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Comparative phytochemical screening and bioactive properties of two aquatic weeds - *Pistia stratiotes* and *Eichhornia crassipes*

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Abstract

Medicinal plants are becoming popular nowadays as more people are practicing herbal remedies in their daily lives because of its less frequent side effects. Natural antioxidants from plants have widely acclaimed nutritional as well as therapeutic value. *Eichhornia crassipes*, commonly known as common Water hyacinth belongs to the family Pontederiaceae; *Pistia stratiotes*, commonly known as water cabbage or shell flower belongs to the family Araceae. *Pistia stratiotes* has often been grown as an ornamental plant in lakes, ponds, aquaria and gardens. It can be beneficial in certain instances as it out competes algae for nutrients in the water, thereby preventing massive algal blooms. The present study investigated and compares the bioactive potential of these plants by evaluating its phytochemical content, antioxidant effects and antibacterial activity and thus validates its uses scientifically. The plants were collected, leaves were separated, washed, shade dried, powdered and extracted using acetone in Soxhlet's apparatus for 6 hours. The phytochemical screening of the leaf extracts revealed the presence of alkaloids, carbohydrates, resin, saponins, flavonoids, etc. The antioxidant effects of acetone extracts were studied by DPPH radical scavenging assay along with ascorbic acid control. Acetone extract of *P. stratiotes* showed higher percentage of inhibition (83.19%) at 1000µg/ml and the IC_{50} value was found to be 462.36µg/ml. Antibacterial properties of acetone extract of the leaves of two plants were tested against *Vibrio cholerae, Xanthomonas campestris,* Staphylococcus *epidermidis, Escherichia coli, Bacillus cereus.*

Keywords: *Pistia stratiotes*, Soxhlet apparatus, Anti- oxidant property, DPPH, *Escherichia coli*.

Introduction

Plants played a vital role in human wealth and economy from ancient times onwards. Many plant derived substances including aromatic compounds and various secondary metabolites have been isolated from the different plants. There are more than thousands of indigenous plants, used for particular treatment processes. Plants were used for medicinal purposes even before 60,000 years. Herbalism is a traditional medicinal or folk medicinal practice based on the use of plant or plant extracts. The scope of herbal medicine is extended to include fungi and bee products, as well as minerals, shells and certain parts of animals (Acharya *et al.*, 2008). Herbs are used as treatment for certain diseases, for maintaining optimal

health and also as an aphrodisiac. It is believed that 80% of the outside industrialized countries depend on herbs for their needs (Muhammed and Musa, 1994).
Plants are used for various purposes which includes dye making, production of cosmetics, perfumes, pest control, food, etc. According to the survey, there were more than 70000 species of plants identified and used as medicines so far. The natural products enhanced the overall function of external organs (Young and Woodside, 2001). Herbal plants are being used as medicine from ancient age and usefulness of them are recorded in human history. Herbal plants are reported to be excellent source of several nutrients. The researchers focused on the significance of traditional medicine from the herbs and medicinal plants. Natural products push on to bestow valuable therapeutic agent, both in traditional and conventional medicines. Because of the less frequent side effects, consumption of natural products is increasing day by day. The use of herbal drugs in treatment of diseases is found widely among the people in India.

The plant *Pistia stratiotes*, commonly known as water cabbage or water lettuce, belongs to the family Araceae, is an edible, aquatic, floating ornamental plant with widely distributed across tropical and sub-tropical areas around the world. *P. stratiotes* is widely distributed widely in Asia and Africa. This plant and its extracts are potentially believed to have medicinal effects. This plant is proven to be antiseptic, anti-tubercular and anti-dysentric. In various parts of the world it is also used as anodyne for eyewash. The leaves are used in eczema, leprosy, ulcers and piles (Kirtikar and Basu 2000). Leaf infusions have been mentioned in the folklore to be used for bladder complaints, kidney afflictions, hematuria, dysentery and anemia. The fresh water aquatic plant *Eichhornia crassipes*, commonly known as water hyacinth is a member of the family Pontederiaceae. This fast growing, free-floating, perennial plant is indigenous to Brazil, Amazon basin and Ecuador region. It was introduced as an ornamental species to adorn the waterbodies. The present study aims to compare the antibacterial and antioxidant activities of acetone leaf extracts of *E. crassipes* and *P. stratiotes*.

