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Research Article

Antibacterial and Antifungal Activity of *Eclipta alba* (l.) Hassk.

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Abstract

This experiment was subjected to investigate antibacterial and antifungal properties of methanolic extract of *Eclipta alba*. Disc diffusion techniques were used to determine the antibacterial and antifungal activity of the Methanolic extract. The antibacterial activity of the both methanolic extract and isolated compound were found to be good (7-18 mm) however the antifungal activity was moderate (11-14 mm). 100 µg/ml concentration of Methanolic extract of *Eclipta alba* showed the minimum inhibitory concentration which compared with standard drug kanamycin and clotrimazole (10 µg). Among the gram negative bacteria tested, *Proteus vulgaris* showed highest zone of inhibition (16 and 17 mm) in the methanolic extract at the dose of 250 and 500 µg per disc respectively whereas *Pseudomonas aeroginosa* showed the lowest zone of inhibition (9 and 10 mm) at the dose of 250 and 500 µg per disc respectively. Among the 4 tested gram positive bacteria, *Staphylococcus aureus* exhibited the highest zone of inhibition (13 and 15 mm) which was followed by *Bacillus cereus* (11 and 12 mm) and *Bacillus clausii* and *Bacillus subtilis* (10 and 11 mm) showed the lowest zone of inhibition at the dose of 250 and 500 µg per disc respectively. Results of fungus exhibited same zone of inhibition (12 and 13 mm at 250 and 500 µg per disc respectively) for both *Aspergillus niger* and *Candida albicans.* This research supports, methanolic extract of *Eclipta alba* has potential antimicrobial activity.

1. INTRODUCTION

Eclipta alba L. Hassk. is an annual herbaceous plant, commonly known as king of hairs. It is an erect or prostrate, much branched, roughly hairy, annual, rooting at the nodes; the leaves are opposite, sessile and lanceolate belonging to family Asteraceae [1-3]. It is also known as Bhringaraja[3] (Sanskrit), Maka (Marathi), Bhangra (Hindi) and Karisilakanni, which is found a common weed throughout India ascending up to 6000 ft [4]. The genus name comes from the Greek word meaning "Deficient" with reference to the absence of the bristles and awns on the fruits. The specific *Eclipta alba* means white which refers to the colour of the flowers. Main active principles consist of coumestans like wedelolactone, desmethyl wedelolactone, furanocoumarins, oleanane and taraxastane glycosides [5-14]. The plant is commonly used in hair oil all over India for healthy black and long hair [6]. It has been reported to show protectiveeffect on experimental liver damage in rats and mice[15]. The plant has been reported for the treatment of liver cirrhosis and infective hepatitis[16]. In Ayurveda, the root powder is used for treating hepatitis, enlarged spleen and skin disorders. Mixed with a little oil when applied to the head, the herb relieves headache. The extract of its leaves is mixed with honey and given to infants, for the expulsion of worms. *Eclipta alba* is also given to children in case of urinary tract infections.

Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world[17, 18]. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumor and antimicrobial agents [19, 20]. The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products[21].

In the present investigation the methanolic extracts of *Eclipta alba* was evaluated for the antimicrobial activity.

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2. MATERIALS AND METHODS

2.1 Collection and authentication of Plant Materials

The plant specimen for the proposed study *Eclipta alba* (L), Hassk was collected from the paddy fields and other irrigated fields in and around Madurai District, Tamil Nadu, India. The herbarium of these plants was identified and authenticated by Dr. D. Stephen, Professor, Department of Botany, American college of Arts and Science, Madurai, Tamil Nadu and the specimen was deposited in Department of Pharmaceutical Chemistry, Ultra College of Pharmacy, Madurai, Tamil Nadu, India**.**

2.2 Preparation of plant extracts

The fresh whole plant of *Eclipta alba* was washed with distilled water to removed unwanted foreign materials like soil and dusts. After, washed plant material was dried under shade at room temperature without direct exposure of sunrays. It was then coarsely grounded by using mechanical device. The powdered plant material was passed through sieve no 40 and stored in an airtight container for further use.

