Free radical scavenging, antidiarrheal and anthelmintic activity of *Pistia stratiotes* L. extracts and its phytochemical analysis

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Abstract: In this phyto-pharmacological screening of *Pistia stratiotes* L leaf and root extracts each separately in two different solvents demonstrated its potential medicinal value. Apparent antioxidant value is demonstrated by DPPH, Nitric oxide scavenging and Ferric ion reducing method. Additionally, total flavonoid and phenolic compounds were measured. The leaf methanolic extract scavenged both nitric oxide (NO) and DPPH radical with a dose dependent manner. But the pet ether fraction of root was found to have highest efficacy in Fe^{3+} reducing power assay. Flavonoid was found to contain highest in the pet ether fraction of root (411.35mg/g) in terms of quercetin equivalent, similarly highest amount (34.96mg/g) of total phenolic compounds (assayed as gallic acid equivalents) were found to contain in the same fraction. The methanolic fractions appeared less cytotoxic compared to pet ether extracts. The plant extracts caused a dose dependent decrease in faecal droppings in both castor oil and magnesium sulphate induced diarrhea, where as leaf extracts in each solvent appeared most effective. Also, the plant extracts showed anthelmintic activity in earthworm by inducing paralysis and death in a dose dependent manner. At highest doses (50 mg/ml) all fractions were almost effective as the positive control piperazine citrate (10 mg/ml). Thus, besides this cytotoxic effect it's traditional claim for therapeutic use can never be overlooked.

Keywords: *Pistia stratiotes* L, free radical scavenging, anthelmintic, anti-dirrhoea.

INTRODUCTION

There are many herbal derived alternative medicines are being used for centuries in Bangladesh. Different types of herbs with their potential therapeutic effects are distributed here. *P. stratiotes* L. (Araceae) is a widespread aquatic stoloniferous herb in this country. This plant is long been used in this subcontinent and in various countries of Africa as a traditional medicine for different purposes. A number of medicinal property has attributed to this plant, including antioxidant (Megha *et al.,* 2010), anthelmentic (Ajaiyeoba *et al.,* 2001) and against leprosy, eczema, piles, ulcers, and syphilis (Kirtikar *et al.,* 2001). A lot of pharmacological investigation has carried out on the basis of its chemical constituents, but still many areas of its therapeutic utility are untapped.

Many folkloric claims of effects of this *Pistia stratiotes* L, insisted us to work extensively and make rational, of its uses and identify its potentiality as a drug candidate. In the course of this screening program, pharmacological properties of methanolic and pet ether extracts of both leaf and root were investigated in different scientifically established model. Experiments included of this plant extracts were antioxidant activity namely DPPH radical scavenging assay, Fe^{3+} ion reducing power (Oyaizu, 1986), nitric oxide scavenging assay (Govindarajan *et al.,* 2003), (Oyaizu, 1986), total phenolic contents (Yu *et al.,* 2002), total flavonoid contents (Kumaran and Karunakaran 2007), cytotoxicity assay by brine shrimp lethality assay (Meyer *et al.,* 1982), antidirrhoeal activity (Shoba and Thomas 2001). and anthelmintic activity (Ajaiyeoba *et al.,* 2009).

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MATERIALS AND METHODS

Collection and identification of plant parts

The semi aquatic whole plant, *P. stratiotes* L., was collected in April 2010, from a pond located in Jahangirnagar University, Savar, Dhaka, after proper identification has done by the National Herbarium, Bangladesh (accession number 35621).

Extract preparation

The whole plant was thoroughly washed into water, sun dried for a day and then the leaves and roots have been separated. Leaves and roots were spread in thin layers in trays and finally placed into a dryer (at 55°C) (Ghani, 2003). The coarse powder of the dried plant parts of *P. stratiotes* L. were extracted separately with the solvent pet ether and methanol in a soxhlet apparatus using 100g powder for each part. When the powders became exhausted of its chemical constituents as evident from cycles of colorless liquid siphoning in the soxhlet

apparatus, extraction was considered to be complete. Extracted liquid was filtered by a sterile cotton plug and reduced in amount by solvent evaporation using water bath and the final sediment was used for the experiments. Standard procedures were followed for the phytochemical screening (Ghani, 2003).

