Research Article

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ANALYSIS OF WEDELOLACTONE IN *ECLIPTA ALBA* AND ITS FORMULATION BY HPTLC

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ABSTRACT

A HPTLC method to determine wedelolactone in *Eclipta alba* and its herbal formulation was developed. The chromatographic separation was performed on silica gel GF 254 precoated HPTLC plates. Ethyl acetate: methanol: water: glacial acetic acid (9: 0.5: 0.5: 0.2) was used as mobile phase. RF value of wedelolactone was 0.72. Calibration plots of peaks area against concentration were linear in the range of 1 µg to 5 µg. The limit of quantification was 0.9µg and limit of detection was 0.3µg. The amount of wedelolactone content in *Eclipta alba* and its formulation was found to contain 0.173%. And 0.131% respectively. The developed HPTLC method is accurate, precise, simple, rapid and selective.

Keywords: *Eclipta alba*, Standardization, Wedelolactone, HPTLC

INTRODUCTION:

Eclipta alba is an erect or prostrate annual herb belonging to family Asteraceae. The plant is distributed throughout India in wet or moist waste lands ascending upto 2000 meters on the hills [1-2]. The plant has a bitter and hot sharp dry taste; anthelmintic; good for complexion, hair, eyes, and teeth; cures inflammations, hernias, eye diseases, bronchitis, asthma, leucoderma, anemia, diseases of heart and skin, itching, night blindness, syphilis; used to prevent abortion and miscarriage, and for uterine pains after delivery. It is principally used as tonic and deobstruent in hepatic and splenic enlargements and in various chronic skin diseases. There is a popular opinion that the herbs taken internally and applied externally will turn the hair black[3]. The drug is also reported to exhibit antifungal, hepatoprotective, hair tonic, for relieving headache and use ful in juandice[4-11]. The plant contain coumestan derivatives, wedelolactone and demethyl wedelolactone, ecliptal, a thiophene derivative, saponins, e.g. eclabosaponins, common sterols and triterpenoids - hentriacontenol, 14-heptacosanol, flavonoids, e.g. leuteolin-7-O-glucoside, alkaloids and polypeptides[12-15]. Because of its widespread use in various geographic region, it is important to standardized the plant material. Therefore we have developed a HPTLC method for standardization of *Eclipta alba* and its formulation using wedelolactone as marker compound.

MATERIAL AND METHOD:

The aerial parts of the material was collected from Chennai and authenticated by Dr. D. Suresh Baburaj, Survey of Medicinal Plants, Collection Unit, Ootacamund. The plant was freed from earthy material, shade dried, cut into small pieces and powdered. Polyherbal formulation were purchased from a medical shop and labeled Formulation. Standard phytoconstituent (wedelolactone) was obtained from Laila impex (A.P), India as a gift sample.

High performance thin layer chromatography:

All organic solvents used in the HPTLC studies were of AR grade.

Standard wedelolactone solution:

1mg/ml stock solution of wedelolactone was prepared in methanol. Various concentration were prepared from the stock solution.

Sample preparation:

One gm of powder was extracted with 20 ml of methanol by heating for 15 minutes. The extract was filtered through the whatmann filter paper. The operation was repeated twice. The combined extract was evaporated to 10 ml.

Powdered polyherbal formulation (equivalent to 1g of *Eclipta alba*) was weighed and extracted with methanol by heating. The methanol extract was filtered through whatmann filter paper. The process was repeated twice and all methanol extracts were combined and concentrated. The final volume was made up to 10ml with methanol in a 10ml standard volumetric flask. Both solutions were used for the HPTLC analysis.

Chromatography:

The chromatography was performed on aluminium backed silica gel GF254 pre-coated HPTLC plates. The plates were prewashed with methanol and dried. Standard solution of wedelolactone and samples were applied to the plate as 8mm bands by means of Linomat IV sample applicator. The plates were developed with ethyl acetate : methanol : water : glacial acetic acid (9 : 0.5 : 0.5 : 0.2) in a twin trough chamber. The plates were dried and the separated compounds scanned in a densitometer at 366nm by means of TLC scanner III controlled by CATS V.4.06 software. The peak areas were recorded for all the peaks. The amount of wedelolactone was calculated from peak areas.

