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Membrane Stabilizing Effect and Antioxidant Potential of Different Extracts from *Eicchornia crassipes* Flowers: An *In Vitro* Assessment

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ABSTRACT

Eicchornia crassipes has been traditionally used as a tonic for the skin of horses, for irritation and inflammation. The aim of this study was to evaluate the total phenolic and flavonoid contents, antioxidant and membrane stabilizing activities of different extracts from *E. crassipes*. *In vitro* antioxidant activity was determined by DPPH radical scavenging, ABTS radical scavenging, hydroxyl radical scavenging and reducing power assays. The total flavonoid and phenolic contents were also investigated. Membrane stabilizing potential of the extracts was studied on human erythrocytes in hypotonic solution and by heat-induced hemolysis procedures. Quantitative analysis revealed the highest amount of total phenols and flavonoids in the ethanol extract while the lowest amount was in the ethyl acetate extract. Ethanol extract exhibited promising antioxidant potential in the DPPH, ABTS, and hydroxyl radical scavenging assays compared to the other extracts. Also, the ethanol extract possesses strong reducing power ability compared to the other extracts. A significant correlation was found between the antioxidant activity of the extracts and their total phenolic and total flavonoid contents. The ethanol extract exhibited the highest percentage hemolysis inhibition in hypotonic solution-induced hemolysis and in heat-induced hemolysis procedures. Results from this study showed that the flowers of *E. crassipes* exhibited *in vitro* antioxidant and anti-inflammatory activities. This study substantiated the folkloric use of *E. crassipes* flowers in inflammatory disorders.

Keywords: *Eicchornia crassipes*, antioxidant, anti-inflammatory, flavonoids, phenolic compounds, *in vitro* assays

Introduction

It is usually accepted that oxidative stress results when reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet -}$), hydroxyl ($OH^{\bullet}$), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) radicals are generated. The ROS have been known to play a vital role in the pathophysiology of various conditions including neurological diseases, cancer, cardiovascular diseases, atherosclerosis, cataracts, and inflammation. In recent years, use of traditional medicine has become popular in the scientific world and plants remain one of the major sources of natural antioxidants that might serve as lead compounds for the development of modern medicine. Several antihepatotoxic, anti-inflammatory, antinecrotic, digestive and neuroprotective drugs have been shown to have an antioxidant/free radical scavenging mechanism as part of their bioactivity. The mechanism of inflammatory injury is attributed, in part, to release of ROS from activated neutrophils and macrophages. This overproduction leads to tissue injury by damaging macromolecules and lipid peroxidation of membranes. In addition, ROS propagate inflammation by stimulating the release of cytokines such as interleukin-1, tumor necrosis factor-α, and interferon-γ, which stimulate recruitment of additional neutrophils and macrophages. Thus, free radicals are important mediators that provoke or sustain inflammatory processes and, consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation.[1] Membrane stabilization is a possible mechanism of action for the anti-inflammatory activity. Several anti-inflammatory medications, such as nonsteroidal anti-inflammatory drugs (NSAIDs) are available to treat the consequences of inflammation. These synthetic medications and some medicinal plants have been extensively studied for their effect on the stabilization of erythrocyte membrane exposed to hypotonic solution and heat. But these studies showed that these drugs have serious adverse effects, such as gastro intestinal erosions that can progress into ulcers. To cope with the problems associated with the use of synthetic anti-inflammatory drugs, several researchers now focus on the discovery of natural products with lesser side effects.[2]

Eichhornia crassipes (Mart.) Solms, a native of South America, is one of the free-floating macrophytes found in the aquatic environment such a ditches, ponds and lakes. It is universally called Water hyacinth. In Chhattisgarh, *E. crassipes* is being used as styptic. Fresh juice of the plant is applied in fresh wounds to stop further spread of infection. In folk medicine, *E. crassipes* has been used to ease swelling, burning, haemorrhage, and goiters. In veterinary medicine, it has been used as a tonic for the skin of horses, for irritation and inflammation.[3] The plant is reported to possess anticoagulant activity,[4] anti-inflammatory activity,[3] wound healing activity,[5] anti-tumour activity,[6] larvicidal activity,[7] antimicrobial activity,[8, 9,10-12] and antioxidant activity.[13,14] To the best of our knowledge, there are no reports on the anti-inflammatory and antioxidant activities of *E. crassipes* flowers. The current study

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investigated the *in vitro* membrane stabilizing and antioxidant activities of extracts prepared from *E. crassipes* flowers.

