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ORIGINAL ARTICLE

An in vivo study of the pharmacological activities of a methanolic acetate fraction of *Pistia stratiotes* L.: An ethno-medicinal plant used in Bangladesh

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Abstract

Background: The present study covered an assessment of methanolic acetate fractions of *Pistia stratiotes*, commonly known as water lettuce, for analgesic, anti-inflammatory, and CNS depressant activities.

Methods: The methanol acetate fractions were extracted from *Pistia stratiotes* and administered to the experimental animals as 200 and 400 mg/kg doses to determine the effect on acetic acid-induced writhing and formalin-induced licking and biting, for the assessment of analgesic activity. The anti-inflammatory assay was done using the carrageenan-induced hind paw edema method, while assessment of CNS depressant activity was done using the open field and hole cross tests. The effects were compared to standard reference drugs.

Results: At both doses (200 and 400 mg/kg body weight), the methanolic acetate extract of *P. stratiotes* showed significant analgesic action ($P < 0.05$) against acetic acid-induced writhing. The extract was also found to give significant protection against licking and biting at both doses. The methanolic acetate extract of *P. stratiotes* showed a significant ($P < 0.05$) anti-inflammatory effect from 0 minutes up to 3 hours in the carrageenan-induced paw edema test. In the CNS depressant assay, the methanolic acetate extract showed significant ($P < 0.05$) depressant activity at both doses from 30 to 120 minutes in both the hole cross and open field tests.

Conclusion: Thus we can conclude that *P. stratiotes* extracts have significant analgesic, anti-inflammatory, and CNS depressant activity, compared to standard compounds, in an animal model.

KEYWORDS
acetic acid, analgesic, anti-inflammatory, CNS-depressant, formalin, *P. stratiotes*

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1 | INTRODUCTION

Plants have been used in conventional medicine all over the world for thousands of years and they continue to provide new medications today.[1] Several current medications used in a number of chronic and severe conditions have been derived from plants and were discovered by the indigenous populations in the course of normal use of the plants. Many such conventional medicines are used in developed and developing countries for primary health care because of their wide biological and medicinal activities, higher safety margins, and low prices.[2] Conversely, it is incontestable that allopathic medicines of all types are liable to exhibit untoward side effects. Thus, in recent times, for many disease conditions, allopathic medicine has replaced by medicines from natural sources that show considerable therapeutic potential.[3] Natural compounds have long been employed in topical medication to treat inflammation, and for pain relief and CNS system-related disorders, as well as for developing new medicines. Plants commonly provide the raw materials for the treatment of many kinds of life-threatening disorder.[4]

Pistia stratiotes (Family: Araceae), commonly known as water cabbage or water lettuce, is a free-floating hydrophone of river, lakes and ponds within the tropical and subtropical regions of Asia, Africa, and America.[5] This plant contains a broad range of constituents such as steroids, sterols, terpenoids, flavone glycosides, lipids, carbohydrates, proteins, vitamins A, B, and C, etc. Historically their leaves have been used for numerous therapeutic purposes, for example as an antiseptic and in the treatment of tuberculosis and chronic dysentery. The ashes of the plant were used for the treatment of ringworm of the scalp. The leaves were employed to treat skin problems such as leprosy, ulcer, piles, and pox, and also have anthelmintic activity.[6-8]

A literature survey shows that while many chemical and biological studies have been carried out on *P. stratiotes*, there has been no specific study of their analgesic, anti-inflammatory, and CNS depressant activities. Thus, the current study was undertaken to gauge the analgesic, anti-inflammatory, and CNS depressant activities of the methanolic acetate fraction of *P. stratiotes* in experimental animal models.

