



## PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL STUDIES OF THE LEAVES OF *ECLIPTA ALBA* (L)

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### ABSTRACT

Globally, medicinal plants have been used for treatment of several ailments. The present study was performed to evaluate anti microbial activity and preliminary phytochemical screening of *Eclipta alba* (L) leaf extracts. The *Eclipta alba* (L.) belongs family Asteraceae. The *Eclipta alba* were shade dried, powered and was extracted using solvents Methanol and other solvents. The antimicrobial activity test performed by the disc diffusion method. Preliminary phytochemical analysis of the plant extracts fractions of Carbon tetrachloride fraction (CTF), Chloroform fraction( CFF) and Aqueous fraction(AQF) showed the presence of alkaloids, tannin, flavonoids phenolic group and flavonoids. The CTF fraction of *E. alba* showed high activity against *E. coli* and *E. faecalis* bacteria.

**KEYWORDS:** Antimicrobial activity, *Eclipta alba*, disc diffusion, MIC.



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## INTRODUCTION

Medicinal plants are a rich source of antimicrobial agents (Kubmarawa *et al.*, 2007; Bakht *et al.*, 2011a). Many plant secondary metabolites are constitutive, existing in healthy plants in their biologically active forms, but others occur as inactive precursors and are activated by tissue damage or pathogen attack. Currently, majority of the pharmaceutically important secondary metabolites are isolated from wild or cultivated plants as their chemical synthesis is not economically feasible (Caldentey & Inze, 2004). Major groups of antimicrobial compounds from plants include simple phenols and phenolic acids, quinones, flavones, flavonoids and flavonols, tannins, coumarins, alkaloids, terpenoids and essential oils. *Eclipta alba* is one of the important medicinal herbs with a role in the traditional medicine systems of the East. It is reported to possess antiseptic, analgesic, antipyretic, antispasmodic, antimicrobial and antiviral properties. *Eclipta alba* is reported to be effective for the retrieval of memory (Banji *et al.*, 2007). It is hepatoprotective (Tabassum & Agrawal, 2004; Malhotra & Singh, 2007), anti-inflammatory (Arunachalam *et al.*, 2009) and antimalarial (Bapna *et al.*, 2007; Chenniappan & Kadarkarai, 2010). This plant is considered rejuvenative and good for hair, and a blackening dye for hair is obtained from this plant. The leaves of *Eclipta alba* are used against snake bites and scorpion stings. This plant is an important constituent of the polyherbal cardioprotective drug called abana (Baliga *et al.*, 2004). *Eclipta alba* is also reported to have antianaphylactic (Patel *et al.*, 2010), antihyperglycemic (Ananthi *et al.*, 2003) and antioxidant (Karthikumar *et al.*, 2007; Veeru *et al.*, 2009) properties. The present study was initiated to investigate the antimicrobial effects of different solvent extracts from *E. alba* on different microorganisms.

## MATERIALS AND METHODS

### (i) Collection of plant material

The selected plant materials used in this study were collected from Parvatagiri Village of

Torrur Mandal, Warangal district, Andhra Pradesh, and identified by Prof.V.Raju, Department of Botany, Kakatiya University, Warangal. The *Eclipta alba* leaves were collected and left at room temperature for two weeks to dry, then ground into powder and extraction with Soxhlet technique with methanol. Obtaining methanolic crude extracts of *Eclipta alba* were then fractionated successively using solvents of increasing polarity, such as, *n*-hexane (HX), carbon tetrachloride (CT), and chloroform (CF) and aqueous fractions (AQ). All the four fractions (HXF, CTF, CFF and AQF) were evaporated to dryness by using a rotary evaporator at low temperature (39<sup>o</sup>C).

### (ii) Preliminary phytochemical investigations

Phytochemical screening of plant extracts were carried out qualitatively for the presence alkaloids, tannin, flavonoids phenolic group and flavonoids were screened according to the common phytochemical methods described by Kokate (1994) and Kokate *et al* (1995).