RESULT AND DISCUSSION:

Chromatography:

The HPTLC chromatogram of wedelolactone is given in figure 1 The Rf value of wedelolactone was 0.72. The absorption spectrum of Wedelolactone is shown in the figure 2.The wavelength 366nm was selected for the detection for both standard and sample. The quantity of wedelolactone in herb was found to be 0.173%. The quantity of wedelolactone in the formulation was found to be 0.131%.

System suitability:

System suitability studies were performed on freshly prepared stock solution of wedelolactone to ascertain the effectiveness of the developed method.

Linearity and limits of quantifications and detection:

Calibration plot of peak area against concentrations were linear in the range 1 µg

to 5 µg for wedelolactone. The calibrations line was represented by the linear equations, Y.wed = 5285.98 x + 3143.44 for this equation the co-relation coefficient, r, was 0.999. The limit of quantification's (LOQ) and limit of detection (LOD) were calculated by the use of the equation LOD=3 x N/B and LOQ = 10 x N/B.

Where N is the standard deviation of peak areas of the drugs (n=3) taken as the measure of the noise and B is slope of corresponding calibration curve.

The limit of quantifications was 0.9µg and limit of detection was 0.3µg finger printing profile of *Eclipta alba extract* and formulation shown in figure 4 and 5.

Accuracy and precision:

The accuracy and precision of the method were studied by performing experiments by standard addition technique. Three different labels of standards were added to a previously analyzed sample, each label being repeated thrice. The amount (µg) of drug found by method (Y-axis) was plotted against the amount of standard drug (X-axis). The intercept on the Y-axis indicates the amount of drug (µg) present in the formulation. The percentage recovery was calculated from amount of drug found. The recovery obtained for wedelolactone was 98.1 and 97.6% respectively as shown in table 2. This shows that there is no interference from the other constituents in the extract and formulation.

Ruggedness and Robustness:

The results of ruggedness testing are reported in table 3 and robustness studies are shown in table 4.

Table 1: Determination of wedelolactone in *Eclipta alba* and its formulation

Herb / formulation	constituent	Percentage of wedelolactone	RSD (%) (n=3)
Herb	Wedelolactone	0.173	0.76
Formulation	Wedelolactone	0.131	0.81

Table -2: Results of recovery analysis

Herb/ Formulation	Amount of wedelo-lactone present in (ng) A	Amount of wedelo-lactone Added to A (ng) B	Total wedelo-lactone taken (A+B) (ng) C	Total wedelo-lactone found (ng) D	% Recovery D/C) x 100 (mean)
Herb	1450	200	1650	1590	98.1
		400	1850	1812	
		600	2050	2065	
Formulation	1135	200	1335	1293	97.6
		400	1535	1520	
		600	1735	1708	

Table-3: Results from ruggedness studies

Analyst	Percentage of wedelolactone from herb and in formulation (assay)	
	Herb	Formulation
I	98.5	96.7
II	97.1	97.3
III	99.3	97.4
Mean	98.3	97.13

Table- 4: Results from robustness studies

Development distance (cm)	Wedelolactone assay (%)	
	Herb	Formulation
7 cm	99.3	100.3
7.5 cm	98.9	102.6
8 cm	98.7	99.9

