

Estimations of *Pistia stratiotes* L. by using Chromatographic Methods

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Abstract

The leaf methanolic extract scavenged each gas NO and DPPH radical with a dose dependent manner. However the pet ether fraction of root was found to possess highest effectuality in Fe^{3+} reducing power assay. Flavonoid was found to contain highest within the pet ether fraction of root in terms of quercetin equivalent, equally highest quantity of total phenolic resin compounds (assayed as acid equivalents) were found to contain within the same fraction. The methanol fractions appeared less cytotoxic compared to pet ether extracts. The plant extracts caused a dose dependent decrease in soiled BM in each cathartic and atomic number 12 sulfate induced looseness of the bowels, wherever as leaf extracts in every solvent appeared only. Also, the plant extracts showed anthelmintic activity in oligochaete worm by causing palsy and death in an exceedingly dose dependent manner. At highest doses all fractions were nearly effective because the positive management helminthic citrate.

Conclusion: Thus, besides this cytotoxic effect its traditional claim for therapeutic use can never be overlooked.

Keywords: *Pistia stratiotes* L; Anthelmintic; Oligochaete worm; Sulfates

Introduction

P. stratiotes L. (Araceae) may be an unremarkably found aquatic plant organ herb in Asian country. As associate ayurvedic medication pistia L. (Araceae) has long been employed in this landmass, in numerous countries of continent and in China. variety of healthful property has attributed to the current plant, as well as antioxidant [1], investigation has dole out on the premise of its chemical constituents, however still thirty two several space of its therapeutic utility or untapped.

Many folkloric claims of effects of this pistia L, insisted us to figure extensively and create rational, of its uses and establish its potentiality as a drug candidate. Within the course of this screening program, medical specialty properties of methanolic and pet ether extracts of each leaf and root were investigated in numerous scientifically established model. Experiments enclosed of this plant extracts were inhibitor activity particularly DPPH radical scavenging assay, Fe^{3+} particle reducing power [2], gas scavenging assay [3-5], total synthetic resin contents [6], total flavonoid contents [7], toxicity assay by brachiopod morbidity assay [8], antidiarrhoeal activity [9], and anthelmintic activity [2] anthelmintic [2], and against Hansen's disease, eczema, piles, ulcers, and syphilis [3], plenty of pharmacological effects.

Plant Collection and Identification

The semi aquatic whole plant, *P. stratiotes* L., was collected in Gregorian calendar month 2010, from a pool placed in Jahangirnagar University, Savar, Dhaka, once correct identification has done by the National Herbarium, Asian country (accession range 35621).

Extract Preparation

The whole plant was completely washed into water, sun dried for daily then the leaves and roots are separated. Once the separation had been completed, the leaves and roots were unfolding in skinny layers in trays and at last placed into an appliance (at 55°C) [10]. The coarse powder of the dried plant components of *P. stratiotes* L. were extracted one by one with the solvent pet ether and wood alcohol during a soxhlet equipment mistreatment 100g powder for every half. Once the powders became exhausted of its chemical constituents as evident from cycles of colorless liquid siphoning within the soxhlet equipment, extraction was thought-about to be complete. Once completion of the extraction, the liquid was filtered by a sterile cotton plug. Then solvent was gaseous mistreatment water tub and therefore the final sediment was used for the experiments. Customary procedures were followed for the phytochemical screening [10].

Assortment and Maintainance of Animals

In order to research the antidiarrhoeal, sedative effects of this plant extracts, three to four weeks aged Swiss anomaly mice of each sex, of around twenty to twenty five g weight, were collected from the International Center for diarrhoeal malady and analysis, Asian nation (ICDDR,B). Animals were caged in teams of five, in cages of (28×22×13 cm) dimension, with dry wadding as flooring. Temperature was maintained 25°C with fifty five to sixty fifth ratio and twelve hrs light/12 hrs dark for the complete study time. Food pellets were provided from ICDDR,B with H_2O and libitum.

