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Full Length Research Paper

Screening of antibacterial and antioxidant activities of leaves of *Eclipta prostrata (L)*

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The hexane, ethyl acetate, ethanol and water extract of aerial parts of the *Eclipta prostrata* were tested for its antibacterial activities against *Escherichia coli, Klebsiella pneumoniae, Shigella dysenteriae, Salmonella typhi, Pseudomonas aeruginosa, Bacillus subtilis,* and *Staphylococcus aureus*. The antioxidant properties of those extracts were also investigated. Highest activity of both antibacterial and antioxidant were observed only in ethanol and ethyl acetate extracts, while hexane and water extracts did not show any significant results. The ethanol extract was fractionated by silica gel G 60-120 mesh column chromatography. Only the last three of 8 fractions of the ethanol extract were found to have significant activity only against *S. typhi*. This result indicates that the fractionated ethanol extract from *Eclipta prostrata* could be used against *S. typhi* pathogen.

Key words: Antibacterial activity, antioxidant activity, *Eclipta prostrata*.

INTRODUCTION

The increased prevalence of antibiotic-resistant bacteria due to the extensive use of antibiotics may render the current antimicrobial agents inefficient to control some bacterial diseases (Tanaka et al., 2006). Herbal medicine is frequently a part of a larger therapeutic system such as traditional and folk medicine. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants might represent an alternative treatment in non-severe cases of infectious diseases. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant. The search and use of drugs and dietary supplements derived from plants have been accelerated in recent years. Ethnopharmacologist, botanist, microbiologist and natural product chemist are combing the medicinal flora for biological substances that could be developed for the treatment of infectious diseases. Several medicinal plants have been extensively studied in order to find more effective and less toxic compounds. Pure extract of an herb's 'active component' are more reliable and safer than administration of the herb itself. Many herbs are now in use whose therapeutic properties and active principle are as yet not well understood.

Plant derived natural products such as flavonoids, terpenoids, and steroids have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity. Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against infection and degenerative. Antioxidants are compounds that help to inhibit many oxidation reactions caused by free radicals such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxy nitrate there by preventing or delaying damage to the cells and tissues. There is some synthetic antioxidant compounds, such as butylated hydroxyl toluene (BHT) and butylated hydroxyanisole (BHA), commonly used in processed foods. However, it has been suggested that these compounds have side effects (Ito Fukushima et al., 1983).

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Medicinal plants possessing natural antioxidant polyphenolics such as anthroquinones, flavonoids, aromatic acids and tannins have been shown to have reactive oxygen species (ROS) scavenging and lipid peroxidation preventing effects (Odukaya et al., 2004). In addition, it has been reported that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of human diseases (Rice-Evans et al., 1997). Therefore, research for the identification of the natural antioxidants source is important.

Eclipta prostrata belongs to Astraceae family and very common in tropical and subtropical regions. The herb has been used in the treatment of infective hepatitis in India (Wagner et al., 1986) and snake venom poisoning in Brazil (Melo et al., 1994). It has been reported that the leaves of this herb are used in the case of gastritis and respiratory disorders like cough and asthma (Kobari et al., 2004). In addition, the crude form of the herb is reported to have anti-inflammatory, anti fungal and anti hepatotoxic properties (Wong et al., 1995). But there is as yet no report concerning the antioxidant effect and antibacterial activity of this plant.

Therefore, the present study is intended to determine the activity guided fractions responsible for antibacterial and antioxidant activity of the crude hexane, ethanol and water extracts of leaves of *E. prostrata*, followed by isolation and identification of active fractions.

MATERIALS AND METHODS

Plant materials

Whole plants of *E. prostrata* were collected from green house attached to the college campus, Kamaraj College of Engineering and Technology, Virudhunagar, and authenticated by Botanical Survey of India, Coimbotore. A voucher specimen was deposited in our departmental laboratory. The whole plant was refluxed in running tap water for 1 – 2 h. Leaves were detached and surface sterilized by 0.1% (w/v) $HgCl_2$ with two drops of Tween 80 for 2 min (Archana et al., 2004), followed by rinsing thrice with sterile distilled water until all traces of sterilent are removed.

Bacterial cultures

Escherichia coli, *Klebsiella pneumoniae, Shigella dysenteriae, Salmonella typhi, Pseudomonas aeruginosa, Bacillus subtilis and Staphylococcus aureus were* purchased from IMTECH, Chandigar, India. Silica gel, organic solvents and other chemicals which were used during this study were from Himedia, Merck and s.d. Fine-Chemicals, Mumbai. Standard discs of tetracycline and chloramphenicol purchased from Himedia.

