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Research Article

Phytochemical Examination of *Corchorus aestuans (Tiliaceae)* Capsule

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ABSTRACT
From the capsule extract of *Corchorus aestuans L*, β-sitosterol, lupeol, betulin, 2-methyl anthraquinone, scopoletin and corchoroside-A were isolated and characterized by spectroscopy and also the hexane, chloroform and methanolic extracts of *Corchorus aestuans* were tested for antimicrobial activity.

Key words: *Corchorus aestuans*, β-sitosterol, lupeol, betulin, 2-methyl anthraquinone, scopoletin and corchoroside-A, antimicrobial activity.

INTRODUCTION

Corchorus aestuans is a *Tiliaceae* member is an erect to procurement of annuval herb grow up to 20 cm long . Capsules are 1.5-2.7 cm long. several important bioactive molecules were reported which includes cardiac glycosides, their aglycones and polysaccharides, triterpenoids, phenolics, sterols and fatty acids [1-96]. Biologically *Corchorus* species are used as diuretic, chronic cystitis, gonorrhoea and dysuria antihistaminic, anti-inflammatory, antimicrobial, cardiotonic, and also to increase the viscosity of the seminal fluid [97-98].

MATERIALS AND METHODS

Plant material collection: The plant material, *Corchorus aestuans* capsules was collected from Warangal in September 2007(2kg). The plant was authenticated by Prof. V.S. Raju , Department of Botany, KaKatiya University, Warangal. A specimen was deposited in the herbarium (Voucher specimen number (CA/07) roots were collected from the plant and dried under shade.

Extraction and Isolation of the compounds: The capsules (2kg) of *Corchorus aestuans* were air dried and coarsely powdered in a Wiley mill and successively extracted with petroleum ether (3×3 l), chloroform (3×3 l) and methanol (3×3 l) and concentrated under reduced pressure. The petroleum ether, chloroform extracts of *Corchorus aestuans* capsules shown similar spots on TLC (1:1 Benzene : Chloroform) and hence combined and column chromatographed over silica gel (Acme 100 mesh), which afforded three compounds designated as CAC-1, CAC-2, and CAC-3. The methanolic extracts showed positive tests for terpenoids and cardiac glycosides. On column chromatography the methanolic extract gave three compounds CAC-4, CAC-5, and CAC-6.

Characterization Of The Compounds

CAC-1(β-sitosterol 200 mg): The compound was crystalized from petroleum ether as a colorless needles, m.p 136-138°C. It showed color reaction for sterols with Liebermann-Burchard test.The UV (MeOH) *λmax* 205 nm; EIMS *m/z* 414 [M]+(calc. for $C_{29}H_{50}O$). ^{1}H NMR ($CDCl_3$, 400 MHz): *δH*3.52 (1H, *m*, H-3), 5.35 (1H, *m*, H-6), 0.68 (3H, *s*, Me-18), 0.98 (3H, *s*, Me-19), 0.91 (3H, *d*, *J* = 6.4 Hz, Me-21), 0.83 (3H, *d*, *J* = 6.8 Hz, Me-26), 0.81 (3H, *d*, *J* = 6.9 Hz, Me-27), 0.85 (3H, *t*, *J* = 7.8 Hz, Me-29). ^{13}C NMR (CDCI3, 100 MHz): *δC*37.4 (C-1), 31.8 (C-2), 72.0 (C-3), 42.5 (C-4), 140.9 (C-5), 121.9 (C-6), 32.1 (C-7), 29.9 (C-8), 50.3 (C-9), 36.7 (C-10), 21.3 (C-11), 40.0 (C-12), 42.5 (C-13), 56.9 (C-14), 24.5 (C-15), 28.4 (C-16), 56.2 (C-17), 12.0 (C-18), 19.6 (C-19), 36.3 (C-20), 19.0 (C-21), 34.1 (C-22), 26.3 (C-23), 46.0 (C-24), 29.3 (C-25), 20.0 (C-26), 19.2 (C-27), 23.2 (C-28), 12.2 (C-29). Based on the spectral data the compound was identified as β-sitosterol and the identity was further confirmed by comparison with authentic sample (m.m.p. and Co-TLC.) The compound was crystallized from hexane as colourless needles with m.p. 212-214^{0}C, $[\alpha]_D^{30}$ + 38° (C, 1.12 in chloroform) and analyzed for the formula $C_{30}H_{50}O$. It gave pink colour with L.B. reaction indicating that the compound was a triterpenoid. The IR spectrum showed bands at 3540 cm^{-1}– OH absorption, 1380 and 1390 cm^{-1} (*gem*- methyls) and at 890 cm^{-1} (vinyl methylene). ^{1}H NMR spectrum ($CDCl_3$, 90 MHz, δ) showed peaks at 0.76 (d, 3H); 0.78, 0.80, 0.90, 1.02 (s,15H); 1.63 (s, 3H); 0.91 (s, 6H) and δ 3.18 (m, 1H). From the above properties **CAC-2** was identified as lupeol and the identity was confirmed by comparison with authentic sample (m.m.p. and co-TLC).[95]