The condition of analgesia is mainly characterized by an unpleasant sensation, sometimes associated with external and internal pernicious stimulation, and analgesics are a class of medicines that block pain signals at the central nervous system.[9] They are divided into two subclasses, opioid analgesics and non-opioid analgesics.[9] Inflammation is an important response of the intrinsic physiological system, acting as protective mechanism against pathogens and initiating a specific train of reactions. Hence, inflammation may be related to pain in some way.[10] CNS depression refers to physiological depression of the central nervous system. Most CNS depressants activate a neurochemical known as gamma-aminobutyric acid (GABA) that helps to decrease brain activity. Neurotransmitters are substances within the brain that carry signals from one somatic cell to another cell, and this property makes them helpful for treating anxiety and sleep disorders. CNS depression is specifically the result of suppressed brain activity.[11]

2 | MATERIALS AND METHODS

2.1 | Chemicals

Indomethacin, ibuprofen, and diazepam were purchased from Square Pharmaceuticals Company Ltd, Bangladesh. Acetic acid was purchased from Merck, Germany. Normal saline water (0.9% NaCl), a product of Beximco Pharmaceuticals Company Ltd, Bangladesh, was purchased from the local market. Other chemicals of analytical grade were supplied by the Department of Pharmacy, Atish Dipankar University of Science and Technology.

2.2 | Collection of plant material

The *Pistia stratiotes* plants were collected from the Mirpur area, Dhaka and identified by a botanist at the Bangladesh National Herbarium, Dhaka. The whole plant was sun-dried (normal daylight) for a week and ground into a powder using an appropriate grinder. The powder was kept in an airtight container in a cool, dark, dry place until use for analysis.

2.3 | Preparation of extract of *P. Sratiotes*

For the preparation of the plant extract, 700 g of a fine-grained powder of *P. sratiotes* was dissolved in a sufficient amount of methanol. After 12 days, the entire mixture was subjected to an initial filtration through a layer of clean white cotton material. Then it was filtered through Whitman filter paper. After a standard drying procedure, the extracted sample consisted of a gluey black concentrate. This methanolic extract was then subjected to fractionated column chromatography to isolate the methanolic acetate fraction of *P. stratiotes*.

2.4 | Experimental animals

For the analysis of the analgesic, anti-inflammatory and central nervous system depressant activities of the methanolic acetate fraction, Swiss albino mice weighing 25-35 g were used. The mice were purchased from the Animal Analysis Branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDRB). The animals were housed under normal laboratory conditions (relative humidity: 55%-65%, temperature: 23 ± 2°C, 12-h dark-light cycle).[12] Experimental animals were fed with a standard rodent diet and safe drinking water; the rules of the Animal Experimentation committee of ICDDRB were followed. The mice were divided into four groups, with five mice in each group.

2.5 | Analgesic activity

2.5.1 | Acetic acid-induced writhing method

The analgesic activity of the samples was studied in the mouse model using the acetic acid-induced writhing method.[13] Test samples

(200 and 400 mg/kg body weight), vehicle (1% Tween 80; control) or indomethacin (10 mg/kg; standard) were administered orally 30 minutes before the intraperitoneal administration of 1% carboxylic acid. Then the mice were observed for specific contractions of the body termed 'writhing' for the succeeding 20 minutes. Continuous wriggling was not usually seen. Typically, the animals initiated wriggling, but failed to continue. The number of 'writhes' in every treated group was compared to the activity produced with standard indomethacin (10 mg/kg) (positive control).

The percentage inhibition (% analgesic activity) was calculated using the equation $(A-B)/A \times 100$, where A = average number of 'writhes' in the control group, and B = average number of "writhes" in the test group.

2.5.2 | Formalin-induced licking and biting

The antinociceptive activity of the methanolic acetate fraction of *P. stratiotes* was determined using the formalin test, as described by Sharma et al.[14] The control group received 2.5% formalin. A 20 μL dose of 2.5% formalin was injected into the dorsal surface of the right hind paw 30 minutes after the administration of the methanolic acetate fraction of *P. stratiotes* (200 and 400 mg/kg) or indomethacin (10 mg/kg). The mice were observed for 30 minutes after the injection of formalin, and the licking of the injected hind paw was recorded. The first 5 minutes after the formalin injection was referred to as the 'early phase' and the period between 15 and 30 minutes as the "late phase." The total time spent of licking and biting the injured paw (pain behaviour) was measured with a stopwatch.