Animal handling

In order to investigate the antidiarrhoeal, sedative and anxiolytic effects of this plant extracts, 3 to 4 weeks aged Swiss Albino mice of both sex, of around 20 to 25g weight, were collected from the International Center for Diarrheal Disease and Research, Bangladesh (ICDDRB). Animals were caged in groups of 5, in cages of (28×22×13 cm) dimension, with dry wood shavings as flooring. Temperature was maintained 25^{o}C with 55 to 65% relative humidity and 12 hrs light/12 hrs dark for the entire study time. Food pellets were provided from ICDDR, B with fresh water *ad libitum.*

DPPH radical scavenging assay

Antioxidant activity by 1, 1-diphenyl, 2-picrylhydrazyl (DPPH, Sigma USA) of plant parts were determined following the method of Oyaizu, (1986). In brief, 3mL methanolic solution containing 0.004% DPPH was added in a tube with an aliquot of 100 µL of either extract or standard (ascorbic acid), and kept in dark for 30min for the reaction to take place, then absorbance taken at 517 nm against a blank (methanol). Percent of activity was determined by - *%* Scavenging = *{(A_0– A_1)/ A_0} X 100*
A_1 is the absorbance of the samples/standard *and* A_0 is the absorbance of the control.

Antioxidant activity test by NO (nitric oxide) scavenging assay

Scavenging of NO of the plant extracts were assayed according to the Govindarajan *et al*. (2003) with slight modified method. Briefly, 1.0mL of 5mM solution of Sodium nitroprusside was mixed with 4.0mL of either extract/ascorbic acid (standard) and kept in incubation at 30^{o}C for 2 hours. Then 1.2mL of Griess reagent (Roch-Light Ltd., Suffolk, England) was mixed with 2.0ml aliquot of that mixture and absorbance taken at 550 nm.

Antioxidant activity test by Fe^{3+} reducing power

The reducing capacity can also be termed as an antioxidant activity of any compound. Reduction of Ferric Chloride ($FeCl_3$, Fine Chemicals, India) to Fe^{2+} determined by measuring the color at 700 nm (Oyaizu, 1986). Briefly, 2.0mL each of extract or ascorbic acid (standard) of various concentrations were taken in test tubes. Then 2.5mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] and 2.5 mL trichloro acetic acid (10%) were added, preceded by 10min incubation at 50^{o}C. After centrifuging the mixture 10min at 3000 rpm, 2.5 ml aliquot was withdrawn and mixed with sterile water (2.5 mL) and 0.1% ferric chloride (0.5mL) solution and finally the absorbance taken at 700 nm.

Phenolic content determination

Phenolic contents of the plant fractions were analyzed according to Yu *et al.* (2002). Dilute (10 fold) Folin–ciocalteu reagent (5 ml) was added in test tube containing 1.0mL each of extracts (200µg/mL) or standard (gallic acid, Sigma Chem.USA) and 4mL sodium carbonate solution and was incubated for 1 hour at 20^{o}C followed by absorbance taking at 765 nm.

Flavonoid content determination

Flavonoid content of the plant fractions were estimated by Kumaran *et al.*, (2007). Where, 1.0 mL of extracts (200 µg/mL) or standard (quercetin, Sigma Chem.USA) was mixed with methanol (3 mL), 10% aluminum chloride (200 µL), 1.0 M potassium acetate (200 µL) and distilled water (5.6 mL). Absorbance was taken at415 nm after 30min keeping the mixture at room temperature. Flavonoid contents of the fractions were calculated and shown as quercetin equivalents (QE) by the following formula:

$C = (c \times V)/m$

C, total flavonoid contents in mg per gm plant extract*; c*, concentration of quercetin calculated by quarcetin standard curve (mg/ml); *V,* sample solution volume (mL); *m*, sample weight (g).

Brine Shrimp lethality bioassay

Cytotoxicity of the plant extracts were determined by Brine Shrimp lethality bioassay described by Meyer *et al.* (1982). Nauplii were collected from brine shrimp eggs after hatching in simulated seawater (38 g/L). Ten nauplii are taken in vials containing 5 mL of simulated seawater treated with extracts dissolved in DMSO. The median lethal concentration, LC_{50} values of the test samples were calculated after 24 hours, and obtained by a plot of percentage of dead Shrimps verses the sample concentration (in Log scale) using Microsoft Excel. Vincristine sulphate was utilized, as a reference cytotoxic molecule (Meyer *et al.*, 1982).