Inhibitor Activity takes a look at by DPPH Radical Scavenging Assay

Antioxidant activities by one, 1-diphenyl, 2-picrylhydrazyl (DPPH, alphabetic character USA) of plant elements were determined following the strategy of Oyaizu [4]. In brief, three milliliter

methanolic resolution containing 0.004% DPPH was further in {an exceedingly in a very} tube with an aliquot of a hundred μL of either extract or standard(ascorbic acid), and unbroken in dark for 30min for the reaction to require place, then absorbance taken at 517 nm against a blank (methanol). % of activity make up my mind by - the concerns Scavenging = X a hundred A1 is that the absorbance of the samples/standard and A0 is that the absorbance of the management.

Inhibitor Activity take a look at by NO (nitric oxide)

Scavenging Assay

Scavenging of NO of the plant extracts were assayed in line with the Govindarajan [5] with slight changed methodology. Briefly, 1.0 milliliter of 5mM resolution of Na nitroprusside was mixed with four.0 milliliter of extract/ascorbic acid (standard) and unbroken in incubation at thirty 0C for two hours. Then 1.2 milliliter of Griess chemical agent (Roch-light Ltd., Suffolk, England) was mixed with two.0 milliliter aliquot of that mixture and absorbance taken at 550 nm. This was explained in Figure 1.

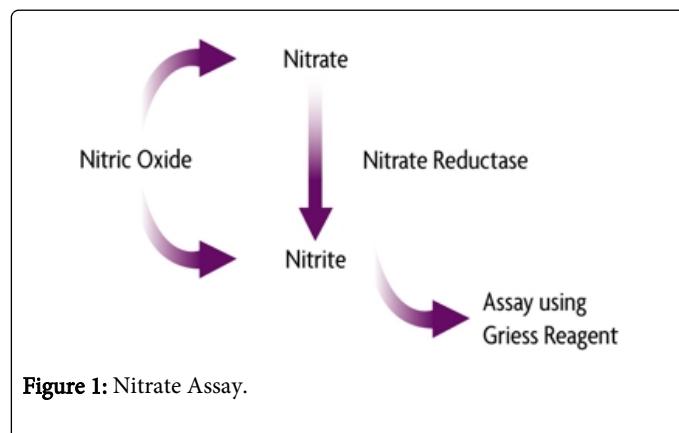


Figure 1: Nitrate Assay.

Inhibitor Activity take a look at by Fe^{3+} Reducing Power

The reducing capability may be termed as associate degree inhibitor activity of any compound. Reduction of metal Chloride (FeCl_3 , Fine Chemicals, India) to Fe^{2+} determined by measure the colour at 700 nm [4]. Briefly, 2.0 milliliter every of extract or antioxidant (standard) of assorted concentration were taken in take a look at tubes. Then 2.5 milliliter of one hundred and twenty fifth K salt [$\text{K}_3\text{Fe}(\text{CN})_6$] and a couple of 0.5 milliliter trichloro carboxylic acid (10%) were further, preceded by 10min incubation at 50°C. when centrifuging the mixture 10min at 3000 rate, 2.5 milliliter aliquot was withdrawn and mixed with sterile water (2.5 mL) and 0.1% metal chloride eighty nine (0.5 mL) resolution and at last the absorbance taken at 700 nm.

Phenoplast Content Determination

Phenolic contents of the plant fractions were analyzed in line with Yu [6]. Dilute (10 fold) Folin–ciocalteu chemical agent (5 ml) was further in tube containing 1.0 milliliter every of extracts (200 $\mu\text{g/mL}$) or customary (gallic acid, Sigma Chem. USA) and 4mL washing soda resolution and was incubated for one hour at 20°C followed by absorbance taking at 765 nm.

Flavonoid Content Determination

Flavonoid content of the plant fractions were calculable by Kumaran [7]. Where, 1.0 milliliter of extracts (200 $\mu\text{g/mL}$) or customary (quercetin, Sigma Chem.USA) was mixed with wood alcohol (3 mL), 100% chloride (200 μL), 1.0 M K acetate (200 μL) and water (5.6 mL). Absorbance was taken at 415 nm when 30min keeping the mixture at temperature. Flavonoid contents of the fractions were calculated and shown as quercetin equivalents (QE) by the subsequent formula:

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

total flavonoid contents in mg per weight unit plant extract, c, concentration of quercetin calculated by quercetin customary curve (mg/ml), V, sample resolution volume (mL), m, sample weight (g).