The compound was obtained as colorless needles, m.p. 253-255° and showed single spot on TLC. It developed pale-yellow coloration with trinitro methane in chloroform indicating unsaturation. It responded positively to Liebermann-Burchard tests characteristic of

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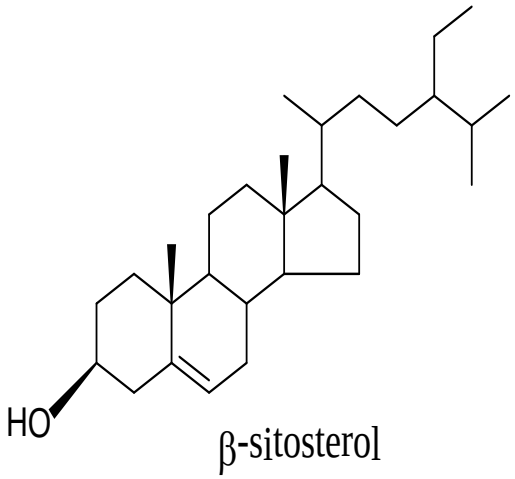
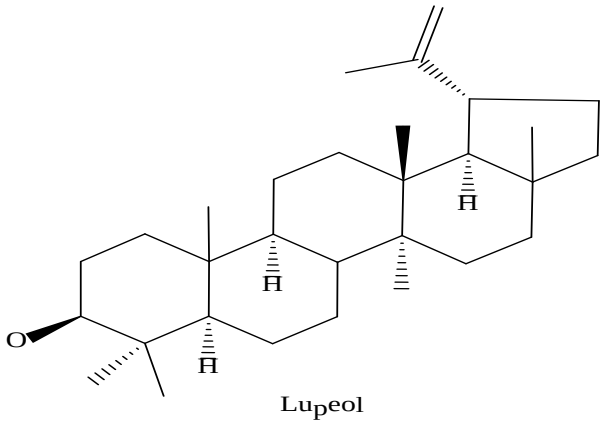


