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Asian Resonance

Phytochemical and Anti-Bacterial Activity of Eclipta Alba

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Abstract

Eclipta alba is an important herb that is widely used in traditional cuisine and in folk medicine for the treatment of various ailments including digestion, headache, asthma, cough and normalizing skin and hair colour. Keeping in view the importance of *E. alba*, preliminary qualitative phytochemical analysis of the extracts was carried out by standard methods. Antimicrobial activity of petroleum ether, ethyl acetate, methanol and aqueous extracts of leaves of *E. alba* was evaluated against selected strains of human pathogenic bacteria, *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella abony* and *Pseudomonas aeruginosa* by agar well diffusion method. Maximum of selected test strain were inhibited by the ethyl acetate extract with zones of inhibition in the range of 8 mm to 16 mm with maximum activity against *Staphyllococcus aureus.* It was followed by methanol extract with zone of inhibition in the range of 8 mm to 10 mm, aqueous extract inhibited the growth of only one bacteria *Bacillus subtilis* with zone of inhibition 11 mm, petroleum ether extract was not effective against any of the selected pathogenic bacteria. Major phytochemicals *viz* alkaloids, carbohydrates, protein, lipid, saponins, resins, tannins, sterols, cardiac glycosides and triterpenes were detected in the extracts. This study justifies the use of the plant in traditional system of medicine and also shows that the plant could be a potential source of new antibacterial agents.

Keywords: Eclipta Alba, Phytochemical Analysis, Antibacterial Activity

Introduction

In India thousands of species of plants are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. Today it is estimated that more than two thirds of the world's population relays on plant derived drugs. About 7000 medicinal compounds used in the Pharmacopoeia are derived from plants. Hence, ethno-pharmacologists, botanists, microbiologists, and natural-product chemists are searching for phytochemicals which could be developed for the treatment of infectious diseases especially in light of the emergence of drug-resistant microorganisms and the need to produce more effective antimicrobial agents (Tanaka *et al*., 2006).

Eclipta alba (L.) Hassk, commonly known as False Daisy and bhringraj is an important medicinal plant belonging to the family Asteraceae (Kusumoto *et al*., 1995). It is usually found in moist tropical regions of the world, throughout India as a common weed in waste lands. It is traditionally used for blackening, promoting hair growth and strengthening the hair. In Ayurveda medicine, the leaf extract is considered a powerful liver tonic and rejuvenative. It is externally used in athlete foot, eczema, dermatitis, on the scalp to address hair loss and the leaves have been used in the treatment of scorpion stings. It is used as anti-venom against snake bite in China and Brazil (Kritikar and Basu, 2004). It is also known for its various pharmacological potentials analgesic (Sawant *et al*., 2004), anticancer (Chaudhary et al., 2011), antidiabetic (Hemlatha *et al*., 2006), antihepatotoxic (Lal *et al*., 2010) and anti-inflammatory activity (Arunachalam *et al*., 2009).

Antimicrobial potential of different medicinal plants is being extensively studied all over the world (Arora and Kaur, 1999; Ahmad *et al.,* 1998; Rojas *et al.,* 2006) but the subject needs in depth studies in a systematic manner since, in the absence of scientific proof of their effectiveness, the validity of these remedies remains questionable and their use locally restricted (Ahmad *et al.,* 1998). Considering the spectrum of medicinal value of *E. alba*, the present study was initiated to investigate

antibacterial potential and to evaluate the effect of different solvents on the extraction of antimicrobial substances from the plant.

Material and Methods

Collection and Identification of Plants

The plants were collected from South Civil Lines area near Govt. Model Science College (Auto.), Jabalpur and R. D. University, Jabalpur. The taxonomic identification of plant was done by available literature (Verma et al., 1993; Oomachan and Shrivastava, 1996; Mudgal and Khanna, 1997) and was confirmed from State Forest Research Institute Polipathar, Jabalpur and deposited the voucher specimen no.-7147/9815.The collected sample were fully grown plants. Only leaves were used for different study. The twigs were washed under running tap water to remove adherent soil and other extraneous matter. After washing the plants were soaked on to a newspaper to get rid of extra water.

