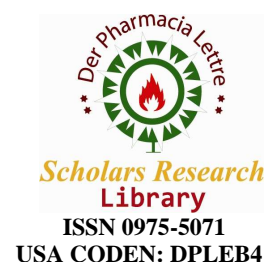




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Comparison of antibacterial activity of three morphotypes of medicinal herb *Eclipta alba* (L.) Hassk

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ABSTRACT

This study was conducted to determine the antimicrobial activity of the three morphotypes of *Eclipta alba* against gram positive bacteria: *Staphylococcus aureus*, *Bacillus subtilis* and gram negative bacteria: *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*. Disc diffusion method was used to test the antimicrobial activity of methanol, acetone and aqueous extracts of the leaves of the three morphotypes of *E. alba*. The study showed that these extracts have antimicrobial activity against all the bacteria except *P. aeruginosa*. Comparison of activity index of various types of extracts has shown that aqueous extracts were least effective. The acetone extracts of all the three morphotypes showed more antimicrobial activity against *S. aureus*, *E. coli* and *K. pneumoniae* than methanol extracts. The methanol leaf extracts of erect and intermediate type of *E. alba* showed maximum activity against *B. subtilis*. Among the three morphotypes the leaf extracts of erect type of plants showed maximum antimicrobial activity against all the bacteria except *B. subtilis* where the extracts of prostrate type showed maximum activity.

Key words: *Eclipta alba*, Antibacterial activity assay, Activity index.

INTRODUCTION

Eclipta alba is a renowned medicinal plant belonging to family Asteraceae and commonly known as False Daisy, 'yerba de tago' and bhringraj. It is an erect to prostrate, much branched, roughly hairy, annual, rooting at the node, leaves are opposite, sessile and lanceolate. Small white flowers are present on small heads. It grows commonly in moist places as a weed all over the world. It is widely distributed throughout India, China, Thailand, and Brazil and grows as a common weed throughout India, ascending up to 1,800m on the hills. *Eclipta alba* has evolved under natural selection in a wide range of agro-climatic areas resulting in wide phenotypic diversity. It is erect, partly erect or prostrate and is described as variable species by systematists. There is intraspecific variation in this polymorphic taxa. The plants are highly

variable in habit, hairiness, size and shape of leaves and size of flower head. Three morphotypes are common among the North Indian populations of *Eclipta alba* i.e. erect, intermediate and prostrate. The erect plants are tall and upright while the prostrate types have all creeping branches. The intermediate type plants have lower parts of branches creeping and tips are ascending.

Eclipta alba is widely used in Ayurveda and traditional Chinese herbal medicines [1]. It is used externally for ulcers and as an antiseptic for wounds in cattle and is reported to treat many microbial infections in rural areas [2]. There are various reports that crude extract from *E. alba* showed antibacterial, antifungal and antiviral activity [3,4,5,6,7,8]. Ethanolic extracts of fruits of *E. alba* has found to possess strong inhibitory effects on acne-inducing bacteria: *Staphylococcus epidermidis* [9]. Girish and Satish in 2008 [10] tested the aqueous and methanol extracts of *E. alba* against some human pathogenic bacteria and found that methanol extracts had wider range of activity. However, the information on the habit of *E. alba* plants used in the above studies is lacking. The present investigation primarily deals with the comparison of the antimicrobial potential of the locally available three morphotypes of *Eclipta alba*.

MATERIALS AND METHODS

Plant Material

The plants of different morphotypes of *Eclipta alba* were collected from different locations at Patiala and were maintained at Botanic gardens, Punjabi University, Patiala. The leaves of plants were used for biochemical quantitative analysis and antimicrobial assay.

Quantitative analysis

Various methods are generally followed for quantitative analysis of carbohydrates, proteins, phenolic compounds and saponins. Carbohydrates were quantitatively determined according to the anthrone method by Ashwell [11]. Briefly, 5 g fresh sample was extracted in 10ml distilled water. To 1 ml of this extract 4 ml of anthrone reagent was added and kept in boiling water bath for 10 min. It is allowed to cool to room temperature and the absorbance was read at 620nm against the blank of distilled water. Standard curve was prepared by using glucose.

To estimate the total protein content, Lowry's method was used [12] Five grams of fresh plant material was homogenized in 5ml of 0.1 N NaOH, Centrifuged at 3000rpm and supernatant was collected. The residue was re-suspended in centrifuge again. The two supernatants were pooled and the final volume was adjusted to 10ml. 2ml of the supernatant was treated with 1ml of 15% TCA and kept at 4°C for 24 hrs. Precipitates of protein were formed which were separated by centrifugation at 5000rpm for 20 min. Supernatant was discarded and precipitate were dissolved in 5ml of 0.1N NaOH and used for estimation. 5ml of soln. C (Prepared by mixing 2.0% Na₂CO₃ in 0.1N NaOH and 0.5% CuSO₄.5H₂O in 1% sodium potassium tartrate in the ratio of 50:1 at the time of use) was added to 1ml of Protein extract taken in a test tube and mixed thoroughly. The solution was left at room temperature for 10 min. and then 0.5ml of Folin-Ciocalteu's phenol reagent was added to it and mixed. After 30 min. absorbance was taken at 520nm against the blank of distilled water replacing the extract. Protein estimation was made by using standard curve prepared by using BSA (10-100µg/ml).

