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Research Article

ANTIBACTERIAL ACTIVITY OF LEAVES AND ROOTS OF *Eclipta alba*

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ABSTRACT

Objective: The present study was aimed to investigate the antibacterial activity of leaves and roots of *Eclipta alba*.

Methods: The antibacterial activity of leaves and roots of *Eclipta alba* was evaluated by agar well diffusion method and minimum inhibition concentration against different bacterial species such as *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas fluorescens, Staphylococcus aureus* and *Shigella flexneri.*

Results: 20 µl of chloroform extract of leaves and root powder of *Eclipta alba* was found to exhibit best inhibitory activity against *Klebsilla pnemoniae* such as 32 mm and 35 mm respectively when compared to other solvents and hot aqueous extract. Leaves of benzene extract exhibited good inhibitory zone of 26mm against *Shigella flexneri* and growth of *Bacillus subtilis* was inhibited up to 28mm by chloroform extract of root. All tested organisms exhibited lowest zone of inhibition between 8-10mm with acetone, methanol and aqueous extract of both leaves and roots of *Eclipta alba*. MIC was between concentration of 8-0.25mg/ 100 µl with all tested bacteria. From the results, it is clear that the extracts of leaves and root of *Eclipta alba* possess potential broad spectrum antibacterial activity.

Keywords: *Eclipta alba*, Micro organisms, Antibacterial activity, Leaves and Root extraction.

INTRODUCTION

The frequency of life threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immun compromised patients in developed countries [1]. The ready availability and economy of plants as direct therapeutic agents make plants more attractive when compared to modern medicine [2]. Medicinal plants have been used for centuries before the advent of orthodox medicine. Leaves, flowers, stems, roots, seeds, fruit and bark can also be constituents of herbal medicines [3]. More than 80 per cent of the world's population in the world still depends on traditional medicine for their primary health care. They continue to provide scientists with invaluable compounds of starting points for the development of new drugs [4]. Conversely, because information on the use of plant species for therapeutic purpose has been passed from one generation to the next through oral tradition, this knowledge of therapeutic plants has started to decline and become obsolete through the lack of recognition by younger generations as a result of a shift in attitude and ongoing socio-economic changes [5].

Historically medicinal plants have provided a good source of anti-infective agents; emetine, quinine and berberine remain highly effective instruments in the fight against microbial infections [6]. The author stated that plant derived medicines have been a part of traditional health care in most parts of the world of thousands of years and there is increasing interest in them as source of agents to fight microbial diseases [7]. *Eclipta alba* is commonly known as Bhringaraja or Maka belonging to the family Asteraceae/Compositae. The herb contain wedelolactone and demethylwedelolactone which possessing potent Antihepatotoxic property. Other prominent chemical constituents present are ecliptal, ecliptine, ecliptalbine, α-terthienylmethanol, β-amyrin and sigmasterol [8]. The main objective of this study is assessment of antibacterial activity *Eclipta alba*.

MATERIALS AND METHODS

Collection of plant samples

The plant *Eclipta alba* was freshly collected from local market at Coimbatore. The plant was duly authenticated by Botanical Survey of India, Tamil Nadu Agricultural University Coimbatore. A voucher specimen was deposited in the Department of Biochemistry, Avinashilingam University for Women, Coimbatore, Tamil Nadu, India (Plate I).

Plate I

Eclipta alba

Collection of microorganisms

The bacterial strains used for the experiment such as *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas fluorescens, Staphylococcus aureus* and *Shigella flexneri* were collected from the Department of Biotechnology, PSG Institute of Medical Science, Coimbatore and stored at -70˚C.

Preparation of extracts

The collected leaves and roots of the plant were washed and air dried in the shade at room temperature for complete drying. The dried sample was crushed with the aid of mechanical grinder to make powder form. The powder obtained was extracted with selected solvents based on polarity using soxhlet apparatus in the 6 hours intervals. The solvents used in sequential order were respectively petroleum ether, benzene, chloroform, acetone and methanol.

