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PHYTOCEUTICAL EVALUATION AND ANTIMICROBIAL PROPERTIES OF *Eclipta alba* AND *Tylophora indica*

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Abstract- Since plants are used as therapeutic agents, the present study was conducted to evaluate the phytochemical profile and antibacterial activities of extracts of. *Eclipta alba* and *Tylophora indica* .Studies on the antibacterial activity of methanol, petroleum ether and aqueous extracts of dry leaf, stem and root of *Eclipta alba* and *Tylophora indica* was evaluated using zone of inhibition studies and minimum inhibitory concentration. The microorganisms used include *Escherichia coli, Micrococcus roseus* and *Pseudomonas flavescens* . The extract exhibited antibacterial activity against these experimental strains. Methanol extracts shows higher degree of antibacterial activity than aqueous and petroleum ether extracts. The minimum inhibitory concentration ranged between 12.5mg/mL and 75mg/mL depending on microorganism and various extract. Screening of the phytochemical extracts revealed the presence of compounds like alkaloids, glycosides, tannins and flavonoids. *Escherichia coli* and *Pseudomonas flavescens* are more sensitive where as *Micrococcus roseus* is less sensitive to extracts of *Eclipta alba* and *Tylophora indica*. The experimental observation shows that *Eclipta alba* and *Tylophora indica* have antibacterial activity and can be used for therapeutic purposes.
Key words -Phytochemical, antibacterial activity, inhibition zone, *Eclipta alba, Tylophora indica* alcoholic and aqueous extracts

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Introduction

Herbal medicines, also called botanical medicines or phytomedicines, refers to the use of any plant's seeds, berries, roots, leaves, bark or flowers for medicinal purposes have long being practiced. As up to-date analysis and research shows their value in the treatment and prevention of disease, herbal drugs are more mainstream products.

Any part of the plant may contain active components like alkaloids, saponins; tannins, essential oils etc.Polyphenols and tannins in plants inhibit insect growth [3] disrupting digestive events of ruminal animals. They are also sometimes added to food meant for pregnant and nursing mothers for medicinal purposes. The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infections- fighting strategies [5].

Aerial parts of a well known Indian hepatoprotective herb, *Eclipta alba* (*Asteraceae*) are used traditionally for the treatment of several diseases of liver, skin and stomach. The shoot extract of *E. alba* showed antimicrobial [1,10,28] antifungal activity [26] and weak cytotoxicity against the M-109 cell lines by alkaloids Verazine [2], antiviral activity against Ranikhet disease [11], antibacterial [12]. The leaves and roots of *Tylophora indica* (Asclepiadaceae) have emetic, cathartic, laxative, expectorant, diaphoretic and purgative properties. It has also been used for the treatment of allergies (asthma), cold, whooping cough, dysentery, hay fever and arthritis. It has reputation as an alterative and as a blood purifier, often used in rheumatism and syphilitic rheumatism. Root or leaf powder is used in diarrhoea, dysentery and intermittent fever. It is regarded as one of the best indigenous substitute for *ipecacuanha.* The roots are suggested to be a good natural preservative of food. Phytochemical literature reveals the presence of tylophorin, alkaloids, tannins, saponins, flavonoids in

Tylophora indica [6,9,23] *T. indica* is used in the preparation of homeopathy medicines.

However, up to date, research has not been done to investigate various pharmacological activities and antimicrobial activities of only crude extracts of these traditionally used plants.

Present investigation reveals the study of antimicrobial activity of *Eclipta alba* and *Tylophora indica* which are found in the medicinal garden of Biotechnology Department of GIET, Gunupur, against *Escherichia coli, Micrococcus roseus* and *Pseudomonas flavescens*.

Materials and Methods

Plant Collection

The leaves, stems and roots of *E. alba* and *T. indica* were collected from medicinal garden of Biotechnology Department, GIET, Gunupur. Fresh plant materials were shade dried then kept in hot air oven at 60°C for 24 hours. and homogenized into fine powder.

Plant Extraction

The fine powder was taken and subjected to different solvents to get phytochemical extracts. The solvents used were methanol, petroleum ether and water. 1gm powder of *E. alba* and *T. indica* was taken for extraction with 100 ml of different solvents in Soxhlet apparatus. The extracts were filtered through muslin cloth and concentrated by evaporation at room temperature until the solvent gets evaporated completely. Extraction was carried out with all solvents separately. Then extracted materials lyophilized by occasional shaking for 24 hrs. The extracts were preserved in refrigerator for further studies.

Phytochemical Screening

Phytochemical screening of plant extracts were carried out qualitatively for the presence of glycosides, alkaloids, flavonoids and tannins.