The coarsely powdered plant materials of *E. alba* (2000 g) were extracted separately to exhaustion in a soxhlet apparatus for 72 hours by using Petroleum ether (60 - 80˚C) and methanol (95%) solvent (Merk & Spectrum Chemicals, India) systems. All the extracts were filtered through a cotton plug followed by whatman filter paper (No.1) and then concentrated by using a rotary evaporator at low temperature (40 - 50˚C) and reduced pressure to get 24.4 g and 108.6 g respectively. The extracts were preserved in airtight containers and kept at 4˚C until further use.

2.3 Isolation of Phytoconstituents from *Eclipta alba*

The isolation of phytoconstituent is done by column chromatographic method. The constituents of methanol extract (MEEA) were isolated by column chromatography. Identification and purity determination were done by thin layer chromatography techniques. The fractions collected were further chromatographed to know the number of constituents present. Silica gel was used as stationary phase. The column was first eluted with 100 % pet. ether. The polarity of mobile phase was gradually increased with chloroform, ethyl acetate, acetone, and methanol. The fractions collected and were concentrated. The dried fraction was kept on vials with suitable label and kept for further use.

2.4 Microbial strains used

Antibacterial effect of *Eclipta alba* was determined against 4 gram negative bacteria *viz., Escherichia coli* MTCC 118*, Proteus vulgaris* MTCC 426*, Klebseilla pneumonia* M 4020 and *Pseudomonas aeruginosa* (Clinical isolate obtained from Vijay Clinical Laboratory, Madurai) and 4 gram positive bacteria *viz., Bacillus cereus* MTCC 1305, *Bacillus subtilis* MTCC 619*, Bacillus clausii (Probiotic spores obtained from medical store)* and *Staphylococcus aureus* MTCC 96. Antifungal effect of *Eclipta alba* was determined against 2 different fungal strains *viz., Aspergillus niger* MTCC 872 and *Candida albicans* MTCC 183.

2.5 Antibacterial assay

Muller Hinton Agar (MHA) was used as the media for culturing of bacterial strains. The spectrum of antibacterial activity was studied using as test agent a range of 8 different strains of human pathogenic bacteria of which there were one standard drug (Kanamycin). *In vitro* antibacterial assay was carried out by disc diffusion technique [22] in whatman No.1 filter paper discs with 4 mm diameter were impregnated with known amount test samples of the *Eclipta alba.* The discs were immersed in different test concentrations (extracts - 250 and 500 µg, new isolated compound from *Eclipta alba* - $EAC_1$25 and 50 µg) allowed to evaporate. The positive control contained a standard drug (Kanamycin) disc. Sterile discs used as negative control. The impregnated discs along with control were kept at the center of agar plates, seeded with test bacterial cultures. The discs were then placed individually using a sterile forceps in appropriate grids which were marked on the undersurface of the plated Petri plates and kept for incubation at room temperature (27°C ± 2) for 24 hrs. After incubation, plates were observed for zones of inhibition and recorded in millimeters.

2.6 Antifungal assay

Stock cultures were maintained in Sabouraud dextrose agar and 2 different species of fungal pathogen were maintained in Sabouraud Dextrose broth for 24hours until used for antifungal activity. *In vitro* antifungal activity was determined by using the disc diffusion technique[(22)]. Two different species of fungal pathogens inoculated by spread plate method using 0.1 ml of 24 hours old culture, maintained in Sabouraud Dextrose broth. The discs were immersed in different test concentrations (extracts - 250 and 500 µg, compounds - $EAC_1$25 and 50 µg) allowed to evaporate. Clotrimazole used as positive control. Incubating the fungal petri plates for 32 hrs at 30°C, then plates were observed for zones of inhibition and recorded in millimeters.

2.7 Minimum Inhibitory Concentration (MIC)

The methanolic extract of *Eclipta alba* antimicrobial activity were further tested against all the organisms for the evaluation of its antibacterial and antifungal efficiency at different concentrations (50µg/ml, 100µg/ml, 250 µg/ml, 500 µg/ml, 1 mg/ml and 10mg/ml) by using the filter paper disc diffusion method. The zone of inhibition was calculated in mm.