Total phenol content estimation have been done on all the accessions of *Eclipta alba* following the procedure of Singleton and Rossi [13]. Two gram fresh leaves were homogenised in 80% aqueous ethanol at room temperature and centrifuged in cold at 10,000rpm for 15 min and the supernatant was saved. The residue was re-extracted twice with 80% ethanol and supernants

were pooled, put into evaporating dishes and evaporated to dryness at room temperature. Residue was dissolved in 5ml distilled water. 100 μ l of this extract was diluted with 3ml distilled Water and 0.5ml Folin Ciocalteau reagent was added. After 3 min, 2ml 20% sodium carbonate was added and the contents were mixed thoroughly. After 60min the absorbance of the solution was taken at 650nm. The results were expressed as mg/g of sample.

Saponin content was estimated by using the method of Obadoni and Ochuko [14]. The leaf sample of *E. alba* was finely ground and Twenty gram of each were put into a conical flask to this 200ml of 20% aqueous ethanol were added. The suspension was heated over a hot water bath for four hour with continuous stirring at about 55°C. The mixture was filtered and the residue was re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90°C. The concentrate was transferred into a 250ml separator funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight. The saponin content was calculated as percentage.

Antimicrobial Assay

Preparation of Plant Extracts

Leaves of *E. alba* were collected and washed under running tap water, air-dried for 4-5 days. Leaves were then homogenized separately to fine powder and stored in airtight container for further use.

Aqueous Extract

Five gram of dried plant material was extracted in 100 ml distilled water for 6 h at 60°C. Extract was filtered and centrifuged at 6000 rpm for 25 min. The supernatant was collected and concentrated in a rotary evaporator and autoclaved at 121°C and dried. Dried extract was dissolved in DMSO (1:1 w/v) and stored at 4°C in airtight bottle.

Solvent Extract

For making solvent extract 5g of dried plant material (leaves) extracted in Methanol or Acetone (100 ml) and kept on a metabolic shaker for 48 h. Thereafter, it was centrifuged and filtered through Whatman filter paper no.1. The filtrate was concentrated in rotary evaporator. The concentrate was dried and dissolved in DMSO and stored at 4°C.

Test Organisms

Organisms used for antimicrobial activity assay were procured from Institute of Microbial Technology (IMTECH), Chandigarh. *Bacillus subtilis* (MTCC ACC NO. 2757), *Escherichia coli* (MTCC ACC NO. 3261), *Klebsiella pneumoniae* (MTCC ACC NO. 3384), *Pseudomonas aeruginosa* (MTCC ACC NO. 1035), *Staphylococcus aureus* (MTCC ACC NO. 740/96). All bacteria were sub-cultured in nutrient broth (13g/L) at 37°C for 24 h.

Disc Diffusion Method

The antimicrobial activities of aqueous, acetone and methanol extracts were performed by Disc Diffusion method [15] using *B. subtilis*, *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus*. All tests were carried out in triplicate. Sterilized petridishes were preseeded with 30 ml of agar containing growth medium and 0.5 ml of inoculation (inoculum's size 10⁴ cells/ml). Sterile paper

discs measured 9 mm diameter that absorbed 20 μ l of the test sample was placed on the solidified plates under aseptic conditions. The inoculated plates were stand for 1h and then incubated at 37 $^{\circ}$ ±1 $^{\circ}$ C for 24 h. The diameter of inhibition zone was measured and compared with those of the standard references i.e. positive control (20 μ l Chloramphenicol), negative control (DMSO), aqueous, acetone and methanol extracts of leaves.

Calculations

Activity index was calculated by comparing the zone of inhibition of by plant extract (leaf extract) with that of chloramphenicol.

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

RESULTS AND DISCUSSION

The leaf extracts in three different solvents viz. acetone, methanol and water were evaluated for antimicrobial activity against five strains of test organisms obtained fro MTCC collection. The result obtained showed that all kind of leaf extracts of *E. alba* exhibit bactericidal effects on the test microorganisms. The results are presented in Fig.1. Among the three morphotypes the leaf extracts of erect type of plants showed maximum antimicrobial activity against all the bacteria except *B. subtilis* where the extracts of prostrate type showed maximum activity. The intermediate type of plants also maintained the intermediate position among the three morphotypes. The most susceptible bacteria were *S. aureus* followed by *E. coli* while the most resistant bacteria were *P. aeruginosa* followed by *K. pneumoniae* and *B. subtilis*.

