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Research Article

Phytochemical Examination of *Corchorus capsularis* Roots

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ABSTRCT

From root extract of *Corchorus capsularis*, a rare Cardiac glycosides like corchoroside-A and cannogenol, Steroids like β-sitosterol and stigmasterol 3-O-β-D-glucoside, Flavonoids like quercetin, Terpenoids like betulinic acid and oleanolic acid were reported from this plant.

Keywords: *Corchorus capsularis*, stigmasterol 3-O-β-D-glucoside, quercetin, betulinic acid and cannogenol.

INTRODUCTION

Corchorus capsularis L, a Tiliaceae membe [1], spread though out India. Most of the compounds isolated from this genus are cardiac glycosides [2], polysaccharides [3,4], triterpenoids [5], phenolics [6], sterols [6,7,8,9] and fatty acids [9,10] The *Corchorus capsularis* is reported to have cardiotonic, carminative, diuretic, antidysenteric, purgative etc [11,12,13,14]

Plant Material: The roots of *corchorus capsularis* (1kg) were collected from Warangal in September 2007. The plant was authenticated by Prof.V.S. Raju, Depatment of Botany, Kakatiya University, Warangal.

Extraction: The roots were air dried, powdered and extracted with petroleum ether, chloroform and methanol and concentrated under vacuum to get the corresponding residues. petroleum ether, chloroform extracts of *corchorus capsularis* roots shown similar spots on Thin layer chromatography (1:1 Benzene: Chloroform) and hence combined and column chromatographed over silica gel (Acme 100 mesh), which afforded four compounds designated as CCR-1, CCR-2, CCR-3 and CCR-4. The methanolic extracts showed positive tests for terpenoids and cardiac glycosides. On column chromatography the methanolic extract gave three compounds CCR-5, CCR-6 and CCR-7. The extracts were tested for triterpenes and sterols (L.B.test), flavonoids (Shinoda test, Mg-Hcl), sugars in cardiac glycosides (Keller killiani-test) and cardenolides (Kedde's test).

CHARACTERIZATION OF COMPOUNDS

CCR-1 (β-sitosterol, 35mg): It was crystallized from chloroform: hexane (1:5) as colourless needles, mp. 134-136^{0}C. $[\alpha_D]$ ^{30} + 36^0 (chloroform) and analyzed for the molecular formula C_{29} $H_{50}O$. It gave a play of colours (pink – blue – green) in Liebermann – Burchard test for sterols. Its IR spectrum showed absorption at 3440 (-OH) and 1385 Cm^{-1}. The ^{1} H NMR (300 MH_z, $CDcl_3$, δ) spectrum showed peaks at δ 0.80-1.25 (methyls), 3.47 1H, broad, C-3H) and 5.32 (1H, m, C-5H). From the above data, the compound CCR-1 was identified as β-sitosterol and further identity was confirmed by comparision with an authentic sample through m.m.p and Co-TLC [6]

