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European Journal of Experimental Biology, 2011, 1 (2):172-177



Anti-inflammatory and antimicrobial activities of the extracts of *Eclipta alba* Leaves

Muthu Kumaran Peraman¹, P. Ramalingam², and Bapatla JNN Sai²

¹Department of Biotechnology, Kumaraguru College of Technology (KCT), Saravanampatti, Coimbatore, Tamil Nadu, India ² Centre for Pharmaceutical Research (CPR), Raghavendra Institute of Pharmaceutical Education and Research (RIPER), Anantapur, AP, India

ABSTRACT

The aqueous and ethanolic extracts of the leaves of Eclipta alba (Astraceae) were evaluated for their anti-inflammatory activity using carrageenan-induced rat paw oedema method in albino rats. The antimicrobial activity was also been performed against the bacteria Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa and the fungi Candida albicans and Aspergillus niger by agar plate disc diffusion method. The results indicated that the ethanolic extracts (200mg/kg) have shown significant anti-inflammatory activity with p value of 0.005 and however, both of the extracts were exhibited moderate antibacterial and antifungal activity against the test organism.

Keywords: Eclipta Alba, Aqueous; Ethanol; Extracts; Anti-Inflammatory, Antimicrobial.

INTRODUCTION

Disease has been an integral part of man from the beginning of his existence. The subject of drugs is also as old as disease and the search for remedies to combat it is perhaps equally old and for more than a millennium, herbal medicine has been extensively used, apparently safely and effectively, in Asian countries, especially in China, Japan and Korea, to alleviate various symptoms of disease [1]. Inflammatory diseases including different types of rheumatic diseases are very common throughout the World [2]. Although the rheumatism is one of the oldest known diseases of mankind and affects a large population of the world and no substantial progress has been made in achieving a permanent cure. The greatest disadvantage of the present remedy of

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synthetic drugs like steroidal and non-steroidal anti-inflammatory drugs lied in their toxicity and relapse of symptoms after discontinuation of treatment. [3]. The research on screening and development of drugs for their activity is therefore, an unending process and there is hope of finding out anti-inflammatory drugs from indigenous plants. Various plant extracts and their isolated compounds have been proved as good as synthetic anti-inflammatory agents [4]. Latest and previous studies have concluded the beneficial aspects of plant derived drugs as good source of antibiotics, antioxidants and anti-inflammatory agents [5,6].

Eclipta alba, family *Compositae* (English: Kadimulbirt) is a mesophytic herb, a common annual weed found throughout India and elsewhere at an altitudes up to 2000m. This tropical annual is a creeping and moisture-loving herb; it has a short, flat or round, brown stem and small white flowers on a long stalk. It grows 3" tall; the leaves are opposite and lance-shaped. Leaves are sessile, 2.5-7.5cm long, oblong, lanceolate, subentire, acute or subacute sparsely strigose with appressed hairs on bothsides and with a tapering base [7,8,9]. The juice of the plant is used as a popular remedy for jaundice, fever, painful swelling, anemia, dysentery, eye diseases, asthma and liver cirrhosis [10]. The juice of Eclipta together with honey is used to treat upper respiratory congestion in children. Root has been reported to possess emetic and purgative property [11]. The tincture of the plant is used for liver and kidney problem [12,13,14] and it is also reported to have therapeutic potential against cardiovascular disorders [15]. The present communication reveals the comparative outcome of both aqueous and ethanolic extract with respect to preliminary phytochemical, anti-inflammatory and antimicrobial activity against Gram –positive bacteria, Gram-negative bacteria and the fungi species.

MATERIALS AND METHODS

Preparation of the Extract

The leaves of *Eclipta alba* were collected during Feb-2008 from Kanigiri, Prakasam district, Andhra Pradesh, India. The plant was identified in the Department of Plant Sciences, Hindu College, Guntur, Andhrapradesh, India where voucher specimen was deposited (Voucher Number: Herbarium/2008/023). The leaves were dried under shade and made into a coarse powder. 300 g each of powdered materials was extracted with 600mLwater and 600 mL (95%) alcohol by cold maceration process to afford aqueous and ethanolic extract. Each extract was concentrated *in vacuum* to dryness and yielded extractive value such as 4.677% and 6.328%, respectively for aqueous and ethanolic extract.

Phytochemical tests

The leaf extracts prepared were evaluated for their chemical constituents as described by the method of Brain and Turner [16]. The plant extract was subjected to TLC and the developed spots showed positive results for terpenoids, alkaloids and flavonoids with reagents such as vanillin - sulphuric acid, and Dragondroffs reagent, respectively.

Animals

The experimental protocols and procedures used in this study were approved by the institutional ethical committee and conform to the rules and regulations of CPCSEA, India of the care and use of animals in research and teaching. Albino rats of either sex (125-150g) were used for the study. Animals were housed in groups of six-per cage at a temperature of $25\pm1^{\circ}$ C and relative humidity

of $45\pm5\%$ under a 12:12 hr light: dark cycle. Animals were allowed free access to feed and water *ad libitum*. The feed was withdrawn six hours before the experiments.

Acute Toxicity Studies

The acute toxicity study was performed as per the DECD guidelines (407). The acute toxicity study was done on albino rats by oral administration of doses viz. 500 mg/kg, 1000mg/kg and 2000 mg/kg and then anatomical and physiological behaviors were observed for 24 h.