Materials and Methods

Collection and extraction of the plant materials

Flowers of *Eicchornia crassipes* were collected from Guntur district, Andhra Pradesh, India and identified by a botanist. A voucher specimen was deposited in the herbarium of Sri Venkateswara University, Tirupathi with reference number 2148. The flowers were shade-dried and was extracted successively using solvents from non-polar to polar (Petroleum ether, Ethyl acetate and Ethanol) in a Soxhlet extractor. All the extracts were vacuum dried to obtain Petroleum ether extract (PEEC), ethyl acetate extract (EAEC) and ethanol extract (EEEC), respectively.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), gallic acid, and quercetin were purchased from Sigma-Aldrich Co., (St Louis, MO, USA). Potassium hexacyanoferrate, Folin-Ciocalteu reagent, and aluminum chloride ($AlCl_3$) were obtained from Merck, Mumbai, India. All the other reagents and chemicals used were of analytical grade.

Quantitative phenolic estimation

The total phenolic content of the extracts obtained from *E. crassipes* flowers was estimated according to the Folin-Ciocalteu procedure.[15] For total phenolic content determination, gallic acid was used to make the standard calibration curve. In brief, 100 µL of each extract or gallic acid standard solution was mixed with 2 mL of 2% (w/v) sodium carbonate solution. The mixture was then incubated for 5 min, and afterward 100 µL of Folin-Ciocalteu reagent was added. After incubation for 30 min at room temperature, absorbance was measured at 750 nm using a spectrophotometer. Results were expressed as gallic acid equivalents (GAE) per gram of crude extract. All determinations were carried out in triplicate.

Quantitative flavonoid estimation

The total flavonoid content of the extracts was determined based on aluminium chloride method.[16] For total flavonoid determination, quercetin was used to make the standard calibration curve. In brief, 250 µL of test extract was mixed with 1.25 mL of distilled water and 750 µL of sodium nitrite. After incubation for 5 min, 150 µL of aluminium chloride solution was added to the flask. After incubation for 6 min, 0.5 mL of sodium hydroxide and 0.3 mL of distilled water were added to the reaction mixture, with final volume made to 10 mL. The absorbance of the reaction mixture was recorded at 510 nm. Results were expressed as quercetin equivalent/g of extract. All determinations were carried out in triplicate.

Antioxidant activity

DPPH radical scavenging assay

The DPPH radical-scavenging activity of the successive extracts from *E. crassipes* was done according to the method by Chang *et al* 2001.[17] Briefly, 10 µL of test samples were mixed with 90 µL of 50 mM Tris-HCl buffer (pH 7.4) and 200 µL of 0.1 mM DPPH-ethanol solution. After 30 min of incubation at ambient temperature, the reduction of the DPPH radical was measured by reading the absorbance at 517 nm. Ascorbic acid was used as positive control. Three replicates were made for each test sample. The inhibition ratio (percent) was calculated according to the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{517}\text{ (control)} - \text{Abs}_{517}\text{ (sample)}}{\text{Abs}_{517}\text{ (control)}} \text{ X } 100$$

ABTS radical scavenging assay

This procedure was performed as described by Re *et al*., 1999 with modifications.[18] In brief, the ABTS solution (7 mM) was reacted with potassium persulfate (2.45 mM) solution and was stored in the dark for 16 h to produce a dark coloured solution containing ABTS radical cation. Before being used in the assay, the ABTS radical cation was diluted with 50% methanol for an initial absorbance of about 0.70 ± 0.02 at 745 nm. Free radical scavenging activity was evaluated by mixing 3 mL of ABTS working standard with 300 µL of test sample in a microcuvette. The absorbance reading was taken after 6 minutes following the reaction. The inhibition percentage was calculated based on the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}} \text{ X } 100$$