From the collected plants leaves were manually separated, washed and shade dried at room temperature. The contaminated leaves having fungal growth during drying were discarded immediately. The dried sample was milled in to powder using the electric blender. The powder was stored in air tight bottles for further analysis.

Extraction of Plant Material with Solvents of Different Polarity

In order to perform a systematic antibacterial activity, powdered leaves of *Eclipta alba* were extracted with an array of solvents by polar solvent directing towards non-polar solvents and for this four solvents were selected water, methanol, ethyl acetate and petroleum ether. Cold percolation method was adopted (Harborne, 1998) for aqueous extraction, while for other solvents successive extraction was done by soxhlet extractor.

Phytochemical Screening

Phytochemical screening was performed in the plant extracts obtained by extraction with different solvents using standard procedure as described by (Trease and Evans, 1983; Harborne, 1998: Thimmaiah, 2004).

Antibacterial Screening

The different extracts were screened for antibacterial activity against five clinically important bacteria. These bacteria were procured from American Type Culture Collection (ATCC), USA through Microbiologics®, USA. Among test bacteria three Gram negative bacteria i.e. *Escherichia coli* ATCC 25922*, Salmonella abony* ATCC 6017 and *Pseudomonas aeruginosa* ATCC 27853 were used while among Gram positive bacteria *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 11774 were used. The agar well diffusion method was used to determine the antibacterial activity using Bauer-Kirby method (Bauer *et al.,* 1966). For antibacterial activity, Muller Hinton Agar media (HiMedia, India) was used.

For agar well preparation, 6 mm of agar wells were punched using the well punch. 50 µl of the plant extract (aqueous, methanol, ethyl acetate and petroleum ether) was placed into this well. Erythromycin (10 µg) was used as a control antibiotic for Gram negative bacteria while Amikacin (30 µg) was used as control antibiotic for Gram positive bacteria. The plates were allowed to stand for 30 min and carefully placed into incubator without spilling the extracts. The plates were incubated for 37 ± 1°C for 48 h. After incubation period the results were expressed as diameter of the clearing zone around the well measured in millimeters using a zone measurement scale (HiMedia, India).

Results and Discussion

The aqueous extract of *Eclipta alba* showed presence of alkaloids, carbohydrates, protein, lipid, saponins, resins, tannins, sterols, cardiac glycosides and triterpenes. In methanol extract lipids, saponins and tannins were present while all other secondary metabolites are absent. Ethyl acetate extract showed positive results only for the presence of carbohydrates, lipids, resins and triterpenes. Petroleum ether extract showed presence of carbohydrates, resins and sterols. But flavonoids were not detected in any extract **(Table 1).** Hussain *et al.* (2011) performed similar studies in *Eclipta alba* and reported the presence of reducing sugars, flavonoids, saponins, alkaloids, tannins in ethanol extract. Prasad *et al*. (2012) performed phytopharmacognostic study of *Eclipta alba.* They reported the presence of carbohydrates, proteins, alkaloids, tannins and steroids.

Eclipta alba leaf aqueous extract showed inhibitory activity only against Gram positive bacteria *Bacillus subtilis* with zone of inhibition (11 mm) but it fails to inhibit the growth of other test bacteria. Methanol extract inhibited the growth of two Gram negative bacteria *Escherichia coli* (7 mm) and *Pseudomonas aeruginosa* (10 mm) and it also showed zone of inhibition against Gram positive bacteria *Staphylococcus aureus* (10 mm). Ethyl acetate extract of *Eclipta alba* leaf showed activity against all the five test bacteria with clear zone of inhibition *Escherichia coli* (12 mm), *Salmonella abony* (8 mm), *Pseudomonas aeruginosa* (11 mm), *Staphylococcus aureus* (16 mm) and *Bacillus subtilis* (14 mm). Petroleum ether extract could not show any activity against any of the selected test bacteria **(Table 2& Fig 1)**. Eclipta alba has significant antimicrobial activity against common pathogens due to the wedelolactone components (Dalal, 2010). Similar studies (Uddin *et al.*, 2010; Chitravadivu *et al*., 2009) also recorded that the organic extracts of aerial parts of *Eclipta alba* revealed high antibacterial activity for *S.aureus, Bacillus subtilis, E.coli* and *Salmonella* spp*.*

