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## Evaluation of Antinociceptive and Antidiarrhoeal Properties of *Pistia stratiotes* (Araceae) Leaves

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### ABSTRACT

*Pistia stratiotes* (Araceae) is a Bangladeshi medicinal plant used traditionally to treat a various diseases. The present study was designed to investigate of antinociceptive and antidiarrhoeal activity of methanolic extract of the leaves of *Pistia stratiotes*. The extract produced significant writhing inhibition in acetic acid-induced writhing in mice at the oral dose of 250 and 500 mg kg<sup>-1</sup> body weight (p<0.001) comparable to the standard drug diclofenac sodium at the dose of 25 mg kg<sup>-1</sup> of body weight. The extract showed antidiarrhoeal activity on castor oil induced diarrhoea in mice, it increased mean latent period and decreased the frequency of defecation significantly at the oral dose of 250 and 500 mg kg<sup>-1</sup> body weight (p<0.01) comparable to the standard drug Loperamide at the dose of 50 mg kg<sup>-1</sup> of body weight. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

**Key words:** Antinociceptive, antidiarrhoeal, Araceae, *Pistia stratiotes*, phytochemical analysis

### INTRODUCTION

*Pistia stratiotes* (Araceae) is commonly known as water cabbage or water lettuce. It floats on the surface of the water and its roots hanging submersed beneath floating leaves. The leaves can be up to 14 cm long and have no stem. Plant parts of Araceae (usually above ground parts) have higher macroelement (nitrogen, phosphorus and potassium) contents (Kilinc *et al.*, 2005). *Pistia stratiotes* has important ecological role to characterize water quality (Ghavzan *et al.*, 2006; Bamidele *et al.*, 2008). The plant is distributed through out the tropical and sub-tropical regions of the world. It is also found in China, Indo-China, Malaya, La Reunion and Brazil. Water lettuce can be possibly used to produce Se-enriched plant for animal nutrition (Chantiratikul *et al.*, 2008). For the healing of ringworm infection of the scalp, syphilitic eruptions, skin infections, boils and wounds, *Pistia stratiotes* leaves are used (Ali *et al.*, 2011; Premkumar and Shyamsundar, 2005). Moreover, the oil extract of *Pistia stratiotes* is used for the treatment of worm infestations, tuberculosis, asthma and dysentery and applied externally to treat skin diseases, inflammation, piles, ulcer and burns (Kirtikar and Basu, 2000). The plant is used as an anodyne for eye-wash in Gambia. The plant's juice is used by Mundas (tribal people in India) in ear complains (Kirtikar and Basu, 1994; Anonymous, 1998). Anthelmintic (Kumar *et al.*, 2010), antidermatophytic and antifungal (Premkumar and Shyamsundar, 2005), diuretic (Pallavi *et al.*, 2011), antiprotease (Jedinak *et al.*, 2010), antitubercular and emollient (Tripathi *et al.*, 2010), antidiabetic (Joy *et al.*, 2001) and antimicrobial properties (Abu Ziada *et al.*, 2008) has been found. A novel stigmastane,

11 $\alpha$ -hydroxy-24S-ethyl-5 $\alpha$ -cholest-22-en-3,6-dione, has been isolated from the aquatic plant *Pistia stratiotes* (Monaco and Previtara, 1991). Plant contains <sup>14</sup>C] Oxalic acid, -[1-<sup>14</sup>C] ascorbic acid, -[6-<sup>14</sup>C] ascorbic acid, -[1-<sup>14</sup>C] erythorbic acid, -[1-<sup>14</sup>C] galactose, or [1-<sup>14</sup>C] glycolate. Specific radioactivities of -Ascorbic acid (AsA), free Oxalic acid (OxA) and Calcium oxalate (CaOx) (Keates *et al.*, 2000). In the present investigation, we evaluated the antinociceptive and antidiarrhoeal activity of methanolic leaf extract of *Pistia stratiotes* (Araceae) in animal model.

## MATERIALS AND METHODS

**Plant material collection and extraction:** The leaves of *Pistia stratiotes* (Araceae) were collected from the Khulna, Bangladesh in December 2009 and were taxonomically identified by experts at the Bangladesh National Herbarium (accession number: 48550). About 400 g of powdered leaves were taken in a clean, flat-bottomed glass container and soaked in 1, 300 mL of 80% methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of cotton followed by a filtration through Whatmann filter paper (Grade 1) and the filtrate thus obtained was concentrated using a rotary evaporator (Bibby RE200, Sterilin Ltd., U.K.) to get the methanolic extract.