Extraction

Surface sterilized leaves were subjected to solvent extraction by various solvents like hexane, ethyl acetate (Et-oAc), ethanol (Et-OH), and water in increasing order of polarity. Samples were extracted with each solvent one after another by soxhlet apparatus for about 24 h (Hoffman et al., 2004). Each solvent extract was distilled and concentrated *in vacuo* with addition of $CaCl_2$. Lyophilized aqueous fractions were further used to test for the antibacterial and antioxidant properties.

Determination of antioxidants

The antioxidant activities of hexane, ethyl acetate, ethanol and water extract of leaves of *E. prostrata* were determined by ferric thiocynate method (Mistuda et al., 1996). 10 mg of each extract was dissolved separately in 99.5% of ethanol and various concentrations (50, 100, 250, 500 µg/mL) were prepared. A mixture of a 2 mL of sample in 99.5% ethanol, 2.052 mL of 2.51% linoleic acid in 99.5% ethanol, 4 mL of 0.05 M phosphate buffer (PH 7.0) and 1.948 mL of water was placed in a vial with a screw cap and placed in an oven at 60°C in the dark. To 0.1 mL of this sample solution 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocynate was added. After the addition of 0.1 mL of 2 x 10^{-2} M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of the red color developed was measured in 3 min at 500 nm (Matook and Hashinaga, 2005). The control and standard were subjected to the same procedures as the sample, except that for the control, only solvent was added, and for the standard, sample was replaced with the same amount of α-tocopherol (reference compound) (Ali Yildirim et al., 2001). The inhibition of lipid peroxidation in percentage was calculated by following equation:

% Inhibition = 1 - (A1/A2) X 100

where A1 was the absorbance of the test sample and A2 was the absorbance control reaction.

Antimicrobial test

Antibacterial assay of the crude extracts of hexane, ethyl acetate, ethanol and water were performed by disc diffusion method using Muller Hinton agar. Wattman No. 1 filter discs were enriched with approximately 50 µg of each solvent extract which was dissolved in n-hexane. Antibacterial activity was determined as diameter of the zone of inhibition (ZOI). ZOI measurements were made three times for each disc at different orientation and the average was recorded. Disc containing n-hexane alone was used as negative control and the standard tetracycline and chloramphenicol were used as positive control at 30 µg concentration.

Determination of minimum inhibitory concentration (MIC)

The samples (hexane, ethyl acetate, ethanol and water extracts) were dissolved in methanol. The solutions were individually added at different concentration from (0 (control) to 100 µg) to Muller Hinton agar and mixed well before being poured into sterile Petri dishes. The bacterial cultures (10 µl) were taken from nutrient broth and added on the medium surface and incubated at 37°C.

Chromatography study

The crude extract which showed maximum antibacterial and antioxidant activities (Ethanol extract) was subjected to fractionation by silica gel G 60-120 mesh column. Ethyl acetate has been used as mobile phase for gradient elution. Elutes were detected and separated simply using UV absorption (254 nm) and TLC spot observation (Ethyl acetate, Methanol, Water, 5:4:1). Each of the eluate of ethanol extract was again subjected to antibacterial and antioxidant activities.

Table 1. Antioxidant activity of hexane, ethyl acetate, ethanol and water extracts of leaves of *E. prostrata*.

Extract	% of Inhibition			
	50 µg/ml	100 µg/ml	250 µg/m	500 µg/ml
Hexane	9.32	10.34	24.30	33.88
Et-oAc	35.36	39.36	45.15	54.86
EtOH	46.85	61.34	68.92	77.62
Water	23.81	27.81	34.21	45.59
α- Tocopherol	66.65	68.28	72.44	80.06

Table 2. Antibacterial activity of hexane, ethyl acetate, ethanol and water extracts of *E. prostrata* leaves on selected bacterial strains.