The proportion index suggests that the antimicrobial activity of the plant extracts varies with the solvent used for the purpose of extraction and hence antimicrobial activity may be regarded solvent-specific (Fig. 2). This variation in the activity of the extracts may be due to the different phytochemicals contained in them (Cowan, 1999; Borkataky *et al.,* 2011; Abdullah *et al.,* 2012; Natarajan *et al.,* 2012)]. The higher activity of the ethyl acetate and methanol

extracts may be due to the different phytochemicals present in different percentages.

The present study shows that the plant extracts prepared in organic solvents - ethyl acetate and Methanol, exhibited higher antibacterial activity than the aqueous extract. These findings support the works of (Vaghasiya and Chanda, 2007). The results clearly indicate that the ethyl acetate extract of the *E. alba* possesses broad-spectrum antibacterial activity.

Conclusion

The results of the present study showed that the selected plant *Eclipta alba* extracts was effective against all the bacterial spp. tested. This can be used to treat various diseases like pimples, typhoid, food borne infections, UTI, sore throat and nosocomial infections. This investigation has opened up the possibility of the use of this plant for formulating a drug for human consumption possibly for the treatment of bacterial infections. 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 antibacterial activity. The results of the present study also support the medicinal usage of the studied extracts which can be used as Antibacterial agents in new drugs for therapy of infectious diseases caused by pathogens. The most active extract can be subjected to identification and isolation of the therapeutic antimicrobials and undergo further pharmacological screening that can be used as sources for new drugs.

Table 1: Phytochemical Analysis of Different Extracts of *Eclipta alba*

Constituents	Test	Extracts			
		Aqueous	Methanol	Ethyl acetate	Petroleum ether
Alkaloids	Mayer's test	-	-	-	-
	Dragendroff's test	+	-	-	-
	Wagner's test	+	-	-	-
Carbohydrates	Molisch's test	-	-	+	+
	Benedict's test	+	-	-	-
	Fehlings test	+	-	-	-
Protein	Xanthoprotic test	+	-	-	-
	Biuret test	+	-	-	-
Lipids	Solubility test	+	+	+	-
	Glycerol test	-	-	-	-
	Sudan III test	+	-	+	-
Saponins	Foam test	+	+	-	-
Flavonoids		-	-	-	-
Resins		+	-	+	+
Tannins	Gelatin test	-	+	-	-
	Lead acetate test	-	-	-	-
	Ferric chloride test	+	-	-	-
Sterols	Salkowski's test	+	-	-	+
Cardiac glycosides	Keller-killiani test	+	-	-	-
Triterpenes		+	-	+	-

Present +
Absent –

Table 2:Anti-Bacterial Activity of Different Extracts of *Eclipta alba*

Name of Bacteria	Aqueous	Methanol	Ethyl-Acetate	Petroleum-Ether	Control Erythromycin /Amikacin
Escherichia coli	0 mm	7 mm	12 mm	0 mm	23 mm
Salmonella abony	0 mm	0 mm	8 mm	0 mm	23 mm
Pseudomonas aeruginosa	0 mm	10 mm	11 mm	0 mm	24 mm
Staphylococcus aureus	0 mm	10 mm	16 mm	0 mm	26 mm
Bacillus subtilis	11 mm	0 mm	14 mm	0 mm	22 mm

Control-Erythromycin for Gram negative bacteria.
Amikacin for Gram positive bacteria.