Antimicrobial Assay

Antimicrobial assay of solvent extracts were performed by Disc diffusion method. Lawn culture of *Escherichia coli, Micrococcus roseus* and *Pseudomonas flavescens* were developed on nutrient agar plates using sterile cotton swabs. The sterile disc (5mm) was saturated with various plant extracts. Disc with solvents were used as control and the respective antibiotic disc Gentamycin used as positive control. The sterile impregnated disc with plant extract were placed on the agar surface with flamed forceps and gently pressed down to ensure complete contact of the disc with the agar surface. After the incubation at 37°C for 24h the size of the inhibition zone were measured.

Antibacterial activity was determined by measuring the diameter of the zone of inhibition For each strain, controls were included that comprised pure solvents instead of the extract. The experiments were repeated three times and the mean values were presented.

Microorganisms Tested

Bacterial Strains used in this study were purchased from MTCC, Chandigarh. These are *Escherichia coli, Micrococcus roseus* and *Pseudomonas flavescens* All these strains were confirmed by cultural & biochemical characteristics and maintained in slants for further use.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration values were determined by broth dilution assay for the plant showing better result. Varying concentrations of the extracts (200mg/mL, 150mg/mL,100mg/mL,75mg/mL,50mg/mL and 12.5mg/mL) were prepared. 0.1mL of each concentration was added to each 9mL of nutrient broth containing 0.1mL of standardized test organism of bacterial cells. The tubes were incubated at 37°C for 24h. Positive controls were equally set up by using solvents and test organisms without extracts. The tube with least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration.

Results and Discussions

The results of the phytochemical analysis of the different alcohol and aqueous plant extract of *Eclipta alba* are shown in Table 1 The extracts mostly contained higher quantities of glycosides, followed by flavonoids and alkaloids. Alkaloids and tannins were entirely absent in most of the tested aqueous extracts.

Table 1- Preliminary Phytochemical Screening of Eclipta alba Alcohol, Aqueous Extract

Sl. No.	Phytochemical Tests	Alcohol Extract	Aqueous Extract
1	Glycosides	+++	+
2	Alkaloids	+	_
3	Flavonoids	+	+
4	Tannins	+	_

Phytochemical tests: +++: quantitative; + : positive; - : negative

The Phytochemical screening of the alcohol and aqueous extracts of *Tylophora indica* revealed the presence of glycosides, alkaloids, flavanoids and tannins in all the plant extracts studied as shown in Table 2. The metabolites are of various pharmacological importance. The presence of tannin in most of plant extract could be responsible for possible antitumor and anti oxidant activities [7].

Table 2- Preliminary Phytochemical Screening of Tylophora indica Alcohol, Aqueous Extract

Sl. No.	Phytochemical Tests	Alcohol Extract	Aqueous Extract
1	Glycosides	+	+
2	Alkaloids	+	+
3	Flavonoids	+	+
4	Tannins	+	+

Phytochemical tests: +++: quantitative; + : positive ; - : negative

Table 3 indicates that methanol, petroleum ether and aqueous leaf extracts of *E. alba* is more sensitive to *E. coli* than to other experimental bacterial strains.

Table 3- Antimicrobial activity of plant leaf extract

Sl. No.	Microorganism	Zone of Inhibition (mm) of *Eclipta alba* leaf extracts in (mm)		
		Methanol	Petroleum ether	Water
1	*Escherichia coli*	15	5	4
2	*Micrococcus roseus*	5	NIL	NIL
3	*Pseudomonas flavescens*	4	3	NIL

In case of *E. coli* methanol leaf extracts shows maximum zone of inhibition (15mm) than that of petroleum ether and aqueous ex-

tracts. Where as *M. roseus* and *P. flaescens* shows more or less zone of inhibition in organic solvents and no zone of inhibition in aqueous extracts.

Table 4 indicates that the methanol, petroleum ether and aqueous stem extracts of *E.alba* is more sensitive to *E.coli* than other two experimental bacterial strains. Here the findings indicate that the aqueous stem extracts is more sensitive i.e. 12mm zone of inhibition. Methanol stem extracts of *E. alba* shows maximum zone of inhibition (20 mm) against *P.flavescens* and no zone of inhibition is seen in aqueous stem extract of *E. alba* against *P. flavescens* and *M. roseus*.

Table 4- Antimicrobial activity of plant stem extract

Sl. No.	Microorganism	Zone of Inhibition (mm) of *Eclipta alba* stem extracts in (mm)		
		Methanol	Petroleum ether	Water
1	*Escherichia coli*	11	5	12
2	*Micrococcus roseus*	10	2	NIL
3	*Pseudomonas flavescens*	20	12	NIL

Table 5 indicates that the methanol root extracts of *E. alba* shows zone of inhibition against the three experimental bacterial strains where as petroleum ether root extracts of *E. alba* does not show any zone of inhibition. Here the methanol root extracts of *E. alba* shows maximum (20mm) zone of inhibition against *P. flavescens*.

