Journal of Current Chemical & Pharmaceutical Sciences

J. Curr. Chem. Pharm. Sc.: 2(1), 2012, 76-80
ISSN 2277-2871

PHYTOCONSTITUENTS OF *SYMPLOCOS PANICULATA* (LEAVES)

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(*Received : 15.10.2011; Revised : 01.11.2011; Accepted : 04.11.2011*)

ABSTRACT

From the petroleum ether (60-80^0) extract of *Symplocos paniculata* (Leaves), Octacos-1- ene **(A)**, Stigmasterol **(B)** and Lupeol **(C)** have been isolated and characterized, where as ethenolic extract yielded Salirepin **(D).**

Key words: Petroleum ether extract, Ethenolic extract *Symplocos paniculata,* Octacos-1- ene, Stigmasterol, Saliredin.

INTRODUCTION

Symplocos paniculata is a species of symplocaceae family which is commonly known as lodh, lodhra and sapphire berry. It is found in montane to submontane Himalayas, Pakistan, South west China, Myanmar, Japan, South west Asia India and locally found on 1600-2000 ft region of Tehri Garhwal[1,2]. Deciduous tree to 8 m high with rough, yellowish brown, corky bark. Leaves ovate or broadly elliptic, 4-10 × 2-5.5 cm, acute, sharply serrate, pilose beneath membranous letral nerves 6-8 pairs. Petioles 3-5 mm long. Flowers white, about 5-8 mm across, pedicelled in terminal or axillaries branched cluster; 2-10 cm long peduncle. Calyx with obovate, ciliated lobes. Corolla with 5 oblong, spreading petals[1,2]. The bark used in folk-medicine to check abortion and species used in ophthalmia, eye diseeses, spongy gums, dysentery inflammations, vaginal discharge and leprosy. Flowers sucked for nectar by bees; yellow dye obtained from the bark, leaves lopped for fodder. It is useful in apiculture as bee forage. The roots, barks, or leaves of many symplocos plants have been used as traditional herbal medicines for treatment of diarrhea, dysentery, menorrhagia, uterine disorders[3] as well as malaria, nephritis and snake bite[4]. A survey of the literature revealed that no work has so far been reported on *symplocos paniculata* except one paper on stem bark of *symplocos paniculata*[5]. In view of interesting medicinal properties and the fact that a very little work has been reported on *S. paniculata* (stem bark) prompted us to carry out the detailed phytochemical investigation on leaves of *symplocos paniculata*.

EXPERIMENTAL

The melting points are uncorrected. The UV spectrums were measured on a Hitachi 320. Perkin Elmer model 202 automatic recording spectrophotometer and Toshinwal manual spectrophotometer. The IR spectra were recorded KBr pellets on Perkin Elmer model 577 and KBr discs (JASCO – IR – 810 spectrometer). The ^{1}H NMR were recorded on UNM-G × 400 JEOL spectrometer at 400 MHz and ^{13}C NMR

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spectra were recorded on same instrument at 100.533 MHz using TMS as internal standard. EI-MS spectra were recorded on JMS – DX 300 (JEOL) with direct inlet at 70 eV.

Plant material

The plant material was collected from Dhanolty, Tehri Garhwal UK (India) in the month of March 2009. The authentification of plant material was made at the Department of Botany, HNB Garhwal University, Campus Badshahithaul, Tehri Garhwal UK, India. A voucher specimen is available at the herbarium of Botany Department.

Extraction and isolation

Shade dried leaves (1 Kg) were exhaustively extracted with petroleum ether ($60\text{-}80^0$) in a 5 Liter R.B. flask (500 g × 2.5 Ltr) followed by repeated extraction with ethanol. Every time, extraction was carried out for to 10 hrs. In each case the extract was filtered and concentrated under reduced pressure when brownish waxy mass (39 g from petroleum ether extract) and brown syrupy mass (110 g from ethanolic extract) respectively were obtained.

