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ISOLATION AND CHARACTERIZATION OF TRITERPENOIDS FROM METHANOLIC EXTRACT OF MEDICINAL PLANT - ECLIPTA ALBA

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Abstract

Pharmacological studies should still be performed using new extract fractions, in order to isolate and characterize the active principle responsible for the Anti inflammatory, anticancer anti hepatotoxic, Antioxident, Antidiabetic Antihyperlipedemic. In present investigations, whole medicinal plant of eclipta Alba was extracted in Soxhlet apparatus using 95% methanol as solvent. The dried extract of Eclipta Alba was further eluted with mobile phase of petroleum ether: chloroform (1:6) using silica gel column to elute compound (Terpenoids) which is identified by thin layer chromatography (Rf : 0.85). The compound was subjected to spectroscopic studies (IR, PNMR, - ^{13}CNMR) for structure elucidation. On the basis of the spectral data analysis and chemical reactions, the structure of compound (PVSN-4) has been investigated as “Lanost-5, 24 dien-3 beta-ol-18,21 olide-3 beta- yl Tetra decanoate”

Key words: Eclipta, Alba, Thin layer Chromatography (TLC), Terpenoids.

Introduction

The branched annual herb Eclipta Alba has been used on the treatment of hepatic diseases, hyperlipidemia and snake venom poisoning in folk medicine. Triterpenoids, Coumestanes and flavonoids have been reported as constituents of Eclipta species (Singh & Bhargave 1992; Yahara et.al.,1997; Wagner et.al.,1986). In addition, Eclipta species have been reported to exert diverse biological activity including hepatoprotective,Antiinflammator,

Antihemorrhagic, Antihyperlipidemic and Antihyperglycemic activities (Saxena et.al.,1973;Melo et.al., 1994; Kumari et.al., 2006)

The Coupling of Liquid Chromatography with Spectroscopic techniques such as UV , IR, NMR Provides a useful tool for rapid data collection and structure Elucidation (snyder et.al.,1997). The use of hyphenated LC/UV/IR/NMR Instrumentation has been reported on numerous applications (Wolfender et.al., 1998). This technique is fast and the short time of exposure of the analysis to light and air limits their degradation.

Here we present this results of a more Comprehensive investigation of chemical composition of E. Alba using reversed phase liquid chromatography.

In recent decades, phytochemicals have been of great interest, as the sours of natural Anti Oxidant, Antitumor and Antimicrobial activities.

The present work highlights the results of studies carried out to identify the constituents (Terpenoids) of the aromatic medicinal plant E. Alba.

We would further synthesize their Transition Metal Complexes especially with platinum, Gold, Palladium, Ruthenium.

The Precursors, their derivatives and the Transition Metal Complexes are all excellent Candidates as Anticancer Drugs.

Materials and Methods

All chemical were purchased from Sigma and all the reagents used were of Analytical grade.

The Medicinal plants of E. Alba were collected from botanical garden of Bio-Technology Department, GITAM University Visakhapatnam, Andhra Pradesh India. The plant was duly authorized and a voucher specimen (PVSN-EA-2011) has been deposited in the Herbarium of the medicinal plant garden. The three month old 950 gm. Of leaves of the plant were dried at room temperature, powdered and extracted with methanol (95 % v/v) in soxhlet apparatus for 3 days. The extract was filtered and reduced the solvent, adsorbed on silica gel (100-200 mesh) and subjected to column ehromatography.

Column Chromatography:

Preparation of Slurry:

The dried extract was dissolved in minimum quantity of methanol in a soxhlet apparatus for 3 days, then the slurry of extract was prepared by adding minimum quantity of silica gel(100-200 mesh) and then air dried. The lower end of a clean dry column was plugged with adsorbent cotton. The cotton 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 thin eluted successively in order of increasing polarity different solvents. The Development and elution of the column was carried out with successively series of solvents in various combinations such as n-Hexane and ethyl acetate (5%, 10%,20%,30%,40%, 50%,70%) all of them were made with help of anhydrous calcium chloride.