Extract	Hexane		Et-oAc		Et-OH		Water	
	A	B	A	B	A	B	A	B
E. coli	1.9	NS	12.2	4.5	13.1	35	NS	NS
K. pneumonia	2.0	NS	5.7	80	8.9	65	2.1	250
S. dysenteriae	2.3	250	4.5	125	4.8	90	NS	NS
S. typhi	NS	NS	15.1	35	20.8	25	NS	NS
P. aeruginosa	2.5	250	6.9	70	8.8	65	1.4	NS
B. subtilis	3.3	175	5.9	80	6.1	80	2.6	250
S. aureus	2.7	250	6.8	65	7.2	70	2.8	250

A: Zone of Inhibition (ZOI, mm).
B: Minimum Inhibitory concentration (MIC; µL/mL).
NS: Non significant.

RESULTS AND DISCUSSION

Antioxidant activity

The antioxidant activity of the hexane, ethyl acetate, ethanol and water extracts of *E. prostrata* were determined by ferric thiocynate (FTC) and the values are presented in Table 1. FTC method was used to determine the amount of peroxide formed and that react with ferrous chloride ($FeCl_2$) to form a reddish ferric chloride ($FeCl_3$) pigment. In this method, the concentration of peroxide decreases as the antioxidant activity increases. hexane, ethyl acetate, ethanol and water extract at various concentration (50, 100, 250 and 500 in µg/mL), showed antioxidant activities in a concentration dependent manner. However, ethanol extract at the concentration of 500 µg/mL showed 77.62%, an antioxidant activity very close to that of 500 µg/mL of α-tocopherol (80.06%), the reference compound. It has been observed that the extract exhibited strong activity with the increase in polarity (with reference to organic solvent), indicating that polyphenols or flavanone or flavanoids may play important roles in the activities. The present findings are in agreement with the report of Tepe et al. (2005). Inspite of water being highly polar the aqueous extract had less activity which might be due to the fact that most of the polar compounds are extracted with ethanol itself.

Antibacterial activities

Table 2 shows the antibacterial activities of hexane, ethyl acetate and water extracts of *E. prostrata* against tested organisms. The extracts exhibited remarkable activity at higher concentration. In this investigation the ethanolic extract of leaves of *E. prostrata* recorded significant antibacterial activities against all tested bacterial strains, while ethyl acetate extract recorded medium activity and no significant results were recorded in both hexane and aqueous extracts as measured by zone of inhibition (ZOI) and minimum inhibitory concentration (MIC) methods. Though ethanol extract showed broad spectrum of activity against all tested organisms (*E. coli, K. pneumoniae, S. dysenteriae, S. typhi, P. aeruginosa, B. subtilis, S. aureus*). *S. typhi* is highly sensitive to ethanol extract even at 25 µl/mL concentration followed by ethyl acetate extract 35 µl/mL. Tetracycline and chlo-ramphenicol were used as reference drugs at 20 µl/mL. Ethyl acetate extract that showed medium activity against almost all organisms need to be tested with high concentration of extracts as suggested by Kumar et al. (2005).

Silica gel G 60 column chromatography analysis of ethanol extract of leaves of *E. prostrata* (toluene and ethyl acetate were used as stationary and mobile phases respectively) showed the presence of at least eight fractions, which were visible under UV light at 254 nm. The antibacterial activities of each of those fractions were

Table 3. Effect of various fractions of ethanolic extract of *E. prostrata* on *S. typhi*.

Et-OH Fractions	*S. typhi*	
	ZOI in mm	MIC in µL/ mL
Fr-I	08	50
Fr-II	08	50
Fr-III	10	50
Fr-IV	11	45
Fr-V	06	75
Fr-VI	13	30
Fr-VII	15	20
Fr-VIII	16	20
Tetracycline	25	20
Chloramphenicol	23	20

A: Zone of Inhibition (ZOI, mm).
B: Minimum Inhibitory concentration (MIC; µL/mL).

tested against *S. typhi* (Table 3). Fractions VI (13 mm), VII (15 mm) and VIII (16 mm) showed the maximum zone of inhibition. The MIC of Fr-VI, Fr-VII and Fr-VIII were recorded as 30, 20 and 20 µl/mL respectively. Based on the results obtained in the antimicrobial studies of ethanol extract of *E. prostrata*, it can be suggested that this plant can be used effectively to treat *S. typhi* infection. However further studies regarding bioactive principle are warranted.

The present study regarding the antibacterial and antioxidant properties of *E. prostrata* indicate that com-pound from this genus could be used against *S. typhi* pathogen. However these extracts and active fraction isolated from leaves of *E. prostrata* must be studied in animal model to determine the efficacy *in-vivo* against *S. typhi* pathogen and positive toxicity, and to elucidate their mechanism of action.

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