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GC/MS STUDY OF DIFFERENT EXTRACTS OF *PYGMAEOPREMNA HERBACEA* ROXB.

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ABSTRACT

The Gas Chromatography Mass Spectroscopic studies of petroleum ether and methanol fractions of *Pygmaeopremna herbacea* Roxb. was carried out. The GC/MS study of petroleum ether fraction afforded eight compounds in which n- Dotriacont-29-en-4-ol was the dement fraction. The methanol fraction gave a mixture of four triterpenoids of steroidal nature in which β –Sitosterol was the major fraction.

INTRODUCTION

Plant *Pygmaeopremna herbacea* Roxb. (Family:- Verbenaceae) is an important less known medicinal plant of old forests of India. It is very small shrub 8-10 cm in height[1]. The roots of *Pygmaeopremna herbacea* Roxb. is claimed to be useful in the Ayurvedic system of medicine for the treatment of several ailments such as rheumatism, snake-bite, scorpion-sting, but it is not an antidote to either snake-venom or scorpion-venom[2-4]. The different parts of the plant are used as laxative, stomachic, alexipharmic, anemia, diabetes chyluria, inflammation, swelling, bronchitis, dyspepsia, piles, fever, tumours, cold, neuralgia and many other diseases[5-10].

Very little work has been reported in literature regarding the phytochemical studies of this plant. The literature survey revealed the isolation of many diterpene, flavonoids and iridoids such as bharangin, bharanginin, sirutekkone[11-15].

MATERIAL AND METHODS

The plant was collected from the forest of Gola Gokaran Nath (District-Lakhempur Kheri,U.P.) in the month January 2009.The plant was identified by HOD Botany & Taxonomist, G.F.(P.G.) College Shahjahanpur. A sample specimen was preserved.

The air dried, coarsely powdered mass was extracted successively using soxhlet with petroleum ether, benzene, chloroform, ethyl acetate and finally with methanol. These extracts were concentrated under reduced pressure. Out of these extracts petroleum ether, ethyl acetate and methanol extracts were considered for further investigation. These extracts were concentrated and chromatographed separately over silica gel using different solvents and their mixtures of increasing polarity. Several fractions were obtained in which the similar fractions were pooled together.

The two different fractions obtained from petroleum ether extract by eluting the column with petroleum ether (fraction: 9 – 20) and petroleum ether – benzene (20:1) (fraction: 46 – 70) showed identical behavior on TLC. These fractions were pooled together. This pooled fraction on TLC gave a broad spot which could not be separated by different solvent system. On concentrating the solution under reduced pressure, a yellowish mass (Compound-**1**) melting at $102^0 – 104^0$C was obtained. The compound was subjected to GC/MS which gave a mixture of eight compounds. The IR Spectrum of compound (**1**), IR (KBr); 3410 cm^{-1} (-OH); 1720 cm^{-1} (-C=O); 1640 cm^{-1} (olefinic bond).

Compound (**2**) was obtained from methanol fraction by eluting with benzene – ethyl acetate (20:1). On GC/MS study the compound was found to be a mixture of four compounds. The mixture of four compounds also identify by the IR, NMR and mass spectral studies. I.R. ν cm^{-1} (KBr) : 3420 cm^{-1} (-OH); 1674 cm^{-1} (double bond strech.); 968 cm^{-1} (trans substituted double bond) ;835, 798 cm^{-1} (tri substituted double bond), ^1H NMR (DMSO), δ 5.31(1H,m,H-6); δ 5.24(1H,d,H-23); δ 5.20(1H,d,H-22); δ 3.52 (1H,m,H-3); δ 1.02(3H,s,CH_3 –19); δ 0.91(3H,s,CH_3 –21); δ 0.87(3H,s,CH_3 –29); δ 0.84(6H,s,CH_3 –26,27); δ 0.67(3H,s,CH_3 –18), ^{13}C NMR (DMSO), δ 37.4(C-1), δ 31.8 (C-2), δ 72.5 (C-3), δ 42.4 (C-4), δ 140.2 (C-5), δ 122.3 (C-6), δ 32.0 (C-7), δ 50.3 (C-9), δ 36.6 (C-10), δ 21.1 (C-11), δ 39.9 (C-12), δ 42.4 (C-13), δ 57.0 (C-14), δ 24.4 (C-15), δ 28.9 (C-16), δ 56.0 (C-17), δ 12.2 (C-18), δ 19.4 (C-19), δ 40.5 (C-20), δ 21.1 (C-21), δ 34.0 & δ 138.4 (C-22), δ 29.3 & δ 129.4 (C-23), δ 51.3 (C-24), δ 31.9 (C-25), δ 19.5 (C-26), δ 21.1 (C-27), δ 25.2 (C-28), δ 12.4 (C-29).

