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Isolation and Characterization of New Lanosteriod from Ethanolic Extract of *Eclipta alba* Linn.

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ABSTRACT

In present investigations, whole plant of *Eclipta alba* was extracted in soxhlet apparatus using 95% ethanol as solvent. The dried ethanolic extract of *Eclipta alba* Linn. was further eluted with mobile phase of petroleum ether: chloroform (1:6) using silica gel column to elute compound 1 i.e. terpenoids (Identified by thin layer chromatography). The compound 1 was subjected to spectroscopic studies (IR, PNMR, ^{13}CNMR and Mass spectroscopy) for structure elucidation. On the basis of the spectral data analysis and chemical reactions, the structure of compound 1 has been established as lanost – 5, 24 – dien – 3 ß – ol – 18, 21 – olide -3 ß- yl tetradecanoate (a lanosteroid).

Keywords: *Eclipta alba*, chromatography, terpenoids, lanosteroid.

INTRODUCTION

Eclipta alba (L.) Hassk (Asteraceae), was commonly known as 'Bhingraj'. It is a small much branched annual herb with white flower heads. Commonly, it is known as 'white bhringraj' when in flower and as 'black bhringraj' when in fruit. It is distributed throughout India, ascending to 1800m. The herb is rich source of ascorbic acid. It is a good source of thiophene derivatives which are effective against nematodes. From this; ecliptal, wedelolactone[1-2], desmethyl wedelolactone [3-5] and its 7-O-glucoside, *ß*- amyrin [6] were isolated. Polypeptides isolated from whole plant gave five amino acids on hydrolysis *viz.*; cystine, glutamic acid, phenylalanine, tyrosine and metionine (leaves/aerial parts) [7]. A new dithienyl acetylene ester isolated from roots and characterized as 5'-isovaleryloxymethylene-2-(4-isovaleryloxybut-3-ynl) dithiophene [8], 5'-tigloyloxymethylene-2-(4-isovaleryloxybut-3-ynyl) ditiophene (III) isolated from aerial parts and roots. It gives glycosides along with eclalbasaponins [9].The plant exhibits anti-inflammatory activity[10]. Wedelolactone and desmethyl wedelolactone possesses potent antihepatotoxic property[11-12]. Alcoholic extract of the herb has antiviral activity against Ranikhet disease. The leaves alone or in combination with ajowan seeds are used in diseases of gall bladder. Plant juice cures skin infections. Bhringraj oil obtained from the plant is applied to scalp before bed time in insomnia. *E. alba* is reported to be effective in the treatment of peptic ulcers. Immunoactive property has also been observed against surface antigen of hepatitis B- virus[2, 13-16]. The fresh plant is used as a tonic and deobstruent in enlargement of the liver and spleen[17]. The plant yields black dye and is used as dyeing agent in textile industries of Assam[18]. The leaf juice boiled with sesamum or coconut oil is used for anointing the head to render the hair black .The root is emetic and purgative[19-20].It is widely used to improve colour and luster of the hair[21-22]. It is a constituent of an Ayurvedic formulation 'Geriforte' which is used in senile pruritus and as antistress[23-24].

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MATERIALS AND METHODS:

Collection of plant material:

The plant material was procured from Khari Baoli Delhi. It was taxonomically identified and authenticated by Dr. M.P. Sharma, Reader, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen has been preserved in Department of Pharmacognosy and Phytochemistry, RIT, Greater Noida (U.P.).

Extraction of plant material:

The plant material was grinded to coarse powder. This coarse powder (3.0 kg) was used for extraction.The exhaustive extraction of plant material was carried out using 95% ethanol in soxhlet apparatus. The ethanolic extract was concentrated to dried residue by distillation. The dark brown mass (210 gm, 7% w/w) thus obtained was subjected to further phytochemical and biological investigations.

