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ISOLATION AND CHARACTERIZATION OF PHYTOCONSTITUENT FROM *ECLIPTA ALBA* HASSK

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ABSTRACT

There are varieties of bioactive compounds in plants, such as alkaloids, tannins, flavonoids, sterols, triterpenes etc., noted to have a major role in nutrition, physiology and control of diseases. The foremost important task in this exemplar is the screening of compound in the plants. The chromatographic study of the compounds serves to be a very valuable and reliable source in the progression of bioactive compounds screening in plants. The repeated fractionation of active ethyl acetate fraction of ethanolic extract of *Eclipta alba* by silica gel column chromatography yielded, white amorphous powder obtained by concentrating the eluent fractions (125 - 169 fractions) and this compound designated as EA-1. The compound EA-I which isolated from this column chromatography was subjected into spectral studies for the determination of the structure. Characterization of isolated compounds by UV, IR, ^{1}H NMR, ^{13}C NMR, DEPT 90, DEPT 135, HMBC, HSQC, ^{1}H - ^{1}H COSY and Mass spectroscopy. Isolated compound from *Eclipta alba* was determined as 2´ acetyl eclalbasaponin II, chemically *O*-[*β-D*- 2' acetyl gluco pyranosyl] Echinocystic acid.

KEYWORDS

Eclipta alba, Column chromatography, TLC, IR, NMR, UV and Mass spectroscopy.

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INTRODUCTION

Plants have the ability to synthesize wide range of chemical compounds, which are used to perform important biological utility and also to protect itself from predators like insects, fungi and herbivorous mammals. Many of these phytochemicals have beneficial effects on long term health and also in the cure of human disease. At least 12000 such compounds have been isolated, which is in point of fact about less than 10 % of the total[1]. These medicinal plants or herbs also show the

antimicrobial and chemo preventive properties[2]. Ethno botany is recognized as the effective way to discover future medicine. In 2001, researchers recognized 122 compounds which are used in modern medicine, 80 % of them have the ethno medicinal use of the active element of the plant[3]. The presence of medicinal activities in medicinal plants is due to presence of dissimilar metabolites that is most important metabolites and secondary bioactive compounds. Primary metabolites are of major significance and are essentially requisite for example sugar, protein, lipid and starch. Many primary metabolites be active as precursor pharmacologically vigorous metabolites. The chemicals which are responsible for colours and smell into the plant are identified as secondary metabolites or phytochemicals. Phytochemicals have been used as drugs since long in the earlier period. Many of the secondary metabolites have been monitored from medicinal plants and have been used in herbal therapy.

Recent studies are involved in the detection and separation of new therapeutic compounds of medicinal importance from the plants for specific diseases[4,5,6]. The secondary metabolites present in the plants such as alkaloids, tannins, flavonoids, sterols, triterpenes *etc.*, well-known to have the main role in nutrition, physiology and control of diseases[7,8]. Knowledge of the isolation and characterization of biologically active constituents of plants is desirable because such information will be of significance for the synthesis of composite chemical substances. Hence chemical constituent from the Ethyl acetate fraction of ethanolic extract of *Eclipta alba* was isolated by column chromatography followed by characterization of isolated compounds by UV, IR, NMR and Mass spectroscopy.

MATERIAL AND METHODS

Collection and confirmation of plant materials

The plant specimens for the proposed study *Eclipta alba* (L), Hassk was collected from the rice fields and other nearby irrigated fields in and around Madurai District, Tamil Nadu, India during the month of October 2009. The herbarium of these plants was identified and authenticated by Dr. D. Stephen,, American college of Arts and Science, Madurai.

PREPARATION OF PLANT EXTRACTS

The fresh whole plant of *E. alba* was cleaned with distilled water to separated unnecessary foreign materials like soil and dusts. After, washed plant material was dried in shadow at room heat without direct exposure of sunrays. It was then coarsely grounded by using automatic device. The pulverized plant substance was passed through sieve no 40 and stored in an airtight container for future use.

