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In-vitro Antioxidant and Antibacterial Properties of Various Extracts of *Eichhornia crassipes*

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Abstract

The water hyacinth, *Eichhornia crassipes* is a harmful aquatic weed found in India and around the world. It is an oceanic macrophyte and also has a rich source of bioactive compounds with therapeutic properties. *Eichhornia crassipes* was found to possess in vitro antioxidant and antibacterial properties in extracts based on different solvents (hexane, chloroform, ethylacetate, methanol, and aqueous). DPPH, FRAP, SOD, H_2O_2, hydroxyl and ABTS radical scavenging assay showed high incidence of scavenging properties of reactive oxygen species. *E. crassipes* leaf extracts showed a significant antibacterial activity against the selected bacterial strains such as *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Streptococcus pyogenes* by using the agar well diffusion method. The results specified that the leaf extracts of *E. crassipes* have prospective activity as bacteriocidial agent and could be used in future for biomedical applications studies.

***Key words*:** *Eichhornia crassipes*, Antioxidant, Antibacterial, Phytocompound

A floating waterweed called "*Eichhornia crassipes*" commonly known as "Water Hyacinth" is. It is becoming more and more clear that plants and hydrophytes, including water hyacinth, are rich sources of naturally occurring bioactive chemicals with antioxidant, antiviral, anticancer, and antibacterial properties. Utilizing spectroscopic techniques on the separated fractions, it was discovered that the crude extract contained a variety of chemicals that worked in concert to produce its highest levels of activity [1]. It has been demonstrated that higher plants may include sources of novel antimicrobial compounds. With their established antibacterial qualities, the application of phytochemicals and plant extracts can be very important to medical interventions [2].

According to Joshi and Kaur [3] and Jayanthi *et al*. [4], water hyacinth is a source of compounds having therapeutic properties. This plant's leaf extract contains alkaloids, flavonoids, phenols and tannins, which have biological activities that include anticancerous, antiviral, antibacterial, antifungal properties [5]. Furthermore, oxidative enzymes and non-enzymatic antioxidant systems are abundant in water hyacinth [6], and the plant also possesses anticancer and wound-healing properties [7]. *E. crassipes* is a convenient and alternative source of antioxidants due to its phytocompounds [8-9].

Natural antioxidants such as flavonoids, phenolic and carotenoids compounds, have a high bioavailability and, as a result, a high level of protection against free radicals and reactive oxygen species (ROS). DNA, proteins, carbs, and lipids are modified as a result of oxidative stress's impact on the biological system's prooxidant and antioxidant balance. Free radicals such as singlet oxygen, superoxide anion radicals, and hydroxyl radicals can attack the unsaturated fatty acids in biomembranes. Lipid peroxidation, fluidity loss, loss of enzyme and receptor activity, damage to membrane proteins, and eventually cell inactivation are all brought on by these radicals [10]. Water hyacinth contains various bioactive chemicals with therapeutic properties [11-13]. The biological activities of the leaf extract include antiviral, antifungal, anticancer, and antibacterial properties due to the incidence of alkaloids, tannins, phenols and flavonoids [14-17]. Furthermore, various antioxidant enzymes present in water hyacinth [18] showed enhanced chemical defense against plant diseases [19-20]. Researchers are becoming more interested identification of alternate method for finding and developing novel and inventive products in common weeds [21-22]. The main objective of the study is to analyze the antioxidant properties of the various solvent (Hexane, Chloroform, Ethylacetate, Methanol and Aqueous) based *E. crassipes* extracts and also their antimicrobial properties were analyzed.

MATERIALS AND METHODS

Experimental plant

The aquatic weed plant *Eichhornia crassipes* belongs to the Family Pontederiaceae was collected from the nearby aquatic bodies. The aerial parts (leaves) of the plants were air dried and powdered and transported to the KIRND laboratory (Tiruchriappalli) for *in vitro* antioxidant studies. By using a Soxhlet apparatus, the plant extraction was performed with various solvents such as Hexane, Chloroform, Ethylacetate, Methanol and Aqueous (1L) extracts for 8hrs at 38°C. The extraction procedure was repeated until the solvent become

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colourless. The obtained supernatant was condensed using a rotary evaporator (Lark Rotary Evaporator, Model RE 100-Pro).

Antioxidant studies

The following antioxidant assay were carried out by the Shashan *et al*. [23].