Preparation of hot aqueous extract

5 grams of dried leaves and root powder were boiled with 100ml of water until it becomes half the volumes. The extracts were kept as such in the room temperature for evaporating until it become semi solid and all of the dried extracts were dissolved in dimethylsulfoxide (DMSO), stored in the refrigeration until required for use.

Agar well disc diffusion method for antibacterial activity

Organic extract were screened for antibacterial activity using the agar disc diffusion method [9]. The test samples dissolved in DMSO were used. Muller - Hinton agar was used as a culture media for bacterial growth. Loaded 20 micro liter of the each extract in the corresponding 6mm diameter in Whatmann filter paper No1. Plates were incubated for 24 h at 37 ° C for bacterial growth. The diameter of inhibition zone around each well was measured and recorded. The antibiotic disc such as penicillin, ampicillin, gentamycin and streptomycin were used as control [10].

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was defined as the lowest concentration of the sample that did not show any growth of the tested microorganism. In the present study 100µl of nutrient broth was taken in the wells of 96 well plate. To this 100µl of these diluents was added in the well making a twofold dilution. 100µl of this dilution was transferred and mixed with 100µl of the diluents in second row making a 4:1 dilution. This proceeds consecutively down the plate making two fold dilutions in each well. After the dilution of the sample is completed and aliquot of test organisms was added to all wells and the microtitre plates were incubated overnight. If the sample concentration was sufficient to kill the organism, no growth appeared and the wells will be clear. At this point there was an insufficient sample to kill the organism and well will be cloudy, indicating growth that is the minimum inhibitory concentration of the sample against that specific organism [11].

Statistical analysis

Values are given in the mean ± SD, and the differences between values were determined by the student's t-test. Values of $P < 0.01$ were considered significant.

RESULTS

The results of antibacterial activity of *Eclipta alba* were given in Table 1 and Table 2. Among all the tested extracts, the chloroform extracts of leaves and root were to be more effective than remaining other solvents and hot aqueous extract against all the tested organisms. In chloroform extract, all test organisms gave good susceptibility with zone of inhibition ranges from 08-35 mm than antibiotic disc. For benzene extract of leaves and root *Shigella flexneri* was most susceptible to the extract and shows 26mm and 12 mm of zone of inhibition respectively. *Bacillus subtilis, Pseudomonas fluorescens* and *Staphylococcus aureus* were exhibited similar activity for both leaves and root extract between 09 -11 mm zone of inhibition. No zone of inhibition was observed in the petroleum ether, acetone and aqueous extracts of leaves and root of *Eclipta alba* against the *Klebsiella pneumonia.* Methanol and acetone extract did not show any inhibition against the microorganism *Escherichia coli* and *Bacillus subtilis* respectively. The hot aqueous extract gave better results against inhibition of *Shigella flexneri* than acetone and methanol. Results of different extracts of the leaves and root of *Eclipta alba* were compared with the control plates. Hence, the leaves and root extracts of the *Eclipta alba* was found to show a good inhibition against the selected bacterial strains.

Table 1: Antibacterial activity of *Eclipta alba*

Extracts		Zone of Inhibition (mm)					
		E.coli	***B.subtilis***	***P. fluorescens***	***S. flexneri***	***K. pneumoniae***	***S. aureus***
PE	**Leaf**	15	13	10	-	**-**	**-**
	Root	08	10	10	09	-	08
BEN	**Leaf**	13	09	09	26	08	09
	Root	08	10	11	12	12	11
CHL	**Leaf**	13	28	22	23	32	10
	Root	08	09	12	12	35	09
ACE	**Leaf**	09	-	-	09	-	08
	Root	08	-	09	09	-	-
MeOH	**Leaf**	-	08	09	10	-	08
	Root	-	09	09	-	12	-
Aqu	**Leaf**	08	08	-	15	-	-
	Root	08	-	08	11	-	09

PE = Petroleum ether, BEN = Benzene, CHL = Chloroform, ACE = Acetone, MeOH = Methanol, Aqu = Aqueous, - = No activity.

#Values are the average of triplicate; includes Whatmann filter paper No1 diameter (6mm)

Antibiotics discs such as penicillin, ampicillin, gentamycin and streptomycin were used as a control for all bacterial strains. The results were shown in the Table 2.