The roughly crushed plant materials of *E. alba* (2000 g) were extracted separately to exhaustion in a soxhlet equipment for 72 hours by using Petroleum ether (60 - 80˚C) and methanol (95%) solvent (Merk and Spectrum Chemicals, India) systems. Extracts obtained were filtered through a cotton plug followed by what mann filter paper (No.1) and concentrated by using a rotating evaporator at low down temperature (40 - 50˚C) and reduced pressure to get 24.4 g and 108.6 g respectively. The extracts were conserved in airtight containers and kept at 4˚C until further use.

COLUMN CHROMATOGRAPHY

The (EEEA) ethyl acetate fraction of ethanolic extract of *Eclipta alba* was subjected to column chromatography based on phytochemical analysis. The collected fractions were further evaluated by Thin Layer Chromatography (TLC) to identify the number of constituents present. Silica gel G was used as stationary phase.

Column chromatography was completed by using a glass column. The proportions of the column were 100 cm in length and 4 mm in diameter. The column was filled with silica gel by wet packing method where in the padding of cotton was placed at the bottom of the column and then it is packed with the eluting solvent of lowest polarity (Pet. ether). Then the required amount of stationary phase (silica gel) was dispensed into the column to form a bed of silica, a thin pad of cotton was placed over it.

The rest of the column was filled with the solvent of the lowest polarity (Pet. ether) and eluted gradually.

THIN LAYER CHROMATOGRAPHY (TLC)

Among the diverse methods of separation of the plant constituents the chromatographic procedure is one of the most commonly used techniques. A chromatography is essentially a technique for the separation of the components of the mixture by a constant distribution of the components between the two phases, one of which is moving faster than other. TLC has turn out to be broadly adopted technique for the standardization of the herbal products. TLC provides drug fingerprint and it is a precious technique for the rapid and reliable monitoring of the identity and clarity of drugs. It is also used for the recognition of adulteration and substitution. Furthermore TLC offers a wide choice of mobile phases. TLC has assured advantages more than paper chromatography. Fractionations can be effected more rapidly with smaller quantities of the mixture; the separated spots are usually more compact and more clearly demarcated from one another and the character of the film is regularly such that drastic reagents such as concentrated sulphuric acid, which would destroy a paper chromatogram, can be used for the position of divided substances.

Two thin layer chromatography methods are available, the traditional method and the micro method, which uses diverse sizes of plates and hence different quantities of solvents.

The ethyl acetate extract concentrate (20 gm) of *Eclipta alba* was chromatographed in silica gel (60 – 120 mesh 300 gm, 100 x 5 cm) column. Elution of the column was carried out with different ratios of toluene and ethyl acetate. The fractions of 100 ml were collected each time. The fractions were monitored by using TLC (pre coated TLC plate, silica gel G_{60} F_{254} with fluorescent indicator, Merck, India). The spots on TLC were observed using UV chamber.

The fractions 96 - 109 yielded a single compound. By comparison of Co TLC with standard compound it was identified as wedel lactone.

The fractions 125 - 169 yielded a single compound. On concentration of these fractions and recrystalization in ethyl acetate and chloroform, it yielded a white amorphous powder. The compound responded to the chemical test for triterpenoid (Noller's test) and glycoside (Molisch's test). The isolated compound was designated as EA-1. The running properties of EA-1 in different solvents were tabulated in Table No.2.

RESULTS AND DISCUSSION

The ethyl acetate soluble fraction of ethanolic extract of EA (EEEA) was subjected to column chromatographic separation and purification to yield a isolated compound EA-1.

Structural Elucidation of Isolated Compound from Ea-1

On column chromatography of EA - 1, 250 mg of a white amorphous powder was obtained.

LC - MS Chromatogram of Ea-1

The eluted fractions 125-169 were subjected to LC-MS analysis. It revealed that eluted fractions contain a single compound. It showed the retention time of 2.595. The chromatogram is given in Figure No.1.

The UV, ^{1}H-NMR, ^{13}C-NMR, DEPT 135, ^{1}H-^{1}H COSY, HSQC, HMBC and ESI-MS were recorded for EA-1.

UV Spectrum of Ea-1

The EA-1 showed an intense λ_{max} at 222, 262 and 268 nm. The spectrum is shown in Figure No.2.

