International Transactions in Applied Sciences
October-December 2013, Volume 6, No. 2, pp. 180-187
ISSN-(Printing) 0974-7273, (Online) 0975-3761

Bio-Chemical investigation of Echinops cornigerus

[1]S. Negi, [1]D. Singhal, [1]H.V. Pant, [1]H. C. Joshi, [1]R. Dhoundiyal & [1]A. K. Gupta
[1]Department of Chemistry, S G R R (PG) College, Dehradun 248001, India
negisandeep1969@gmail.com

ABSTRACT

Echinops species are medicinally important, so we take unexplored species, Echinops cornigerus. Seven components Betulinic acid, Olenolic acid, β-amyrin, Luteolin, Lupeol, Apigenin and Apigenin-7-O- β-D-glucoside are isolated first time from Echinops cornigerus and identified from their physicochemical data.

Keyword: Echinops cornigerus, Betulinic acid, Olenolic acid, β-amyrin,Luteolin, Lupeol, Apigenin and Apigenin-7-O- β-D-glucoside

INTRODUCTION

Echinops cornigerus is annual, spinescent herbs of 70 cm height with simple stem distributed in submontane and montane region of west Himalayas. Leaves are sessile, ovate-oblong or pinnatifid, 10-20 cm long. Heads 6-7.5 cm across, with or without projecting spines. In Indian system of medicine, the plant is well known as kantela, kantalu (Sanskrit) and commonly known as Globe-Thistle (English).As per the traditional claims roots extracts given to infants to promote emergence of teeth; root juice given in fever and urinary trouble[1]. Phytochemically the root of *Echinops Latifolia* is diuretic, anti-inflammmatory and haemostatic[2]. *E. echinates* is antifungal, anti androgenic and anti inflammatory[3-5]. Root of *E. giganteus* contain Silphiperfol-6-ene, pusilphiperfol-7-ene, cameroonin-7-ol, nopsan-4-ol, echinan-8-ol [6]. Although plenty of researches have been carried out about the other species of *Echinops*, the works on *Echinops cornigerus* are restricted. We isolated and elucidated six components Betulinic acid, Olenolic acid, β-amyrin, Lupeol, Apigenin and Apigenin-7-O- β-D-glucoside from ariel parts of *Echinops cornigerus*. These components are known but they were isolated from that at first. The compounds were isolated by

column chromatographic technique and identified by UV, IR NMR and mass spectroscopic data and in comparison of their physical and spectral properties reported from the literature.

MATERIALS AND METHODS

General

Melting points were measured on Buchi 545 B, UV Spectra was recorded on Shimadzu 1801, IR spectrum was recorded on FT-IR 8100 Shimadzu and Pye-Unicam SP-3-200 spectrophotometer, EI-MS was recorded on a JMS-SX 102A 5890 series II mass spectrophotometer, FAB-MS was recorded on a JEOL JMS-AX 505 mass spectrophotometer and ^{1}H-NMR and ^{13}C NMR spectra were recorded on JEOL JNM-A600 spectrometer with TMS as an internal standard and chemical shifts were expressed on the δ (ppm) scale.

Plant material

The leaves of Echinops cornigerus were collected from Srinagar garhwal, Distt. Pauri Garhwal, and authenticated by Department of Botany, HNB Garhwal University with voucher specimen no. GUH 1704 herbarium was also deposited for future reference.

Extraction and isolation

The leaves were shade dried for a week and powdered. Powdered material (500 g) was extracted using Soxhlet apparatus with 95% ethanol for about 36 h. The extract was filtered and concentrated *in vacuum* under reduced pressure using rotary flash evaporator. The ethanol extract was further partitioned with light petroleum (60-80^{0}C) and petroleum extract was concentrated *in vacuum* under reduced pressure using rotary flash evaporator. The petroleum extract and petroleum free mass were chromatograph over silica gel (60-120 mesh) as adsorbent and elution was carried out with various solvent in order of their increasing polarity. The light petroleum extract on CC with light petroleum (60-80 ^{0}C) and ethyl acetate (99:1-94:6) afforded compound Betulinic acid (1), Oleanolic acid(2) and β-amyrin. The petroleum free mass on CC with chloroform and methnol (98:2-90:10) gives compound Luteolin (4), Lupeol(5), Apigenin(6) and Apigenin-7-O- β-D-glucoside(7).