CCR-2 (Stigmasterol 3-O-β-D-glucoside, 28mg): It was crystallized from hexane: chloroform (9:1). The molecular formula was established as $C_{35}H_{58}O_6$ by HR-FAB-MS which showed molecular ion peak at *m/z* 574.4231 (calcd. for $C_{35}H_{58}O_6$, 574.4233), MP: 289 - 290°C. [α]D 25 - 51.40 (CH_3OH c 0.22); IR (KBr) λmax cm^{-1}: 3458 (OH), 1646 (C=C). EIMS *m/z* (rel. int. %): [M-Glu]+ 412 (72), 397 (15), 394 (22), 379 (28), 369 (35), 351 (71), 300 (67), 327 (55), 301 (67), 273 (21), 271 (26). ^{1}H-NMR ($CDCl_3$, 400 MHz), δ: 5.23 (1H, br d J = 5.4 Hz,H-6), 5.14 (1H, dd, J = 15.2, 8.0 Hz, H-22), 5.02 (1H, dd, J = 15.3, 8.0 Hz, H- 23), 4.78 (1H, d, J = 7.5 Hz, H-1/), 3.83 (1H, m, H-3), 3.84 - 4.44 (m, Glc-H), 1.01 (3H, s, Me-19), 0.90 (3H, d, J= 6.2 Hz, Me-21), 0.83 (3H, d, J = 6.6 Hz, M-26), 0.82 (3H,t, J = 7.0 Hz, Me- 29), 0.80 (3H, d, J = 6.5 Hz, Me-27), 0.66 (3H, s, Me-18). ^{13}C-NMR (CD_3OD ,400 MHz), δ: 141.5 (C-5), 138.9 (C-22), 129.1 (C-23), 121.1 (C-6), 102.8 (C-1), 79.8 (C-3), 76.7 (C-5), 74.2 (C- 2), 70.6 (C-4), 62.2 (C-6), 57.0 (C-14), 56.1 (C-17), 52.1 (C-24), 50.8 (C-9), 43.9 (C-4), 43.1 (C-13), 40.5 (C-20), 39.9 (C-12), 37.8 (C-1), 36.9 (C-10), 32.9 (C-25), 32.8 (C-2), 31.9 (C-7), 31.7 (C-8), 28.9 (C-16), 25.6 (C-28), 24.5 (C-15),21.9 (C-21), (C-24), 21.7 (C- 27), 21.5 (C-11), 19.5 (C-19), 19.1 (C-26), 12.6 (C-18), 12.1 (C-29). Based on the data the compound was identified as stigmasterol 3-O-β-D-glucoside. [3]

CCR-3 (Quercetin, 20mg): The compound was obtained from methanol-chloroform mixture and was crystallized from methanol as yellow crystalline solid, melting point 312-314ºC and analyzed for the formula $C_{15}H_{10}O_7$. On paper chromatography it was yellow and intense yellow under U.V/NH_3, with ferric chloride it gave green colour, characteristic for flavonoids, an orange red precipitate, with neutral lead acetate indicating the presence of free 3-hydroxyl group. U.V spectrum in ethanol shows absorptions at λ^{EtOH}_{max} 255, 267sh, 301sh, 374 nm. gave 10nm bathochromic shift in band-II indicating the presence of a free 7-hydroxy group, with aluminum chloride, it formed a complex and showed a shift of 55nm

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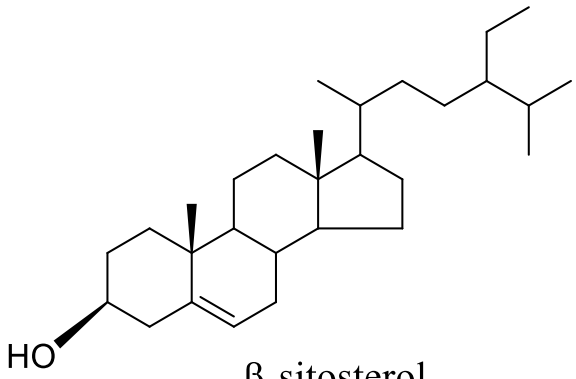
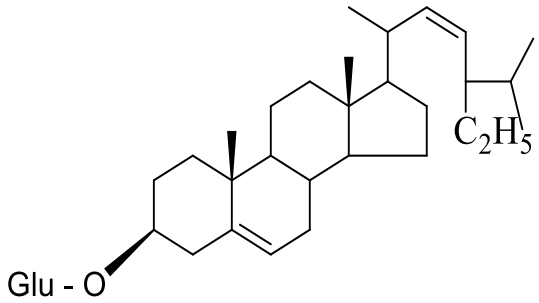