Different types of leaf extracts showed varying degrees of antimicrobial activity. The acetone extracts of all the three morphotypes showed more antimicrobial activity against *S. aureus*, *E. coli* and *K. pneumoniae* than methanol extracts. But for *B. subtilis*, the methanol extracts of erect and intermediate plants showed higher antimicrobial activity than prostrate type which showed more antimicrobial activity for acetone extracts. Thus among the three morphotypes the leaf extracts of erect type of plants showed maximum antimicrobial activity against all the bacteria except *B. subtilis* where the extracts of prostrate type showed maximum activity. The widest inhibition zones were observed on *S. aureus* and minimum against *B. subtilis*. The methanol and acetone extracts showed good results against all the bacteria except *P. aeruginosa*. Aqueous extract proved to be the least effective in all the cases. To evaluate the effectiveness of antimicrobial activity of different extracts of *E. alba*, AI (activity index) was also estimated using inhibition zone of Chloramphenicol as standard (+ve) control. Comparison of AI of various types of extracts has shown that aqueous extracts were least effective (Table 1). The study showed that these extracts have antimicrobial activity against all the bacteria except *P. aeruginosa*.

The biochemical composition of plants is the most common parameter used for the characterization of plants. The slight variation in biochemical compositions of the *E. alba* studied here, however, could be well attributed to genetic rather than environmental and seasonal factors. Quantitative analysis of phytochemicals in three morphotypes of *E. alba* showed the presence of carbohydrates, proteins, phenols and saponins. The biochemical compositions of *E. alba* leaves of the three morphotypes are presented in Table 2. It is observed that the levels of biochemicals in the leaves of erect and intermediate *E. alba* plants were comparatively higher than those in the leaves prostrate plants.

