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Research Article

PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF LEAF EXTRACTS OF *ECLIPTA ALBA L.*

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ABSTRACT

Eclipta alba L. is commonly known as false daisy belonging to the Asteraceae family. The present study was designed to study the phytochemical composition and antimicrobial potential of ethanol and methanolic leaf extracts of *Eclipta alba* L. Phytochemical analysis was done using standard phytochemical methods. The crude leaf extracts of the plant were tested against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC BAA1705 and *Pseudomonas aeruginosa* ATCC 27853 by agar-well diffusion method. Ethanol and methanol leaf extract revealed the presence of carbohydrates, reducing sugars, alkaloids, fixed oil, saponins and phenolic compounds. The methanolic leaf extract showed the highest zone of inhibition compared with ethanolic leaf extract against all the tested bacterial pathogens. The results of the present study conclude that the studied plant possesses broad spectrum antibacterial properties.

INTRODUCTION

Antibiotic resistance has become a serious and common problem in developing countries, both in hospitals and the community, causing high mortality every year (Uddin *et al*., 2021). Improper usage of antibiotics is the most important factor of antibiotic resistance and the global emergence of multi-drug resistant bacteria is increasingly limiting the effectiveness of current medications. Moreover, wide use of antibiotics in the animal industry has also resulted in the emergence of antibiotic resistant microorganisms. By means of increasing patient movement and travel throughout the world, transmission of the drug resistant microorganisms from one nation to another nation also increased (Bokhary *et al*., 2021).

Antibiotic resistance results in reduced efficacy of antibacterial drugs, making the treatment of patients difficult, costly, or even impossible (Shrestha *et al*., 2018). The impact on particularly susceptible patients is apparent, resulting in prolonged illness and increased mortality (Djeussi *et al*., 2013). The possible solution for the above-mentioned problems could come from medicinal plants which have been reported to have antimicrobial properties. During the last few decades there has been an increasing interest in the study of traditional plants and their medicinal value in different parts of the world. The medicinal properties of plants have been investigated due to their potent pharmacological activities, low toxicity and economic viability (Chew *et al*., 2012).

Eclipta alba (L.) belongs to the Asteraceae family and is commonly known as false daisy in English. The plant is useful in the treatment of atherosclerosis, hyperlipidemia, inflammatory conditions, memory disorders, edema, rheumatic joint pains, hepatitis, enlarged spleen, and skin problems (Karthikumar *et al*., 2007). The plant is commonly used in hair oil all over India for healthy black and long hair (Roy *et al*., 2008). The plant is used in the treatment of minor cuts and burns and the fresh leaf-juice is considered very effective in stopping bleeding (Khan and Khan, 2008). Leaf juice mixed with honey is also used for children with upper respiratory infections and also used in eye and ear infections. Root has been reported to possess emetic and purgative property (McGuffin *et al*., 1997). The present study is aimed at phytochemical analysis and evaluating the antimicrobial properties of leaf extracts of *Eclipta alba* L. in order to give credence to its acclaimed ethnomedical usage.

MATERIALS AND METHODS

Collection and Processing of Leaves of *Eclipta alba* L.

Fresh leaves of *Eclipta alba* L. were collected from Puliyur, Cuddalore district, South India. The collected plant was authenticated by Dr. L.Mullainathan, Department of Botany, Annamalai University, Chidambaram. A voucher specimen of the plant (Acc.No. 367) was deposited at the herbarium of the department for future reference. The leaves were washed under running tap water, air dried, and homogenized to

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powder form using a mechanical grinder and then stored in a sterile air-tight container until further use.

Preparation of Crude Leaf Extracts

Leaf powder (10 g) was soaked separately in 100 ml of ethanol (90%) and methanol (90%), in a conical flask, plugged with cotton and left at room temperature for 3 days. The obtained extract was filtered through Whatman No.1 filter paper. Each filtrate was evaporated in glass Petri dishes at room temperature for 2-3 days till golden viscous mass was obtained and then stored at 4℃ in air tight bottles (Swaminathan and Santhi, 2019).

Phytochemical Screening of Leaf Extracts

The ethanolic and methanolic leaf extracts of *Eclipta alba* L. were tested for the presence of phytochemicals using standard phytochemical methods (Parkavi *et al*., 2020).

Determination of Antimicrobial Activity of Leaf Extracts

Ethanol and methanol extracts of leaves of *Eclipta alba* were tested for antibacterial activity against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC BAA1705 and *Pseudomonas aeruginosa* ATCC 27853 using agar-well diffusion method (Mahida and Mohan, 2006). The test bacterial strain was inoculated in to Mueller-Hinton broth and incubated at 37°C for 6 hours. After incubation, the inoculum was spread evenly over the entire Mueller-Hinton agar surface by a sterile cotton swab and allowed to dry for about 3 minutes. Wells measuring the diameter of 6 mm were punched in to the agar medium using a sterile cork borer and filled with 100 µl of crude leaf extracts (100 mg/ml 10% DMSO) with the help of micropipette. Gentamicin antibiotic was used as positive reference standard to determine the sensitivity of the strains. For negative control, 100 µl of 10% dimethyl sulfoxide (DMSO) was added to one of the well. The plates were incubated in an upright position at 37°C for overnight in an incubator. Antibacterial activities were evaluated by measuring the diameter of zone of inhibition in mm against the test organism.