Column chromatography:

Preparation of slurry:

The dried extract was dissolved in minimum quantity of methanol in a china dish kept on water bath, then slurry of extract was prepared by adding minimum quantity of silica gel (column chromatography, 60-120mμ) and then air dried. The lower end of a clean dry column was plugged with adsorbent cotton. The column was then half filled with petroleum ether. Silica gel was added in small proportions and allowed to settle down gently until the necessary length of column was attained. All the air bubbles were allowed to escape by running the column blank thrice with solvent. The dried silica gel slurry of the extract was packed in the column and plugged with the adsorbent cotton and then eluted successively in order of increasing polarity with different solvents. The development and elution of the column was carried out with successively series of solvents in various combi-

Table 1: ^{13}CNMR spectrum values of compound 1(VS-KTC09)

Position	dc	Position	dc
1	35.81	16	31.81
2	26.10	17	55.32
3	81.72	18	173.14
4	38.46	19	20.52
5	138.52	20	33.01
6	123.61	21	61.92
7	67.20	22	36.45
8	46.55	23	28.93
9	54.87	24	112.32
10	36.76	25	136.81
11	22.52	26	23.10
12	23.41	27	22.93
13	54.30	28	23.33
14	49.13	29	18.61
15	32.75	30	16.32

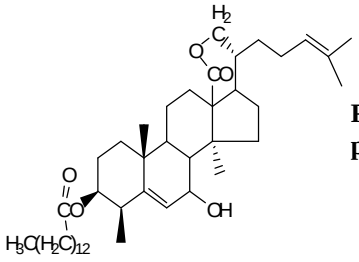

Fig. 1: Structure of isolated compound VS-KTCO9 (Lanosteroid)

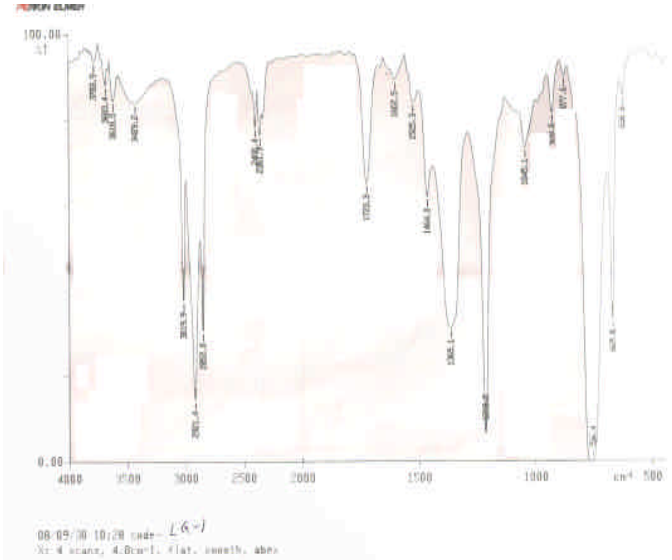

Fig 2: IR Spectrum of compound VS-KTCO9 (Lanosteroid)

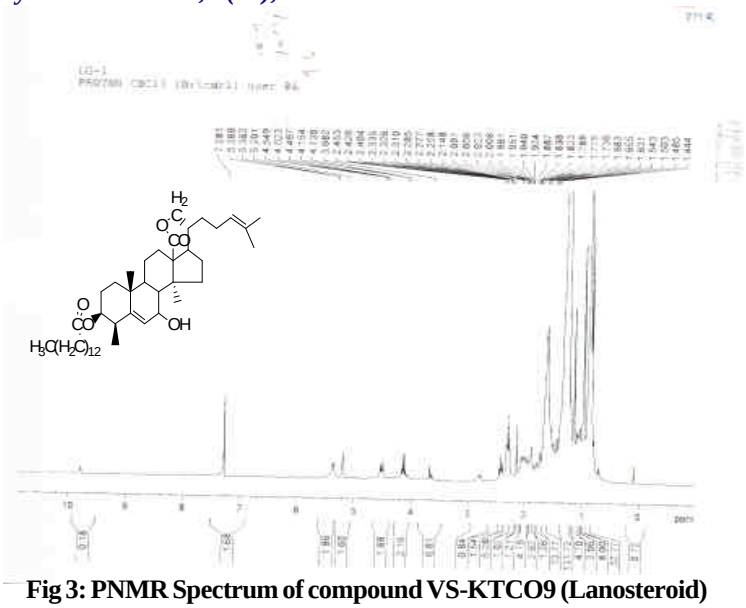

Fig 3: PNMR Spectrum of compound VS-KTCO9 (Lanosteroid)