Identification

Betulinic acid (1). Colorless amorphous powder. This compound was crystallized from $CHCl_3$, m.p. : 315-316^{0}C. EI-MS : m/z 456[M]$^+$ 441, 438, 423, 411, 248, 220, 219, 207 and 189 (base peak). IR (ν_{max} KBr) : cm-1 3400, 2935, 2850, 1690, 1640, 1460, 1392, 1381, 1370, 1360, 1295, 1274, 890. ^{1}H NMR (500MHz, $CDCl_3$, δ ppm): 0.81, 1.04, 1.06, 1.08, 1.26, 1.82 (each 3H, *s*, Me × 6),3.42 (1H, brt, *J* = 7.2 Hz, H-3), 4.72 (1H, brs, H-29a), 4.94 (1H, brs, H-29b); ^{13}C NMR (pyridine-*d5*): _ 178.4 (C- 28), 152.0 (C-20), 109.4 (C-29), 78.2 (C-3), 56.4 (C-17), 56.2 (C-5), 51.0 (C-9), 49.8 (C-19), 47.8 (C-18), 42.9 (C-14), 41.2 (C-8), 39.6 (C-4), 39.1 (C-1), 38.7 (C-13), 37.9 (C-10), 37.4 (C-22), 34.9 (C-7), 32.6 (C-16), 31.3 (C-15), 30.6 (C-21), 28.5(C-23), 28.3 (C-2), 26.2 (C-12), 21.3 (C-11), 19.3 (C-30), 18.8(C-6), 16.8 (C-25), 16.4 (C-26), 16.2 (C-24), 15.0 (C-27) [7].

Oleanolic acid (2). White amorphous powder. m.p.: 271-273°C. ^{1}H-NMR (500MHz, $CDCl_3$, δ ppm): 0.75, 0.77, 0.90, 0.91, 0.93, 0.98 (each 3H, *s*, CH3 ×6), 1.13 (3H, *s*, H-27),2.82 (1H, *dd*, *J*= 3.6, 13.2 Hz, H-18), 3.23(1H, *dd*, *J*= 11.2, 4.4 Hz, H-3), 5.27 (1H, *t*,*J*=3.5 Hz, H-12). ^{13}C-NMR (125 MHz, Pyridine-*d*5, δ ppm): δC (from C-1 to C-30) 39.0, 28.2,78.1, 39.4, 55.8, 18.8, 33.3, 39.8, 48.2, 37.4,23.7, 122.6, 144.8, 42.2, 28.4, 23.8, 46.7, 42.0,46.5, 31.0, 34.3, 33.2, 28.8, 16.6, 15.6, 17.5,26.2, 180.2, 33.3, 23.8.

β-amyrin (3). White crystalline needles, m.p. : 196-198°C. EI-MS : m/z 426[M]$^+$, 411, 392, 315, 257, 234, 218 (base peak), 208, 207, 203, 198, 189, 175, 169, 147 and 133. IR (ν_{max} KBr) (cm^{-1}): 3550, 2940, 2880, 1650, 1385, 1370 and 1055. ^{1}H-NMR (500MHz, $CDCl_3$, δ ppm): 1.68(3H, 20-CH3), 3.21(1H, dd, J=6.2 Hz, 3-H), 4.57, 4.67 (2H, dd, 24-CH2).

Lupeol (4). Found C- 84.50, H -11.73%, $C_{30}H_{50}O$, Cal. C, 85.51, H 11.72% . EI-MS**:** m/z 426 [M]$^+$, 218, 189, 135, 121, 109, 95. IR: (ν_{max} KBr) (cm^{-1}): 3440, 2970, 2959, 2930, 2859, 1463, 1380, 1055 cm-1,^{1}H**-** NMR **(**$CDCl_3$, δ ppm) : 1.68 (3H, s, 20 –CH3), 3.21 (1H, dd, *J* = 6.2 Hz, 3 α-H) , 4.57, 4.67 (2H, bd, s, 24-H2)**.**

Apigenin (5). A pale yellow solid. m.p. : 346-349 °C. . Molecular formula: $C_{15}H_{10}O_5$; Cal. C-66.60%, H-3.700% found; C-66.60%, H-3.80%.UV-Vis: (ethanol) λ_{max}; 270,335 IR: (ν_{max} KBr) (cm^{-1}): 3420, 1650, 1600, 1520, 1450, 1360, 1310, 1270, 1180, 1070, 1030, 850, 750. ^{1}H-NMR (500 MHz, $CDCl_3$

, δ ppm) : 6.80(s, 1H, H-3), 6.52(d, J=2Hz, 1H, H-6), 6.82(d, J=2 Hz, 1H, H-8), 6.90(d, J=8.5Hz, 1H, H-3′, H-5′), 7.88(d, J=8.5Hz, 1H, H-2′, H-6′), 12.90 (s, 1H, C5-OH, exchangeable with D_2O), 10.10 (s, 1H, C7-OH, exchangeable with D_2O) and 5.70 (s, 1H, C′4-OH, exchangeable with D_2O).