IR Spectrum of Ea - 1

The IR spectrum is shown in Figure No.3. The spectral data of compound EA-1 and their group assignments are tabulated in Table No.3. The IR spectrum of EA-1 showed a shallow peak at 3407.6 cm^{-1} indicating the presence of OH stretching. The intense peak at 2937.06 cm^{-1} showed the presence of aliphatic CH stretching in EA-1. The peak at 1454.06 cm^{-1} confirmed the presence of C = C stretching. The peaks at 1078.0 and 1029.8 cm^{-1} were due to the presence of OH and C – O stretching in the molecule EA-1.

^{1}H-NMR Spectrum of Ea-1

The ^{1}H-NMR spectral data of EA-1 and corresponding signal assignments are given in Table No.4.

The ^{1}H NMR spectrum displayed a one proton broad singlet at δ 5.34 ppm, which indicated the presence of olefinic proton. Seven singlets, each of three proton intensity at δ 0.82, 0.88, 0.95, 1.05, 1.25, 1.16 and 1.80 ppm in the ^{1}H NMR spectrum suggested the presence of seven tertiary methyl groups in compound EA - 1. The signals observed at δ 4.47, 3.28, 2.99 and 2.19 ppm were assigned for the protons at H-16, H-3, H-18 and H-9 position. A downfield proton signal at δ 4.36 was assigned for the anomeric proton of the glucose moiety.

^{13}c – NMR Spectrum of Ea-1

In ^{13}C NMR spectrum a resonance at δ 178.8 ppm was characteristic for a carboxylic acid group. In the HSQC spectrum the proton signals at δ 5.34, 4.47, 4.36, 3.18, 2.99 and 2.19 ppm showed chemical shifts of directly bonded carbon at δ 121.5, 75.0, 104.7, 88.9, 39.89 and 46.1 ppm respectively. The anomeric proton at δ 4.36 ppm and the sugar proton signals and five carbon signals at 60 – 70 ppm revealed the presence of sugar residue in the compound EA-1.

The methyl proton at 1.04 (H-27) showed correlation with the carbon signals at 40.8 (C-14) δ 34.7 ppm (C-15). The proton signal at 0.86 ppm (H-7) have correlation with carbon signals δ 39.8 (C-8) and δ 36.1 (C-10). The proton signal at 0.75 ppm (H-5) showed correlation with carbon signals at δ 47.6 (C-9) δ 39.8 (C-8) δ 32.4 (C-7).

Mass Spectrum of Isolated Compound Ea-1

The ESI - MS mass spectral data are given in Table No.5.

The mass spectrum of EA-1 showed the molecular ion peak at *m/z* 675.64 $(M - 1)^{-}$ in the negative ion mode, a fragment ion at 633.64 ($M - 1 - CO - CH_3$) and 453 (M - Glu) by the loss of glucose moiety.

The chemical and spectral studies indicated the presence of echinocystic acid. IR, ^{1}H-NMR, ^{13}C-NMR, HSQC and HMBC spectral data indicated the presence of carboxylic acid group and the ester linkage in the EA - 1. On the basis of chemical test, R_f value, UV, IR, ^{1}H-NMR, ^{13}C-NMR, DEPT 90, DEPT 135, ^{1}H-^{1}H COSY, HSQC, HMBC and ESI-MS the isolated compound EA1 has been 2´ acetyl eclalbasaponin II.

Molecular formula: $C_{38}H_{60}O_{10}$

Table No.1: Chromatographic fraction of *e.Alba*

S.No	Fractions Collected	Eluent Composition	Remarks
1	1 - 40	100 % Toluene	Mixture of compounds
2	41 - 79	90 : 10 Toluene : Ethyl acetate	Mixture of compounds
3	80 - 95	80 : 20 Toluene : Ethyl acetate	Double compound
4	96 - 109	80 : 20 Toluene : Ethyl acetate	Single compound
5	110 - 115	70 : 30 Toluene : Ethyl acetate	Mixture of three compounds
6	116 - 124	70 : 30 Toluene : Ethyl acetate	Mixture of two compounds
7	125 - 169	70 : 30 Toluene : Ethyl acetate	Single compound
8	170 - 182	70 : 30 Toluene : Ethyl acetate	Single compound but low yield