β-sitosterol



Stigmasterol 3-O-β-D-glucoside

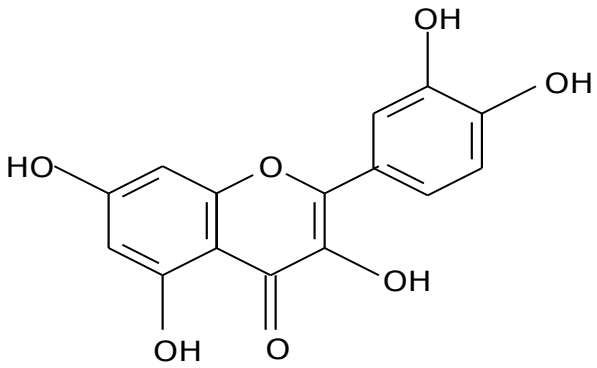
band-I further confirmed the presence of free 3-hydroxyl group. Sodium acetate, boric acid reagent showed a bathochromic shift of 20nm indicating the presence of free 3', 4' – di hydroxyl groups (ortho dihydroxy system). ^{1}HNMR exhibits peaks at δ 7.60 (d, 6'H) and δ 7.75 (d, 2'H). From the above properties CCR-3 was identified as quercetin and its identity was confirmed by comparison with authentic sample (m.m.p and Co - T.L.C).

CCR-4 (Betulinic acid, 25mg): It was crystallized from chloroform as white fluffy needles of m.p 276-278°C. It gave positive Liebermann-Burchard test (pink colour) for terpenoids. This observation was supported by ms: *m/z* 456[M] +, 423, 411, 410, 342, 248, 220, 207, 203, 189, 143, 69 suggested the molecular formula $C_{30}H_{48}O_3$. IR (KBr, cm-1) : 3385 (OH), 3350 (COOH), 1715 cm-1 (C=O); ^{1}H NMR (δ, $CDCl_3$) : 4.56 and 4.68 (=CH2), 1.68 (s, =C-CH3), 2.30 (m, H-19) 3.27 (dd, H-3α), 0.76 (s, 3H), 0.78 (s, 3H), 0.82 (s, 3H), 0.96 (s, 3H), 1.03 (s, 3H) for five tertiary methyl groups; ^{13}C NMR (δ, $CDCl_3$) : 38.7 (C-1), 27.4 (C-2), 78.9 (C-3), 38.8 (C-4), 55.3 (C-5), 18.3 (C-6), 34.3 (C-7), 40.7 (C-8), 50.5 (C-9), 37.2 (C-10), 20.8 (C-11), 25.5 (C-12), 38.4 (C-13), 42.4 (C-14), 30.5 (C-15), 32.1 (C-16), 56.3 (C-17), 46.8 (C-18), 49.2 (C-19), 150.3 (C-20), 29.7 (C-21), 37.0 (C-22), 27.9 (C-23), 15.3 (C-24), 16.0 (C-25), 16.1 (C-26), 14.7 (C-27), 180.5 (C-28), 09.6 (C-29), 19.4 (C-30). Based on the data the compound was identified as betulinic acid [15].