2.6 | Anti-inflammatory activity

2.6.1 | Carrageenan-induced paw edema method

The mice were divided into four groups, each containing five mice. Acute inflammation was induced by injecting 0.1 ml of (1%) carrageenan into the plantar surface of one of the hind paws.[15] The extract (200 and 400 mg/kg), normal saline (1 ml/kg), or ibuprofen (10 mg/kg) were administered 30 minutes before the carrageenan injection. The paw volume was measured at 0, 1, 2, and 3 hours using a Vernier caliper to determine the diameter of edema. The difference between the reading at 1 hour and subsequent readings at different time intervals was taken as the thickness of edema.

2.7 | CNS depressant activity

2.7.1 | Hole cross test

The hole cross test was carried out as described by Takagi et al. (1971).[16] A 30 × 20 × 14 cm steel partition was fixed in the middle of a cage. A hole 3 cm in diameter was made at a height of 7.5 cm in the center of the partition. Twenty mice were divided into four groups of five mice. Group I received vehicle (1% Tween-80) at 10 ml/kg body weight, group II received diazepam at 1 mg/kg body weight, and groups III and IV were treated with 200 and 400 mg/kg body weight, respectively, of the extract. The number of mice that passed through the hole from one chamber to the other was counted for a period of 3 minutes at 0, 30, 60, 90, and 120 minutes after oral administration of the test samples.

2.7.2 | Open field test

The open field CNS depressant activity tests were evaluated using the method described by Gupta et al.[17] The cage floor was marked with a grid, dividing it into a series of square open 'fields'. Accordingly, mice were randomly divided into four groups: standard, control, and two sample groups. The control group was treated orally with distilled water (10 ml/kg), the two sample groups were treated orally with 200 and 400 mg/kg of plant extract and the standard group was treated intraperitonially with diazepam (1 mg/kg body weight). Each mice was observed over a timed interval of 3 minutes (at 0, 15, 30, 45 and 60 minutes after administration) to note the number of fields crossed by each mouse in all groups. The mean number of open fields crossed by the mice in each group was compared with the standard group to detect neuropharmacological activity.

2.8 | Statistical analysis

All the results were expressed as means ± SEM (standard error of the mean). The *P* value was calculated by one-way ANOVA using SPSS software, version 22.0 (IBM Corporation, New York, NY, USA). Asterisks indicate increasing levels of significance: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (one-way ANOVA followed by Dunnett's test).

3 | RESULTS

3.1 | Analgesic activity

3.1.1 | Acetic acid-induced writhing in mice

The analgesic effect of the methanolic acetate fraction of *P. stratiotes* on acetic acid-elicited writhing in mice is shown in Table 1. An approximately 78.62% inhibition of writhing was found in group II mice (indomethacin, 10 mg/kg). In groups III and IV (200 and 400 mg/kg methanolic acetate fraction), the acetic acid-induced abdominal constrictions and stretching were significantly ($P < 0.05$) reduced compared to the control and standard groups in a dose-dependent manner.

3.1.2 | Formalin-induced hind paw licking in mice

The experiment was conducted to check whether or not an extract of *P. stratoites* had any impact on formalin-evoked hind paw licking in mice. The extract given at a dose of 400 mg/kg

weight resulted in an "early phase" 71.25% reduction in hind paw licking in Group IV mice (Table 2). The extract given at 200 mg/kg body weight dose yielded a slightly lower, non-significant, reduction in formalin-evoked hind paw licking in comparison to the reference standard (indomethacin, 10 mg/kg).