DPPH radical scavenging assay

2.5 mL of a 0.1 mM methanolic solution of DPPH was combined with 0.5 mL of the plant extracts at different concentrations (25, 50, 75, and 100 µg/mL in hexane) and the ascorbic acid standard. At 30°C, the reactants were incubated for thirty minutes. Methanol was used as a blank to test the absorbance at 517 nm. The extracts' antioxidant activity was determined and represented as the percentage of DPPH free radicals that were inhibited.

Ferric reducing antioxidant power assay

The antioxidant activity of FRAP was examined at different concentrations of plant extracts (25, 50, 75, and 100 µg/mL). One millilitre of each was combined with two millilitres of the FRAP (ferric tripyridyltriazine) reagent, and carefully mixed. After 30 minutes of incubation at 37°C, the absorbance at 593 nm was measured with acetate buffer serving as the blank.

Superoxide radical scavenging assay

NBT decrease was used to gauge SOD activity. Phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH): A 1 ml reaction mixture comprising phosphate buffer (20 mM, pH 7.4), NADH (73 µM), NBT (50 µM), PMS (15 µM), and different quantities of sample solution (25, 50, 75, and 100µg/mL). The absorbance at 562 nm was measured after 5 minutes of incubation at room temperature.

Hydrogen peroxide scavenging assay

The plant extract (25, 50, 75, and 100 µg/mL in distilled water) was combined with a 40 mM hydrogen peroxide solution in 2 mL of distilled water. The absorbance of the reaction mixture was measured at 230 nm following a 10-minute incubation period at 30°C. The standard utilized was ascorbic acid. The extracts' and the standard's percentage of H_2O_2 scavenging activity were computed.

Hydroxyl radical scavenging assay

Fe^{3+}-ascorbate-EDTA-H_2O_2 reaction mixture and various concentrations (25, 50, 75 and 100 µg/mL) of the plant extracts were incubated for 1hr at 37°C. 0.5 ml of the reaction mixture was added to 1ml TCA, then 1 ml aqueous TBA were incubated at 90°C for 15min to develop the color. After cooling, the absorbance was measured at 532 nm.

ABTS radical scavenging assay

Free radical scavenging activity of plant samples was determined by ABTS radical cation decolorization assay. Diluted $ABTS^{\cdot+}$ solution was mixed with various concentrations (25, 50, 75 and 100 µg/mL) of plant extracts. Percent inhibition of absorbance at 734 nm.

Antimicrobial activity

Using the disc diffusion method, methanolic extracts of *E. crassipes* leaves were tested for antibacterial activity (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*). Pathogens were procured from KIRND laboratory (Tiruchirappalli). For the inoculation, the overnight culture was utilized. The concentration of the plant extracts that were going to be tested was 10 mg/ml. Using framing forceps, the sterile impregnated discs containing plant extracts were put on the agar surface and carefully pressed down to guarantee full contact between the disc and the surfaces of the agar and dextrose. The same protocol was used to prepare positive control discs using 10 µg/ml of Gentamicin. For a full day, all of the plates—including the control plates—were incubated at 37°C. The inhibitory zones' diameter was evaluated after incubation. The inhibitory zone's diameter was used to express the results [24].

Statistical analysis

All the assays were carried out in triplicate. The means and standard deviation (SD) were determined using SPSS version 20. Antioxidant activities, and antimicrobial activity are expressed as mean values ± SD, and the analysis of variance was performed to determine significant ($P<0.05$) differences.

RESULTS AND DISCUSSION

In vitro antioxidant activities

DPPH activity

Free radical scavenging potential of various extracts and ascorbic acid at different concentrations (25, 50, 75, 100µg/ml) was tested by DPPH method (Table 1). Radical scavenging activity increases with increasing percentage of the free radical inhibition in the tested solvents. Among the tested various solvent based extracts, methanolic extracts of the fresh plant *Eichhornia crassipes* reduces the radical to corresponding hydrazine when it reacts with hydrogen donors in antioxidant activity by increased radical scavenging activity as 10.01±0.16, 20.82±0.33, 25.73±0.58 and 39.59±0.59% for 25, 50, 75, 100µg/ml respectively.

FRAP activity

Ferrozine produces a violet complex with Fe^{2+} in the presence of a chelating complex, the absorbance of the solution is varied due to the fading of the violet colour. In higher concentration (100µg/ml), the FRAP activities were 43.41±0.32, 65.04±0.89, 63.56±0.28, 79.96±1.06 and 85.73±1.56% for hexane, chloroform, ethylacetate, methanol and aqueous extracts of *Eichhornia crassipes*. Among the tested various solvent based extracts, methanolic extracts showed high FRAP activities which ranged as 10.01±0.16 to 39.59±0.59%.