This was detailed in chromatographic spectrum of *Pistia Stratois* in below figure:

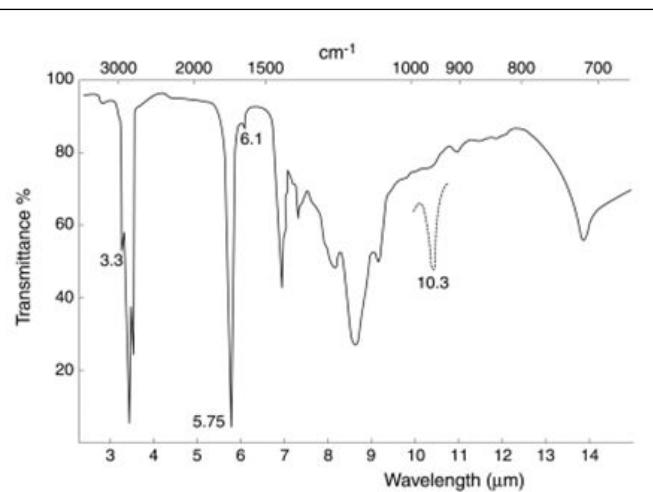


Figure 2: Brand spectrum of *Pistia Stratois*.

Artemia Salina Deadliness Bioassay

Cytotoxicity of the plant extracts were determined by Artemia salina deadliness bioassay delineated by Meyer) [8]. Nauplii were collected from Artemia salina eggs when hatching in simulated water (38 g/L). 10 nauplii area unit taken in vials containing five milliliter of simulated water treated with extracts dissolved in DMSO. The median fatal concentration, LC50 values of the take a look at samples were calculated when twenty four hours, and obtained by a plot of proportion of dead Shrimps versus the sample concentration (in Log scale) victimization Microsoft surpass. Periwinkle plant derivative sulfate was utilized, as a reference cytotoxic molecule [8].

Antidiarrhea Activity take a look at Induced by Physic / Atomic Number Sulfate

Shoba and Thomas [9] have incontestable the strategy. Briefly, 10 teams of mice having five animals in everywhere fasted for twenty-four h then fed 0.5 milliliter of physic, and people animal with watery stool were selected for the ultimate experiment. Treated cluster mice received four completely different fractions of pistia at the doses of two hundred mg/kg and four hundred mg/kg, whereas loperamide (3 mg/

kg) was as positive and solely vehicle as negative management and every one were administered orally. the complete teams of animal were fed 0.5 milliliter every of physic orally to provide looseness of the bowels when 1h and placed in a personal cage 119 lined by paper that was replaced with new one on each hour for a 4h observation of diarrhoeatic dropping. On the opposite hand, in atomic number 12 sulfate (2 g/kg) was orally administered thirty min later from the drug/ control treatment to provide looseness of the bowels.

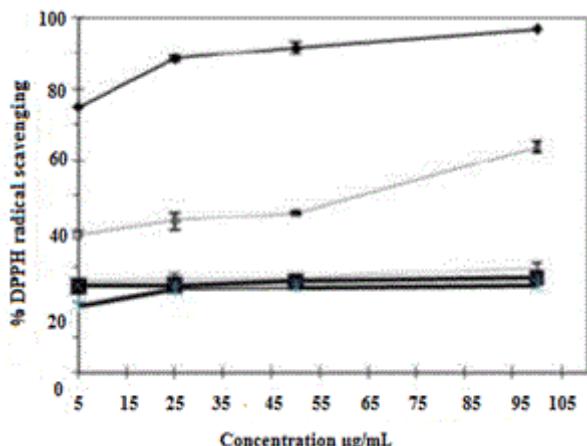


Figure 3: Inhibitor activity of various fractions of *P. stratiotes*.

the cell in vivo [11]. Therefore, NO scavenging activity of *P. stratiotes* L. fractions may impart a cytoprotective impact.

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

Table 1: Extracts containing different chemical constituents. (+, Present, -, Absent, PSLM, Methanolic fraction of leaf, PSLP, Pet ether fraction of leaf, PSRM, Methanolic fraction of root, and PSRP, Pet ether fraction of the root in *P. stratiotes* L.)