Materials and Methods

Preparation of Plant extracts

E. crassipes and *P. stratiotes* leaves were collected during the November season from the backwaters of Alappuzha. The leaves were separated, washed with distilled water and dried under shadow. Then the leaves were chopped into small pieces and powdered. 20g of powdered leaf were extracted using 350ml of acetone as solvent in a Soxhlet apparatus for 7 hours. The excess solvent in the extracts were removed by using Rotary evaporator. The concentrated samples were weighed, kept in screw capped bottles and stored at 4^{o}C.

Phytochemical screening

Preliminary phytochemical screening was done for the identification of phytoconstituents present in the acetone leaf extract of *E. crassipes* and *P. stratiotes* (Harborne, 1999; Sofowara, 1993).The extracts were analysed for the presence of alkaloids, flavonoids, phenols, steroids and saponins.

Libermann-Burchard test: Few drops of acetic anhydride were added to the extract dissolved in chloroform, followed by concentrated H_2SO_4through the sides of the test tube. Presence of red coloured ring indicated terpenoids and the change in red colour to green marked the presence ofsteroids.

NaOH test: Take 2-3 ml of extract, add few drops of sodium hydroxide (NaOH) solution into a test tube. Formation of intense yellow colour that became colourless on addition of few drops of dilute HCl indicates the presence of flavonoids.

Wagner's test: A fraction of extract was treated with Wagner's test reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml of water). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Ferric Chloride Test: To 200 µl of the extract, 2ml distilled water was added followed by the addition of few drops of 5 % ferric chloride along the sides of the test tube. A dark green coloured showed the presence of phenolic compounds.

Foam test: 10 ml of distilled water was added to the extract and shaken well for few minutes. Formation of frothing that persisted for 60-120 seconds indicated the presence of saponins.

Antioxidant assay

DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay

The DDPH radical scavenging activity of extract was measured using modified method of Blois (Blois,

1958). Different concentrations of acetone extract of *E. crassipes* and *P. stratiotes* were tested for radical scavenging activity. DPPH (20 mg) was dissolved in methanol (250 mL) to obtain the concentration of 80 µg/mL. 1 ml DPPH solution was added to 3 ml of leaf extracts in acetone at different concentrations (200, 400, 600, 800 µg/ml). The mixture was shaken vigorously and allowed to stand in dark at room temp for 30 min. Then, absorbance was measured at 517 nm by using spectrophotometer (UV-VIS Systronics). Ascorbic acid was used as standard and experiment was done in triplicate. The IC 50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity. The percent DPPH scavenging effect was calculated by using following equation: DPPH scavenging effect (%) or Percent inhibition = A0 - A 1 / A0 × 100. Where A0 was the Absorbance of control reaction and A1 was the Absorbance of test or standardsample.

Antibacterial activity

Acetone extract of leaves of *E. crassipes* and *P. stratiotes* were tested for its anti bacterial activity.

Test micro organisms

Bacterial stock cultures of *Vibrio cholera, Xanthomonas campestris, Staphylococcus epidermidis, Escherichia coli and Bacillus cereus* maintained at the Department of Biotechnology, KVM College, Cherthala were used.

Preparation of plant extracts

Acetone extract at a concentration of 100 mg/ml and 250 mg/ml DMSO were used for the antibacterial activity.

Disc diffusion method

The antibacterial sensitivity testing of the extract was determined using disc diffusion method. 100µl of the inoculated microorganisms from nutrient broth were spread over the plates containing Mueller-Hinton agar using sterile cotton swabs. The sterile discs having a diameter of 6 mm were impregnated with 20µl of a leaf extracts and were placed on inoculated surface of agar plate with the help of sterile forceps. Control experiments were carried out under similar condition by using Streptomycin (10mg) as positive control and DMSO as negative control. The antibacterial activity was observed after incubating the plates for 24 hrs at 37° C and the zone of inhibition surrounding the disc were noted in millimetres(mm).

Results

Yield of extract of leaves of *Eichhornia crassipes* and *Pistia stratiotes*

20 gm of the leaf of *E. crassipes* and *P. stratiotes* were extracted using acetone. The yield of extraction was presented in **Table 1**. The acetone extraction of *E. crassipes* leaf yield 3.24 gm extract while yield of *P. stratiotes* yield 2.8 gm of extract.