SOD and H_2O_2 scavenging activity

The superoxide radicals generated from dissolved oxygen by PMS-NADH coupling can be measured by their ability to reduce NBT which indicated their abilities to quench superoxide radicals in the reaction mixture. In higher concentration (100µg/ml), the SOD activities were 37.32±1.76, 60.32±0.88, 67.9±0.64, 87.0±1.21 and 74.61±0.63% for hexane, chloroform, ethylacetate, methanol and aqueous extracts of *Eichhornia crassipes*. Among the tested various solvent based extracts, methanolic extracts showed high SOD activities which ranged as 22.82±0.08 to 87.0±1.21%. In higher concentration (100µg/ml), the H_2O_2 activities were 47.56±2.03, 64.72±0.60, 65.72±1.09, 76.66±0.96 and 67.72±1.77% (Table 1) for hexane, chloroform, ethylacetate, methanol and aqueous extracts of *Eichhornia crassipes*. Among the tested various solvent based extracts, methanolic extracts showed high SOD activities which ranged as 20.53±0.37 to 76.66±0.96%.

Hydroxyl radical scavenging and ABTS radical scavenging activity

Hydroxyl radical scavenging assay showed the abilities of the extract and standard ascorbic acid to inhibit hydroxyl radical-mediated deoxyribose degradation in Fe^{3+}-EDTA-ascorbic acid and H_2O_2 reaction mixture.

In higher concentration (100 µg/ml), the hydroxyl activities were 29.19±1.54, 38.82±1.41, 70.02±1.25, 78.22±1.75 and 65.72±0.61% (Table 1) for hexane, chloroform, ethylacetate, methanol and aqueous extracts of *Eichhornia crassipes*. Among the tested various solvent based extracts, methanolic extracts showed high hydroxyl activities which ranged as 20.93±0.62 to 78.22±1.75%. Similarly, among the tested different extracts, methanolic extracts of *Eichhornia carssipes* ABTS radical scavenging activities were significantly increased as 25.86±0.34, 47.47±0.99, 70.12±1.25 and 94.31±0.33% for 25, 50, 75 and 100µg/ml respectively. The methanolic extract of Eichhornia crassipes possesses notable antioxidant properties, as evidenced by its high hydroxyl activities and ABTS radical scavenging activities.

Table 1 Antioxidant activity of the various concentrations of *Eichhornia carssipes* by different solvents

Concentration (µg/ml)	Hexane solvent	Chloroform solvent	Ethylacetate solvent	Methanol solvent	Aqueous solvent
			DPPH radical scavenging activity (%)		
25	5.65±0.08	5.20±0.05	7.91±0.13	10.01±0.16	8.59±0.06
50	8.06±0.06	6.45±0.11	9.77±0.12	20.82±0.33	15.39±0.48
75	8.41±0.10	9.19±0.05	11.26±0.09	25.73±0.58	19.61±0.58
100	10.47±0.05	11.73±0.14	13.54±0.04	39.59±0.59	25.4±0.20
			FRAP activity (%)		
25	12.67±0.19	15.82±0.52	19.2±0.21	22.06±0.25	27.31±0.8
50	22.9±0.60	27.48±0.75	33.64±0.49	38.07±0.31	49.07±0.85
75	32.7±0.44	47.15±0.89	48.29±1.85	54.01±0.67	57.73±0.58
100	43.41±0.32	65.04±0.89	63.56±0.28	79.96±1.06	85.73±1.56
			SOD activity (%)		
25	10.99±0.16	19.66±0.26	17.87±0.57	22.82±0.08	18.99±0.12
50	18.98±0.07	36.06±0.48	34.46±0.41	43.21±0.33	34.48±0.34
75	26.53±0.34	48.93±1.19	49.0±0.09	64.5±0.36	49.56±0.65
100	37.32±1.76	60.32±0.88	67.9±0.64	87.0±1.21	74.61±0.63
25	12.64±0.41	16.87±0.13	17.81±0.35	20.53±0.37	18.2±0.06
50	24.05±0.30	33.31±0.10	32.39±0.37	38.07±0.48	33.91±0.41
75	32.78±0.21	47.13±0.56	44.56±0.81	53.62±0.32	48.36±0.21
100	47.56±2.03	64.72±0.60	65.72±1.09	76.66±0.96	67.72±1.77
			Hydroxyl activity (%)		
25	8.48±0.37	10.95±0.07	17.84±0.05	20.93±0.62	18.87±0.08
50	14.52±0.37	18.85±0.07	33.77±0.59	37.41±0.66	34.49±0.16
75	20.99±0.10	29.56±0.64	48.95±0.11	60.38±0.77	50.28±0.73
100	29.19±1.54	38.82±1.41	70.02±1.25	78.22±1.75	65.72±0.61
			ABTS radical scavenging activity (%)		
25	17.15±0.45	21.79±0.42	20.7±0.14	25.86±0.34	24.51±0.28
50	29.18±0.59	40.42±0.29	38.44±0.86	47.47±0.99	45.45±0.32
75	45.58±0.29	59.0±0.61	55.59±0.31	70.12±1.25	69.09±0.59
100	58.7±0.69	81.15±0.52	75.01±0.60	94.31±0.33	87.10±1.27