The reducing power displayed by all the fractions of *P. stratiotes* L. was found to rise with increasing concentrations (Figure 1). Since it absolutely was antecedently mentioned the role of reductants [12], in reducing ability by donating atom to interrupt the radical chain [13]. Therefore, it absolutely was speculated that the presence of reductants (i.e. antioxidants) in *P. stratiotes* L. fractions could elicit the reduction of the Fe^{3+} /ferricyanide complicated to the metallic element kind.

Several reports have concerned the role of flavonoids and phenolic resin compounds in NO scavenging [14-16] and therefore the relationship between antioxidative activity and total phenolic resin contents [17]. The total flavonoid and phenolic resin contents determination discovered the very best quantity gift within the pet ether root extract, 34.96 mg/g and 411.35 mg/g phenolic resin and flavonoid content severally. however during this study, we tend to found to possess the NO scavenging and antioxidative activity was higher correlative with methanolic leaf extracts, that contains less flavonoid (153.93 mg/g) and phenolic resin compounds (21.23 mg/g) than the foundation extracts, different constituents may synergistically elicit the impact here [18].

The plant extract was found to possess dose dependent antidiarrheal drug activity within the take a look at animals. Leaf extracts of each wood spirit and pet ether square measure shown to be most efficacious ($P < 0.05$) than different components in each physic and MgSO_4 induced diarrhetic model (Tables 2 and 3). But root extracts additionally possess some anti diarrhetic effects too, that seems in its higher dose (400 mg/kg) in those ways. antecedently it absolutely was reportable that antidiarrhoeal properties of healthful plants may attributed with alkaloids, tannins, saponins, flavonoids, sterols and reducing sugars [19] and flavonoids, could contribute to inhibit unharness of autacoids and prostaglandins (ricinoleic acid of physic induce irritation and inflammation of enteric mucosa) [20], and stop motility.

Values square measure mean \pm SEM, ($n = 5$), * $P < 0.05$, Dunnet take a look at as 183 compared to manage. Vehicle, one hundred and twenty fifth Tween eighty in water, 0.4 mL/mouse, PSLM, Methanolic fraction of leaf, PSLP, Pet ether fraction of leaf, PSRM, Methanolic

Anthelmintic Activity

Anthelmintic potential of the plant fractions make up my mind in line with the given methodology of Ajaiyeoba [2]. *Pheretima posthuma*, associate degree anatomically similar species of roundworm was taken for this assay. Earthworms of spherical four to eight cm long and 0.2 to 0.4 cm broad were placed 3-in every dish containing 25mL every often, twenty five and fifty mg/mL extract in water containing one hundred and twenty fifth tween eighty. anthelmintic turn (10 and twenty mg/mL) and one hundred and twenty fifth tween eighty in water, used as positive and negative management, severally. Time for causation dysfunction was measured by visual observation with occasional shaking, finally death was confirmed by losing the whole movement with vigorous shaking and exposing to heat water of five hundred C temperature and pale look.

Discussions

In the gift phytochemical and pharmacologic screening of various fractions of root and leaf of *P. stratiotes* L discovered the presence of assorted bioactive elements like flavonoids, alkaloids, glucosides and steroids (Table 1), that square measure correlative with some observations of others. The take a look at for inhibitor by DPPH radical scavenging capability of neither fractions of this plant extracts were shown pretty much as good effectivity as in gas (NO) scavenging assay, but methanolic leaf extracts had very little improved activity (Figure 1). In gas (NO) radical scavenging assay, each methanolic and pet ether fractions of leaf extracts were shown to possess the NO scavenging ability near the reference customary, antioxidant, in an exceedingly dose dependent manner. This *invivo* scavenging of NO, generated from atomic number 11 nitroprusside to additional forestall peroxynitrite particle formation is anticipated to require place within

fractions of the root, and PSRP, Pet ether fraction of the root in *P. stratiotes* L.

Group	Treatment	Dose	No. of feces in 4 h	% inhibition defecation
I	Vehicle	0.4 mUmouse	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
		400 mg/kg	10.8 ± 2.479'	52.41
V	PSLP	200 mg/kg	13.3 ± 2.346'	41.68
		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 2: Effects of the different fractions on castor oil-induced diarrhea in mice.