Reducing power assay

The Ferric reducing power of extracts was determined by the method of Oyaizu, 1986.[19] The extracts (0.75 mL) at various concentrations (62.5-1000 µg/mL) was mixed with 0.75 mL of phosphate buffer (0.2 M, pH 6.6) and 0.75 mL of potassium ferricyanide ($K_3Fe(CN)_6$) (1%, w/v), followed by incubation at 50°C in a water bath for 20 min. The reaction was stopped by adding 0.75 mL of trichloroacetic acid (TCA) solution (10%) and the mixture centrifuged at 800 g for 10 min. The supernatant (1.5 mL) was mixed with 1.5 mL of distilled water and 0.1 mL of ferric chloride ($FeCl_3$) solution (0.1%, w/v) for 10 min. The absorbance of reaction mixture was taken at 700 nm. Higher value absorbance of the reaction mixture indicated greater reducing power.

Hydroxyl radical scavenging assay

The scavenging activity for hydroxyl radicals was studied by following the method of Yu *et al*., 2002 and Ganu *et al*., 2010.[20,21] The reaction mixture contained 60 µL of 1.0 mM $FeCl_3$, 90 µL of 1 mM 1,10-phenanthroline, 2.4 mL of 0.2 M phosphate buffer (pH 7.8), 150 µL of 0.17 M H_2O_2, and 1.5 mL of the extracts at various concentrations (62.5-1000 µg/mL). After incubation at room temperature for 5 min, the absorbance of reaction mixture was noted at 560 nm. The hydroxyl radicals scavenging activity was calculated according to the following equation and compared with ascorbic acid as standard:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{560}\text{ (control)} - \text{Abs}_{560}\text{ (sample)}}{\text{Abs}_{560}\text{ (control)}} \text{ X } 100$$

Membrane stabilizing activity

The membrane stabilization by hypotonic solution and heat-induced hemolysis method was used to assess anti-inflammatory activity of the extracts by following standard procedure.

Erythrocyte suspension

To prepare the erythrocyte suspension, whole blood was collected in syringes (containing anticoagulant) from healthy human volunteers. The blood was centrifuged at 3000 rpm for 10 min and blood cells were washed three times with isotonic solution (0.9% saline). The volume of saline was measured and reconstituted as a 40% (v/v) suspension with isotonic buffer solution (pH 7.4) which was contained in 1 liter of distilled water: NaH_2PO_4. $2H_2O$, 0.26 g; Na_2HPO_4, 1.15 g; NaCl, 9 g (10 mM sodium phosphate buffer). Thus, the suspension finally collected was the stock human erythrocyte (RBC) suspension.

Hypotonic solution induced hemolysis

The membrane stabilizing activity of the extracts was evaluated by using hypotonic solution induced human erythrocyte hemolysis, designed by Sikder *et al*., 2010.[22] The test sample, consisted of stock erythrocyte suspension (0.50 mL), was mixed with 5 mL of hypotonic solution (5 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing either the extracts (1.0 mg/mL) or acetylsalicylic acid (ASA) (0.1 mg/mL). The control sample (0.5 mL of RBCs) was mixed with hypotonic-buffered saline alone. The mixture was incubated for 10 min at room temperature, centrifuged for 10 min at 3000 x g and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of either hemolysis or membrane stabilization was calculated using the following equation:

$$\% \text{ inhibition of hemolysis} = 100 \text{ x } (OD_1 - OD_2/OD_1)$$

Where,

OD_1 = Optical density of hypotonic-buffered saline solution alone (control) and,

OD_2 = Optical density of test sample in hypotonic solution.