Apigenin-7-O-β-D-glucoside (6). A yellowish brown amorphous solid. m.p.: 178-180 °C. . Molecular formula: $C_{21}H_{20}O_{10}$; Cal. C-58.282%, H-4.662% found; C-58.10%, H-4.59%.UV-Vis: (ethanol) λ_{max}; 247, 352. FAB-MS: *m/z*; 433 $[M+H]^+$,432$[M]^+$, 271(glucose residue). IR: (KBr) (cm^{-1}); 3402, 2920, 2850, 1631, 597. ^{1}H-NMR (500 MHz, TMS, DMSO d6, δ ppm): 3.5 (t, *J*=3.6, H-3, CH), 3.8 (t, *J*=1.8,2-H, CH_2OH), 4.0 (q, *J*=31.5,1-H, CH-O), 4.7 (t, *J*=19.5,1-H, CH_2OH), 4.9 (d, *J*=22.5, 3-H, OH), 6.0 (d, *J*=18.3,1-H, CH-O), 6.7 (s, 3-H, Ar C-H), 7.1 (d, *J*=6.9 4-H, ArC-H), 8.5 (s, 2-H, Ar-OH).

RESULTS AND DISCUSSION

Compound 1: Compound 1 was isolated as a colorless amorphous powder and its mass spectral data suggested the molecular formula as $C_{30}H_{48}O_3$. It was positive to LB[8] and Noller's reagent[9] testes and developd yellow colour with TNT, thereby indicating it to be a triterpenoid system with an unsaturation in the compound. The ^{1}H NMR spectrum showed six tertiary methyl singlets at 0.81, 1.04, 1.06, 1.08, 1.26, 1.82; and one secondary hydroxyl group as a broad triplet at 3.42, and two olefinic protons at 4.72 and 4.94 representing the exocyclic double bond. In the absence of an additional methyl singlet as in 1 and 2 together with the appearance of a carbonyl group at 178.4 in the ^{13}C NMR spectrum of compound 1 suggested the presence of an acid group in its structure which was identified at C-28 position by the key HMBC correlations. Based on the above spectral data, the structure of 1 was assigned as betulinic acid further supported by the physical and spectral data reported from the literature [7].

Compound 2: Compound 2 gave a positive Liebermann-Burchardt[8] and anisaldehyde test and the mass spectrum of it showed a molecular ion at *m/z* 456 corresponding to $C_{30}H_{48}O_3$. The ^{1}H-NMR spectrum of compound 2 showed seven tertiary methyl groups at δ 0.75, 0.77, 0.90, 0.91, 0.93, 0.98 and 1.13 on an oleanane skeleton. A doubletdoublet of one proton at δ 2.82 and a triplet of one vinyl proton at δ 5.27 were assigned to H-18 and H-12, respectively, suggesting an olea-12-ene skeleton. One methine proton at δ 3.23 (*dd*, *J*= 11.2 and 4.4 Hz) showed that **2** has at least one

hydroxyl group. In ^{13}C-NMR spectrum, the signal corresponding to the carboxyl C-28 appeared at δ 183.8. The spectral data were similar to the ones reported for oleanolic acid[7] .

Compound 3: Molecular ion at $[M]^+$ 426 showed its molecular weight. It gave positive to LB [8]and Noller's test[9]. The positive TNT test showed the presence of unsaturation in the molecule. The characteristic absorption bands in IR were observed at 3550 (OH, stretching), 2940, 2880 (C-H, stretching), 1650 (C=O, stretching), 1385, 1370 [C-(CH_2) deformation] ,1055 (C-O, stretching). In EI-MS is showed the molecular ion peak at m/z 426$[M]^+$. The base peak was observed at m/z 218. The identification of compound 3 as β-amyrin was unambiguously confirmed by co-TLC, co-IR and mixed mp.