According to Surendraraj *et al*. [25], ethanolic extracts of *E. crassipes* had high phenolic acid contents, while water extracts had lower concentrations of a variety of phenolic acids. As a result, the ethanolic extract of water hyacinth leaves had a high Fe^{2+} chelating activity and also inhibited the process of lipid peroxidation in both fish oil and liposomes.

Total alkaloid content in the *Pistia stratiotes* leaves and stems [26] reported as 0.163±0.041 mg. alkaloid/gm d.w. and 0.096±0.041 mg. alkaloid/gm d.wt. respectively. DPPH antioxidant activity confirmed that leaves extract showed high IC50 value (2.463±0.018 mg/ml) when compared to stems (4.098±0.030 mg/ml). Dalimunthe *et al*. [27] have also isolated alkaloid chemicals as antioxidants from Litsea cubeba plants. At pH 7, the chloroform fraction contained the antioxidant chemicals DPPH and ABTS, with IC50 values of 23.81±0.01µg/mL and 56.43±0.06µg/mL respectively.

Table 2 Antimicrobial activity of *Eichhornia crassipes* against pathogenic bacteria

Bacteria	Hexane solvent	Chloroform solvent	Ethylacetate solvent	Methanol solvent	Aqueous solvent	Control (Gentamicin)
Escherichia coli	10.9±0.5	11.4±0.3	12.5±0.6	15.9±0.9	13.5±0.6	14.4±0.7
Bacillus subtilis	15.7±0.4	16.5±0.4	17.5±0.3	21.7±0.6	18.5±0.9	19.8±0.5
Staphylococcus aureus	11.4±0.6	12.9±0.3	13.1±0.5	16.9±0.5	15.3±0.5	15.8±0.9
Streptococcus pyogenes	12.6±0.5	13.1±0.5	14.9±0.6	17.9±0.7	16.0±0.4	16.4±0.7

Antimicrobial activity

An agar well diffusion assay was performed using 100μg/mL of various extracts of *Eichhornia crassipes*. (Table 2) showed the antimicrobial activity of the *E. crassipes* against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus pyogenes*. Among the six solvent-based *E. crassipes* extract, methanolic extracts showed high incidence of antimicrobial activity against the tested all four bacterial species. Methanolic extracts of *E. crassipes* showed the zone of inhibition as 15.9±0.9, 21.7±0.6, 16.9±0.5 and 17.9±0.7mm for *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus pyogenes* respectively. Yin *et al.* [28] have reported on additional investigations. The alkaloid molecule (+)- orientaline was effectively isolated, and it was found to exhibit antioxidant activity against DPPH and ABTS, with IC50 values of 1.53±0.05µg/mL and 6.64±0.19µg/mL respectively. This condition is obtained due to the rich free hydroxyl groups in alkaloid whereas hydroxyl groups that are connected to the structure in non-steric states, and hydroxyl groups that are abundant, they can scavenge radicals and cationic radicals. The antiradical activity of alkaloids increases with the number of hydroxyl groups and their places that are unblocked by other groups [29]. Several *E. crassipes* extracts antimicrobial activity matched those from other writers [30].

CONCLUSION

Many literatures have been studying water hyacinth, initially it was considered as a weed, lately because of its potential uses in phytochemistry and medicine. Water hyacinth leaf extracts prepared using various solvents demonstrated strong antioxidant and antibacterial properties against every tested bacterium. Phytocompounds showed significantly increased scavenging activities as increased concentrations. Various solvent based extracts from the leaves of *E. crassipes* are newly discovered to have antibacterial and radical-scavenging properties.

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