Antidiarrhea activity test induced by castor oil / magnesium sulphate

Shoba and Thomas (2001) have demonstrated the method. Briefly, ten groups of mice having 5 animals in each were fasted for 24h then fed 0.5mL of castor oil, and those animals with watery stool were selected for the final experiment. Treated group mice received four different fractions of *Pistia stratiotes* at the doses of 200mg/kg and 400 mg/kg, whereas loperamide (3mg/kg) was as positive and only vehicle as negative control and all were administered orally. The entire groups of animal were fed 0.5ml each of castor oil orally to produce diarrhea after 1h and placed in an individual cage lined by blotting paper which was replaced with new one on every hour for a 4h observation of diarrheal dropping. On the other hand, in magnesium sulphate (2g/kg) was orally administered 30 min later from the drug/control treatment to produce diarrhea.

Anthelmintic activity test

Anthelmintic potential of the plant fractions was determined according to the given method of Ajaiyeoba *et al.* (2009). *Pheretima posthuma*, an anatomically similar species of roundworm was taken for this assay. Earthworms of around 4 to 8 cm in length and 0.2 to 0.4 cm in width were placed 3-in each Petri dish containing 25mL each of 10, 25 and 50mg/mL extract in distilled water containing 1% tween 80. Piperazine citrate (10 and 20mg/mL) and 1% tween 80 in distilled water, used as positive and negative control, respectively. Time for inducing paralysis was measured by visual observation with occasional shaking; finally death was confirmed by losing the complete movement with vigorous shaking and exposing to warm water of 50^{o}C temperature and pale appearance.

STATISTICAL ANALYSIS

ANOVA and Dunnet's tests were performed by SPSS 11.5 software to compare the groups. Results are given in mean ± SD, where the threshold value for the statistically significant was considered 0.05.

RESULTS

In vitro antioxidant test

Our results shows that the leaf methanolic extracts of *P. stratiotes* L have the antioxidant effect with IC_{50} values 96.84, 76.25 and 46.11 (µg/mL) for DPPH free radical scavenging, NO scavenging, and Fe^{3+} reducing method, respectively. The ethereal root extract shows the NO scavenging IC_{50} value 254.97µg/mL and Fe^{3+} reducing activity IC_{50} value 47.97µg/mL. Whereas, for the methanolic root extract IC_{50} value was 61.09 (fig. 1). Total antioxidant capacity of the different fractions of P. stratiotes L. was evaluated by the phosphomolybdenum method and was expressed as ascorbic acid equivalents (AAE) per gram of plant extract. Total antioxidant capacity of the test samples was calculated using the standard curve of ascorbic acid (*y = 0.0043x + 0.1503; R2 = 0.887*).

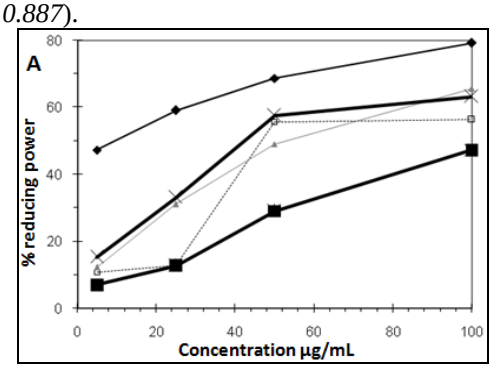

Fig. 1: Antioxidant activity of different fractions of *P. stratiotes*, PSLM (-□-), Methanolic fraction of leaf; PSLP (—■—), Pet ether fraction of leaf; PSRM (—▲—), Methanolic fraction of root; PSRP (—×—), Pet ether fraction of root; and AA (—◆—), ascorbic acid.

Quantitatively Phenolic and Flavonoid content assay

Study reveals pet ether fraction of root extract contains 34.96mg/g and 411.35mg/g phenolic and flavonoid content respectively, where as methanolic leaf extracts contains 153.93mg/g and 21.23mg/g.