### (iii) Bacterial species

Bacterial species selected for the study were the four pathogens, namely, two Gram-positive *Staphylococcus aureus* and *Enterococcus faecalis* and two Gram-negative *Psuedomonas aeruginosa* and *Escherichia coli*. All the cultures were maintained on Mueller-Hilton agar at 40<sup>o</sup>C. The cells were inoculated and incubated at 37<sup>o</sup>C in broth for 12 hours prior to the screening procedure.

### (iv) Minimal inhibitory concentration (MIC)

The serial microplate dilution method developed by Eloff (1998) was used to determine the minimum inhibitory concentration (MIC) for plant extracts using tetrazolium violet reduction as an indicator of growth. Residues of the different extracts were re-dissolved in methanol to a concentration of 1 mg/ml. for each of the four bacteria used, 100µl of each plant extract tested were two-fold serially diluted with 100 µl sterile distilled water in a sterile 96-well microtitre plates. A

similar two- fold serial dilution of gentamicine (0.1mg/ml) was used as a positive control against each bacterium. One hundred microlitres of each bacterial culture were added to each well. The plates were covered and incubated overnight at 37<sup>o</sup> C. To indicate bacterial growth 40 µl of 0.2 mg/ml p-iodonitrotetrazolium violet (INT) were added to each well and the plates incubated at 37<sup>o</sup> C for 30 minutes. Bacterial growth in the wells was indicated by a red colour, where as clear wells indicated inhibition of the bacterial growth by the plant extracts.

## RESULTS AND DISCUSSION

### (v) Percentage yield

The yield of the methanol crude extract of *Eclipta alba* was 500gr (16.4%) . The percentage yield of these fractions of the methanolic extract of *Eclipta alba* were showed in the table-1. The CTF fractions obtained highest yield (2.2%) when compared to other fractions. 0.8% yield obtained in HXF fraction which is lowest.

**Table-1**  
**Percentage of yield**

S.No	Fractions	Yield (%)
1	HXF	0.8
2	CTF	2.2
3	CFF	1.5
4	AQF	1.2

### (vi) Phytochemical screening in *E. Alba*

The phytochemical analysis of *Eclipta alba* showed the presence of different groups of secondary metabolites viz, alkaloids, tannin, flavonoids which are of medicinal importance of the test extracts, aqueous fraction showed positive results for most of the test compounds. The phenolic group and flavonoids were rich in CTF, CFF and AQF fractions when compared to other metabolites (Table -2). Alkaloids are rich in all fractions of *E. alba*.

**Table-2**  
**Phytochemical screening for presence of different phytoconstituents in *Eclipta alba* leaves extract fractions**

Sl. No.	Phytochemicals test	HXF	CTF	CFF	AQF
<b>I</b>	<b>Test for Alkaloids</b>				
a.	Mayer's Test	+	+	+	+
b.	Wagner's Test	+	+	+	+
c.	Hager's Test	++	++	++	+++
D	Dragendorff's Test	+	+	+	+
<b>II</b>	<b>Test for Carbohydrates</b>				
A	Molish's Test	+	+	++	+++
B	Fehling's Test	-	-	++	+++
C	Barfoed's Test	-	-	++	+++
D	Benedict's Test	-	-	+	++
<b>III</b>	<b>Test for Glycosides</b>				
a.	Borntrager's Test	+	+	+	++
B	Legal's Test	+	+	++	+++
<b>IV</b>	<b>Test for Saponin</b>				
A	Foam Test	+	+++	+++	-
<b>V</b>	<b>Test for Proteins and Amino acids</b>				
A	Millon's Test	-	++	+	-
B	Biuret's Test	-	++	++	-
C	Ninhydrin Test	-	++	+	-
<b>VI</b>	<b>Test for Phytosteroids</b>				
A	Libermann – Burchard's Test	-	-	+++	++
<b>VII</b>	<b>Test for fixed oils and fats</b>				
A	Spot Test	+	++	-	++
B	Saponification Test	-	+	-	+++
<b>VIII</b>	<b>Tests for Phenolic Compounds and Flavonoides</b>				
A	Ferric chloride Test	-	+	+	-
B	Gelatin Test	-	+	-	-
C	Lead acetate Test	-	+	-	++
D	Alkaline Rgt. Test	-	++	++	+
E	Magnesium Test	-	++	+	+