Heat-induced hemolysis

Aliquots (5 mL) of the isotonic buffer, containing 1.0 mg/mL of different extracts of the plant were put into two duplicate sets of centrifuge tubes.[23] The vehicle, in the same amount, was added to another tube as the control. Erythrocyte suspension (30 mL) was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 54°C for 20 min in a water bath. The other pair were maintained at 0-5°C in an ice bath. The reaction mixture was centrifuged for 3 min at 1300 x g and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition or

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acceleration of hemolysis in tests and control was calculated using the following equation:

$$\% \text{ inhibition of hemolysis} = 100 \times [1-(OD_2-OD_1/OD_3-OD_1)]$$

Where,
OD_1 = test sample unheated,
OD_2 = test sample heated and,
OD_3 = control sample heated.

Results and Discussion

In the present investigation, results of *in vitro* antioxidant activity demonstrated that *E. crassipes* possess antioxidant and free radical scavenging activity. In the *in vitro* membrane stabilization study, *E. crassipes* protected human red blood cell (HRBC) membrane against lysis induced by heat and hypotonic solution. The ethanol extract demonstrated highest antioxidant and membrane stabilizing activity when compared to other extracts studied.

Oxidative stress is involved in the pathophysiology of inflammation, degenerative disease, diabetes, cancer, ischemic heart disease and many more. Antioxidants interrupt the production of ROS and play a key role to inactivate them. Although, all human cells protect themselves against oxidative damage by some antioxidant mechanism, these sometimes are not sufficient to prevent the ROS damage totally. Several plant-based natural antioxidants have already been reported which react with free radicals to prevent the oxidation of substrate.[24] In the present investigation, the successive extracts prepared from *E. crassipes* flowers were tested for antioxidant potential in four classical *in vitro* methods. DPPH is a stable free radical because of its spare electron delocalization over the whole molecule. The donation of H^+ to the DPPH radicals made a corresponding change from violet colour to pale yellow in the solution.[25] The results of the DPPH radical scavenging activity of *E. crassipes* flower extracts are presented in Table 1**.** This activity was increased by increasing the concentration of the sample extract. At higher concentrations (1000 µg/mL), the ethanol extract showed highest percentage inhibition of DPPH when compared to the other extracts. The IC_{50} values of the extracts were >1000 µg/mL for PEEC, 723.89 µg/mL for EAEC; and 403.53 µg/mL for EEEC. The DPPH scavenging by the successive extracts of *E. crassipes* was found to be in the order: EEEC > EAEC > PEEC. In $ABTS^{\bullet+}$ scavenging assay, the reaction between ABTS and potassium persulfate results in the production of a blue colored chromophore, $ABTS^{\bullet+}$. After addition of the extracts of *E. crassipes*, this pre-formed radical cation was converted to ABTS in a dose-dependent fashion.[24] The mean IC_{50} values for ABTS radical of PEEC, EAEC, and EEEC of *E. crassipes* were found to be >1000 µg/mL, 757.97 µg/mL, and 524.60 µg/mL, respectively and the results are presented in Table 2. The mean IC_{50} value of ascorbic acid was found to be 114.41 µg/mL. The order of $ABTS^{\bullet+}$ scavenging by the extracts of *E. crassipes* was found to be EEEC > EAEC > PEEC. Reducing power indirectly evaluates the antioxidant activity. It is used to determine the extent of reduction of ferricyanide to ferrocyanide by antioxidants.[26] The absorbance values at the differentconcentrations of extracts are given in Table 3**.** The absorbances of the successive extracts at 1000 µg/mL were 0.080 ± 0.003 for PEEC, 0.108 ± 0.003 for EAEC; and 0.197 ± 0.002 for EEEC, indicating that compounds containing reducing properties were present in all these extracts. The ethanol extract exhibited the highest reducing potential when compared with the other extracts.