Activity index was calculated by comparing the zone of inhibition by plant extract with that of standard drug.

$$\text{Activity Index} = \frac{\text{Inhibition zone of test sample (extract)}}{\text{Inhibition zone of standard antibiotic}}$$

2.8 Statistical Analysis

Tests were carried out in triplicates. The mean values were calculated from the triplicate values. Values are expressed as the Mean ± SD and differences between groups were considered to be significant if $p < 0.05$.

3. RESULTS AND DISCUSSION

Antibacterial and antifungal activity was done for methanolic extract and isolated compound of *Eclipta alba.* During antimicrobial study the methanolic extract and isolated compound showed concentration dependent increase in zone of inhibition against Gram positive, Gram negative bacteria and fungus by disc diffusion method. The results of antibacterial and antifungal activity are shown in Table 1 and Figure 1.

The antibacterial activity of the both methanolic extract and isolated compound were found to be good (7-18 mm) however the antifungal activity was moderate (11-14 mm). Minimun inhibitory concentration of the methanolic extract of *Eclipta alba* at the concentration of 100µg/ml as compared with standard drug kanamycin and clotrimazole (10 µg). Among the gram negative bacteria tested, *Proteus vulgaris* showed highest zone of inhibition (16 and 17 mm) in the methanolic extract at the dose of 250 and 500 µg per disc respectively whereas *Pseudomonas aeruginosa* showed the lowest zone of inhibition (9 and 10 mm) at the dose of 250 and 500 µg per disc respectively. Among the 4 tested gram positive bacteria, *Staphylococcus aureus* exhibited the highest zone of inhibition (13 and 15 mm) which was followed by *Bacillus cereus* (11 and 12 mm) and *Bacillus clausii* and *Bacillus subtilis* (10 and 11 mm) showed the lowest zone of inhibition at the dose of 250 and 500 µg per disc respectively. Results of fungus exhibited same zone of inhibition (12 and 13 mm at 250 and 500 µg per disc respectively) for both *Aspergillus niger* and *Candida albicans* (Table 1).

In four tested gram negative bacteria, *Pseudomonas aeruginosa* showed the highest zone of inhibition (15 mm in 25 µg per disc) whereas *Klebseilla pneumonia* showed the lowest zone of inhibition (12 mm in 25 µg per disc)in the isolated compound of *Eclipta alba.* Highest zone of inhibition (18 mm in 50 µg per disc) occurred in *Pseudomonas aeruginosa* which was followed by *Escherichia coli* and *Proteus vulgaris* (15 mm) and lowest zone of inhibition (14 mm in 50 µg per disc) exhibited in *Klebseilla pneumonia*. Among the 4 tested gram positive bacteria, *Staphylococcus aureus* exhibited the highest zone of inhibition (10 and 11 mm) which was followed by *Bacillus cereus* (8 and 10 mm), *Bacillus subtilis* (8 and 9 mm) and *Bacillus clausii* (7 and 9 mm) in the dose of 25 and 50 µg isolated compound per disc respectively. Results of fungus exhibited same zone of inhibition (11 and 14 mm at 25 and 50µg of isolated compound per disc respectively) for both *Aspergillus niger* and *Candida albicans.*

The antibacterial activity of standard kanamycin showed the maximum zone of inhibition against *Proteus vulgaris* and minimum zone of inhibition was observed against *Pseudomonas aeruginosa, Bacillus cereus* and *Bacillus clausii.* Table 2 shows the activity index of methanolic extract and isolated compound of *Eclipta alba*. From the activity index results, the methanolic extract showed good activity against all the bacteria except *Bacillus subtilis.*