+++ Prominently Present, ++ Moderately Present, + Slightly Present, - Absent

**(vi) Antibacterial activity**

The MIC values and total activity of the four fractions of methanol crude extract of *Eclipta alba* plant against all the tested bacteria are presented in Table 3. *E. alba* AQF fractions also had noteworthy MIC values of 0.08 mg/ml and 0.63 mg/ml against *S. aureus* and *E. faecalis* respectively

**Table 3**

MIC values (mg/ml) of the four plant extracts fractions of the study: *P. emblica*, *E. alba*, *T. cordifolia* and *C. occidentalis* against four bacteria: *Escherichia coli* (Ec), *Enterococcus faecalis* (Ef), *Pseudomonas aeruginosa* (Pa) and *Staphylococcus aureus* (Sa). Gentamicin was used as a positive control.

Plant species	Ec	Ef	Pa	Sa
<i>E. alba</i>				
HXF	0.15	0.63	0.63	0.63
CTF	0.15	0.15	0.31	0.63
CFF	1.25	0.62	1.25	1.25
AQF	0.63	0.63	1.25	0.63
Gentamicin ( $\mu$ g/ml)	8.0	1.6	0.2	0.3

HXF=n-hexane fraction, CTF=Carbon tetra chloride fraction, CFF=Chloroform fraction and AQF=Aqueous fraction.

In this study the highest total activity was obtained on *Eclipta alba* (Table-4) from HXF fraction across *Enterococcus faecalis* (Ef), *Pseudomonas aeruginosa* (pa) and *Staphylococcus aureus* (Sa) bacteria. CTF fraction of *E. alba* showed high total activity against *E. coli* (146 ml/mg) and *E. faecalis* (146 ml/g) bacteria.

**Table-4**

Total activity (ml/g) of four plant extracts fractions of this study: *P. emblica*, *E. alba*, *T. cordifolia* and *C. occidentalis* against four bacteria: *Escherichia coli* (Ec), *Enterococcus faecalis* (Ef), *Pseudomonas aeruginosa* (Pa) and *Staphylococcus aureus* (Sa).

Plant species	Ec	Ef	Pa	Sa
<i>E. alba</i>				
HXF	48	11	11	11
CTF	146	146	70	34
CFF	11	22	11	11
AQF	15	15	8	15

HXF=n-hexane fraction, CTF=Carbon tetra chloride fraction, CFF=Chloroform fraction and AQF=Aqueous fraction.

**DISCUSSION**

The primary and secondary metabolites were analyzed in methanolic extracts. In an earlier study the extracts of the leaves of *E. alba* tested positive for alkaloids, phenolics, saponins and tannins, but no anthraquinones and flavonoids were detected (Caldentey et al.,2004). In Gujrat and Punjab, *E. alba* is used externally for ulcers and as an antiseptic for wounds in cattle and is reported to treat many microbial infections in rural areas (Warrier, 1994). The results from the current studies revealed that the wedelolactone may be the main constituent responsible for antimicrobial activity. There are various reports that crude extract from *E. alba* showed antibacterial, antifungal and anti viral activity (Kosuge et al.,

1985; Wiart et al., 2004; Karthikumar et al., 2007). In this study the CTF fraction of *E. alba* showed high activity against *E. coli* and *E. faecalis* bacteria.

**CONCLUSION**

Different extracts from the aerial parts of *Eclipta alba* showed antimicrobial activity against four microbial species. Most of the antimicrobial compounds of *Eclipta alba* are soluble in butanol and ethyl acetate. The antimicrobial compounds present in *Eclipta alba* may serve as an affordable and new source for the treatment of infectious diseases.

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