Research Article



Evaluation of Antibacterial, Anti-inflammatory and Antioxidant Activities of Methanolic Extract of Whole Plant of *Eichhornia crassipes*

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ABSTRACT

This study was subjected to investigate in vitro antibacterial, anti-inflammatory and antioxidant activities of methanol extracts of *Eichhornia crassipes*. In antibacterial activity the methanolic extract exhibited the best antibacterial activity against *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli*, shows the positive result. Out of these three organisms *E.coli* shows the high zone of inhibition. Inhibition of albumin denaturation technique was used to determine *in-vitro* anti-inflammatory activity. DPPH free radical scavenging method and hydrogen peroxide scavenging method were used for antioxidant activity. Antibacterial activity was determined by disc diffusion method. In in-vitro anti-inflammatory investigation there is a linear relation of %inhibition for the *Eichhornia crassipes* which indicates having positive anti-inflammatory property. In DPPH free radical was calculated by using log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity and compared with standard ascorbic acid. *Eichhornia crassipes* may pocesss potent antibacterial activity, anti-inflammatory, as well as good antioxidant.

Keywords: DPPH, Anti-inflammatory, Ascorbic acid, Eichhornia crassipes, Escherichia coli, methanolic extract.

INTRODUCTION

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The roots of *Eichhornia crassipes* naturally absorb pollutants, including lead, mercury and strontium-90, as well as some organic compounds believed to be carcinogenic, in concentrations 10,000 times that in the surrounding water ⁵. Water hyacinths can be cultivated for waste water treatment ⁶. Wolverton and McDonald (1981) note ⁷" cultivation of higher plants for use in wastewater treatment, and incorporation of these plants into a system where the biomass is harvested for fuel production is economically appealing at the present time. Since this biomass is a by-product of wastewater treatment, it has a positive environmental impact, and thus poses no threat as competitor to food, feed, or fiber-producing plants."

Water hyacinth leaf extract has been shown to exhibit phytotoxicity against another invasive weed Mimosa pigra. The flowers are used for medicating the skin of horses. The species is a "tonic"^{8,9}. The plant is used as a carotene-rich table vegetable in Taiwan. Along with vinegar, it is being used in treatment of septic wounds 10 . In the animal kingdom, it has been used as a tonic for the skin of horses, for irritation and inflammation. Several authors have reported favourable results with herbal drugs (mostly in the form of extracts) either in animal or in human studies ¹¹. The uses of plant-derived products as disease control agents have been studied, since they tend to have low toxicity to mammals, less environmental effects and wide public acceptance ¹². Plant products are rich sources of phytochemicals which have been found to possess variety of biological activities including antioxidant, cytotoxic, and hepatoprotective potential. They act as reducing agents and reverse oxidation by donating electrons and/or hydrogen ions ^{13, 14}

Pathak Aditi Kannan C. described Water hvacinth (Eichhornia crassipes) is one of the most predominant, persistent and troublesome aquatic weeds. Periodical surveys of various water bodies in and around Jabalpur were under taken with the objective to isolate and evaluate the indigenous strains of fungal pathogens as myco-herbicides to manage water hyacinth Antioxidants include beta-carotene, phenolic acids, phytate and phyto estrogens lycopene, vitamins A, C, and E, and other natural and manufactured substances. Antioxidants exert their effects via several basic mechanisms, which include: scavenging the species that initiate peroxidation, quenching singlet oxygen, chelating metals, breaking free radical chain reactions, and reducing the concentration of O₂¹⁶. *E.crassipes* has been used to ease swelling, burning, haemorrhage, and goiters. In the animal kingdom, it has been used as a tonic for the



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skin of horses, for irritation and inflammation ¹⁷. They are widely used in the human therapy, veterinary, agriculture, scientific research and in countless other areas ¹⁸. This indigenous art of healing has to be transformed to an exact science ¹⁹. Many plants have been studied for their antibacterial activity^{20, 21}, in past few years indicating the surge of the researchers to identify a plant with good antimicrobial. The use of crude extracts of plants parts and phytochemicals of known antimicrobial properties can be of great significance in the therapeutic treatments. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as communicable diseases. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency²²

MATERIALS AND METHODS

Collection and Preparation of whole Plant material

The whole plant of *Eichhornia crassipes* was collected in the month of August from local area of Korangi, East Godavari District, and Andhra Pradesh, India. The plant was identified and authenticated (specimen no.BSI/DRC/16-17/Tech./506) by L.RASINGAM, scientist In-charge Botanical survey of India, Deccan regional center, Hyderabad-500048, Telangana State.