RESULTS

Phytochemical Screening of Leaf Extracts

Preliminary phytochemical analysis of ethanolic and methanolic extracts of leaves of *Eclipta alba* L. is shown in Table 1. Ethanol extract revealed the presence of carbohydrates, reducing sugars, alkaloids, phytosterols, fixed oil, saponins and phenolic compounds, whereas flavonoids, proteins and free aminoacids were absent. Methanol extract showed the presence of carbohydrates, reducing sugars, alkaloids, fixed oil, saponin, phenolic compounds and flavonoids, whereas phytosterols, proteins and free aminoacids were absent.

Determination of Antimicrobial Activity of Leaf Extracts

The antibacterial activity of ethanolic and methanolic leaf extract of leaves of *Eclipta alba* L. was tested against four standard bacterial cultures with gentamicin as positive control and DMSO as negative control (Table 2). The inhibitory activities of leaf extracts were compared with standard antibiotic gentamicin. Ethanolic leaf extract of the plant showed highest antibacterial activity against *Escherichia coli*, followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Methanolic leaf extract of the plant recorded highest antibacterial against *Escherichia coli*, followed by *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Table 1 Phytochemical screening of leaf extracts of *Eclipta alba* L.

S.No.	Phytochemicals	Test	Ethanol Extract	Methanol Extract
1	Carbohydrates	Molisch's test	+	+
2	Reducing sugar	Benedict's test	+	+
		Fehling's test	+	+
3	Alkaloids	Dragendorff's test	+	+
		Mayer's test	-	-
4	Phytosterols	Salkowski test	+	-
5	Fixed oil	Spot test	+	+
6	Saponins	Froth test	+	+
7	Protein and free aminoacids	Biuret test	-	-
		Ninhydrin test	-	-
8	Phenolic compounds	Ferric chloride test	+	+
9	Flavonoids	Alkaline reagent test	-	+

+: Positive - : Negative

Based on the results, it is concluded that the methanolic leaf extract showed the highest zone of inhibition compared with ethanolic leaf extract against all the tested bacterial pathogens. Gentamicin showed zone of inhibition ranging from 20 mm – 35 mm. DMSO was used as negative control which showed no zone of growth inhibition.

Table 2: Antibacterial activity of ethanol and methanolic leaf extracts of *Eclipta alba* L.

S. No	Bacterial Strains	Diameter of Zone of Inhibition (mm)			
		Gentamicin	DMSO	Ethanolic Leaf extract	Methanolic Leaf extract
1	*Staphylococcus aureus* ATCC 25923	30	-	17	27
2	*Escherichia coli* ATCC 25922	35	-	24	28
3	*Klebsiella pneumonia* ATCC BAA1705	20	-	18	19
4	*Pseudomonas aeruginosa* ATCC 27853	27	-	18	22

- : No zone of inhibition DMSO: Dimethyl sulfoxide

DISCUSSION

The antibacterial activity of leaf extracts of *Eclipta alba* documented in the present study may be due the presence of phytochemicals i.e., carbohydrates, reducing sugars, alkaloids, phytosterols, saponins, phenolic compounds and flavonoids. The zone of inhibition varied suggesting the varying degree of efficacy and different phyto-constituents of the plant on the test organism (Elumalai *et al*., 2011). The zone of inhibition

produced by gentamicin, was larger than those produced by ethanol and methanol leaf extracts of *Eclipta alba.* It may be attributed to the fact that the plant extracts being in crude form contain smaller fraction of bioactive compounds (Zuraini *et al*., 2007).

Many plants release phenolic compounds that are toxic to microbial pathogens (Aboaba *et al*., 2006). Flavonoids are reported for their antimicrobial, antiviral, and spasmolytic properties. Saponins has the property of precipitating and coagulating red blood cells. Some of the characteristics include formation of foams in aqueous solution, haemolytic activity, cholesterol binding properties and bitterness (Okwu, 2004). Alkaloids isolated from plants are commonly found to have antimicrobial properties. The antibacterial activities of these compounds might be due to their ability to complex with bacterial cell wall and therefore, inhibiting the microbial growth.

The outer membrane found in the Gram-negative cell wall is composed of structural lipopolysaccharides which render the cell wall impermeable to lipophilic solutes, unlike Gram-positive bacteria which do not have this outer membrane. This morphological difference influences their reaction to antibacterial agents (Scherrer and Gerhardt, 1971). The result obtained in the present study showed that the ethanolic and methanolic leaf extracts of *Eclipta alba* L. are effective against both Gram positive and Gram-negative bacteria.

The results of the present study are in agreement with Dalal, 2010 who have reported that ethanol extract of leaves of *Eclipta alba* showed significant antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* due to the wedelolactone components. Uddin *et al.*, 2010 and Chitravadivu *et al.,* 2009 also recorded that the ethanolic extract of aerial parts of *Eclipta alba* revealed high antibacterial activity for *Staphylococcus aureus* and *Escherichia coli.*

CONCLUSION

In the present study, ethanol and methanolic leaf extract of leaves of *Eclipta alba* L. showed very good antibacterial activity against a battery of standard bacterial strains. The obtained results could serve as a primary basis for further pharmacological studies in the discovery of potential natural bioactive compounds.

CONFLICT OF INTEREST: Nil

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