Compond 4: The molecular formula of compound 4 (m.p. 215-216^{o}C) found homogeneous in TLC was assigned as $C_{30}H_{50}O$, on the basis of elemental analysis and molecular weight determination (M^+ 426) the compound responded to Liebermann–Burchard[8] test (violet colour changing to blue green) and Noller's test[9] (Produced red colour) these colour reactions indicated it to be a triterpenoid red colour with chlorosulphonic acid and violet colour in the Brieskorne test also suggested that the compound was triterpenoid. It gave positive test with TNM (yellow colour) thereby indicating the presence of unsaturation in the molecule. The characteristic absorption bands observed in the IR spectrum were at KBr max ν 3400 (OH), 2970, 2959, 2920, 2859 (-C-H stretching), 1463, 1380, (dimethyl groups) and 1055 cm-1 (C-O stretching). NMR ($CDCl_3$) of Lupeol exhibited characteristic signals at δ 81.68 (3H, s, 20- CH3), 3.21 (1H, dd, *J* = 6.2 Hz, 3 α-H), 4.57, 4.65 (2H, bd, s, 24-H2). EI-MS showed peaks at m/z 426 (M^+), 218, 189, 121, 109 and 95. Compound gave monoacetate (m.p. 216^0) on acetytation with the appearance of acetyl band at KBr max ν 1735 cm-1 showing the presence of hydroxyl group in the molecule, which was further confirmed by the characteristic absorption band at 3400 cm^{-1} in it's IR spectrum. From all these observation Lupeol was characterized to be lupeol (β- viscol). It was identified by direct comparisons (mixed m.p., co-tlc and superimposable IR) with an authentic sample of Lupeol and also confirmed by the preparations of it's acetyl derivatives (reported m.p. 216^{0}C) as described above.

Compound 5: A pale yellow solid with m.p. 346-349 °C. Elemental analysis suggested the molecular formula $C_{15}H_{10}O_5$ and molecular ion at $[M]^+$ showed its molecular weight. It gave colour reaction.

Yellow c olour with aq. NaOH.

Yellow colour with few drops of conc. H_2SO_4.

Pink colour with Mg/HCl (Shinoda's test)[10].

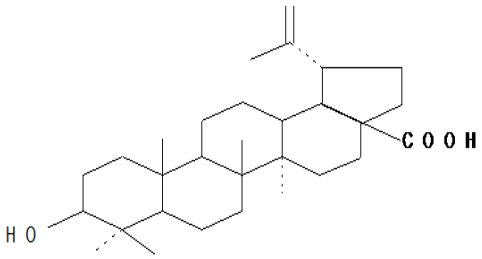
The above tests indicates the usual flavonoidal character of the compound.

In the IR spectrum, characteristic absorption were observed at 3420, 1650, 1600, 1520, 1450, 1360, 1310, 1270, 1180, 1070, 1030, 850, 750 cm^{-1}. ^{1}H-NMR spectrum showed signals at δ ppm at 6.80(s, 1H, H-3), 6.52(d, J=2Hz, 1H, H-6), 6.82(d, J=2 Hz, 1H, H-8), 6.90(d, J=8.5Hz, 1H, H-3', H-5'), 7.88(d, J=8.5Hz, 1H, H-2', H-6'), 12.90 (s, 1H, C5-OH, exchangeable with D2O), 10.10 (s, 1H, C7-OH, exchangeable with D2O) and 5.70 (s, 1H, C'4-OH, exchangeable with D2O).

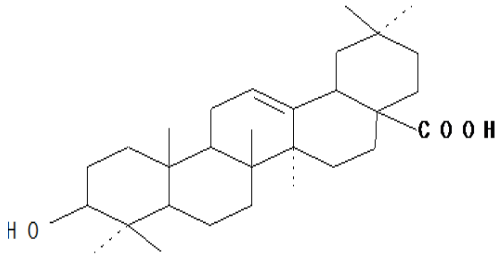
From the above spectral data (Harborne et al., 1975) and comparison with authentic sample (mp, co-TLC, co-Ir) compound 5 was identified as Apigenin.