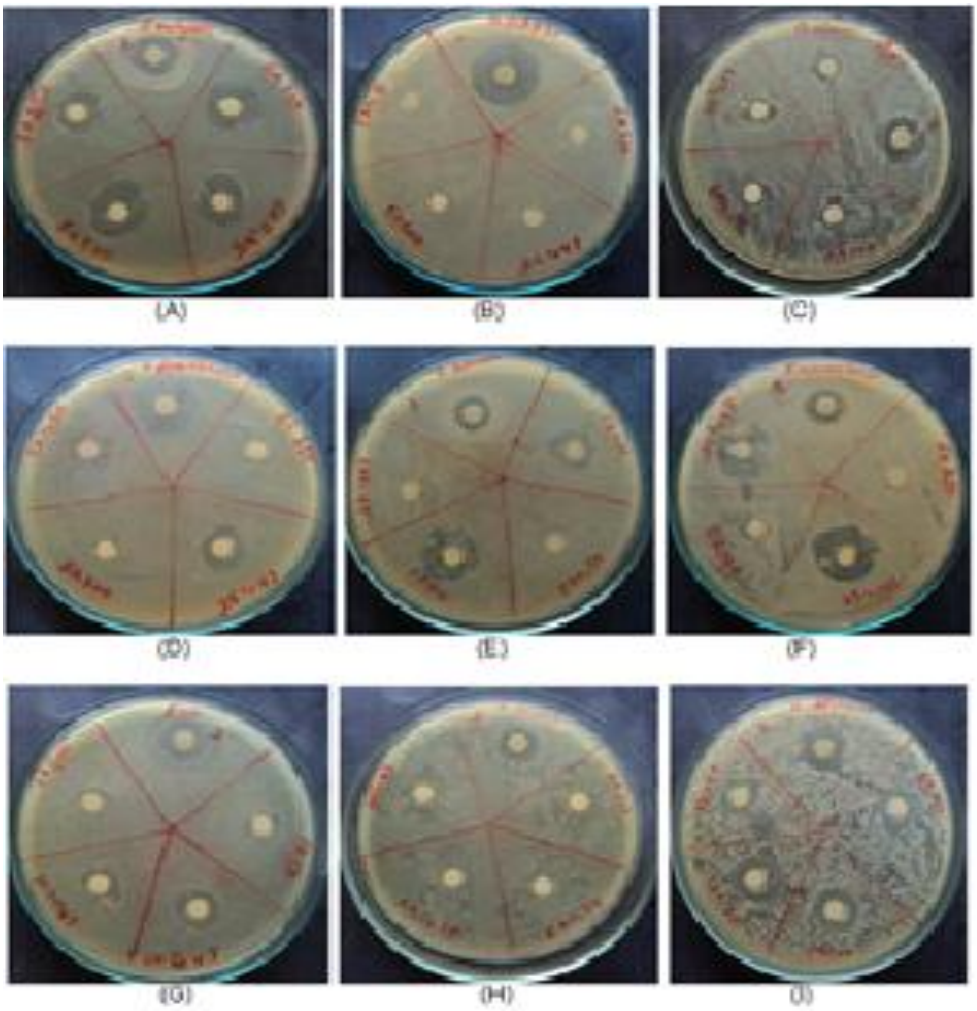
With reference to the chemical constituents of *Eclipta alba* such as alkaloids (Ecliptine, Nicotine, steroidal alkaloids), Flavanoids (Apigenin, Luteolin, Luteolin – 7 – glucoside), terpenoids and the results of the present evaluation, it suggests that antimicrobial potential may be due to flavanoids and alkaloids. The present results are in accordance with the findings of the researcher who found saponin, alkaloid and flavanoid crude extracts of *E. alba* was found active against *Fusarium solani, Aspergillus flavus* and *Aspergillus niger*[23].The minimum inhibitory concentration of antibacterial and antifungal of the methanolic extract of *Eclipta alba* at a concentration of 250 µg/ml as compared with standard drug tetracycline and streptomycin (100µg/ml)[24]. Hexane extract of *E. alba* showed high antibacterial activity against *S. aureus, B. cereus, E. coli, S. typhi, K. pneumonia, S. pyogenes* and *P. aeruginosa* whereas acetone, ethanol, methanol and aqueous extract showed intermediate activity against the *P. mirabilis* and *S. pyogenes*[25].