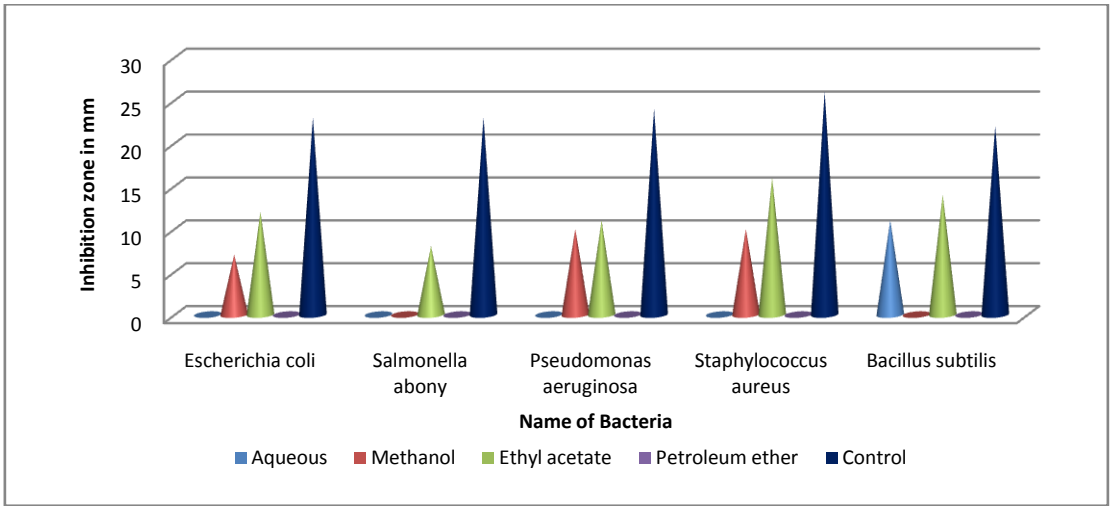

Fig 1: Antibacterial Activity of Different Extracts of *Eclipta alba*

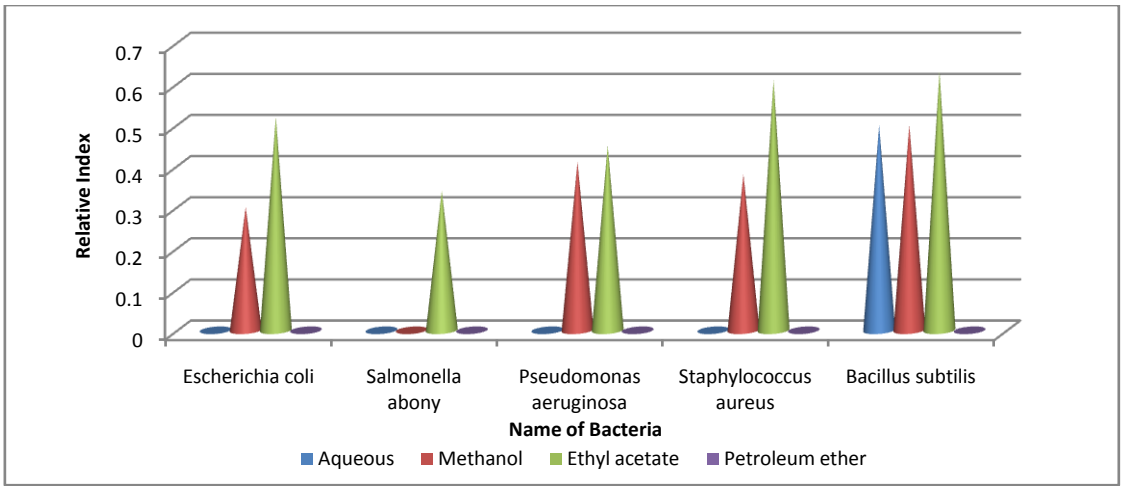

Fig 2: Relative Activity Index of Different Extracts of *Eclipta alba*

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