Table 5- Antimicrobial activity of plant root extract

Sl. No.	Microorganism	Zone of Inhibition (mm) of *Eclipta alba* root extracts in (mm)		
		Methanol	Petroleum ether	Water
1	*Escherichia coli*	10	NIL	6
2	*Micrococcus roseus*	3	NIL	NIL
3	*Pseudomonas flavescens*	20	NIL	NIL

The observations of Table 6 shows that the methanol and aqueous leaf extracts of *T. indica* is more sensitive to *E. coli* than the other two bacterial strains studied. Methanol leaf extract of *T.indica* shows maximum zone of inhibition (8mm) against *E. coli.* Where as neither of the leaf extracts of *T. indica* shows any zone of inhibition against *M. roseus*. Also the aqueous leaf extracts of *T. indica* shows zone of inhibition (2mm) against *P. flavescens*.

Table 6- Antimicrobial activity of plant leaf extract

Sl. No.	Microorganism	Zone of Inhibition (mm) of *Tylophora indica* leaf extracts in (mm)		
		Methanol	Petroleum ether	Water
1	*Escherichia coli*	8	NIL	5
2	*Micrococcus roseus*	NIL	NIL	NIL
3	*Pseudomonas flavescens*	NIL	NIL	2

The findings of Table 7 indicates that the methanol, petroleum ether & aqueous stem extracts of *T. indica* is more sensitive to *E.coli* than the other two experimental bacterial strains. Among them the methanol stem extracts of T.indica shows maximum zone of inhibition (12mm) against *E. coli*, but no zone of inhibition is found against *M. roseus*. Only the petroleum ether stem extracts shows zone of inhibition against *P. flvescens* where as it is resistant to the other stem extracts.

Table 7- Antimicrobial activity of plant stems extract

Sl. No.	Microorganism	Zone of Inhibition (mm) of *Tylophora indica* stem extracts in (mm)		
		Methanol	Petroleum ether	Water
1	*Escherichia coli*	12	4	3
2	*Micrococcus roseus*	NIL	NIL	NIL
3	*Pseudomonas flavescens*	NIL	2	NIL

The observations of Table 8 suggests that methanol root extracts of *T. indica* shows zone of inhibition against all the three experimental bacterial strain showing maximum zone of inhibition (15mm) against *P. flavescens* and minimum zone of inhibition (4mm) against *M. roseus*.

Table 8- Antimicrobial activity of plant root extract

Sl. No.	Microorganism	Zone of Inhibition (mm) of *Tylophora indica* root extracts in (mm)		
		Methanol	Petroleum ether	Water
1	*Escherichia coli*	10	NIL	12
2	*Micrococcus roseus*	4	NIL	NIL
3	*Pseudomonas flavescens*	15	NIL	NIL

**The wider the diameter of zone of inhibitions, the higher the antimicrobial activity of the extract. NIL: No zone of inhibition*

The minimum inhibitory concentration ranged between 12.5mg/mL and 75mg/mL depending on microorganism and various extracts are shown in Table 9. The result of minimum inhibitory concentration suggests that methanol, petroleum ether and aqueous extract of *E.alba* leaf, stem and root could possibly act as a bactericidal agent to these microorganisms. Except *P. flavescens* all other microorganisms were inhibited by the lowest concentration (12.5mg/mL) of methanol and petroleum ether extract of leaf, stem and root.

Table 9- Minimum inhibitory concentration of methanol, petroleum ether and aqueous extracts of leaf, stem and root of Eclipta alba against microorganisms (values are mean of three replicates)

Organisms	Leaf (mg/mL)			Stem (mg/mL)			Root (mg/mL)		
	ME	PE	AE	ME	PE	AE	ME	PE	AE
Escherichia coli	12.5	12.5	25	12.5	12.5	25	12.5	12.5	25
Pseudomonas flavescens	25	25	50	12.5	25	25	12.5	25	50
Micrococcus roseus	12.5	12.5	25	12.5	12.5	75	12.5	12.5	25

ME = Methanol extract, PE= Petroleum ether extract, AE = Aqueous extract.

Conclusion

The present study exhibited the antibacterial effect of various extracts of *Eclipta alba* and *Tylophora indica* against *Escherichia coli, Micrococcus roseus* and *Pseudomonas flavescens*. As a result it is`sure that these extracts can surely inhibit the growth of these microorganisms there by preventing various diseases such as asthma, whooping cough, dysentery etc. *Eclipta alba* and *Tylophora indica* extract thus provides safe, easy, effective and practical solutions to every day ailments leaving behind no toxins and creating a clean, pleasant atmosphere. The inhibitory effect of the extracts justified the medicinal use of *Eclipta alba* and *Tylophora indica* and further study is required to find out the active component of medicinal value.

Conservation of genetic resources of crop species is of prime importance for suitable production and maintenance of diversity for future generation. Approaches are made for the production of low cost medicines for rural people. This *in vitro* study demonstrated that folk medicine can be used as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. However, none of the plants are recommended in the treatment of infections produced by *E.coli*, *M. roseus* and *P.flavescens.*

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