Isolation of Octacos-1- Ene (Compound –A): Molecular formula $C_{28}H_{56}$, FT-IR: 2924, 1640 cm^{-1} ^1H NMR**:** ($CDCl_3$): (δ ppm), 0.86 (t; 3H, CH_3-, *J* = 6.1 Hz), 1.23 (br s, 48H, CH_2-aliphatic), 2.0 (m; 2H, CH_2-CH = CH, *J* = 6.7 Hz), 4.93, 4.98 (dd; 2H, CH_2 *J* = 14, 1.5 Hz), 5.97, 5.90 (m; 1H, CH_2 = CH -CH_2), ^{13}C NMR: ($CDCl_3$), δ ppm: 138.6 (CH = CH_2), 113.4 (CH_2 = CH), 33.23 (CH_2-CH = CH_2), 28.37 -29.11 (CH_2), 22.11 (CH_2 CH_3), 13.53 (CH_3), EI-MS: (70 eV) m/z 391 (M^+), 279 (M-C_8H_{17})$^+$, 167 (M-Z C_8H_{17}).

Isolation of Stegmasterol (Compound –B)found: C, 84.70 H, 11.16%**,** Cald for: $C_{29}H_{48}O$**,** C, 84.68 H, 11.17%**,** IR: ν_{max}^{KBr} 3440, 2960, 1640, 1465**,** 1445, 1375, 1070, 990, 958, 824 cm^{-1}**,** NMR: ($CDCl_3$) δ (ppm)**,** 0.70 (CH_3), 0.90(s), 0.93 (d), 0.96(d), 1.0 (s) and 5.3 (t) for OH group , M/S: m/e 412 (M^+) 398, 380, 360, 271,229, 197

Isolation of Lupeol (Compound –C): Found: C, 84.50, H 11.73%, Calcd for: C_{30} H_{50} O**,** C, 85.51, H 11.72% **,** IR: ν_{max}^{KBr} 3440, 2970, 2959, 2930, 2859, 1463, 1380, 1055 cm^{-1}**,** NMR: **(**$CDCl_3$) δ (ppm) 1.68 (3H, s 20 –CH_3), 3.21 (1H, dd, *J* = 6.2 Hz, 3 α-H) , 4.57, 4.67 (2H, bd, s, 24-H_2)**,** EIMS**:** m/z 426 (M^+), 218, 189, 135, 121, 109, 95

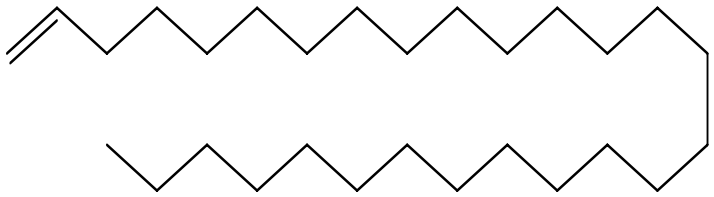
Isolation of Saliredin (Compound D): White powder (300 mg)**,** [α] D^{23} = -45% (C=O 368, MeOH)**,** UV: λ_{max}^{MeOH} nm (log ε) (MeOH)**,** 285.8 (2.92), 251.2 (2.30), 225.6 (3.36)**,** 212.0 (3.29), 203.2 (3.70) nm**,** IR: ν_{max}^{KBr} 3408 (OH), 2921 (C-H), 1665-1443 (C=C, Ar)**,** 1215 (C-O-C), 1084, 1040 (C-O) 992, 671 cm^{-1}**,** ^1H-NMR: (CD_3OH) (400 MHz) δ ppm 7.07 (1H, d, *J* = 8.7Hz, H-6), 6.78 (1H, d, *J* = 2.9 Hz, H-3), 6.65 (1H, dd, *J* = 8.7, 2.9 Hz, H-5), 4.69 (1H, d *J* = 13.0 Hz H_β-7), 4.67 (1H,d, *J* = 6.9 Hz, H-1'), 4.51 (1H, d, *J* = 13.0 Hz, H_α-7), 3.88 (1H, dd, *J* = 11.6, 2.8 Hz, H_β-6'), 3.68 (1H, dd, *J* = 11.8, 7.2 Hz, H_α-6') , 3.46 (1H, m, H-5), 3.43 (1H, t, *J* = 8.8 Hz, H-2'), 3.40 (1H, t, *J* = 8.6 Hz, H-3), 3.35 (1H, t, *J* = 8.3 Hz, H-4'). ^{13}C NMR: ($CDCl_3$) (100 MHz) δ (ppm) 154.0 (C-4), 150.2 (C-1), 133.8 (C-2), 119.5 (C-6), 116.4 (C-3), 115.8 (C-5', 104.7 (C-1'), 78.1 (C-3'), 78.0 (C-5'), 75.1 (C-2'), 71.4 (C-4'), 62.6 (C-6'), 61.0 (C-7').