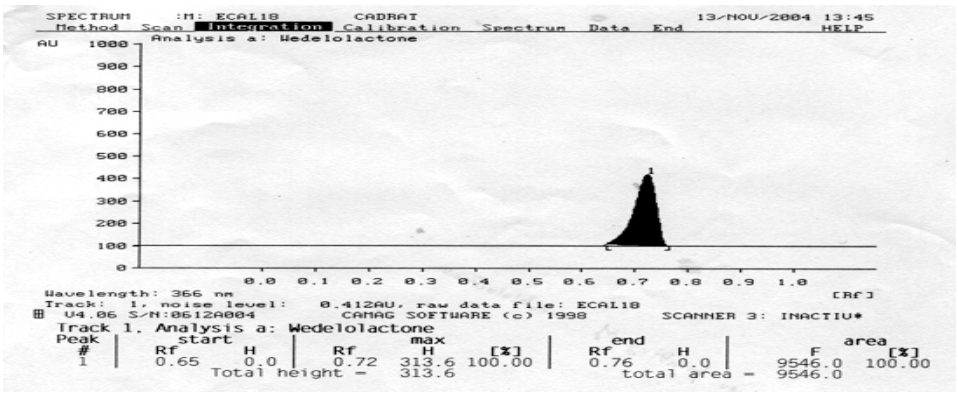

Figure-1: Chromatogram of wedelolactone

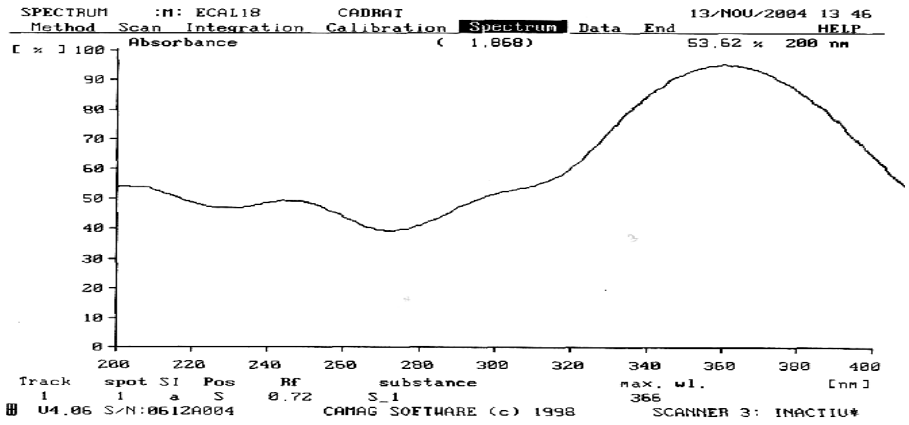

Figure-2: Absorption spectrum of wedelolactone

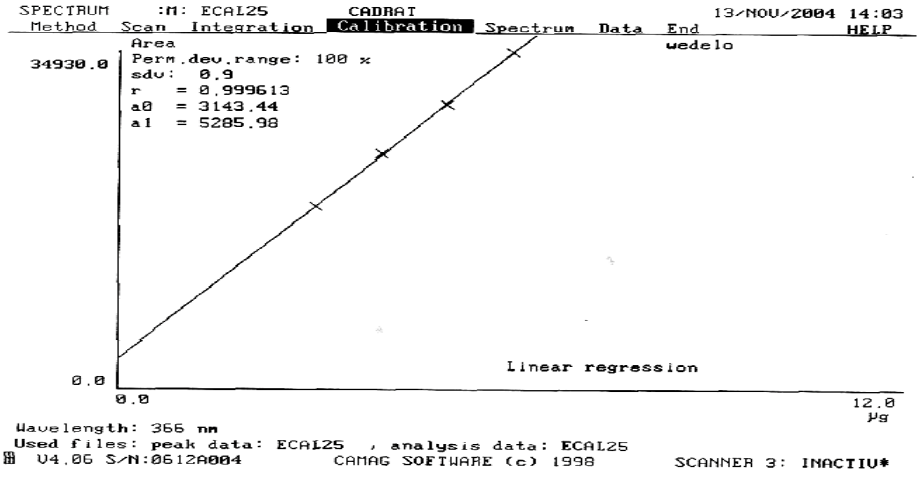

Figure -3: Calibration curve of wedelolactone

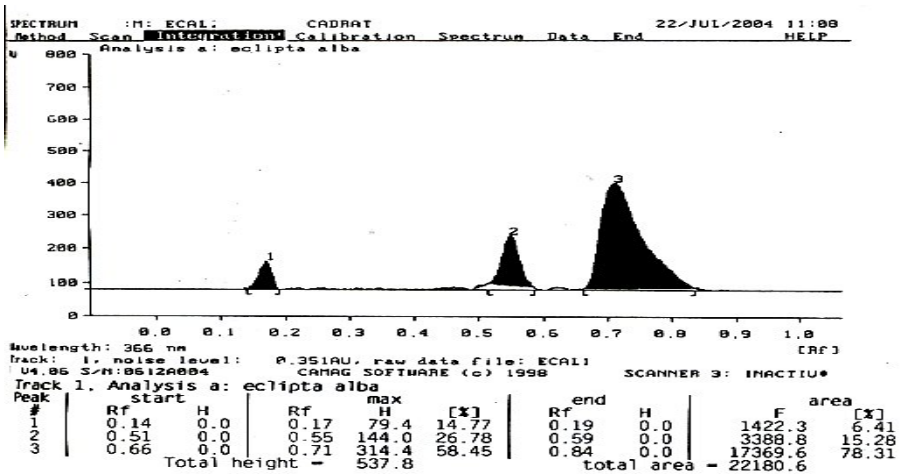

Figure -4: Finger printing profile of *Eclipta alba*