Group	Treatment	Dose	No. of feces in 4 h	% inhibition of defecation
I	Vehicle	OA mUmouse	16.833.597	
II	Loperamide	3 mg/kg	5.732.019	65.89
III	PSLM	200 mg/kg	11.322.347'	32.98
		400 mg/kg	9.031.271'	46.57
V	PSLP	200 mg/kg	10.833.175'	35.98
		400 mg/kg	8.2 ± 1.067*	51.04
VII	PSRM	200 mg/kg	14232.457	15.45
		400 mg/kg	13.831.917	17.74
IX	PSRP	200 mg/kg	14.632.421	12.89
		400 mg/kg	11.631.245'	31.07

Table 3: Effects of the various fractions of *P. stratiotes* on MgSO₄-induced symptom in mice. Values square measure mean ±SEM, (n = 5), *P < 0.05, Dunnet take a look at as compared to manage.

Vehicle, one hundred and twenty fifth Tween eighty 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 the root in *P. stratiotes* L. to boot we tend to additionally found a dose dependent anthelmintic property of this plant extracts within the in vitro anthelmintic assay victimization adult earthworm (Table 4). Of its highest dose (50 mg/mL) oil root extract had taken virtually same time (6.5 min) like the positive management anthelmintic citrate (10 mg/mL) however it circle with the foundation methanolic extract (13.5 min). Shows that the anthelmintic

compound was largely extracted in pet ether fraction. Several polyphenolic compounds as well as niclosamide, oxyclozanide and bithionol square measure concerned with anthelmintic activity by uncoupling 196 biological process of parasites [21]. The anthelmintic activity of *P. stratiotes* L. could also be ascribed by the presence of flavonoids, tannins, steroids (Table 1) etc in *P. stratiotes*, and every one square measure gift within the root pet ether extract.

The pet ether fractions of each leaf and root extracts were shown to possess higher toxicity within the *Artemia salina* Assay [22], LC50 values square measure 121.99 and 45.80 µg/mL for leaf and root, severally. On the opposite hand the methanolic fractions appeared relatively less toxicant, LC50s square measure 424.58 and 6217.75 µg/mL for leaf and root, severally. The study displayed the cytotoxic action increasing with the increment of doses indicating the plant could have some cytotoxic principles. many phytochemical constituents were known during this plant components (Table 1). Stigmastane, a brand new hormone cytotoxic part is reportable to gift during this plant structure [23].

Treatment	Concentration (mg/mL)	Time taken for	
		paralysis(min)	death(min)
Vehicle (1% Tween 80 in Water)			
PSLM	10	41.511.243	59.010.627
	25	13.010.957	36.0t 1.256
	50	8.511.054	19.510.548
PSLP	10	43.510.692	66.510.873
	25	16.0t 1.274	36.511.115
	50	8.0t 1.283	17.010.725
PSRM	10	58.5t 1.025	77.010.927
	25	32.010.628	46.510.974
	50	13.511.174	25.011.034
PSRP	10	39.510.959	61.511.361
	25	12.511.026	31.0t1.148
	50	6.510.869	17.511.017
Piperazine citrate	10	7.0t 1.023	25.010.681
	20	4.510.717	16.0 ± 0.826

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

Conclusion

In conclusion, it seems from this preliminary study that the leaf methanolic extracts of *P. stratiotes* L have best inhibitor impact with IC50 values ninety six.84, 76.25, and 46.11(µg/mL) for DPPH radical scavenging, NO scavenging, and Fe³⁺ reducing technique, severally. The ethereal root extracts were shown the dose dependent increase in NO scavenging (IC50, 254.97 µg/mL) and Fe³⁺ reducing activity (IC50, 47.97 µg/mL) and for the methanolic root extract IC50 (Figure 1). Leaf extracts appeared pharmacologically effective as an anti-diarrheal, and each leaf and root extracts could also be effective against

worm by induction palsy and death. additional investigation is required for the potential chemical constituents gift in it.

Competing Interests

Authors have declared that no competitive interests exist.

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