Hydroxyl radical being highly reactive can react especially with proteins, DNA, and lipids and extract the hydrogen atoms thereby initiating the lipid peroxidation process. Using the Fenton reaction model system, the assay of hydroxyl radical scavenging activity was conducted. The reversible reaction between Fe^{2+} and 1,10-Phenanthroline formed Fe $(Phen)_2^{2+}$, which react with H_2O_2 and formed OH^- free radicals.[25]

$$Fe\ (Phen)_3^{2+} <----> Fe\ (Phen)_2^{2+} + Phen$$

$$Fe\ (Phen)_2^{2+} + H_2O_2 \rightarrow Fe\ (Phen)_2^{3+} + OH^- + {\cdot}OH$$

The PEEC, EAEC and EEEC of *E. crassipes* showed concentration-dependent scavenging activity on hydroxyl radicals and the results were given in Table 4. The mean IC_{50} values for hydroxyl radical scavenging activity of PEEC, EAEC, and EEEC of *E. crassipes* were found to be >1000 µg/mL, 864.35 µg/mL, and 567.73 µg/mL, respectively. The mean IC_{50} value of ascorbic acid was found to be 118.28 µg/mL. The order of hydroxyl radical scavenging activity was found to be EEEC > EAEC > PEEC. In the above-mentioned procedures for screening for antioxidant activity, EEEC has shown effective antioxidant potential, although lesser in potency than that of ascorbic acid.

The antioxidant potential of *E. crassipes* may be attributed to the high phenolic and flavonoid contents of the plant as revealed from their quantitative estimation. It is also found that EEEC showed a high amount of flavonoids and phenolic contents. The ethanol extract showed high amount of flavonoid content (96.26 quercetin equivalent/g of extract) than ethyl acetate extract (87.83 quercetin equivalent/g of extract). The ethanol extract showed high amount of phenolic content (109.41 mg GAE/g of crude extract) than ethyl acetate extract (65.33 mg GAE/g of crude extract). The results of total flavonoid and phenolic contents are presented in Table 5. Flavonoids show their antioxidative action through scavenging or chelating process. Phenolic compounds are also very important active constituents because of their scavenging ability due to their hydroxyl groups. Both compounds have good antioxidant potential and their effects on human nutrition and health are significant.[24]

The HRBC membrane stabilization has been used as a method to study the anti-inflammatory activity. Membrane stabilization is a process, which act by retaining the integrity of biological membranes such as erythrocyte and lysosomal membranes against hypotonic solution and heat-induced lysis by interfering with the release of inflammatory mediators like histamine, serotonin, prostaglandins, leukotrienes, etc.[27] During inflammation, lysosomal enzymes are released into the cytosol, producing damage to the surrounding tissues, thereby triggering inflammation. Most of NSAIDs stabilize lysosomal membrane and inhibit the inflammatory process by restricting the release of lysosomal enzymes.[28] The extracts of the flowers of *E. crassipes*, were subjected to assay for membrane stabilizing activity and the results are presented in Tables 6 and 7. For hypotonic solution induced hemolysis, at a concentration of 1.0 mg/mL, EEEC inhibited 65.78% hemolysis of RBCs as compared to 77.77% produced by ASA (0.10 mg/mL). PEEC and EAEC showed 18.12% and 46.19% inhibition of hemolysis of RBCs. For heat-induced hemolysis, at a concentration of 1.0 mg/mL, EEEC inhibited 41.06% hemolysis of RBCs as compared to 66.76% produced by ASA (0.10 mg/mL). PEEC and EAEC showed 17.06% and 21.21% inhibition of hemolysis of RBCs. From the results of the present study, it is evident that *E. crassipes* flower extract can protect

Table 1: DPPH scavenging activity of *E. crassipes* flower extracts

Test Sample	% Inhibition/Concentration					IC_{50} (µg/mL)
	62.5 µg/mL	125 µg/mL	250 µg/mL	500 µg/mL	1000 µg/mL	
PEEC	11.31 ± 0.58	20.23 ± 0.54	30.37 ± 0.97	35.57 ± 0.58	45.88 ± 0.96	>1000
EAEC	15.29 ± 0.55	25.39 ± 0.77	35.88 ± 1.09	44.84 ± 0.96	55.23 ± 1.27	723.89
EEEC	18.62 ± 0.39	29.30 ± 0.82	46.34 ± 0.51	52.67 ± 1.19	64.30 ± 0.52	403.53
Ascorbic acid	44.77 ± 1.23	62.80 ± 1.30	74.75 ± 0.97	84.04 ± 1.22	95.84 ± 0.95	80.34