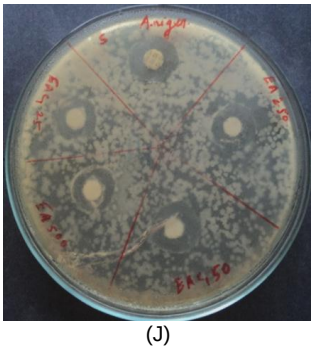
Table 1: Antibacterial and antifungal activity of methanolic extract and isolated compound of *Eclipta alba*(L) Hassk.

Microorganism	Mean ± SEM of diameter of zone of inhibition (in mm)				
	Methanolic extract of *E. alba*		Isolated compound of *E. alba*		Standard
	EA250µg/disc	EA500µg/disc	$EAC_1$25µg/ disc	$EAC_1$50µg/disc	
Escherichia coli	11±0.30	12±1.14	14±0.54	15±1.15	20±1.11
Proteus vulgaris	16±0.17	17±1.12	14±0.57	15±0.94	23±0.56
Klebseilla pneumonia	10±1.12	11±0.97	12±1.12	14±0.91	18±0.32
Pseudomonas aeruginosa	9±1.15	10±0.65	15±1.16	18±0.34	13±0.78
Bacillus cereus	11±0.97	12±1.16	8±0.98	10±0.76	13±0.45
Bacillus subtilis	10±0.45	11±2.01	8±0.39	9±1.13	21±1.19
Bacillus clausii	10±0.37	11±0.99	7±0.26	9±2.16	13±1.16
Staphylococcus aureus	13±0.28	15±0.33	10±1.14	11±2.06	16±0.78
Aspergillusniger	12±1.14	13±0.52	11±2.01	14±0.96	15±0.33
Candida albicans	12±0.94	13±0.46	11±2.97	14±0.43	14±0.75

Table 2: Activity index of methanolic extract and isolated compound of *Eclipta alba*(L) Hassk.

Microorganism	Activity index			
	Methanolic extract of *E. alba*		Isolated compound of*E. alba*	
	EA250µg/disc	EA500µg/disc	$EAC_1$25µg/ disc	$EAC_1$50µg/disc
Escherichia coli	0.55	0.60	0.70	0.75
Proteus vulgaris	0.70	0.74	0.61	0.65
Klebseilla pneumonia	0.56	0.61	0.67	0.78
Pseudomonas aeruginosa	0.69	0.77	1.15	1.38
Bacillus cereus	0.85	0.92	0.62	0.77
Bacillus subtilis	0.48	0.52	0.38	0.43
Bacillus clausii	0.77	0.87	0.54	0.69
Staphylococcus aureus	0.81	0.94	0.63	0.69
Aspergillusniger	0.80	0.83	0.73	0.93
Candida albicans	0.86	0.93	0.79	1.00





(J)

Fig. 1: Antimicrobial activity of Methanolic extract and isolated compound of *Eclipta alba*

A – P. vulgaris; B – B. subtilis; C – B. clausii; D – K. pneumonia; E – S. aureus; F – P. aeruginosa; G – E. coli; H – B. cereus; I – C. albicans; J - A. niger

4. CONCLUSION

Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus there has been a continuing search for new and more potent antibiotics. Our studies can be strong scientific evidence to use this plant as a useful source of both antibacterial and antifungal properties.

Eclipta alba is important plant among many traditional herbs in the treatment of many diseases, which are usually free from side effects, are economical and also easily accessible to humans. On the basis of our results of the present study, it is concluded that the methanolic extract of *Eclipta alba* have significant antibacterial and antifungal activity. *Eclipta alba*be considered for preparation of Nutraceuticals with potent antimicrobial activity suitable for the prevention of human disease. These findings support the traditional knowledge of local users about their selection of this plant sample as antimicrobial agents and it is a preliminary scientific validation for the use of this plant for antimicrobial activity.

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