (s, 3H), 1.65 (s, 3H), 2.10 (d, $J = 17.1$ Hz, H-22), 2.78 (d, $J = 11.8$ Hz, H-19), 3.05 and 3.57 (each 1H, d, $J = 10.2$ Hz, H-28), 3.96 (t, $J = 2.4$ Hz, H-3), 5.27 (t, $J = 5.7$ Hz, H-12), 5.47 (s, H-29); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 38.24 (C-1), 26.73 (C-2), 78.90 (C-3), 38.52 (C-4), 55.56 (C-5), 18.31 (C-6), 33.93 (C-7), 41.62 (C-8), 50.61 (C-9), 36.82 (C-10), 23.50

(C-11), 124.66 (C-12), 145.01 (C-13), 49.66 (C-14), 26.22 (C-15), 29.56 (C-16), 47.31 (C-17), 47.89 (C-18), 48.90 (C-19), 150.23 (C-20), 29.66 (C-21), 34.52 (C-22), 28.71 (C-23), 15.99 (C-24), 16.92 (C-25), 16.51 (C-26), 14.73 (C-27), 61.52 (C-28), 109.60 (C-29), 19.73 (C-30); FAB-MS m/z (rel.int): 440 [M^+] ($C_{30}H_{48}O_2$) (78), 340 (61), 326 (29), 232 (21), 219 (60.5), 208 (56.5), 201(100), 189 (30).

Compound III (urs-12-ene-3 β ,22 β -diol): White crystalline mass; R_f 0.61 in chloroform-methanol (9.5:0.5); m.p. 194-196 °C; IR ν_{max} (KBr): 3434, 2925, 2857, 1642, 1458, 1378, 1260, 1189, 1096, 1035 cm^{-1} ; 1H NMR ($CDCl_3$, 400 MHz): δ 1.67 (H-1), 1.59 (H-2), 3.22 (1H, dd, $J = 10.52, 4.93$ Hz, H-3), 1.79 (1H, dd, $J = 11.86, 1.62$ Hz, H-5), 1.56 (m, H-6), 1.39 (H-7), 1.57 (H-9), 1.97 (H-11), 5.17 (t, $J = 3.53$ Hz, H-12), 1.07 (H-15), 1.38 (H-16), 1.35 (m, H-18), 1.05 (H-19), 1.39 (m, H-20), 1.03 (m, H-21), 3.34 (1H, dd, $J = 12.21, 4.14$ Hz, H-22), 1.02 (s, 3H, Me-23), 0.82 (s, 3H, Me-24), 0.97 (s, 3H, Me-25), 1.04 (s, 3H, Me-26), 1.10 (s, 3H, Me-27), 0.98 (s, 3H, Me-28), 0.81 (3H, d, $J = 7.12$ Hz, Me-29), 0.99 (3H, d, $J = 6.71$ Hz, Me-30); ^{13}C NMR ($CDCl_3$, 400 MHz): δ 39.09 (C-1), 27.52 (C-2), 78.91 (C-3), 39.19 (C-4), 55.54 (C-5), 19.01 (C-6), 33.03 (C-7), 41.02 (C-8), 47.93 (C-9), 37.13 (C-10), 23.72 (C-11), 124.42 (C-12), 139.01 (C-13), 43.02 (C-14), 26.91 (C-15), 20.92 (C-16), 39.33 (C-17), 59.01 (C-18), 37.45 (C-19), 39.46 (C-20), 39.01 (C-21), 78.99 (C-22), 28.39 (C-23), 15.98 (C-24), 15.81 (C-25), 17.01 (C-26), 23.56 (C-27), 24.82 (C-28), 17.69 (C-29), 21.29 (C-30); FAB-MS m/z (rel.int): 442 [M^+] ($C_{30}H_{50}O_2$) (70.5), 234 (56.3), 216 (43.5), 207 (73.5), 201 (50.5), 189 (71.5), 133 (93.5), 95 (100).

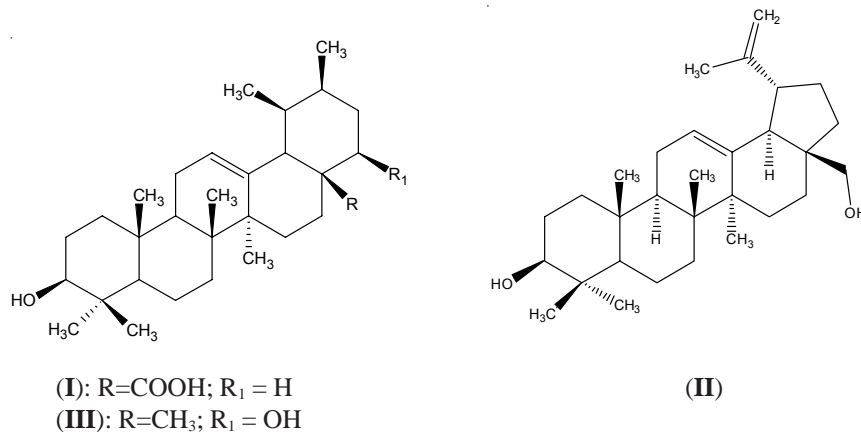
RESULTS AND DISCUSSION

Compound **I** was obtained as pale yellow powder. The FAB-MS of **I** showed a molecular ion at m/z 456 corresponding to a molecular formula $C_{30}H_{48}O_3$. It was unambiguously identified as ursolic acid on the basis of its m.p., IR, 1H NMR and ^{13}C NMR spectral data⁷.

Compound **II** was isolated as white amorphous powder. Its molecular formula was determined by FAB-MS to be $C_{30}H_{48}O_2$, m/z : 440 [M^+]. The 1H NMR spectrum of compound **II** showed five tertiary methyl groups at δ 0.84-1.01 (s, 3H each) and two olefinic protons δ 5.47 (H-29) and 5.27 (H-12), an isoprenyl group (δ 1.65) for the presence of terpenoid compound, which is further confirmed by the ^{13}C NMR signals at δ 19.73 (C-30), 109.60 (C-29) and 150.23 (C-20). On the basis of its m.p., IR, 1H NMR and ^{13}C NMR spectral data, the compound **II** has been established as lup-12,20(29)-dien-3 β , 28-diol⁸.

Compound **III** was isolated as white crystalline mass. It gave a characteristic colour reaction for triterpenoids. The molecular formula was established as $C_{30}H_{50}O_2$, m/z : 442 [M^+] from FAB-MS spectrum. The mass spectrum displayed the characteristic retro-Diels-Alder fragment peak at m/z 234 indicating a C-12/C-13 double bond which suggested an ursane structure substituted by two hydroxyl groups, one located at A/B rings and one on D/E rings. These results are in good agreement

with the IR spectrum of compound **III**, which showed absorption bands assigned to the hydroxyl groups (3434 cm^{-1}) and a double bond (1642 cm^{-1}). The ^1H NMR and ^{13}C NMR spectral data reported in the literature confirmed that compound **III** was urs-12-ene-3 β ,22 β -diol⁹.



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