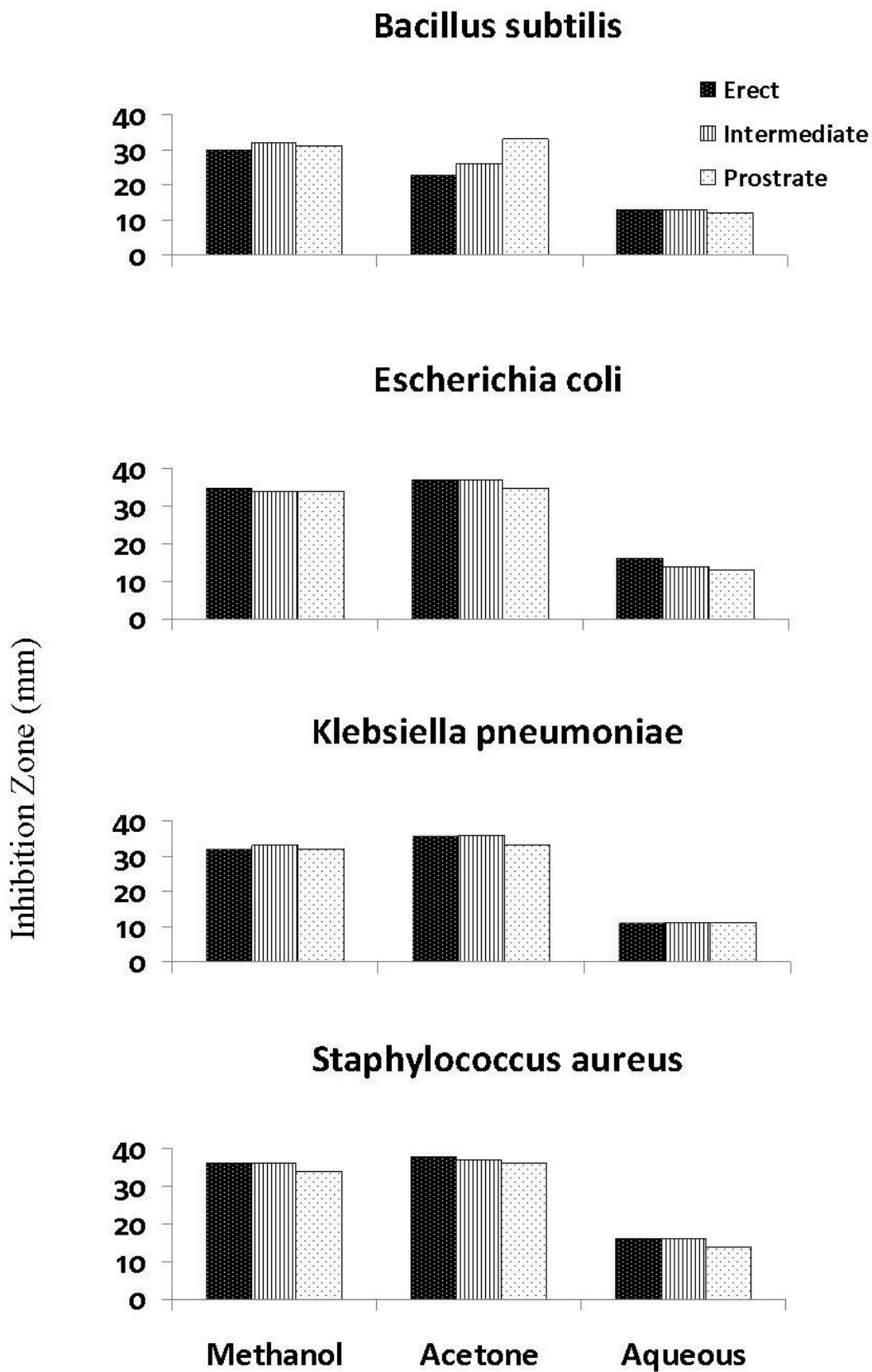


Fig. 1: Histograms showing antibacterial activity of three different morphotypes of *E. alba* against different organism

Phytochemical screening of the extracts of *E. alba* by earlier workers revealed the presence of tannins, coumestans, saponins, alkaloids, etc. [16,17,18,19]. However, no flavonoids and anthraquinones were detected in *E. alba* [20]. Of various phytoconstituents, Wedelolactones (coumestan), present in all *E. alba* is believed to be responsible for almost all the antibacterial activities [16]. In addition, other secondary metabolites such as phenolics could be held partially responsible for some of these biological activities.

Table 1: Activity index of various extracts of erect, intermediate and prostrate morphotypes of *Eclipta alba*.

Sr. No.	Organism	Erect type			Intermediate type			Prostrate type		
		M	Ac	Aq	M	Ac	Aq	M	Ac	Aq
1.	<i>Bacillus subtilis</i>	0.65	0.50	0.28	0.69	0.56	0.28	0.67	0.71	0.26
2.	<i>Escherichia coli</i>	0.81	0.86	0.37	0.69	0.86	0.32	0.69	0.81	0.30
3.	<i>Klebsiella pneumoniae</i>	0.72	0.81	0.25	0.75	0.81	0.25	0.72	0.75	0.25
4.	<i>Pseudomonas aeruginosa</i>	00	00	00	00	00	00	00	00	00
5.	<i>Staphylococcus aureus</i>	0.83	0.88	0.37	0.83	0.86	0.37	0.79	0.83	0.32

Abbreviations: Aq – Aqueous, Ac – Acetone, M – Methanol

Table 2: Biochemical compositions of leaves of three morphotypes of *Eclipta alba*

Biochemicals (mg/g)	Erect Mean \pm SD	Intermediate Mean \pm SD	Prostrate Mean \pm SD
Carbohydrates	18.38 \pm 0.0027	17.89 \pm 0.0055	17.16 \pm 0.0032
Proteins	14.41 \pm 0.0031	14.19 \pm 0.0025	13.69 \pm 0.0030
Phenols	19.98 \pm 0.0058	19.23 \pm 0.0045	18.26 \pm 0.0036
Saponins	4.2%	4.1%	3.9%

The antibacterial response of three types of extracts of *Eclipta alba* showed good antibiotic activity against both gram positive and gram negative strains except *P. aeruginosa*. The reason for the different sensitivity between gram-positive and gram-negative bacteria could be ascribed to the morphological differences between these micro-organisms, Gram-negative bacteria having an outer phospholipids membrane carrying the structural lipo-polysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while porins constitutes a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da [21]. The Gram-positive bacteria should be more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier [22]. Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and herbivores [23,24]. The demonstration of antibacterial activity of *E. alba* leaf extracts against both gram positive and gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds [25]. Out of the three solvents used for extraction, the acetone extracts showed the highest antibiotic activity against the test organisms, followed by the methanol extracts and water extracts. Different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent. Acetone extracts in this study might have had higher solubility for more phytoconstituents, consequently the highest antibacterial activity.

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 [26]. According to World Health Report on infectious diseases (WHO, 2000), overcoming antibiotic resistance is the major issue of the WHO for 21st century. The quality and quantity of these secondary metabolites can differ within the same species having different morphotypes or chemotypes.

CONCLUSION

The results obtained from the present investigation of screening three morphotypes of *E. alba* confirm the therapeutic potency of this plant used in traditional medicine. This study shows that the erect plants have higher potential to be used in antibiotic drug formulations. The antimicrobial response of three morphotypes can be attributed to the different proportions of constituents in the plants. This is probably the first report which compares the antibacterial action of three different morphotypes in this native medicinal plant.

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