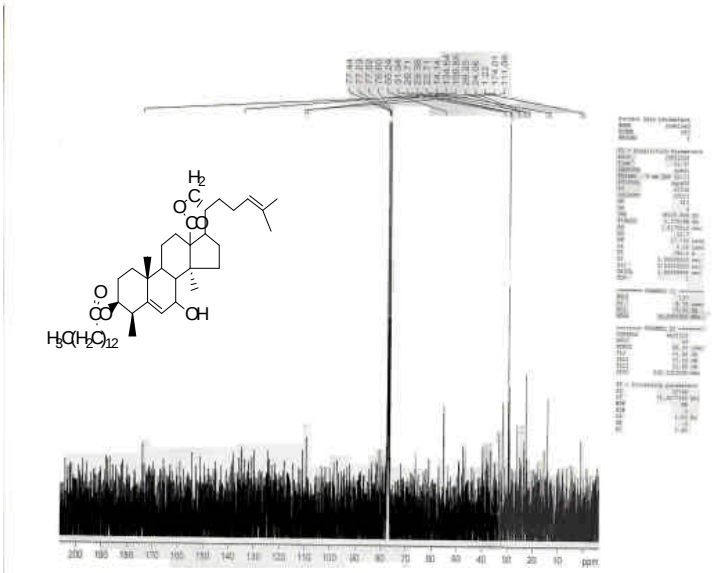

Fig 4: C^{13}NMR Spectrum of compound VS-KTCO9 (Lanosteroid)

nations, *viz.*, Petroleum ether, petroleum ether in chloroform, chloroform, chloroform in methanol and methanol. Before using solvents in column chromatography, all of them were made dried with help of anhydrous sodium sulphate/ calcium chloride. The completion of the elution of the components was confirmed via evaporating a small fraction of the elution.

Homogeneity of the fractions:

The fractions collected were subjected to thin layer chromatography to check homogeneity of various fractions. Chromatographically identical fractions were combined and concentrated.

Isolation of compound:

Elution of the column with Petroleum ether and chloroform (1:6) furnished pale yellow amorphous powder of **(VS-KTCO9)**, recrystallized from methanol, mp 130-131^{0}C, % YIELD 0.12 w/w, Rf - 0.85 **(**Chloroform: Methanol: Petroleum ether- 9: 0.5: 0.5).

IR ?$_{max}$ (KBr): 3429, 3019, 2921, 2852, 1723, 1602, 1464, 1365, 1216, 1045, 756 Cm^{-1} (Fig 2).

^{1}HNMR (CDCl$_3$) *d* 5.38 (1H, d, J = 7.2Hz, H - 6), 5.20 (1H, m, H -24), 4.49 (1H, dd, J =7.2, 10.4 Hz, H -7, *a*), 4.15 (1H, d, J = 11.3 Hz, H$_2$ – 21 a), 4.13 (1H, d, J= 11.3 Hz, H$_2$ – 21 b), 3.68 (1H, dd, J =5.3, 8.5 Hz, H-3*a*), 1.73 (6H, brs, Me -26, Me -271), 1.13 (3H, brs, Me -19), 0.91 (3H, brs, Me – 29), 0.82(3H, brs, Me - 28), 0.79 (3H, brs, Me -30), 2.27 (1H, d, J = 7.6 Hz, H$_2$ – 2'a), 2.25 (1H, d, J=7.6 Hz, H$_2$ – 2' b), 1.53 (2H, brs, CH$_2$), 1.25 (20 H, brs, 10 X CH$_2$), 0.83 (3H, Ê, J = 6.1 Hz, Me - 14). (Fig 3)

^{13}CNMR (CDCl$_3$)

The values of spectra have been shown in Table 1**.**

ESI MS MHz 680 [M]$^+$ ($C_{44}H_{72}O_5$) (Fig 5)

RESULTS AND DISCUSSION:

Compound (VS-KTC09) was obtained from petroleum ether and chlo-

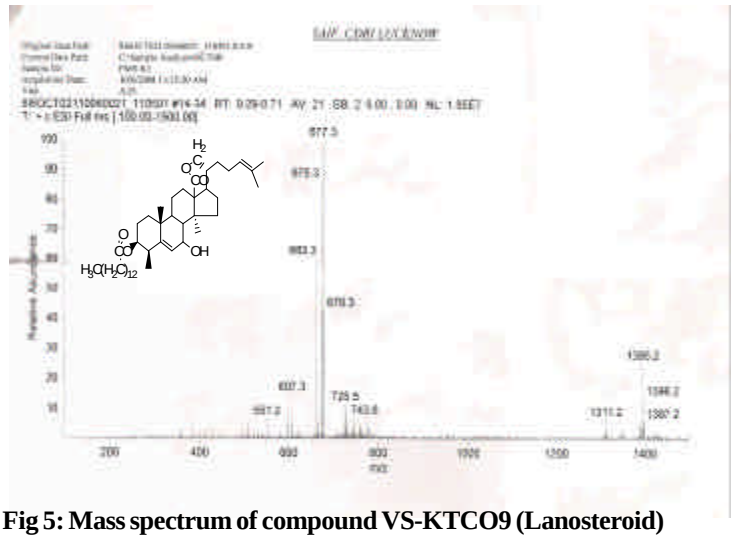

Fig 5: Mass spectrum of compound VS-KTCO9 (Lanosteroid)

roform (1:6) eluents. It responded tests for triterpenic glycosides positively. Its IR spectrum showed characteristic absorption bands for hydroxyl group (3429 cm^{-1}), ester group (1723 cm^{-1}), unsaturation (1602 cm^{-1}), and long aliphatic chain (756 cm^{-1}). Its mass spectrum exhibited molecular ion peak at MHz 680 corresponding to triterpenic ester a d – lactone function, $C_{44}H_{72}O_5$. It indicated nine double bond equivalent; four of them were adjusted in the tetracyclic carbon skeleton of the triterpenoid, two in the vinylic linkage, two in d –lactone and in the ester group. The ^{1}H NMR spectrum of compound 1(VS-KTC09) displayed a one – proton doublet at d 5.38 (J= 7.2 Hz) and a one - proton multiplet at d 5.20 assigned to vinylic H- 6 and H -24 protons. Two one-proton double doublets at d 4.49 (J= 7.2, 10.4 Hz) and 3.68 (J = 5.3, 8.5 Hz) were attributed a – oriented carbinol H -7 and H – 3 protons, respectively. Two, one proton doublets at d 4.15 (J = 11.3 Hz) and 4.13 (J = 11.3 Hz) were ascribed to oxygenated methylene H_2 – 21 protons. A six proton broad signal at d 1.73 was accounted to C -26 and C- 27 methyl protons located on a vinylic carbon. Four broad signals at d 1.13, 0.91, 0.82 and 0.79 were associated with C- 19, C -29, C- 28 and C-30 tertiary methyl protons. Two, one –proton doublets at d 2.27 (J = 7.6 Hz) and 2.25 (J = 7.6 Hz) were due to methylene H_2- 2' protons adjacent to the ester function. Two broad signals at d 1.53 ($_2$H) and 1.25 ($_{20}$ H) were accommodated in the aliphatic chain of fatty acid group. A three proton triplet at d 0.83 (J = 6.1 Hz) was assigned to C- 14' primary methyl protons. The ^{13}CNMR spectrum of compound 1 exhibited signals for ester carbon at d 168.30 (C- 1'), lactone carbon at d 173.14 (C- 18), carbinol carbons at d 81.72 (C - 3) and 67.20 (C - 7), oxygenated methylene carbon at d 61.92 (C - 21), vinylic carbons at d 138.52 (C - 5), 123.61 (C -6), 112.32 (C - 24) and 136.81 (C -25) and methyl carbons between d 23.33 – 14.31[25-26]. Alkaline hydrolysis of compound 1 yielded triterpenic moiety and myristic acid. On the basis of the spectral data analysis and chemical reactions, the structure of compound 1 (VS-KTC09) has been established as lanost – 5, 24 – dien – 3 ß – ol – 18, 21 – olide -3 ß- yl tetradecanoate (m.p. ^{0}C 130-131) shown in Fig. 1.

The results of present study demonstrated that the compound 1 isolated from ethanolic extract of *Eclipta alba* is lanost – 5, 24 – dien – 3 ß – ol – 18, 21 – olide -3 ß- yl tetradecanoate.

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