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ESTIMATION OF WEDELOLACTONE IN *ECLIPTA ALBA* (LINN.)HASSK. AND ITS ANTIMICROBIAL EFFICACY

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A simple and sensitive spectrophotometric method was opted for estimation of wedelolactone, a furanocouramin, present as a major active constitute in the methanol extract of the plant *Eclipta alba*. Linear relationship was obtained in the range 5-30ng/ml at wavelength 384nm. This active constituent was also examined for the antimicrobial activities against certain pathogenic bacteria and fungi.

Keywords : Antimicrobial activity; *Eclipta alba;* Spectrophotometric; Wedelolactone.

Eclipta alba (L.) Hassk.(Synonym: *Eclipta prostrate* L.)(Asteraceae), is a small branched annual herb with white flower heads, is native to the tropical and subtropical regions of the world. It has been used in India traditionally for liver disorders[1,2]. The plant is an active ingredient of many herbal formulation prescribed for liver ailments and show effect on liver cell enlargement. *Eclipta alba* leaves showed antihyperglycemic activity[3]. The root of *Eclipta alba* were found effective in wound healing[4]. It is a source of coumestan type compounds used in pytopharmaceutical formulations of medicines prescribed for the treatment of cirrhosis of the liver and infectious hepatitis[5] . Coumestan-type compounds, wedelolactone[6] and dimethylwedelolactone[7] have been isolated as the main active principles of *Eclipta alba*, both constituents exhibiting antihepatotoxic activity[8,9]. *In vivo* tests indicate that the wedelolactone neutralizes the lethal and myotoxic activities of rattlesnake venom[10]. Wedelolactone and Dimethylwedelolactone showed potent activity when were tested in trypsin inhibition bioassay (*in vitro*).

Earlier reports indicate the presence of wedelolactone, as active constituent, but not the quantification and its antimicrobial efficacy against pathogenic bacteria and fungi, therefore, it was aimed to quantify and study the antimicrobial activity of wedelolactone.

Plants of *Eclipta alba* with actively growing shoots were collected in March, 2008 from university garden, University of Rajasthan, Jaipur. The plant was authenticated at herbarium, Department of Botany, University of Rajasthan, Jaipur. Voucher, specimen of the sample was deposited in the herbarium.

Preparation of standard solution: Standard solution (100µg/ml) was prepared by dissolving 5mg wedelolactone in 50ml methanol. Standard stock solution of the concentration 5µg/ml was prepared by diluting 2.5 ml of the above solution to 50 ml with methanol. Appropriate dilutions were prepared in methanol to produce working stock solution of 5, 10, 15, 20, 25, 30mg/ml. Later, the standard curve was plotted by measuring the optical density.

Preparation of sample solution:- 50 gm of powdered sample was extracted with methanol in Soxhlet apparatus for 6h at, 70°C. Methanol extract was concentrated to about 25 ml and then dried *in vacuo*. A solution of extract (1mg/ml) was prepared in methanol. With the use of marked capillary, the resultant solution was applied on the chromatographic plate as a band along with the reference spot of standard wedelolactone. Thin layer chromatographic plate was run using Silicagel G as a stationary phase and a mobile phase[11] consisting of Toluene: acetone: formic acid (11: 6: 1, by volume). Visualization of wedelolactone was performed under U.V. Chamber, having Rf value 0.63 and distinct bluish green fluorescence. The band of wedelolactone was scrapped off using sharp blade, extracted with methanol and filtered through Whatman filter paper no. 42. The residue on the filter paper was washed with methanol and final volume of the solution was made up to 50ml. It was considered as a sample stock solution. 5ml of this solution was adjusted to 10 ml and optical intensity was measured.

Estimation procedure: Standard and sample solution of wedelolactone was quantified by taking optical density at 384nm. Three replicates were examined in each case and their mean value were recorded.

Antimicrobial efficacy:

a. Bacteria: Pure cultures of all tests organism, namely *Escherichia coli* (Gram negative) and *Staphylococcus*

aureus (Gram positive), the human pathogen, were obtained through the courtesy of SMS Medical College, Jaipur, India, which are maintained on Nutrient Broth Medium.

b. Fungi : The pure cultures of test fungi *Aspergillus niger, Fusarium oxysporum* were obtained from the Seed Pathology Laboratory, Departmentt of Botany University of Rajasthan, Jaipur which are maintained on Potato Agar Medium. Disc diffusion method[12] was adopted (Disc of whatman no.1 paper,6mm containing 4 mg of test drug).

The optical density of wedelolactone has linear relationship in the concentration range of 5-30mg/ml. Stability proved that the intensity of the solution was stable upto 3h at room temperature, but after that the intensity started gradual diminishing. The concentration of wedelolactone in all samples under investigation was calculated with the help of standard curve. The method was found to be simple, accurate, specific and precise (Table 1).

T.L.C. of methanol extract showed separation, having three colored spots with Rf value 0.67, 0.62, 0.54 (Table 3). The standard wedelolactone showed bluish green spot with Rf value of 0.63.

The results of bactericidal and fungicidal efficacy of wedelolactone was tested against pathogenic bacteria and fungi. The wedelolactone sample against *E. coli* was more active and among fungi, it was more active against *Fusarium oxysporum* (Table 2).

Based on the results of phytochemical investigations it can be concluded that the methanolic extract of *Eclipta alba* contains a good amount of wedelolactone and possess antimicrobial activity which support the use of these plants for human and animal disease therapy and reinforce the importance of the ethanobotanical approach as a potential sources of bioactive substances.

Table 1. Data of calibration curve for wedelolactone.

Concentaration of wedelolactone (mg/ml)	Optical density (384 nm)
5	20.37
10	29.94
15	43.21
20	58.02
25	73.15
30	87.35

Table 2. Antimicrobial efficacy of Wedelolactone.

Test Microorganisms	Inhibition area (in mm) of standard-streptomycin (10 µg)	Inhibition Zone (IZ) in mm	Activity Index (AI)
Escherichia coli	16.0	11.0	0.687
Staphylococcus aureus	15.0	10.0	0.666
Aspergillus flavus	14.0	5.0	0.357
Fusarium oxysporum	11.0	6.0	0.545

IZ = Inhibition Zone in mm;

AI = Activity Index = Inhibition area of the sample / Inhibition area of the Standard

Table 3. TLC studies.

Solvent system	Rf values	
Toluene:Acetone: Formic acid (11:6:1)	Standard Wedelolactone	Natural plant
	0.63	0.67, 0.62, 0.54

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