Table 1: Yield of acetone leaf extracts of *E. crassipes* and *P. stratiotes*

Sample	Weight of sample	Weight of Acetone extract
Eichhornia crassipes	20gm	3.24gm
Pistia stratiotes	20gm	2.8gm

Phytochemical screening of acetone leaf extracts of *E. crassipes* and *P.stratiotes*

The results of phytochemical screening of acetone leaf extracts of *E. crassipes* and *P. stratiotes* were showed in

Table 2. The phytochemical composition of *E. crassipes* leaf extract showed the presence of saponins, phytosterols, carbohydrates, phenols, terpenoids, coumarin glycoside. However alkaloids, resins, flavonoids were absent. The leaf extract of *P. stratiotes* showed the presence of saponins, flavonoids, carbohydrates, phenols, terpenoids, coumarin glycoside.

Table 2. Phytochemical analysis of *E. crassipes* and *P. stratiotes* leaf extracts

Tests	*E. crassipes*	*P. stratiotes*
Alkaloids	-ve	-ve
Carbohydrates	+ve	+ve
Resin	-ve	-ve
Saponins	+ve	+ve
Flavinoids	-ve	+ve
Phytosterols	+ve	-ve
Phenols/tannins	+ve	+ve
Terpenoids	+ve	+ve
Coumarin glycoside	+ve	+ve

Antimicrobial activity

The agar disc diffusion method was employed for testing the antimicrobial susceptibility of acetone extracts of *E. crassipes* and *P. stratiotes* against five microorganisms *Vibrio cholerae, Xanthomonas campestris, Staphylococcus epidermidis, Escherichia coli, Bacillus cereus.* The results of the antibacterial activity of acetone extracts of *E. crassipes* and *P. stratiotes* were shown in **Table 3**. Both acetone extracts of *E. crassipes* and *P. stratiotes* showed varying degrees of antibacterial potential only at 250mg/ml.

The acetone extract of *E. crassipes* showed maximum antimicrobial activity against *Escherichia coli* with a zone of inhibition of 16.33 ± 0.577mm and minimum antimicrobial activity against *Vibrio cholerae* with a zone of inhibition of 8.33 ± 1.52mm. The extract also showed comparatively high antimicrobial activity against *Staphylococcus epidermidis* with a zone of inhibition of 11.66 ± 1.52mm.

P. stratiotes showed antimicrobial activity against five pathogens. Its acetone extract showed maximum antimicrobial activity against *Staphylococcus epidermidis* with a zone of inhibition of 13 ± 1.73mm. *Escherichia coli* also showed high range of antimicrobial activity with a zone of inhibition 12.33 ± 1.15mm. The test extracts showed lower zone size when compared with that of the streptomycin control **(Fig: 1- 5).**

Table 3: Antimicrobial activity of *E. crassipes* and *P. stratiotes* against different bacteria

Microorganism	*E. crassipes*	*P. stratiotes*	+ve control
Vibrio cholerae	8.33 ± 1.52mm	8 ± 1mm	31.33±1.15mm
Xanthomonas campestris	10 ± 1mm	8.33 ± 0.57mm	19±1.73mm
Staphylococcus epidermidis	11.66± 1.52mm	13 ± 1.73mm	24.33±1.52mm
Escherichia coli	16.33 ± 0.577mm	12.33 ± 1.15mm	32.66±2.08mm
Bacillus cereus	8.66 ± 1.15mm	11. 66 ± 1.52mm	19.66±2.08mm

Fig 1: ***Vibrio cholerae***

Fig 2: ***Xanthomonas campestris***

Fig 3: ***Bacillus cereus***

Fig 4: ***Escherichia Coli***

Fig 5: ***Bacillus epidermidis***

Antioxidant activity

The antioxidant activity of acetone extracts of *E. crassipes* and *P. stratiotes* leaf were determined using DPPH reagent. DPPH is a very stable free radical. The effect of an antioxidant on DPPH radical scavenging is due to their hydrogen donating ability or radical scavenging activity. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form diphenyl picryl hydrazine with the loss of its violet color.