DISCUSSION

In the present phytochemical and pharmacological screening of different fractions of root and leaf of *P. stratiotes* L revealed the presence of various bioactive components like flavonoids, alkaloids, glucosides and steroids (table 1). The quantitative determination of chemical constituents would give better correlation with the bioassay, unfortunately that has not done. In the course of antioxidant test by DPPH radical scavenging capacity of neither fractions of this plant extracts were shown as good efficacy as in nitric oxide (NO) scavenging assay, however methanolic leaf extracts had little improved activity (data not shown). In nitric oxide (NO) radical scavenging assay, both methanolic and pet ether fractions of leaf extracts were shown to have the NO scavenging ability close to the reference standard, ascorbic acid, in a dose dependent manner. This in vivo sodium nitroprusside generated NO scavenging ability of this plant extract to prevent highly reactive peroxynitrite ion formation is expected to take place *in vivo* as well (Joseph *et al.*, 2009). Therefore, NO scavenging activity of *P. stratiotes* L. fractions could impart a cytoprotective effect.

The reducing power displayed by all the fractions of *P. stratiotes* L. was found to rise with increasing concentrations (fig. 1A). Since it was previously mentioned the role of reductants (Duh *et al.*, 1999) in reducing ability by donating hydrogen atom to break the free radical chain (Boone., 1990). Therefore, it was speculated that the presence of reductants (i.e. antioxidants) in *P. stratiotes* L. fractions may elicit the reduction of the Fe3+/ferricyanide complex to the ferrous form.

Several reports have implicated the role of flavonoids and phenolic compounds in NO scavenging (Kim *et al.*, 1999; Crozier *et al.*, 2000; Jagetia *et al.*, 2004) and the relationship between anti-oxidative activity and total phenolic contents (Vinson *et al.*, 1998). The total flavonoid and phenolic contents determination revealed the highest amount present in the pet ether root extract, 34.96mg/g and 411.35mg/g phenolic and flavonoid content respectively. But in this study, we found to have the NO scavenging and anti-oxidative activity was better correlated with methanolic leaf extracts, which contains less flavonoid (153.93mg/g) and phenolic compounds (21.23mg/g) than the root extracts, other constituents could synergistically elicit the effect here (Khare, 2007).

Table 1: Chemical constituents present in different extracts.

Contents	PSLM	PSLP	PSRM	PSRP
Alkaloid	+	+	+	+
Glycoside	+	-	-	-
Glucoside	+	+	+	+
Carbohydrate	+	-	+	-
Tannin	-	+	+	+
Flavonoid	+	+	+	+
Steroid	+	+	-	+
Saponin	+	-	-	-

+, Present, -, Absent; PSLM, Methanolic fraction of leaf; PSLP, Pet ether fraction of leaf; PSRM, Methanolic fraction of root; and PSRP, Pet ether fraction of root of *P. stratiotes* L.

The plant extract was found to have dose dependent anti-diarrheal activity in the test animals. Leaf extracts of both methanol and pet ether are shown to be most efficacious ($p<0.05$) than other parts in both castor oil and $MgSO_4$-induced diarrheal model (tables 2 and 3). However root extracts also possess some anti-diarrheal effects too, which appears in its higher dose (400mg/kg) in those methods. Previously it was reported that anti-diarrhoeal properties of medicinal plants might attributed to tannins, alkaloids, saponins, flavonoids, sterols and reducing sugars (Longanga *et al.*, 2000) and flavonoids, may contribute to inhibit release of autacoids and prostaglandins (ricinoleic acid of castor oil induce irritation and inflammation of intestinal mucosa) (Mora *et al.*, 1990) and prevent motility.

Additionally we also found a dose dependent anthelmentic property of this plant extracts in the *in vitro* anthelmintic assay using adult earthworm (table 4). Of it's highest dose (50 mg/mL) petroleum root extract had taken almost same time (6.5 min) as with the positive control piperazine citrate (10mg/mL) but it turn around with the root methanolic extract (13.5min) which shows that the anthelmintic compound was mostly extracted in pet ether fraction. Many polyphenolic compounds including niclosamide, oxyclozanide and bithionol are implicated with anthelmintic activity by uncoupling oxidative phosphorylation of parasites (Martin, 1997). The anthelmintic activity of *P. stratiotes* L. may be ascribed by the presence of flavonoids, tannins, steroids (table 1) etc in P. stratiotes, and all are present in the root pet ether extract.