Table 2: Antibiotic sensitivity against control

Control	***E.coli***	***B.subtilis***	***P.fluorescens***	***S.flexneri***	***K.pneumoniae***	***S.aureus***
PCN	07	10	-	16	-	22
AMP	13	-	11	26	-	22
GEN	17	24	20	18	-	21
STREP	10	23	18	15	11	20

PCN - Pencillin, AMP - Ampicillin, GEN - Gentamycin , STREP - Streptomycin

Table 3: Antibacterial activity of *Eclipta alba* against *Escherichia coli* and *Bacillus subtilis* by minimum inhibitory concentration (MIC) method

Extracts		***Escherichia coli* (mg/100µl)**							***Bacillus subtilis* (mg/100µl)**						
		0	**8**	**4**	**2**	**1**	**0.5**	**0.25**	**0**	**8**	**4**	**2**	**1**	**0.5**	**0.25**
Benzene	Leaves	**-**	**-**	**+**	**-**	**+**	**-**	**-**	**-**	**+**	**-**	**-**	**-**	**-**	**-**
	Root	-	+	+	-	-	-	-	-	+	+	+	+	-	-
Chloroform	Leaves	-	+	+	+	-	-	-	-	+	+	+	+	+	+
	Root	-	-	+	+	-	-	-	-	+	+	+	+	-	-

+ indicates positive -indicates negative

Table 4: Antibacterial activity of *Eclipta alba* against *Pseudomonas fluorescens* and *Shigella flexneria* by minimum inhibitory concentration (MIC) method

Extracts		*Pseudomonas fluorescens* (mg/100µl)							*Shigella flexneria* (mg/100µl)						
		0	8	4	2	1	0.5	0.25	0	8	4	2	1	0.5	0.25
Benzene	Leaves	-	+	+	+	-	-	-	-	+	-	+	+	+	-
	Root	-	+	+	+	+	-	-	-	-	+	+	-	-	-
Chloroform	Leaves	-	+	+	+	+	-	-	-	+	+	+	-	-	-
	Root	-	+	+	-	-	-	-	-	+	-	-	+	-	-

+ indicate positive - indicate negative

Table 5: Antibacterial activity of *Eclipta alba* against *Klebsiella pmeumoniae* and *Staphylococcus aureus* by minimum inhibitory concentration (MIC) method

Extracts		*Klebsiella pmeumoniae* (mg/100µl)							*Staphylococcus aureus* (mg/100µl)						
		0	8	4	2	1	0.5	0.25	0	8	4	2	1	0.5	0.25
Benzene	Leaves	-	+	+	+	-	+	-	-	+	-	+	+	-	-
	Root	-	+	+	-	+	+	-	-	-	+	+	-	-	-
Chloroform	Leaves	-	-	+	+	-	-	-	-	+	+	-	-	-	-
	Root	-	+	+	-	+	+	-	-	-	+	+	+	-	-

+ indicate positive - indicate negative

Minimum inhibitory concentration (MIC)

Those extracts giving zone of inhibition for all the selected bacterial strains were chosen to assay the minimum inhibitory concentration with agar dilution method and it is given in Table 3-5.

Eclipta alba extracts were inoculated against *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas fluorescens, Staphylococcus aureus* and *Shigella flexneri* in the concentration of 8-0.25 mg / 100µl. The growth of *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas fluorescens, Staphylococcus aureus, Shigella flexneri* were inhibited by benzene, chloroform extracts of leaves and root of *Eclipta alba* of the concentration of 8-1.0 mg / 100µl. 8 -2 mg /100µl of benzene and chloroform extract of leaves and root were found to have significant killing effects.

DISCUSSION

Among the tested organisms *Klebsiella pmeumoniae* showed higher percent of resistance against the different extracts of leaf and root. These observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings[12]. With no antibacterial activity, extracts may be active against other bacterial species which were not tested [13]

CONCLUSION

In the present study *Eclipta alba* has shown significant antibacterial activity against *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas fluorescens, Staphylococcus aureus, Shigella flexneri.* Hence the antibacterial activity might be due to the presence of active compounds in *Eclipta alba* against the growth of bacteria.

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