PEEC: Petroleum ether extract of *E. crassipes;* EAEC: Ethyl acetate extract of *E. crassipes;* EEEC: Ethanol extract of *E. crassipes.*
Values are expressed as Mean ± SEM of triplicate measurement.

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Table 2: ABTS scavenging activity of *E. crassipes* flower extracts

Test Sample	% Inhibition/Concentration					IC_{50} (µg/mL)
	62.5 µg/mL	125 µg/mL	250 µg/mL	500 µg/mL	1000 µg/mL	
PEEC	9.35 ± 0.62	15.66 ± 0.33	23.51 ± 0.69	31.16 ± 0.60	43.34 ± 0.65	>1000
EAEC	14.59 ± 0.49	23.75 ± 0.89	32.31 ± 0.64	41.25 ± 0.55	58.24 ± 0.79	757.97
EEEC	19.66 ± 0.87	26.45 ± 0.61	38.67 ± 0.61	49.23 ± 0.83	70.28 ± 0.64	524.60
Ascorbic acid	33.75 ± 0.97	53.34 ± 0.63	72.38 ± 0.54	85.52 ± 1.19	97.33 ± 0.93	114.41

PEEC: Petroleum ether extract of *E. crassipes;* EAEC: Ethyl acetate extract of *E. crassipes;* EEEC: Ethanol extract of *E. crassipes.* Values are expressed as Mean ± SEM of triplicate measurement.

Table 3: Reducing power assessment of *E. crassipes* flowers

Concentration (µg/mL)	Absorbance at 700 nm			
	PEEC	EAEC	EEEC	Ascorbic acid
62.5	0.034 ± 0.001	0.046 ± 0.001	0.072 ± 0.003	0.175 ± 0.005
125	0.044 ± 0.002	0.053 ± 0.000	0.096 ± 0.002	0.200 ± 0.004
250	0.057 ± 0.002	0.066 ± 0.002	0.129 ± 0.005	0.234 ± 0.003
500	0.069 ± 0.002	0.076 ± 0.005	0.147 ± 0.005	0.252 ± 0.003
1000	0.080 ± 0.003	0.108 ± 0.003	0.197 ± 0.002	0.290 ± 0.005

PEEC: Petroleum ether extract of *E. crassipes;* EAEC: Ethyl acetate extract of *E. crassipes;* EEEC: Ethanol extract of *E. crassipes.* Values are expressed as Mean ± SEM of triplicate measurement.

Table 4: Hydroxyl radical scavenging activity of *E. crassipes* flower extracts

Test Substance	% Inhibition/Concentration					IC_{50} (µg/mL)
	62.5 µg/mL	125 µg/mL	250 µg/mL	500 µg/mL	1000 µg/mL	
PEEC	8.75 ± 0.51	11.55 ± 0.33	20.85 ± 0.81	27.33 ± 0.35	40.23 ± 0.57	>1000
EAEC	10.73 ± 0.25	20.28 ± 0.51	30.35 ± 0.42	41.93 ± 0.69	53.03 ± 0.40	864.35
EEEC	15.56 ± 0.44	25.76 ± 0.65	38.11 ± 0.21	48.28 ± 0.36	60.78 ± 0.73	567.73
Ascorbic acid	37.37 ± 0.51	51.56 ± 0.50	71.90 ± 0.89	81.53 ± 0.40	91.52 ± 0.90	118.28

PEEC: Petroleum ether extract of *E. crassipes;* EAEC: Ethyl acetate extract of *E. crassipes;* EEEC: Ethanol extract of *E. crassipes.* Values are expressed as Mean ± SEM of triplicate measurement.