RESULTS AND DISCUSSION

Characterization of 'A' as Octacos-1- ene

The molecular formula to **'A'** (homogeneous in TLC) as $C_{28}H_{56}$ was assigned on the basis of elemental analysis and molecular weight determination (M^+ 391). It gave yellow colour with TNM[6] there by indicating it's unsaturated nature. The FT-IR spectrum showed alkyl (2924 cm^{-1}) and double bond

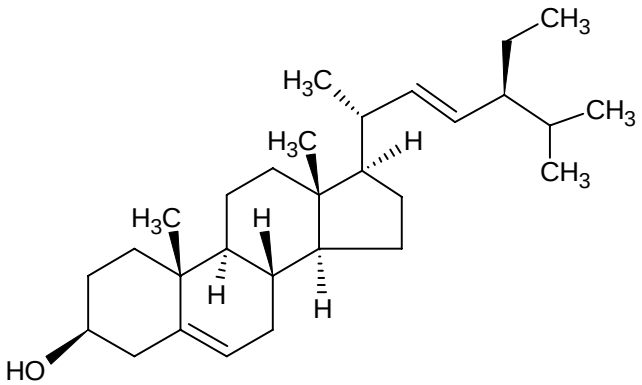
(1640 cm^{-1}) groups. By EIMS a molecular ion at m/z 391 was observed. The mass spectrum of '**A**' showed peaks of ions at m/z 279 and 167 resulting from successive elimination of $C_8H^+_{37}$ fragments. The ^1H NMR spectrum of **'A'** cleanly showed one methyl signal of δ_H 0.86 (3H, t H-28), a long chain methylene protons at δ_H 1.23 (br, s) and an olefinic group at at J = 1.4 and 1.5 Hz indicate the presence of a vinyl group. In addition the ^{13}C–NMR data of **'A'** showed correlation were observed between H-1/H-2/H-3 in COZY spectrum. Thus the structure of **'A'** was determined to be octacos -1- ene.



A

Characterization of 'B'as Stigmasterol

'B' (found homogeneous in TLC) was assigned the molecular formula is $C_{29}H_{48}O$. On the basis of elemental analysis and molecular weight determination by mass spectral studies (M^+ 412). It responded to the positive Liebermann –Burchard[7] test and Noller's test[8]. These colour reaction indicate it's steroidal nature. It gave yellow colour with TNM indicating the presence of unsatration in the molecule. From molecular formula and characteristic colour reactions compound '**B'** appeared to be unsaturated sterol. The compound gave monoacetate $C_{29}H_{47}O.COCH_3$ (m.p. 144-145^{o}C) an acetylation. This indicates the presence of one hydroxyl group in the molecule. It was further confirmed by a characteristic absorption band for hydroxyl group observed in IR spectrum at ν_{max}^{KBr} 3440 cm^{-1} and 1055 cm^{-1} (for C-O stretching). The NMR (solvent $CHCl_3$, TMS an internal reference) spectrum of the compound gave a singlet for 3H at δ 0.70 for a methyl group at C-13. another singlet for 3H at δ 0.90 for methyl group at C-28, at δ 0.93 a doublet for two methyl group at C-25, at δ 0.96 a doublet for one methyl group at C-20 and δ 1.0 a singlet for methyl group at C-10.