The pet ether fractions of both leaf and root extracts were

Table 2: Effects of the different fractions of *P. stratiotes* on castor oil-induced diarrhea in mice

Group	Treatment	Dose	No. of feces in 4 h	% inhibition of defecation
I	Vehicle	0.4 mL/mouse	22.8±3.148	-
II	Loperamide	3 mg/kg	5.9±1.872	73.82
III	PSLM	200 mg/kg	14.4±1.346*	36.65
IV		400 mg/kg.	10.8±2.479*	52.41
V	PSLP	200 mg/kg	13.3±2.346*	41.68
VI		400 mg/kg.	9.2±1.453*	59.54
VII	PSRM	200 mg/kg.	19.6±2.864	13.83
VIII		400 mg/kg	19.1±2.245	16.23
IX	PSRP	200 mg/kg.	18.9±3.012	16.92
X		400 mg/kg	14.88±2.429*	34.85

Table 3: Effects of the different fractions of *P. stratiotes* on MgSO4-induced diarrhea in mice

Group	Treatment	Dose	No. of feces in 4 h	% inhibition of defecation
I	Vehicle	0.4 mL/mouse	16.8±3.597	-
II	Loperamide	3 mg/kg	5.7±2.019	65.89
III	PSLM	200 mg/kg	11.3±2.347*	32.98
IV		400 mg/kg.	9.0±1.271*	46.57
V	PSLP	200 mg/kg	10.8±3.175*	35.98
VI		400 mg/kg.	8.2±1.067*	51.04
VII	PSRM	200 mg/kg.	14.2±2.457	15.45
VIII		400 mg/kg	13.8±1.917	17.74
IX	PSRP	200 mg/kg.	14.6±2.421	12.89
X		400 mg/kg	11.6±1.245*	31.07

Values are mean ±SEM, (n = 5); * $p<0.05$, Dunnet test as compared to control. Vehicle, 1% Tween 80 in water, 0.4 mL/mouse; PSLM, Methanolic fraction of leaf; PSLP, Pet ether fraction of leaf; PSRM, Methanolic fraction of root; and PSRP, Pet ether fraction of root of *P. stratiotes* L.

Table 4: Anthelmintic activity of the different fractions of *P. stratiotes* L. on the Indian earthworm

Treatment	Concentration (mg/mL)	Time taken for paralysis (min)	Time taken for death (min)
Vehicle (1% Tween 80 in Water)	-	-	-
PSLM	10	41.5±1.243	59.0±0.627
	25	13.0±0.957	36.0±1.256
	50	8.5±1.054	19.5±0.548
PSLP	10	43.5±0.692	66.5±0.873
	25	16.0±1.274	36.5±1.115
	50	8.0±1.283	17.0±0.725
PSRM	10	58.5±1.025	77.0±0.927
	25	32.0±0.628	46.5±0.974
	50	13.5±1.174	25.0±1.034
PSRP	10	39.5±0.959	61.5±1.361
	25	12.5±1.026	31.0±1.148
	50	6.5±0.869	17.5±1.017
Piperazine citrate	10	7.0±1.023	25.0±0.681
	20	4.5±0.717	16.0±0.826

shown to possess higher cytotoxicity in the Brine Shrimp Assay (Mongelli *et al.,* 2002), LC_{50} values are 121.99 and 45.80µg/mL for leaf and root, respectively. On the other hand the methanolic fractions appeared comparatively less toxic, LC50s are 424.58 and 6217.75µg/mL for leaf and root, respectively. The study displayed the cytotoxic action increasing with the increment of doses indicating the plant may have some cytotoxic principles. Several phytochemical constituents were identified in this plant parts (table 1). Stigmastane, a new steroidal cytotoxic component is reported to present in this plant part (Ayyad, 2002).

In conclusion, it appears from this preliminary study that the leaf methanolic extracts of *P. stratiotes* L have best antioxidant effect with IC_{50} values 96.84, 76.25 and 46.11 (µg/mL) for DPPH free radical scavenging, NO scavenging and Fe^{3+} reducing method, respectively. The ethereal root extracts were shown the dose dependent increase in NO scavenging (IC_{50}, 254.97µg/mL) and Fe^{3+} reducing activity (IC_{50}, 47.97µg/mL) and for the methanolic root extract IC_{50} value was 61.09 (fig. 1). Leaf extracts appeared pharmacologically effective as an anti-diarrheal and both leaf and root extracts may be effective against helminth by inducing paralysis and death. Further investigation is needed for the potential chemical constituents present in it.

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