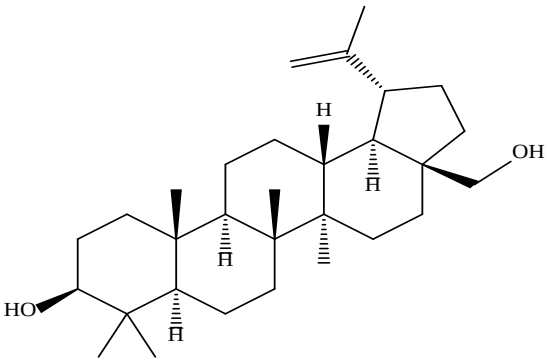
β-sitosterol

CAC-2 (Lupeol, 20mg**)**

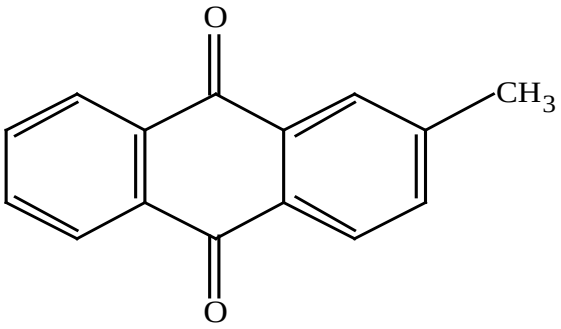


Lupeol

CAC-3 (Betulin, 30mg**)**



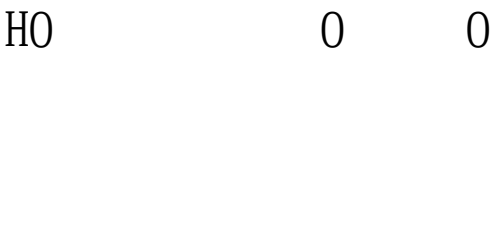
Betulin



2-Methylanthraquinone

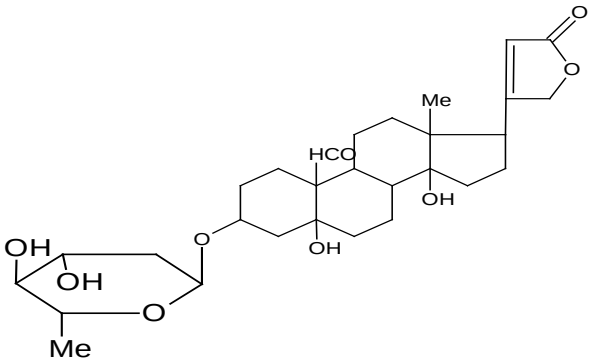
CAC-4 (2-methylanthraquinone, 25mg

CAC-5 (Scopoletin, 80mg**)**



Scopoletin

CAC-6 (Corchoroside A, 20mg)



C**orchoroside A**

triterpenoids. Its infrared spectrum showed characteristic absorption bands at 3460-3400 (broad, OH stretching), 2970-2880 (C-H stretching), 1650 cm-1 (C=C stretching). ^{1}H-NMR **:** (δ, $CDCl_3$): 4.53 and 4.67 (=CH_2), 3.33 and 3.85 (d, *J* = 11 Hz each – CH_2OH), 3.18 (dd, *J* = 12, 5Hz H-3α), 2.44 (m, H- 19), 1.67 (s, =C-CH_3), 0.75 (s, 3H), 0.85 (s, 3H), 0.96 (s, 3H), 0.98(s, 3H), 1.02 (s, 3H) for five tertiary methyl groups. ^{13} C-NMR **:** (δ, $CDCl_3$): 38.8 (C-1), 27.4 (C-2), 79.0 (C-3), 38.3 (C-4), 55.4 (C-5), 18.3 (C-6), 34.3 (C-7), 41.0 (C-8), 50.6 (C- 9), 37.4 (C-10), 20.9 (C11), 25.6 (C-12), 37.0 (C-13), 42.8 (C-14), 27.1 (C-15), 29.3 (C-16), 46.4(C-17), 47.8 (C-18), 48.8 (C-19), 150.3 (C-20), 29.8 (C-21), 34.0 (C-22), 28.0 (C-24), 6.1 (C-25), 6.1 (C-26), 14.7 (C- 27), 60.8 (C-28), 109.6

(C-29), 19.4 (C-30). Based on above data and by comparing with an authentic sample, the compound was identified as betulin.