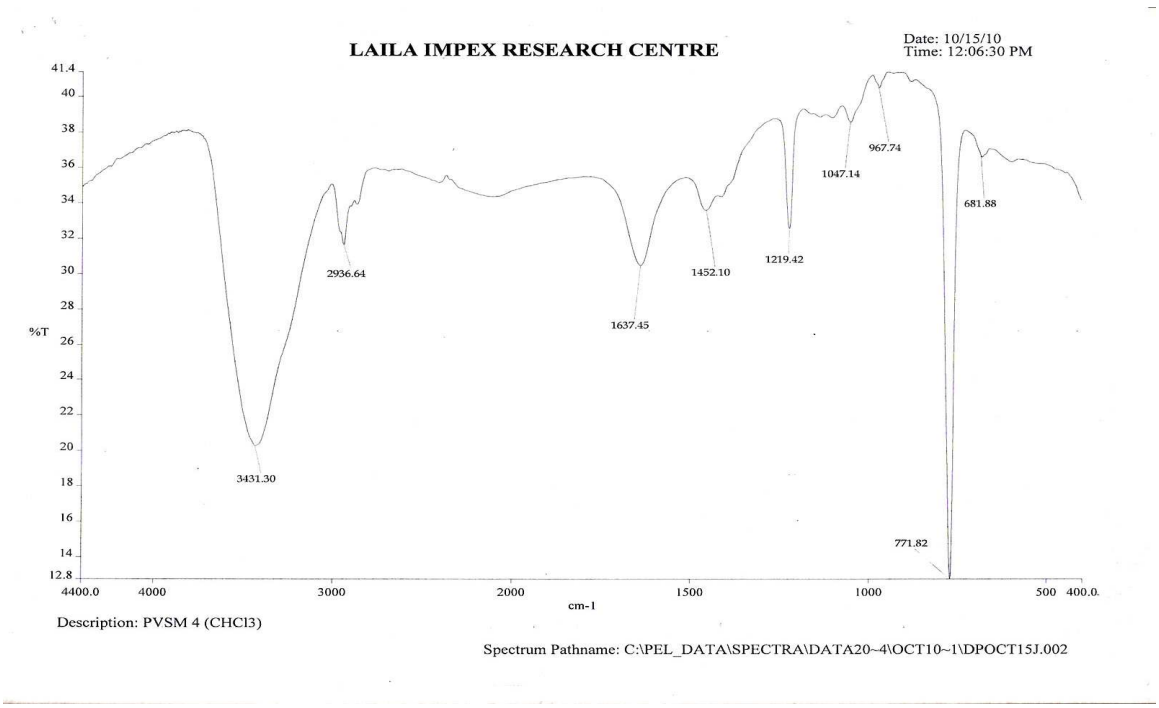
The Completion of the elution of the components was confirmed via evaporating a small fraction of the elution.

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

Results and Discussion

Elution of the column with different solvents, furnished pale yellow amorphous powder of (PVSN-4) obtained. Recrystalized from methanol,