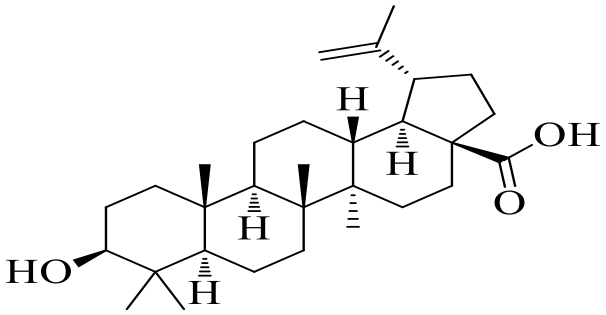
CCR-5 (Oleanolic acid, 35 mg): The compound was crystallized from methanol as white flakes, 271-273°. UV (MeOH) *λmax*215 nm; EIMS *m/z* 456 [M]+(calc. for $C_{30}H_{48}O_3$). ^{1}H NMR ($CDCI_3$, 400 MHz): *δH*5.24 (1H, *t, J* = 3.6 Hz, H-12), 3.21 (1H, *dd, J* = 10.2/4.4 Hz, H-3), 2.82 (1H, *dd, J* = 12.7/4.3 Hz, H-18), 0.96 (3H, *s*, Me-23), 0.78 (3H, *s*, Me-24), 0.84 (3H, *s*, Me-25), 0.76 (3H, *s*, Me-26), 1.25 (3H, *s*, Me-27), 0.87 (3H, *s*, Me-29), 0.93 (3H, *s*, Me-30). ^{13}C NMR ($CDCI_3$, 100 MHz): *δC*38.6 (C-1), 26.7 (C-2), 78.5 (C-3), 39.2 (C-4), 55.5 (C-5), 18.3 (C-6), 32.6 (C-7), 39.6 (C-8), 48.1 (C-9), 37.0 (C-10), 22.7 (C-11), 122.4 (C-12), 144.1 (C-13), 42.0 (C-14), 27.7 (C-15), 22.8 (C-16), 46.7 (C-17), 41.5 (C-18), 46.1 (C-19), 30.4 (C-20), 33.7 (C-21), 32.3 (C-22), 28.8 (C-23), 14.7 (C-24), 15.1 (C-25), 16.5 (C-26), 25.2 (C-27), 180.4 (C-28), 32.8 (C-29), 23.3 (C-30). Based on chemical tests and spectral data the compound was identified as oleanolic acid [16].

CCR-6 (Corchoroside-A, 28mg): It crystallized from methanol-chloroform as colour less prisms, with one molecule of water, m.p. 164-168°C; $[\alpha]_D$ = +19.7° (methanol), it showed positive Kedde and Legal reactions indicating the cardinolide nature of the compound. This observation was supported by U.V. spectrum (ethanol) which showed maxima at 218 nm and 298 nm confirming the presence of α,β unsaturated γ-lactone group and a carbonyl group. It was 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). Based on the spectral data, the compound was identified as corchoroside –A. [17]

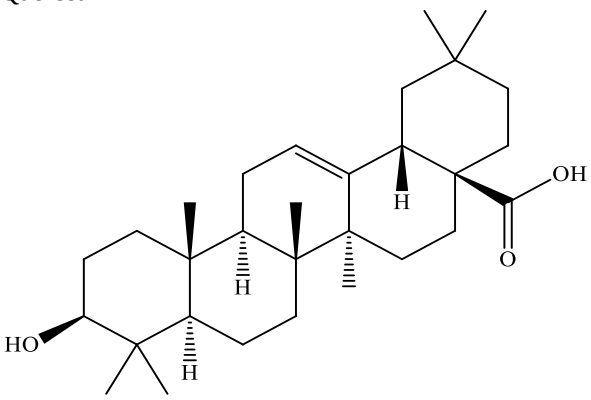
CCR -7 (Cannogenol, 30mg): It was obtained as a white amorphous powder in 30% methanol: chloroform. It showed positive kedde and legal reactions indicating the cardinolide nature of the compound**.** In the high resolution negative ion FAB mass spectrum , CCR-7 showed a $[M-H]^-$ ion peak at m/z 681.3436 .The fragment ion peaks of low resolution FABMS,m/z 519 for $[M-H-162]^-$ and 389 for $[aglycone –H]^-$, were observed. CCR-7 had more mass units from digitoxigenin, and one extra hydroxyl group in the aglycone. The C-19 signal of CCR -7 was observed at δ 66.0 shifted by +41.7 ppm. The signals of C-1 (δ 24.8, -6.6 ppm), C-5 (δ 30.2 7.7 ppm) and C-10 (δ 40.4,-4.1 ppm) were significantly shifted. The ^{1}H NMR, H-H COSY and HMQC spectra, two protons at the 19-position were assigned at δ3.41 and 3.81 .These data indicated that the aglycone was cannogenol which had one hydroxyl at C-19. The ^{1}H NMR showed signals at ^{1}H NMR δ 0.88 (3H,s, H_3 -18),1.25(3H.d,J=6.5H_z, bio H_3 -6),2.82 (1H, m, H-17), 3.41, 3.81 (1H,d,J=11H_z, H_2-19), 3.45 (1H, m, boi H-4), 3.65 (1H, dd, J=5.5, 12.0 H_z , glc H-6b), 4.04 (1H, m, H-3), 4.15 (1H, br.q, boi H-3), 4.31 (1H, d, J=8.0Hz, glc H-1), 4.88 (1H, dd, J=1.5,18.5Hz, H_2-21), 5.89 (1H,s,H-22). Based on the spectral data the compound was identified as cannogenol.