It was crystallized as yellow needles in methanol in chloroform mixture, mp 170 - 173° C and gave color reaction with Kedde's reagent, negative with LB reaction and showed a red-orange color under UV 366 nm at Rf 0.72 in $CDCl_3$:MeOH (19:1) and Rf 0.37 in petroleum ether : acetone : acetic acid (75:25:1.5) .The ^{1}HNMR showed signals at (300 MHz, $CDCl_3$): 2.47 (3H, s, –CH_3), 7.53, (1H,d, J=7.8 Hz, H-3), 7.71–7.73 (2H, m, H-5, 8), 8.04 (1H,s ,H-1), 8.14 (1H, d, J = 7.8 Hz, H-4), 8.23–8.25 (2H, m, H-6, 7). ^{13} C-NMR**:** (125 MHz, $CDCl_3$): d = 127.8 (C-1), 145.5 (C-2), 135.3 (C-3), 127.7 (C-4), 127.4 (C-5), 134.3 (C-6), 134.2 (C-7), 127.5 (C-8), 183.7 (C-9),183.3 (C-10), 22.1 (CH_3). The data and the result correspond with 2-methylanthraquinone, and is in good agreement with that of 2-methylanthraquinone and further the identify was confirmed by comparison with an authentic sample by m.m.p and co-TLC.

This compound was obtained as yellow crystal, mp: 202-204 °; IR (KBr) λ max cm^{-1}: 3340, 3106, 3031, 2990, 1710, 1600; UV max (MeOH) nm: 230, 254, 260, 298, 346 ; FAB-MS *m/z*:193 [M-H]$^-$; ^{1}H-NMR and ^{13}C-NMR spectral data were in accord with the molecular formula $C_{10}H_8O_4$. The ^{1}H-NMR spectrum revealed the presence of two doublets at δH 7.90 (H-4, *J* = 6.0 Hz) and 6.25 (H-3, *J* = 6.0 Hz). The two singlet signals appeared at δ H 6.80 and 7.25 were assigned for the two protons H-5 and H-8, respectively. ^{1}H-NMR: (CD_3OD): d 3.90 (3H, s, 6-OCH3), 6.19 (1H, d, J = 9, H-3), 6.75 (1H,s, H-8), 7.09 (1H, s, H-5), 7.84 (1H, d, J = 9, H-4). ^{13} C-NMR : (CD_3OD): d 56.8 (OMe), 104.0 (C-8), 109.9 (C-5), 112.6 (C-3 and C-4a), 146.1 (C-4), 147.1 (C-6), 151.4 (C-8a), 153.0 (C-7), 164.1 (C-2). Based on the spectral data and chemical tests, the compound was identified as scopoletin.

The compound was crystallized from methanol-ethyl acetate as colourless prisms m.p. 164-168°C; $[\alpha]_D$ = +19.7° (methanol), it showed positive Kedde and Legal reactions indicating the cardinolide nature of the compound. The U.V. spectrum (ethanol) showed two maxima at 218 nm and 298 nm confirming the presence of α,β unsaturated γ-lactone group and a carbonyl group. It analyzed for the formula ($C_{29}H_{42}O_9$) and formed a diacetate. ^{1}H NMR : δ 0.84 (3H,s,H_3 -18) , 1.22 (3H, d, J=6.5H_z ,boi H_3-6),2.82 (1H, m,H-17), 3.21 (1H,m, boi H-3), 4.17 (1H,m, H-3), 4.87 (1H,dd,J=2.0 , 10.0 H_z ,boi H-1). 4.90. 5.02 (each 1H, dd, J=2.0. 18.5 H_z , H_2 -21), 5.89 (1H,s, H-22), 10.04 (1H, s, H_3-19). From the above properties CAC-6 was identified as corchoroside-A and the identity was confirmed by comparison with authentic sample (m.m.p. and co-TLC).

RESULTS AND DISCUSSION

The chemical examination of the capsules of *C.aestuans* yielded six compounds from the capsules. The compounds were identified as β-sitosterol, lupeol, betulin, 2-methylanthraquinone, scopoletin and corchoroside-A These isolates betulin 2-methylanthraquinone and Corchoroside -A were reported for the first time from *C.aestuans* capsules.

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