Compound 6: Compound 6 was obtained as yellowish brown amorphous solid having the melting point 178-180 ^{o}C. The compound also gave positive color reactions for a hydroxyl flavone with several reagents[11]. On hydrolysis (7% H2SO4) it gave apigenin and glucose (co-PC and co-TLC). UV-Vis spectrum showed characteristic absorption at ethanol λ_{max}; 247, 352. The IR spectrum indicates absorption at 3402, 2920, 2850, 1631, 597. ^{1}H-NMR spectrum, characteristic signals were exhibited at (ppm) δ 3.5 (t, *J*=3.6, H-3, CH), 3.8 (t, *J*=1.8,2-H, CH2OH), 4.0 (q, *J*=31.5,1-H, CH-O), 4.7 (t, *J*=19.5,1-H, CH_2OH), 4.9 (d, *J*=22.5, 3-H, OH), 6.0 (d, *J*=18.3,1-H, CH-O), 6.7 (s, 3-H, Ar C-H), 7.1 (d, *J*=6.9 4-H, ArC-H), 8.5 (s, 2-H, Ar-OH).

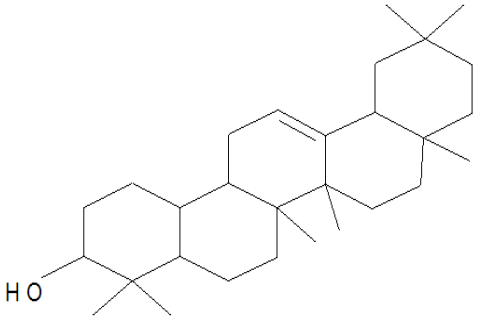
From the above spectral data and comparison with authentic sample (mp, co-TLC, co-Ir) compound 6 was identified as Apigenin-7-O-β-D-glucoside.



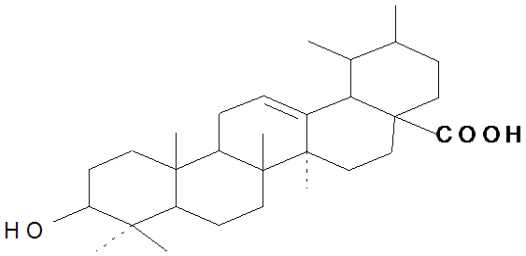
Compound 1



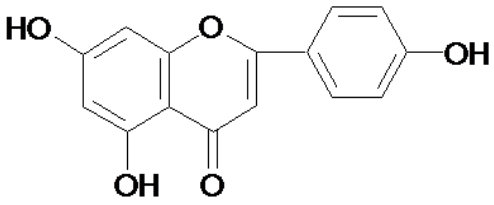
Compound 2



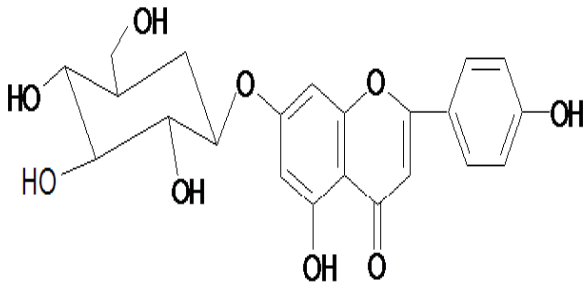
Compound 3



Compound 4



Compound 5



Compound 6

REFERENCES:

Gaur, R.D., " Flora of the District Garhwal Himalaya", TransMedia, Srinagar, pp. 571(1999).

Batorava,S.M., Rabshain, K.V., Bagdanova, T.V. and Shantanova, L.N., *Rastit. Resur.*, **16**(1), 134 (1988).

Singh, U.P., Pandey, V.B. and Singh, K.N., *Canad. J. Bot*., **66**(9), 1901 (1988).

Singh, B., Pandey, V.B. and Gambhir, S.S., *Phyto., Res*., **5**(3), 103 (1991)..

Chaturvedi, M., Mali, P.C., and Dixit, V.P.J., *Phyto. Res.*, **8**(2), 115 (1995).

Weyerstahl, P., Seelmann, I., Marschall, H. and Menut, C., "Twenty eight International symposium on essential oil, Eskischir", Turkey, Pl-8, 1-3 September (1997).

Baek M.-Y., Cho J.-G., Lee D.-Y., Ahn E.-M., Jeong T.-S. and Baek N.-I., *Korean Soc. Appl. Biol. Chem.,* **53**, 310-315 (2010)

Libermann, C., *Ber Dausch. Chem. Ges*, **18**, 1804 (1985).

Noller, C.R., Smith, A.K., Harris, G.H. and Walker, J.M., *J. Am. Chem. Soc.*, **64**, 3047 (1942).

Shinoda, J., *J. Pharm. Soc. Japan*, **48**, 214 (1928).

Kaneta, M., *Fukushima Tech Jr Col Bull,* **7**, 40-44(1971).