Anti-inflammatory test

Carrageenan-induced paw edema

The animals weighing 125 -150 g of either sex Wistar albino rats were divided into six groups, each of six animals. Group I (normal control) received 0.5% carboxy methyl cellulose (CMC), group II standard drug (diclofenac sodium) (20mg/kg), ethanolic extract 100 mg/kg, 200 mg/kg, aqueous extract 100 mg/kg and 200 mg/kg, respectively. The test extracts (100 and 200 mg/kg) were administered orally to the test animals (III, IV, V and VI) suspended in 0.5% carboxy methyl cellulose (CMC). After 30 mins of drug administration, 0.1 ml of 1% carrageenan (sigma) in normal saline was injected into the sub-plantar region of right of the hind paw. The paw dorsiventral thickness was recorded using a Zeitlin's apparatus [17] at 30 mins,1 hr, 2 hrs and 3 hrs.

Antimicrobial activity

Both of the extracts were evaluated for their *invitro* antibacterial activity against *S.aureus B.subtilis* (Gram – positive bacteria), *P.vulgaris, P.aeruginosa* (Gram – negative bacteria) and the antifungal activity against *A. Niger* and *C. albicans* by agar plate disc diffusion method [18]. The antimicrobial susceptibility discs (Himedia, 1993) were used in the study. The discs were place aluminum foil and added 10μ L of the resinous extracts in dimethyl formamide (DMF) at concentrations of 5mg/mL and 10 mg/mL equivalent to a concentration of 50μ g/disc and 100μ g/disc, respectively. The method was performed at triplicate. Nalidixic acid (50μ g/disc) and clotrimazole (10μ g/disc) were used as standard drugs respectively for antibacterial and antifungal screening. DMF was employed as blank. The results of the test were interpreted as per Kirby-Bauer method [18].

RESULTS AND DISCUSSION

The extractive values of ethanolic and aqueous extracts were good and exhibited required solubility in the solvents for their biological evaluation. Both 100 and 200 mg/kg doses of ethanolic and aqueous extracts of the leaf of *Eclipta alba* have demonstrated considerable anti-inflammatory activity with the statistical significance of p<0.05. The ethanol extract at 200mg/kg dose have shown better anti-inflammatory response (p<0.005) among the study doses and are shown in Table1 and Figure1. The antimicrobial screening revealed that the extracts exhibited moderate antibacterial activity against bacteria, however, the extracts shown good antifungal activity nearer to the activity of standard drug (clotrimazole). The results are shown in Table 2.

The plant extracts of *Eclipta alba* have been extracted by maceration as per British Pharmaceutical codex and preserved under refrigeration until the experimental usage to avoid

microbial contaminations. The extracts found to be as resinous and their DMF solutions were used for the experimental purpose owing to the good solubility nature. However DMF was proven to be biologically inert for the current study. The anti-inflammatory study on Carageenan induced rat paw edema, the results after 3 hrs revealed the potent activity (51% of the standard; p<0.005) of ethanolic extract at 200 mg/kg than the aqueous extract where as the dose 100 mg/kg of both the doses have demonstrated the similar potency such as 38.50% and 35.33%, respectively (p<0.05). The antibacterial activity of both the extracts were found to be moderate (8- 13 mm) however the antifungal activity was good (14 – 19mm). With reference to the Chemical constituents of *Eclipta alba* such as alkaloids, flavanoids, terpenoids and the present results of the present biological evaluation, it suggests that anti-inflammatory potential and antimicrobial potential may be due to the potential flavanoids and/ or Alkaloids. Further work may be extended to isolate the active constituents for their biological potential and is continuing our interest too.

Table 1. Anti-inflammatory	activity of lea	af Extracts of	Eclipta alba
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S No	Group	Increase in paw thickness by Zeitlin's apparatus				inhibition	
3.110		30 min	1hr	2hr	3hr	after 3 hrs	
1	Disease Control	2.5±0.3416	3.7±0.33	7.9 ± 0.2582	9.2±0.3801		
2	Standard	1.6 ± 0.2007	1.1±0.1537	0.83±0.1537	0.5±0.1291	94.56%	
3	EE 100 mg/Kg	2.5 ± 0.1826	2.2±0.4014	5.25±0.3096	5.7±0.1118	38.50%*	
4	EE 200 mg/Kg	2.5±0.1927	1.9 ± 0.4104	4.81 ±0.3377	4.44 ± 0.1809	51.74%**	
5	EA 100 mg/Kg	2.5 ± 0.1861	2.4±0.4441	5.54±0.3443	5.95±0.1768	35.33%*	
6	EA 200 mg/Kg	2.5 ± 0.1798	2.1±0.4670	5.03 ± 0.3665	4.75±0.1209	44.56%*	
$EE = E(1, \dots, 1)$, $EA = A$, $EA = $							

EE = Ethanolic extract; EA = Aqueous extract ; * p < 0.05 ; **p < 0.005

Table 2. Antibacterial and antifungal activity of leaf extracts of Eclipta alba

Contents of	Antibacterial				Antifungal	
sterile disc	S.aureus	B.subtilis	P.vulgaris	P.aeruginosa	A. Niger	C. albicans
EE 50 µg/disc	12	11	08	12	15	14
EE 100 µg/disc	14	13	09	10	19	16
AE 50µg/disc	10	10	09	11	15	18
AE 100µg/disc	13	12	13	12	18	17
Std ^a	24	25	34	22	21	20
Blank ^b	06	06	06	06	06	06

The above results mean of triplicate and are presented as zone inhibition in millimeter (Disc diameter 5 mm). ^a Nalidixic acid ($50\mu g/disc$) and clotrimazole ($10\mu g/disc$) were used as standard respectively for antibacterial and antifungal screening.



Figure 1. Effect of *Eclipta alba* leaf extracts on inflammation of rat paw oedema

Acknowledgements

The authors are grateful to the Pharmacology Division, Centre for Pharmaceutical Research (CPR), Raghavendra Institute of Pharmaceutical Education and Research (RIPER), Andhra Pradesh, India for providing facility for animal Studies.

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