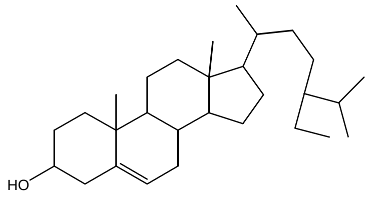
RESULT AND DISCUSSION

The compound (1) was obtained by petroleum ether extract. The identical pooled fractions obtained by eluting the column with petroleum ether and petroleum ether – benzene (20:1) on concentration yielded a yellowish mass. The I.R. spectrum of this compound showed characteristic absorption band for hydroxyl group (3410 cm^{-1}), carbonyl (1720 cm^{-1}) and olefinic bond (1640 cm^{-1}). These findings suggested it to be a long chain hydrocarbon bearing alcohol, carbonyl and unsaturation in the molecule. The compound was further investigated by GC/MS which proved to be a mixture of eight compounds.

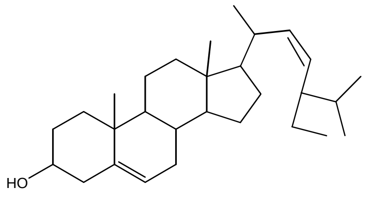
On the basis of the molecular composition, retention time and the percentage of the different compounds observed in GC/MS it was evident that

n- Dotriacont-29-en-4-ol (45.3%) was dominant component of the mixture followed by n-Tetracosan-4-ol (24.7%) and n- Pentacosan-4-ol (16.4) . The n-Untriactontan-5, 21, 26-triol (5.7%), n-Octacosa-21, 25-dien-4-ol (4.1%), n- Tritriaconta – 19, 22, 27-trien – 3 – ol (2.6) and n-Eicosan-9-one (2.6) were found as minor components while n- Docosan-5-ol (0.5%) was found in traces.

The compound (2) was obtained from the methanol extract by eluting the column with benzene – ethyl acetate (20:1) responded Liebermann Burchard test for triterpenoid. The combusted data of the compound ($C_{29}H_{48}O$) suggested it to be a sterol. The I.R. spectrum showed the absorption bands for hydroxyl group (3420 cm^{-1}), double bond stretching (1674 cm^{-1}), trans substituted double bond (968 cm^{-1}) and tri substituted double bond (835, 798 cm^{-1}). The ^1H NMR spectrum of the compound revealed the presence of vinylic and olefinic protons at the range δ 5.31 – δ 0.67 along with the signals of methyl, methylene and methine protons. The ^{13}C NMR spectrum showed more than twenty-nine signals. The detailed analysis of ^1H and ^{13}C NMR spectra of this sample revealed it to be a mixture of two or more sterols. Further identification of constituents was preformed by GC/MS studies which showed that it was a mixture of four components. Thus, on the basis of their mass fragments, GC retention time the four compounds were characterized as Ergosterol, Campesterol, Stigmasterol and β -Sitosterol in which β –Sitosterol (70.8%) was found to be the prominent component followed by Stigmasterol (28.2%). The mass fragmentation showed important molecular ion fragments of major components.



β –Sitosterol



Stigmasterol

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