Preparation of crude extract

500gm of whole plant of *Eichhornia crassipes* was washed with distilled water to remove dust particles, then the parts of the plants was shade dried at room temperature. Then shade dried plant materials were powdered, and passed through 60 mesh size sieves. 150g of plant powdered were weighed accurately and extracted with 600ml methanol solvent using cold maceration method. Thus obtained extract were filtered through whattman filter paper and the filtrate was concentrated. The extract (3.5g) were transferred to sterile screw cap bottles, labelled and stored in room temperature until use.

Chemicals material

DPPH (1, 1-diphenyl, 2-picrylhydrazyl), Hydrogen peroxide, Ascorbic acid, Bovine albumin, Ibuprofen, Ciprofloxacin. All chemicals and reagents used were of the highest commercially available purity.

Antibacterial activity

Test Organisms:

Culture Medium: Mueller Hinton Agar (MHA) medium was used to study the antibacterial activity and Potato Dextrose Agar (PDA) was used to study the antifungal activity.

Antibacterial Activity assay

Antibacterial test was performed as described ²³ by 0.1ml of inocculum was poured into petridish and then added 20ml of sterilized liquid agar nutrient media, and

spread homogenized and let it stand in order to obtain a solid media. The paper discs with diameter of 6 mm was soaked into various concentrations of test solutions, dried and place them on the surface of agar nutrient media. It was then incubated for 18-24 hours at the temperature of 36-37°C. The inhibition zone diameter around the paper disc was then measured and recorded. The tests were respectively done 3 times. The standard antibiotic Gentamycin were used as a positive control and compared with extracts, fractionates and compound of waterhyacinth under identical conditions. The tests of antibacterial activities of methanol extract of *Eicchornia crassipes* whole plant against *Staphylococcus aureus, Bacillus substilis* and *Escherichia coli* are depicted.

In-vitro Anti-inflammatory activity

The anti-inflammatory activity of *Eichhornia crassipes* was studied by using inhibition of albumin denaturation technique which was studied according to Mizushima ²⁴and Sakat ²⁵ the reaction mixture was consists of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl. The sample extracts were incubated at 37°C for 20 min and then heated to 51°C for 20 min, after cooling the samples the turbidity was measured at 660 nm. (UV Visible Spectrophotometer, Elico India Ltd) The experiment was performed in triplicate.

The Percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition = (Abs Control – Abs Sample) X 100/ Abs control

Antioxidant Activity

Evaluation of antioxidant activity by DPPH radical scavenging method ^{26, 27}

The DPPH assay measure hydrogen atom (or one electron) donating activity and hence provided an evaluation of antioxidant activity due to free radical scavenging. DPPH, a purple colored stable free radical, was reduced into the yellow-colored di-phenyl picrylhydrazine which is measured spectrophotometrically at 517 nm. Free radical scavenging activity of methanol extract of whole plant of Echhornia crassipes was measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH). In brief, 0.1 mM solution of DPPH in methanol was prepared. This solution (1 ml) was added to 3 ml. of different extracts in methanol at different concentration (50, 100, 150, 200, 250 µg/ml). The mixture was shaken vigorously and allowed to stand at room temp for 30 min. then, absorbance was measured at 517 nm. by using UV Visible spectrophotometer (ELICO). Reference standard compound being used was ascorbic acid. The percent DPPH scavenging effect was calculated by using following equation.

% scavenging activity = Absorbance of control - Absorbance of sample ×100



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Hydrogen Peroxide Scavenging Method²⁸

All the compounds and the standard were dissolved in DMSO as a solvent-stock solution (1mg/ml) and from stock solution various concentrations of 50, 100, 150,200 and 250μ g/ml of methanol extracts were prepared in different volumetric flasks. To each solution 2 ml hydrogen peroxide was added and the volume was made

to 10 ml with phosphate buffer saline (pH-7.4). A control solution was prepared with DMSO in phosphate buffer saline without drug. The absorbance at 230nm was recorded using U.V spectrophotometer against blank (Phosphate buffer saline). The standard drug is Ascorbic acid. The % inhibition by hydrogen peroxide scavenging activity was calculated using the following formula:

Percentage inhibition = <u>absorbance of control-absorbance of test</u> × 100 Absorbance of control

RESULTS AND DISCUSSION

Antibacterial activity

The antibacterial activities test was conducted in various concentrations to investigate their relationship. The results of antibacterial activities examination showed that methanol extract fraction gave antibacterial effect againts *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus*. They were denoted by the existence of inhibition zone around the paper disc. The results of the average of inhibition zone diameter of methanol extract of *E.crassipes* for *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli* can completely be seen on Tabel-1 below.