The percentage inhibition of the plant extracts of *E. crassipes* and *P. stratiotes* were represented in **Table 4**. The results revealed that both plant extracts showed antioxidant activity. The results indicate that the antioxidant activity of the crude extract of both the plants was lower than that of the standard. The results of DPPH radical scavenging activity of *E. crassipes* and *P. stratiotes* leaf extracts were presented in **Fig: 6.** Among the two extracts studied, acetone extract of *P. stratiotes* showed higher percentage of inhibition (83.19%) at 1000µg/ml and the IC50 value was found to be 462.36µg/ml. The IC50 value of *E. crassipes* was found to be 643.57µg/ml. Ascorbic acid was used as a standard and the percentage inhibition of ascorbic acid at 100µg/ml was 96.38±0.76% **(Fig: 7)** and its IC50 value was found to be 28.59±0.24µg/ml. It was found that the DPPH radical scavenging activities of the extracts increased with increase in concentration.

Table 4: The antioxidant activity of the plant extracts of *E. crassipes* and *P. Stratiotes*

Conc. of plant extract (µg/ml)	Percentage inhibition of *P. stratiotes*	Percentage inhibition of *E. crassipes*
200	25.37%	22.85%
400	48.39%	45.18%
600	66.48%	56.95%
800	77.94%	58.13%
1000	83.19%	58.67%

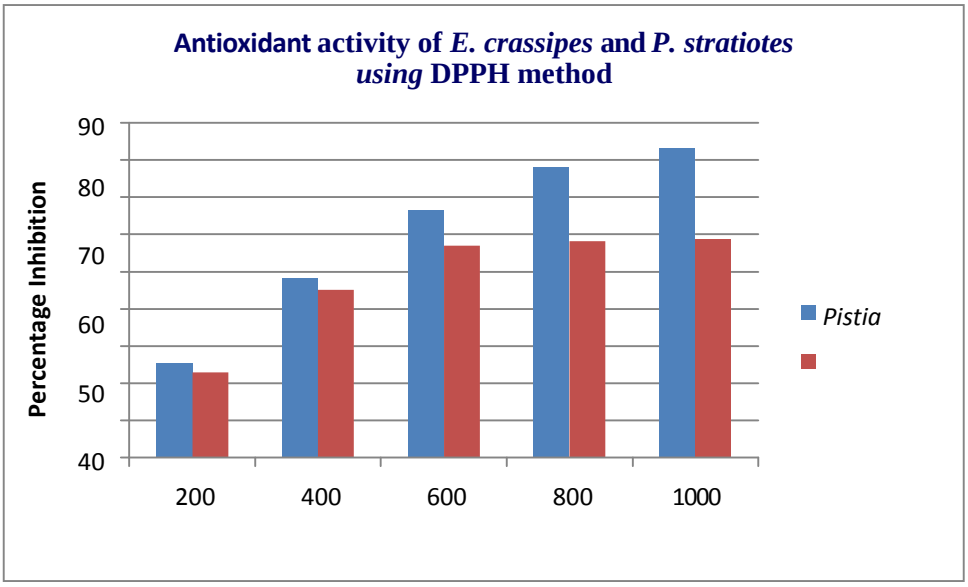

Fig 6: Graph showing the antioxidant property of the acetone extract of *E. crassipes* and *P. stratiotes*