**Drugs:** Diclofenac sodium (Opsonin Chemical Industries Ltd., Bangladesh), Loperamide (Square Pharmaceuticals Ltd., Bangladesh).

**Animals:** Young Swiss albino mice (20-30 g) of either sex were obtained from the animal house of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). Ten animals per group were used for the study. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature 23 $\pm$ 2 $^{\circ}$ C and 12 h light/dark cycle). The animals were fed with standard diet and water *ad libitum*. The University Animal Research Ethical Committee approved the experimental protocol.

**Preliminary phytochemical analysis:** The crude extracts were subjected to preliminary phytochemical screening for the detection of major chemical groups. In each test 10% (w/v) solution of the extract in methanol was used unless otherwise mentioned in individual test (Evans, 1989; Ghani, 1998).

## Pharmacological studies

**Antinociceptive activity:** The antinociceptive activity of the extracts was determined following the method described by Ahmed *et al.* (2004) and Whittle (1964).

## Antidiarrhoeal activity

**Castor oil-induced diarrhoea:** The antinociceptive activity of the extracts was determined following the method described by Chatterjee (1993).

**Statistical analysis:** Student's t-test was used to determine a significant difference between the control group and experimental groups. The statistical analysis of data was done by using the SPSS software. p<0.01 was considered as significant.

## RESULTS

**Preliminary phytochemical analysis:** Results of different chemical tests on the methanolic extract of *Pistia stratiotes* (Araceae) showed the presence of alkaloids, glycosides, flavonoids, saponins and tannins (Table 1).

**Antinociceptive activity:** Table 2 showed the effect of the methanol extract of *Pistia stratiotes* (Araceae) on acetic acid-induced writhing in mice. At dose of 250 and 500 mg kg<sup>-1</sup> of body weight, the extract produced about 35.80% and 47.94% writhing inhibition in test animals, respectively. The results were statistically significant (p<0.001) and were comparable to the standard drug diclofenac sodium which showed about 60% writhing inhibition at the dose of 25 mg kg<sup>-1</sup> (p<0.001).

**Antidiarrhoeal activity:** Antidiarrhoeal activity of the methanolic extract of *Pistia stratiotes* (Araceae) was tested by castor oil-induced diarrhoea in mice. Diarrhoeal initiation time and the number of stools excreted by the animals in 4 h were collected. The extract caused an increase in latent period (0.7 h) and (0.9 h) i.e., delayed the onset of diarrhoeal episode of 250 and 500 mg kg<sup>-1</sup> body of weight significantly (p<0.01) which was comparable to the standard drug loperamide at the dose of 50 mg kg<sup>-1</sup> body weight in which the resulted value was 1.5 h (p<0.01) (Table 3). The selected concentration of the extract also showed a good diarrheal inhibition with 27.78% and 41.67%. Loperamide, standard antidiarrhoeal agent showed an inhibition of 72.22%. The latent period for the initiation of stool excretion was noted. This was 0.75 and 0.94 h consecutively which is 0.75 and 0.55 h earlier than loperamide treated mice but 0.1 h and 0.34 h delayed than experimental control mice.

Table 1: Phytochemical properties of *Pistia stratiotes* (Araceae) methanolic extract

Compound	Alkaloids	Glycosides	Steroids	Gums	Flavonoids	Saponins	Reducing sugars	Tannins
Observation	+ ve	+ ve	- ve	- ve	+ ve	+ ve	- ve	+ ve

(-): Absence, (+): Presence

Table 2: Effects of *Pistia stratiotes* (Araceae) methanolic extract on writhing effect on acetic acid induced mice

Treatment	Dose (kg <sup>-1</sup> )	Mean writhing	% Inhibition	SD	p-value (One way anova)*
Experimental control (1% Tween 80)	10 mL	34.0±1.33	-	2.97	p<0.001
Positive control (Diclofenac sodium)	25 mg	13.6±1.22	60.00	2.73	p<0.001
Test sample	250 mg	21.8±1.00	35.80	2.23	p<0.001
Test sample	500 mg	18.8±1.21	47.94	2.71	p<0.001

\*- (Vassar stats); Test sample- *Pistia stratiotes* (Araceae). Crude extract. 30 min after treatment, 0.7% acetic acid was injected i.p. 10 min after injection writhing responses was recorded for 10 min. (n = 5)

Table 3: Effects of *Pistia stratiotes* (Araceae) methanolic extract on inhibition of castor oil induced diarrhoea