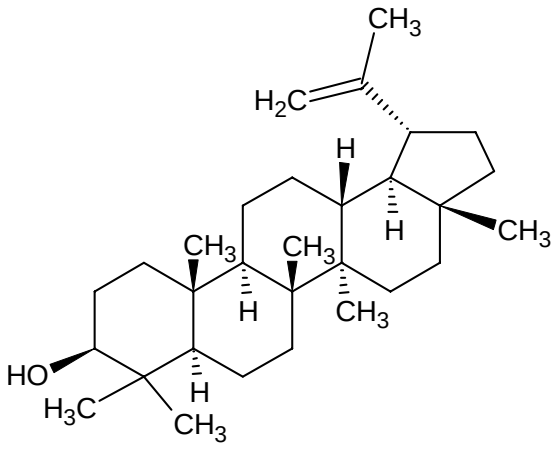
B

From the all these fact it appeared that the properties of **'B'** and its derivatives corresponded to those of stigmasterol reported in the literature[9]. It was confirmed by mixed m.p. co-tlc and superimposable infrared spectrum with that of the authentic sample of stigmasterol. The structure was further confirmed by mass spectrum of compound, giving prominent peaks at m/z 412 (M^+) 398, 380, 360, 271, 229 and 197 etc.

Characterization of 'C' as "Lupeol (β-VISCOL)

The molecular formula of **'C'** (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 test[10] (violet colour changing to blue green) and Noller's test[8] (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 ν_{max}^{KBr} 3400 (OH), 2970, 2959, 2920, 2859 (-C-H stretching), 1463, 1380, (dimethyl groups) and 1055 cm^{-1} (C-O stretching). NMR ($CDCl_3$) of **'C'** exhibited characteristic signals at δ 81.68 (3H, s, 20- CH_3), 3.21 (1H, dd, J = 6.2 Hz, 3 α-H), 4.57, 4.65 (2H, bd, s, 24-H_2). 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 ν_{max}^{KBr} 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[11]. From all these observation **'C'** 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[12] m.p. 216^0) as described above.

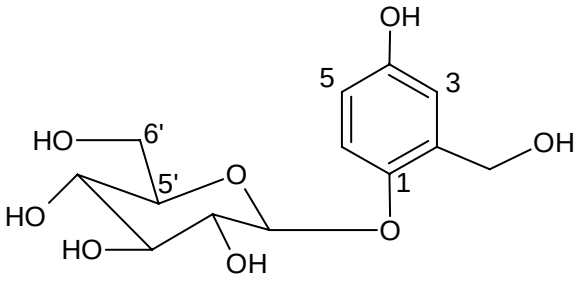


C

Characterization of 'D' as Salirepin

Salirepin **'D'** was isolated as a white powder from the ethanolic extract of *Symplocos peniculata* by column chromatography.

The FAB –MS of Salirepin **'D'** showed a $[M]^+$ ion peak at m/z 302, corresponding with the molecular formula $C_{13}H_{18}O_8$, which indicate 5 degrees of unsaturation. It exhibited a UV absorption band (λ_{max}^{MeOH} 285. 8 nm) typical of phenolic compounds. The glycone formed by acid hydrolysis of **'D'** identified as glucose as TLC comparison with an authentic sample of this sugar. The IR absorption bands observed at 3408 (OH), 2921 (C-H), 1665-1443, (C =C, Ar), 1268, 1215 (C-O-C) revealed the presence of hydroxyl groups methines, aromatic double bonds and ether linkage while the broad (C-O) stretching bands in the region of 1084-1040 cm^{-1} suggested its glycosidic nature. The EI-MS spectrum of **'D'** exhibited the following characteristic fragments. $[C_6H_3(OH)_2\ CH_2OH]^+$ (m/z 140, 77.6%, $[C_6H_3(OH)_2\ CH_2]^+$ m/z 123, 36.5 %) [gentisyl alcohol $–H_2O]^+$ (m/z 122 100%), which indicated the presence of gentisyl alcohol moiety in the molecule. In the ^{1}H-NMR spectrum, the usual ABx spin system of the gentisyl alcohol group was readily indentified by signals observed at δ 7.07 (1H,d, J = 7, 8.7 H_2 H-6), 6.78 (1H, d, J = 2.9 Hz, H-3) and 6.65 (1H,dd, J = 8.7, 2.9 Hz, H -5). The other signals observed were assignable to a β-D–glucose moiety. The ^{13}C NMR spectrum and 2D-NMR experiment, confirmed the structure of **'D'** to be 2(oxymethyl)–4-hydroxyl phenyl–β-D glucopyranoside[13].



D

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