Table 5: Total phenolic content and flavonoid content of different solvent extracts of *E. crassipes* flowers

Extract	Total Phenolic Content (mg/g) GAE	Flavonoid content (mg/g) quercetin equivalent
PEEC	NP	NP
EAEC	65.33 ± 2.11	87.83 ± 2.59
EEEC	109.41 ± 2.21	96.26 ± 3.27

PEEC: Petroleum ether extract of *E. crassipes;* EAEC: Ethyl acetate extract of *E. crassipes;* EEEC: Ethanol extract of *E. crassipes.* Values are expressed as Mean ± SEM of triplicate measurement. GAE – Gallic acid equivalent, NP - not performed.

Table 6: Effect of *E. crassipes* flowers on h**ypotonic** solution-**induced hemolysis of erythrocyte membrane**

Treatment	Concentration	Optical density of the sample	% Inhibition of haemolysis
Control	1 mg/mL	0.684 ± 0.002	-
PEEC	1 mg/mL	0.560 ± 0.010	18.12
EAEC	1 mg/mL	0.368 ± 0.013	46.19
EEEC	1 mg/mL	0.234 ± 0.010	65.78
ASA	0.1 mg/mL	0.152 ± 0.011	77.77

PEEC: Petroleum ether extract of *E. crassipes;* EAEC: Ethyl acetate extract of *E. crassipes;* EEEC: Ethanol extract of *E. crassipes;* ASA: Acetyl Salicylic acid. Values are expressed as mean±SEM of triplicate measurement.

Table 7: Effect of *E. crassipes* flowers on heat-**induced haemolysis of erythrocyte membrane**

Treatment	Concentration	Optical density of the solution		% Inhibition of haemolysis
		Heated solution	Unheated solution	
Control	1 mg/mL	0.651 ± 0.013	0.254 ± 0.017	-
PEEC	1 mg/mL	0.430 ± 0.013	0.229 ± 0.015	17.06
EAEC	1 mg/mL	0.339 ± 0.015	0.255 ± 0.010	21.21
EEEC	1 mg/mL	0.301 ± 0.010	0.276 ± 0.012	41.06
ASA	0.1 mg/mL	0.543 ± 0.014	0.326 ± 0.010	66.76

PEEC: Petroleum ether extract of *E. crassipes;* EAEC: Ethyl acetate extract of *E. crassipes;* EEEC: Ethanol extract of *E. crassipes.* ASA: Acetyl Salicylic acid. Values are expressed as Mean ± SEM of triplicate measurement.

the HRBC membrane against lysis induced by hypotonic solution and heat. The ethanol extract showed the highest protection when compared to other extracts tested. Since HRBC membranes are analogous to lysosomal membrane components, protection against hypotonicity or heat-induced lysis of RBC is often extrapolated to stabilization of lysosomal membranes and used as a biochemical index of anti-inflammatory activity.[28]

Previous studies have shown that flavonoids and phenolic compounds can exhibit anti-inflammatory effects because of their membrane stabilizing actions.[29] Preliminary phytochemical analysis revealed the presence of flavonoids, saponins, and phenolic compounds in the extracts of *E. crassipes* flowers. Therefore, it is not unreasonable to speculate that the presence of these chemical components may be responsible for the observed membrane stabilizing action of the plant extracts. Free radicals attract various inflammatory mediators in the inflammatory process. They cause release of proinflammatory cytokines and initiate inflammation.[26] It seems that the antioxidant effect of *E. crassipes* might have a contributing role to its anti-inflammatory activity.

Conclusion

E. crassipes flowers exhibited significant antioxidant and anti-inflammatory activities. The anti-inflammatory effect could be attributed to antioxidant potential of *E. crassipes* flowers which may be due to the presence of flavonoids and phenolic compounds present in the flowers extract. The results of the present study have supported the claimed traditional uses of *E. crassipes* flowers in inflammation.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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