Treatment	Dose (kg <sup>-1</sup> )	Latent period (h)	Mean number of stools*1	% Inhibition	SD	p-value (One way anova)*
Experimental control (1% Tween 80)	10 mL	0.684±0.19	3.6	-	0.91	
Positive control (Loperamide)	50 mg	1.522±0.57	1.0	72.22	0.67	p<0.01
Test sample	250 mg	0.758±0.07	2.6	27.78	0.75	p<0.01
Test sample	500 mg	0.944±0.08	2.1	41.67	0.62	p<0.01

\*- (VassarStats); Test sample- *Pistia stratiotes* (Araceae) methanolic extract. 40 min after treatment, 0.3 mL castor oil was administered orally. Latent period of castor oil induced diarrhea was noted. Number of stools excreted for the next 4 h were noted. \*1 - Mean number of stools was an average number of stools for 4 h for each treatment. % Inhibition, SD and p value was also calculated with respect to the number of stools (n = 5)

## DISCUSSION

Among several traditional claims, the usefulness of *Pistia stratiotes* in dysentery (Kirtikar and Basu, 2000) and applied externally to treat skin diseases (Premkumar and Shyamsundar, 2005), inflammation, cytotoxicity (Ali *et al.*, 2011), burns have been emphasized more in literature. Hence, it was considered that investigations for these medicinal properties might give scientific authentication to the traditional claims. Moreover, this plant has not been subjected to above-mentioned systemic pharmacological screening so far.

Phytochemical screening of methanolic extract of *Pistia stratiotes* (Araceae) showed the presence of alkaloids, glycosides, flavonoids, saponins and tannins (Table 1). Antinociceptive effects of flavonoids were also reported by Meotti *et al.* (2006) and Asmawi *et al.* (2011). Preliminary studies have demonstrated that various flavonoids possessed significant antinociception *in vivo* (Calixto *et al.*, 2000).

Antinociceptive activity of the methanol extract of *Pistia stratiotes* (Araceae) was tested by acetic acid-induced writhing model in mice. At dose of 250 and 500 mg kg<sup>-1</sup> of body weight, the extract produced about 35.80 and 47.94% writhing inhibition in test animals, respectively. The results were statistically significant (p<0.001). Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid which is used to induce writhing, causes algnesia by liberation of endogenous substances which in turn excite the pain nerve endings (Taesotikul *et al.*, 2003). Increased levels of PGE2 and PGF2 $\alpha$  in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid (Deraedt *et al.*, 1980). The extract produced significant writhing inhibition comparable to the standard drug diclofenac sodium (Table 2). Based on this result it can be concluded that the methanolic extract of *Pistia stratiotes* (Araceae) might possess antinociceptive activity.

At doses of 250 and 500 mg kg<sup>-1</sup>, the methanol extract of *Pistia stratiotes* (Araceae) showed significant antidiarrhoeal activity against castor oil-induced diarrhoea as compared with the control group. It significantly (p<0.01) reduced the frequency of diarrhoea and an increase in latent period (0.75 h) and (0.94 h). Previous studies have suggested that the anti-diarrhoeal activity of medicinal plants may be due to flavonoids (Galvez *et al.*, 1991, 1993; Di-Carlo *et al.*, 1993), alkaloids (Shoba and Thomas, 2001), tannins (Mukherjee *et al.*, 1998), saponins, sterols and reducing sugars (Otshudi *et al.*, 2000). Antidiarrhoeal activity of the extract of *Pistia stratiotes* (Araceae) was tested by using the model of castor oil-induced diarrhoea in mice. Castor oil mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglycerides upon oral administration. Most of the ricinolic acid remains in the intestine and produces its absorptive or secretory effect. The ricinolic acid thus liberated readily forms of ricinoleate salts with sodium and potassium in the lumen of the intestine. The salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface. Generally ricinoleate salts stimulates the intestinal epithelial cells adenylyl cyclise (Racusen and Binder, 1979) or released prostaglandin (Beubler and Juan, 1979). The extract caused and increased in latent period and decreased the frequency of defecation as well as the number of total stool count. On the basis of this result it can be concluded that the ethanol extract of *P. stratiotes* possesses antidiarrhoeal activity.

## CONCLUSION

It could be concluded that the methanol extract of *Pistia stratiotes* (Araceae) leaves possesses antinociceptive and antidiarrhoeal activities. These facts indicate the scientific basis of *Pistia stratiotes* (Araceae) being used as a traditional medicine. However, further experiments may help to determine the pharmaceutical potentialities of the plant as a medicine.

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