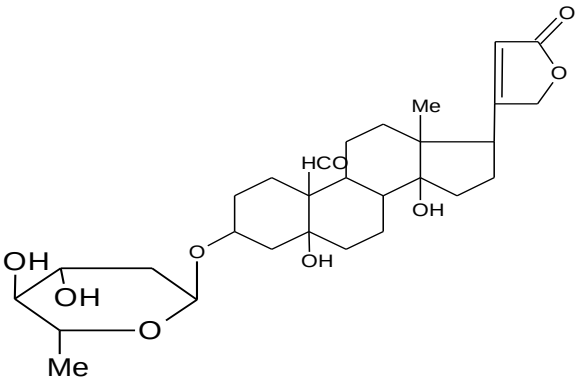
Quercetin



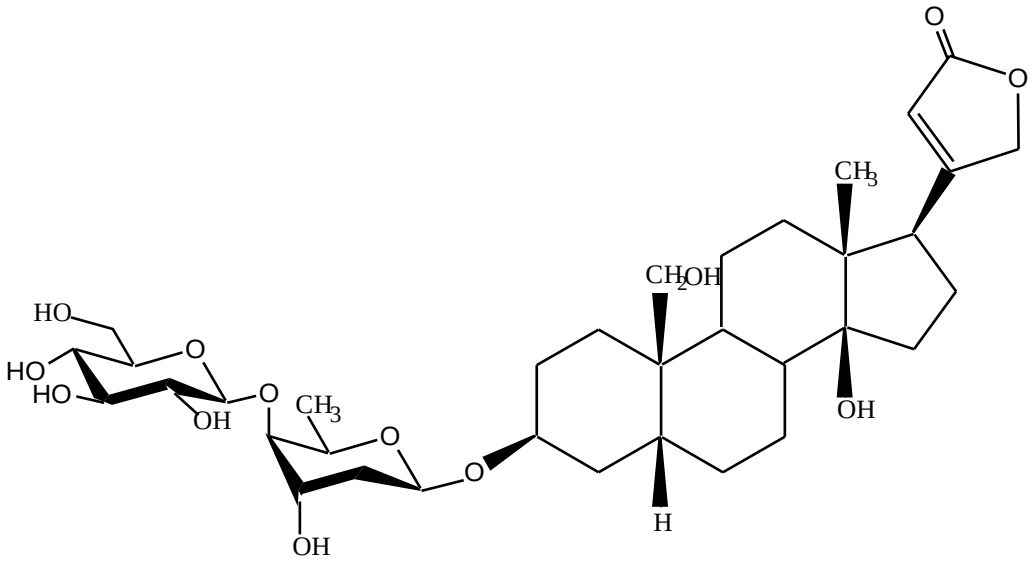
Betulinic acid



Oleanolic acid



Corchoroside - A



Cannogenol

RESULTS AND DISCUSSION

The chemical examination of the roots of *C.capsularis* by usual extraction methods and on column chromatography of the residue gave seven compounds. The compounds were identified as β-sitosterol, stigmasterol -3-0- β-D-glucoside, quercetin, betulinic acid, oleanolic acid, corchoroside-A and cannogenol. From these isolates stigmasterol-3-β-D- glucoside**,** quercetin**,** betulinic acid, cannogenol were reported for the first time from this root of *C.capsularis.*

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