Table No.2: Running properties of isolated compound ea - 1

S.No	Solvent system	R_f x (100)
1	Toluene : Chloroform (7 : 3)	10
2	Toluene : Ethyl acetate (8 : 2)	18
3	Toluene: Ethyl acetate : Methanol (7 : 2 : 1)	70
4	Ethyl acetate : Methanol : Acetic acid (9 : 1 : 0.1)	96

Table No.3: IR spectral data of isolated compound ea-1

S.No	Peak (cm^{-1})	Group assignment
1	3407.6	OH –Stretching
2	2937.06	Aliphatic C-H Stretching
3	1687.41	C=O stretching
4	1454.06	C=C stretching
5	1078.0,1029.8	OH-Primary alcohol stretching and C-O stretching

Table No.4: ^{1}H – NMR spectral data of isolated compound ea-1

S.No	Chemical shift value (δ_H ppm)	Signal assignment
1	5.34	s, 1 H, H-12
2	4.47	1 H, s H-16
3	4.36	1 H d, (J=7.9 Hz) Anomeric proton H-1´
4	3.811	d, (J = 11.8 Hz) H-6´
5	3.46	1H, m H-3´
6	3.57	1H, m H-4´
7	3.20	1H, (d, J = 5.0 Hz) H-5´
8	3.70	1H, (d, J = 4.9 Hz) H-2´
9	3.283	1H, m, H-3
10	2.990	1H, (dd, J = 4.7 Hz) H-18
11	2.199	1H, (d, J = 5.0 Hz) H-9

Table No.5: Mass spectral data of isolated compound ea-1

S.No	Mass Peak *m/z*	Assignment
1	*m/z* 675	$(M - 1)^-$
2	*m/z* 633	$(M - 1 - CO - CH_3)$
3	*m/z* 453	$(M - Glu)^-$