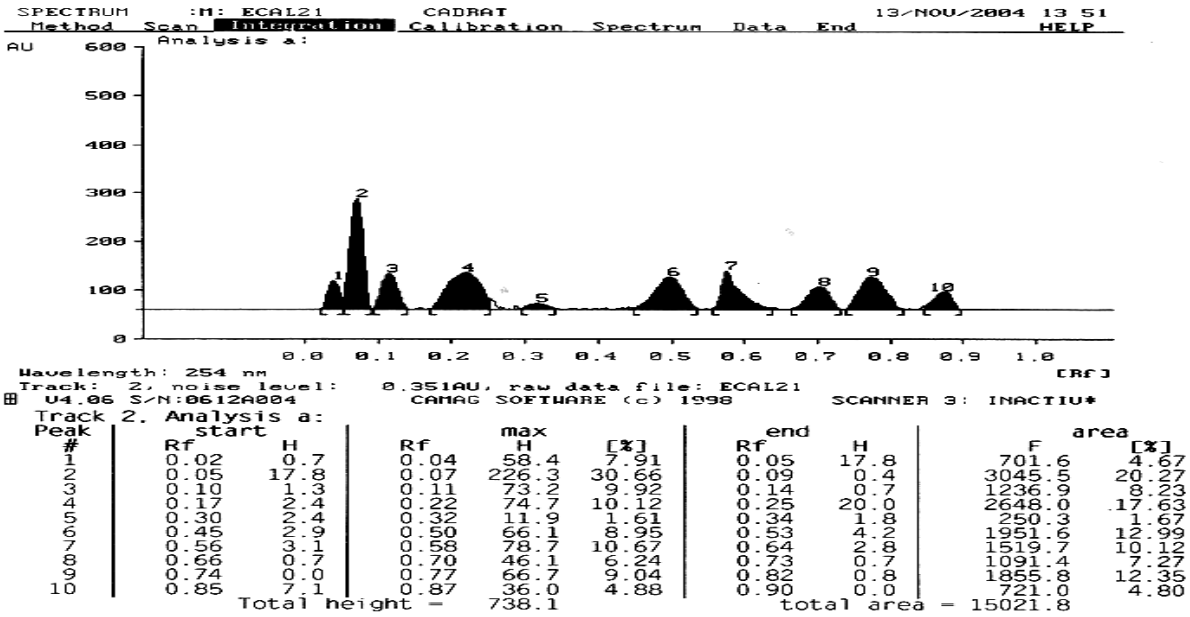

Figure- 5: Finger printing profile of formulation

CONCLUSION:

The HPTLC method proposed for determination of wedelolactone in the polyherbal formulation are accurate, precise, rapid and selective. It can, therefore, be easily and conveniently adopted for routine quality control analysis.

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