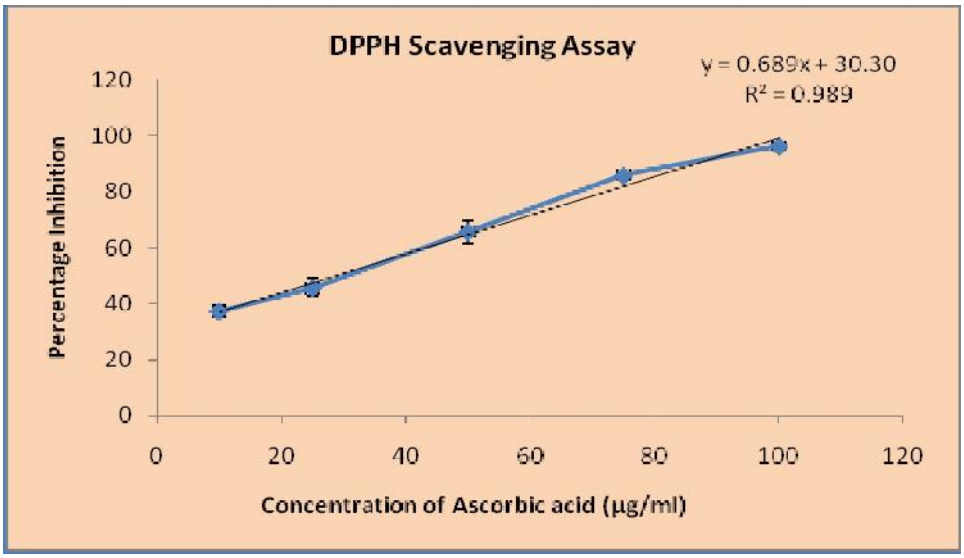

Fig 7: Ascorbic acid standard curve

Discussion

The extract of leaves of *E. crassipes* showed the presence of saponins, phytosterols, phenols or tannins, terpenoids, coumarin glycosides. However, alkaloids, resin, flavonoids were not found. The phytochemical composition of *P. stratiotes* showed the presence of carbohydrates, saponins, flavonoids, phenols or tannins terpanoids and coumarin glycoside. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties (Kumar, 2009). Different phytochemicals have been found to possess a wide range of activities. The phytochemicals are known to have antimicrobial activity (Ebana, 1995). Tannins has been found to possess astringent properties to hasten the healing of wounds and inflamed mucous membranes. Tannins and flavonoids are thought to be responsible for antidiarrhoeal activity (Enzo, 2007). Similarly, tannins have been reported to have antibacterial potential as they react with proteins in the cell membrane thereby killing the bacteria (Elmarie and Johan, 2001). Phytochemicals such as terpenoid, flavonoid and tannins have anti- inflammatory effects (Manach., 1996). Flavonoid and tannins have hypoglycemic activities (Cherian and Augusti, 1995).

Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that actas primary antioxidants or free radical scavengers. Flavonoids exhibit pharmaceutical activities like anti-allergic, anti inflammatory, antimicrobial and anticancer activity. Tannin has been widely used to sprains, bruises and superficial wounds and has antibacterial property. Saponins are widely used for Anti-inflammatory, anti-hepatotonic, hypoglycemic, anti- microbial and anti-viral; used in detergents and molluscicides. Also terpenoids possess anti- viral, anti-bacterial, anti- malarial, anti-inflammatory, anti-cancer; inhibits cholesterol synthesis (Nostro et al., 2000). *P. stratiotes* showed antimicrobial activity against five pathogens. Its acetone extract showed maximum antimicrobial activity against *Staphylococcus epidermidis* with a zone of inhibition of 13± 1.73mm. *Escherichia coli* also showed high range of antimicrobial activity with a zone of inhibition 12.33 ± 1.15mm. The test extracts showed lower zone size when compared with that of the streptomycin control.

The antioxidant activity of acetone extracts of *E. crassipes* and *P. stratiotes* leaf were determined using DPPH radical scavenging activity. DPPH is a very stable free radical. The effect of an antioxidant on DPPH radical scavenging is due to their hydrogen donating ability or radical scavenging activity. The DPPH assay is used to predict antioxidant activities by mechanism in which antioxidants act to inhibit lipid oxidation, so scavenging of DPPH radical and therefore determinate free radical scavenging capacity. Leaves of *P. Stratiotes* have less IC50 value confirmed better antioxidant activity. The therapeutic potential of natural medicinal plants as an antioxidant in reducing free radical induced tissue injury. The antioxidants play significant role in maintaining integrity of the cell membrane by prevention of lipid peroxidation. Acetone extracts of leaf, which contained the highest total phenolic content, were found to have high DPPH radical scavenging activity and reducing power (Soares, 1997).

Conclusion

Plants are rich in primary and secondary metabolites are widely used in traditional medicine to combat and cure various ailments. *E. crassipes* and *P. stratiotes* are the plants used in medicine from the time of Ayurveda, the ancient system of Indian medicine. The extracts of both the plants contain many bioactive chemical constituents including alkaloids, steroids, terpenoids, saponins and tannin. The anti-inflammatory, antispasmodic, antianalgesic and diuretic effects can be attributed to the high steroids, tannins, terpenoids, saponins and glycosides present in these plants. These plants has been used successfully in Ayurvedic medicine for centuries, more clinical trials should be conducted to support its therapeutic use. Thus the study can be used in future for the economical formulation of the active chemical ingredients in natural drugs against a variety of diseases. These bioactive compounds should be separated and studied in detail the action of each of the compounds as well as the bioactivities of a mixture of compounds.

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