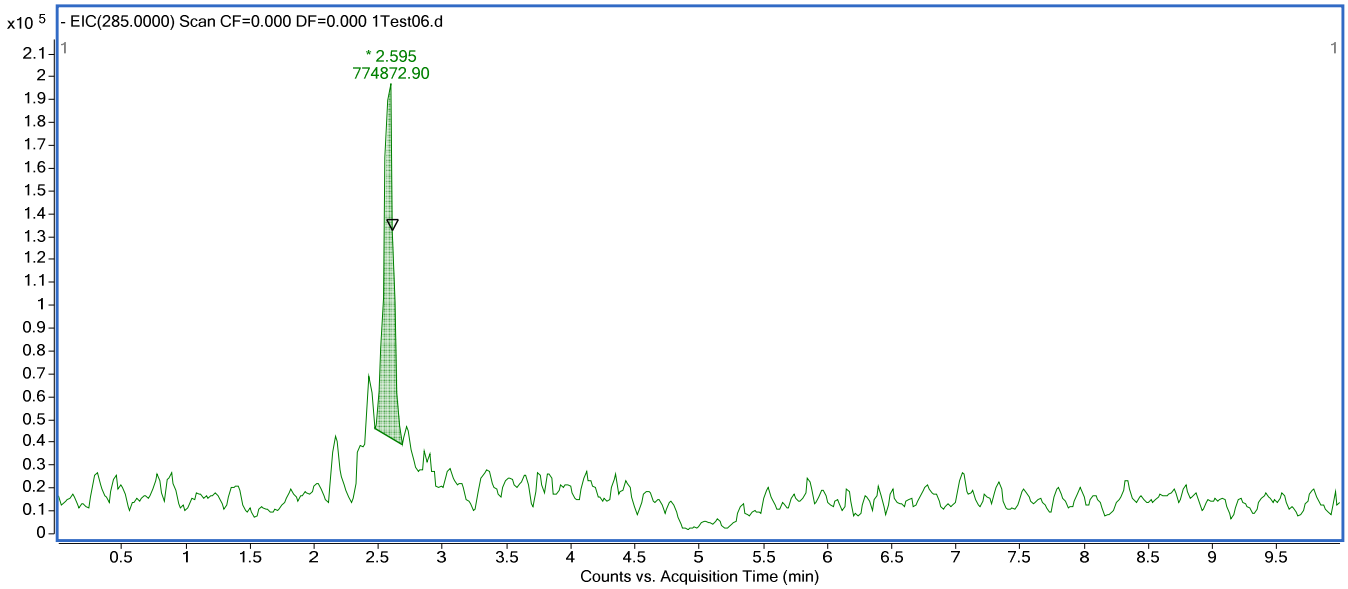

Figure No.1: LC – MS chromatogram of isolated compound from *E. alba*

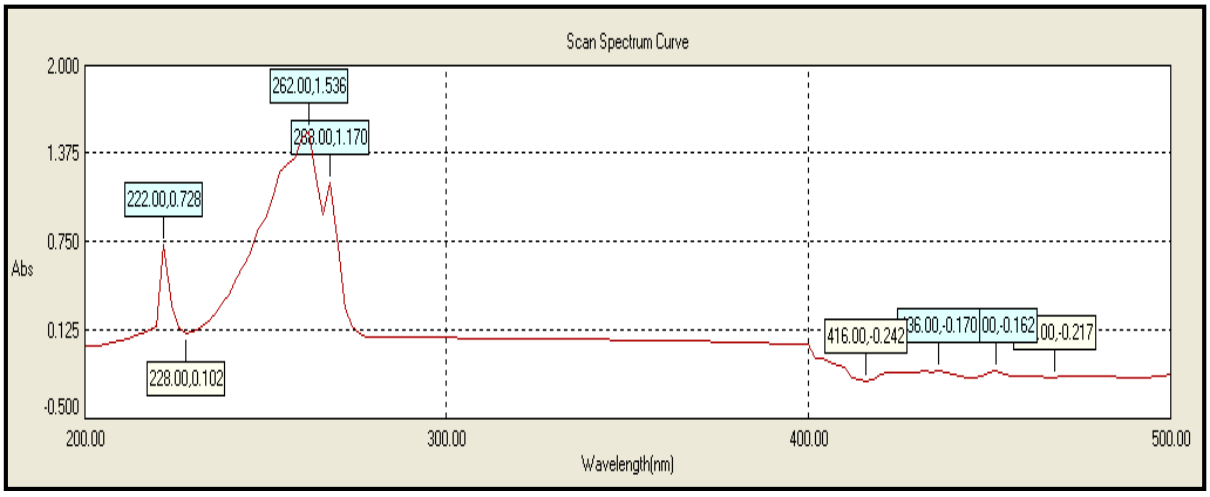

Figure No.2: UV spectrum of the isolated compound from *E. alba*

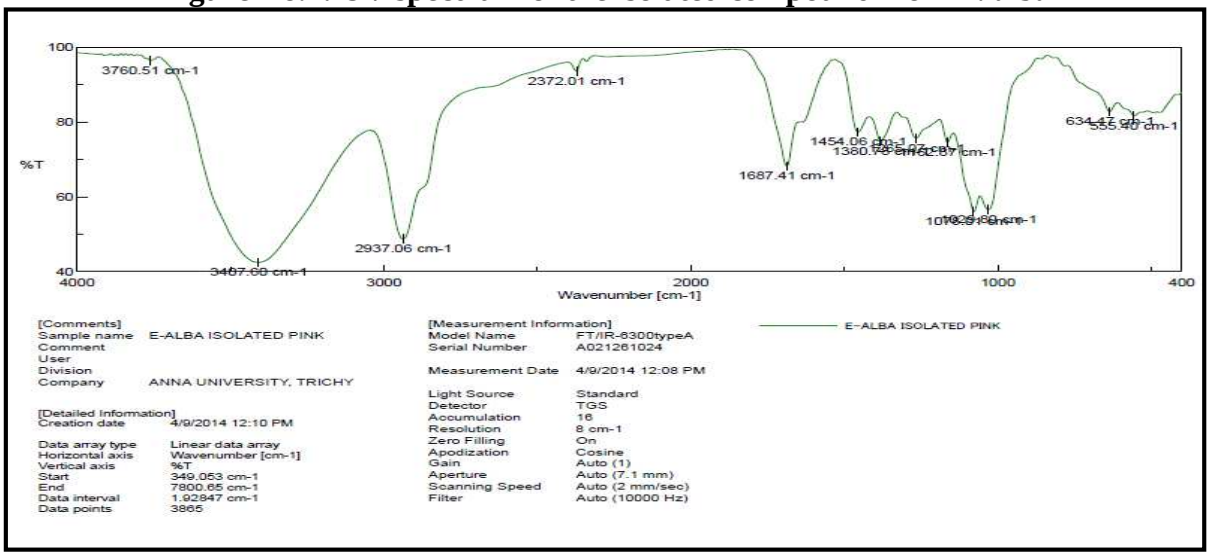

Figure No.3: IR spectrum of the isolated compound from *E. alba*

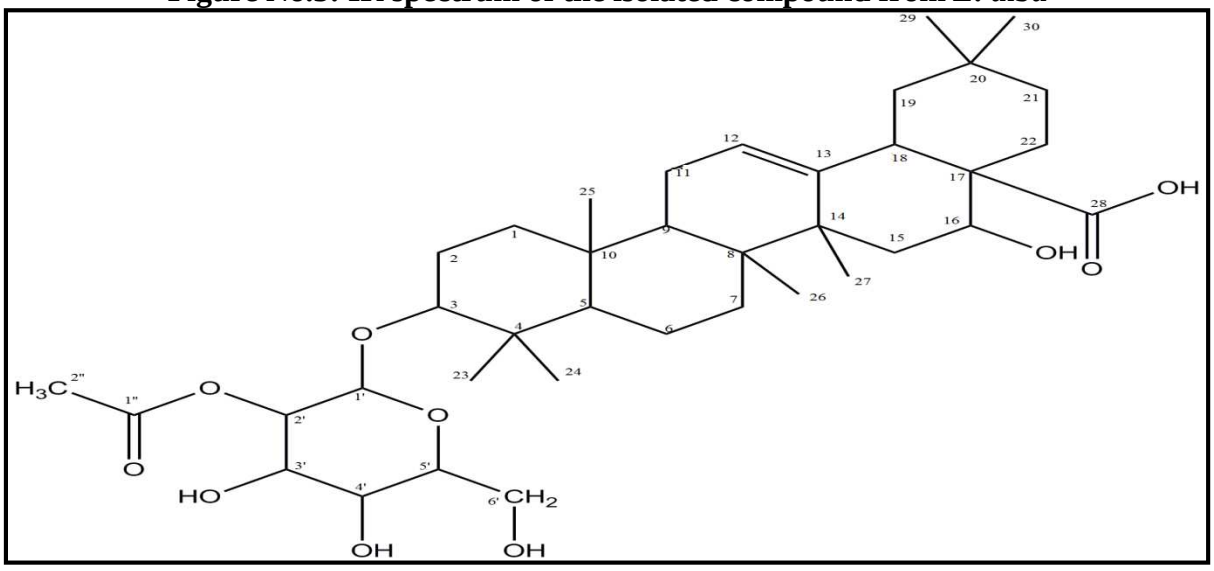

Figure No.4: Structure of isolated compound 2´ acetyl eclalbasaponin II

CONCLUSION

The present study was projected to isolate and characterize the phytoconstituent from the medicinal plant *Eclipta alba.* From the above procedural workout, it can be clearly concluded that the plant *E. alba* does contain the compound EA -1.Characterization of isolated compound EA -1 by UV, IR, ^{1}H NMR, ^{13}C NMR, DEPT 90, DEPT 135, HMBC, HSQC, ^{1}H - ^{1}H COSY and Mass spectroscopy. Based on the spectral studies, isolated compound from *Eclipta alba* was determined as 2´ acetyl eclalbasaponin II, chemically *O*-[*β-D*- 2' acetyl gluco pyranosyl] Echinocystic acid.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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