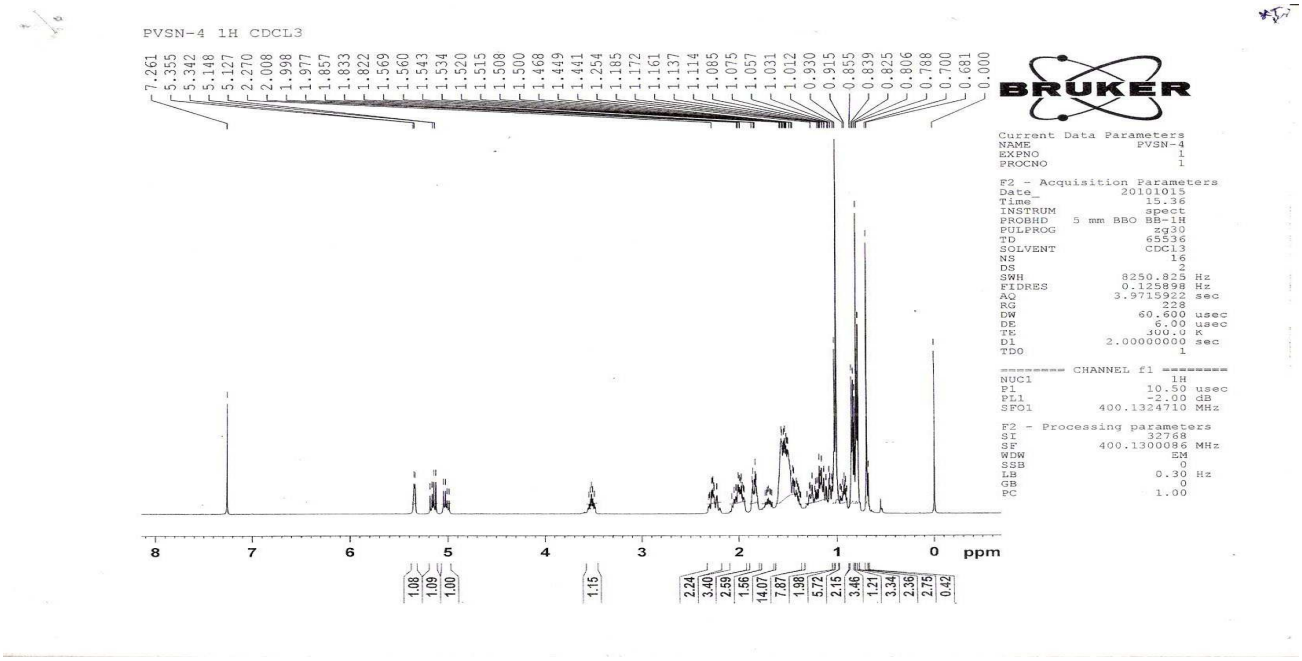
m.p:131 ^{0}C, Rf (TLC):0.85



From IR-SPETRUM (PVSN-4($CHCL_3$)

3431.30,2936.64, 1637.45, 1452.10,

1219.42, 1047.14, 771.82 CM^{-1}(FIG.1)



From PNMR (PVSN-4 1H $CDCL_3$)

D 5.38 (1H,d J=7.2 H_2, H-6), 5.20(1Hm H.24)

3.68 (1H dd J=5.3,8.5 H2, H-3a)

1.73 (6H, brs, Me-26,M-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)

2.24 (1H, d, J-7.6 H_2, H_2-2a)

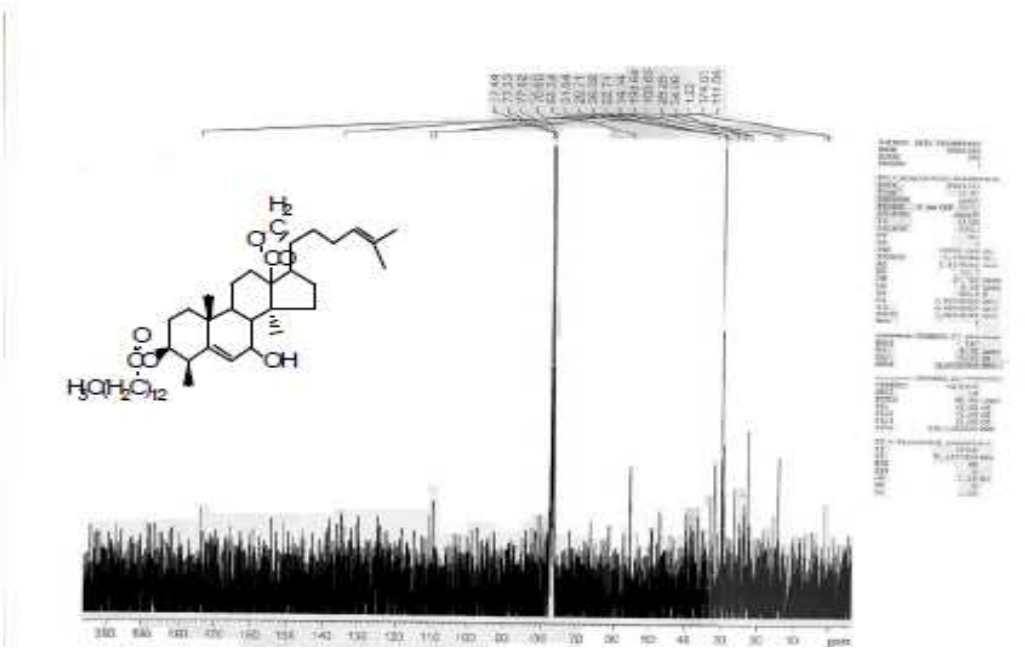
1.53 (2H,brs,CH_2)