Table 1: Antibacterial activity of methanolic activity of Eichhornia crassipes

Extract/Drug	Concentration	Z	Zone of Inhibition (mm)*		
	(µg/ml)	S.aureus	E.coli	B.subtills	
	50	6	8	5	
Methanolic extract	100	9	9	8	
of whole plant extract	150	10	11	9	
	200	11	13	9	
	250	13	15	11	
Ciprofloxacin	50	13	16	12	
Blank	-	-	-	-	

Informations: (*) = The average results of triple measurements, (-) = no inhibition



Figure 1: Antibacterial activity of methanolic activity of Eichhornia crassipes

Anti inflammatory activity

The anti inflammatory activity of whole plant of methanolic extact of *E.crassipes* was investigated by inhibition of albumin denaturation Protein. Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied. It was effective in

inhibiting heat induced albumin denaturation. Maximum inhibition of 79% was observed at 500 μ g/ml. Ibuprofen, a standard anti inflammatory drug showed the maximum inhibition 42% at the concentration of 100 μ g/ml compared with control.







Table 2: Anti inflammatory activity of methanolic activity of *Eichhornia crassipes*

Treatments	Concentration (µg/ml)	%inhibition of protein denaturation
Control	-	-
Methanolic Extract	100	20
Methanolic Extract	200	42
Methanolic Extract	300	60
Methanolic Extract	400	72
Methanolic Extract	500	79
Ibuprofen	100	42
Ibuprofen	200	54
Ibuprofen	300	74
Ibuprofen	400	80
Ibuprofen	500	96

Antioxidant activity

The antioxidant activity of methanolic extract of *E.crassipes* was studied by two different methods:

- 1. DPPH radical scavenging method
- 2. Hydrogen Peroxide Scavenging Method.

DPPH radical scavenging method

Free radical scavenging potential of methanol extract and ascorbic acid at different concentrations was tested by DPPH method. The result demonstrated good free radical scavenging activity of the extract.



Figure 3: Anti oxidant activity of methanolic activity of Eichhornia crassipes

Solutions of various concentrations (50, 100, 150, 200,250 μ g/ml) were taken and the absorbance was measured at 517 nm. DPPH free radical was calculated by using log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity. Maximum inhibition of 78% was observed at 250 μ g/ml. Ascorbic

acid, a standard antioxidant drug showed the maximum inhibition 69% at the concentration of 100 $\mu g/ml$ compared with control.

Table 3: Antioxidant activity of methanolic activity of

 Eichhornia crassipes

Treatments	Concentration (µg/ml)	%inhibition of DPPH
Control	-	-
Methanolic Extract	50	41
Methanolic Extract	100	60
Methanolic Extract	150	65
Methanolic Extract	200	70
Methanolic Extract	250	78
Ascorbic acid	50	53
Ascorbic acid	100	69
Ascorbic acid	150	72
Ascorbic acid	200	79
Ascorbic acid	250	83

Hydrogen Peroxide Scavenging Method:

Free radical scavenging potential of methanol extract and ascorbic acid at different concentrations was tested by hydrogen peroxide scavenging method. The result demonstrated good free radical scavenging activity of the extract. Solutions of various concentrations (50, 100, 150, 200,250 μ g/ml) were taken and the absorbance was measured at 517nm. Hydrogen peroxide scavenging activity was calculated by using log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity. Maximum inhibition of 80% was observed at 250 μ g/ml. Ascorbic acid, a standard antioxidant drug showed the maximum inhibition 68% at the concentration of 100 μ g/ml compared with control.

Table 4: Antioxidant activity of methanolic activity of

 Eichhornia crassipe

Treatments	Concentratio n (µg/ml)	%inhibition of DPPH
Control	-	-
Methanolic Extract	50	44
Methanolic Extract	100	59
Methanolic Extract	150	65
Methanolic Extract	200	72
Methanolic Extract	250	80
Ascorbic acid	50	52
Ascorbic acid	100	69
Ascorbic acid	150	74
Ascorbic acid	200	82
Ascorbic acid	250	90



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Figure 4: Anti oxidant activity of methanolic activity of *Eichhornia crassipes*

CONCLUSION

The antibacterial screening carried out for the whole plant extract of water hyacinth showed significant activity against the test organisms. Antibacterial activity of the plant extract are, may be, due to the presence of phenols and flavonoids. The anti inflammatory activity was investigated by inhibition of albumin denaturation protein maximum inhibition was observed. And it was concluded that the extract possesses the significant antioxidant activity. This study suggests that the plant can be productively used in the pharmaceutical area because of its possible activities reported.

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