1.25 (20H, brs, 10 X CH_2)

0.83(3H,E, J-6.1 H_2, Me-14)

^{13}CNMR ($CDCL_3$)

^{13}CNMR Spectrum of Compound (PVSN-4).



^{13}CNMR spectrum values of compound (PVSN-4).

Position	DC	Position	DC
1	35.77	16	31.90
2	26.14	17	55.40
3	82.00	18	173.10
4	38.50	19	21.10
5	137.93	20	33.14
6	124.00	21	62.10

7	67.14	22	36.50
8	46.61	23	29.10
9	54.90	24	112.41
10	37.10	25	137.10
11	22.64	26	23.14
12	24.10	27	23.10
13	54.33	28	23.30
14	49.20	29	18.70
15	33.10	30	16.40

The compound PVSNL-4 was responded for triterpenic glycosides test positively its IR – spectrum showed characteristic absorpsion bands for hydrogel group (3431 cm^{-1}), maturation (1637 cm^{-1}) and long aliphatic chain (771 cm^{-1}).

The PNMR Spectrum of compound (PVSN-4, 1H $CDCl_3$) dinplayed a one-proton doublet at 5.38 (J=7.2M_2) and a one-proton multiplet at d 5.20 assigned to rinylic H-6 and H-24 protons.

One proton double doublets at d 3.68 (J=5.3, 8-5 H_2) was attributed, α-oriented carbinol H-7 and M-3 protons respectively. A six proton broad signal at d 1.73 was accounted to C-26 and C-27 methyl proton 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. One-motion doublets at 2.24 (J-7.6H_2) was done to methyline H_2-2a motions adjacent to the ester function. Two broad signals at d 1-53 (2H) and 1.25 (20M) were accommodated in the aliphatic chair of fatty acid group. A three protons triplet at 0.82 (J-6.1 H_2) was assigned to C-14 primary methyl protons.

The ^{13}CNMR spectrum of compound (PVSN-4) exhibited signals for ester carbon d 35.77 (C-1) lactone carbon at d 173.10 (C-18), carbinol carbon at a 82.0 (C-3) and 67.14 (C-7) oxygenated methylene carbon at d 62.10 (C-21), virylic carbons at d 137.93 (C-5), 124.00 (C-6), 112.41 (C-24) and 137.10 (C-25) and methyl carbon between d 23.3-14.31 Alkaline hydrolysis of compound (PVSN-4) yielded triterpenic moiety and myristic acid. On the basis of the spectral data analysis and chemical reactions, the structure of the compound (PVSN-4) as "Lanost-5,24 dien-3 beta-ol-18,21 olide-3 beta- yl Tetra decanoate"

Because of having the Carbonyl group, there is a chance to synthesize the Anticancer Drugs. Work in progress towards.

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