3.2 | Anti-inflammatory activity

3.2.1 | Carrageenan-induced paw edema in mice

The methanolic acetate fraction of *P. stratiotes* (200 and 400 mg/kg) exerted a significant ($P < 0.05$) anti-inflammatory effect from 0 minutes to 3 hours after administration, which was comparable to that of the control and reference standard groups (Table 3).

TABLE 1 Effects of methanolic acetate fraction of *P. stratiotes* on acetic acid-induced writhing in mice

Groups	Dose (mg/kg)	No. of "writhes"	% inhibition
Group I	Vehicle	39.75 ± 2.14[b]	
Group II	10 (standard)	8.5 ± 1.14	78.62
Group III	200	18.75 ± 1.76[a]	52.83
Group IV	400	12.75 ± 2.09[c]	67.92

Dunnett's *t* test compared to control (one-way ANOVA followed by Dunnett's test). Group I animals received vehicle (1% Tween 80 in water), group II received indomethacin (10 mg/kg body weight), and groups III and IV were treated with, respectively, 200 and 400 mg/kg (p/o) methanolic acetate fraction of *P. stratiotes*.
Values are means ± SEM (n = 5).
[a]$P < 0.05$.

3.3 | CNS depressant activity

3.3.1 | Hole cross test

Table 4 shows a hole cross test of the CNS depressant activity of the methanolic acetate fraction of *P. stratiotes* in mice. At doses of 200 and 400 mg/kg, the CNS depressant effect of methanolic acetate fraction of *P. stratiotes* was statistically significant ($P < 0.05$) at 90 minutes in a dose-dependent manner.

3.3.2 | Open field test

The methanolic acetate fraction of *P. stratiotes* fractions produced a decrease in the movements of the test animals at both dose levels. The effects of the standard and extract (200 mg/kg) were statistically significant ($P < 0.05$) at 0-120 minutes in a dose-dependent manner (Table 5).

4 | DISCUSSION

Pistia stratiotes is used as a universal flavoring agent and has a wide spectrum of medicinal properties. We tested a methanolic acetate fraction of *P. stratiotes* for analgesic, anti-inflammatory and CNS depressant activity.

Acetic acid induces pain by enhancing levels of PGE2 and PGF2α at the receptor sites of the organ cavity,[18-20] which means that carboxylic acid acts indirectly by increasing the discharge of

TABLE 2 Effects of methanol acetate fraction of *P. stratiotes* on hind paw licking in the formalin test in mice

Groups	Dose (mg/kg)	Early phase	% protection	Late phase	% protection
Group I	Methanol	40 ± 3.29	—	25.50 ± 2.41	
Group II	10 (standard)	11.25 ± 0.98	71.88	8.25 ± 0.98[b]	67.65
Group III	200	20 ± 1.19	50	11.50 ± 1.82	54.90
Group IV	400	11.5 ± 1.82[b]	71.25	7.25 ± 2.33[a]	71.57

Values are expressed at means ± SEM (n = 5).
[a]$P < 0.05$, and [b]$P < 0.01$, compared with control (one-way ANOVA followed by Dunnett's *t* test). Group I animals received vehicle (1% Tween 80 in water), group II received indomethacin (10 mg/kg body weight), and groups III and IV were treated with, respectively, 200 and 400 mg/kg methanol acetate fraction of *P. stratiotes*.

TABLE 3 Effect of methanolic acetate fraction of *P. stratiotes* on carrageenan-induced paw edema in mice

Groups		Edema diameter (mm)				% Inhibition			
Group	Dose (mg/kg)	0 min	1 h	2 h	3 h	0 min	1 h	2 h	3 h
Group I	Vehicle	3.85 ± 0.24	3.88 ± 0.41	3.86 ± 0.45	3.68 ± 0.63		-	-	
Group II	10	2.48 ± 0.45	1.90 ± 0.46[b]	1.65 ± 0.46	1.18 ± 0.31	35.71	50.97	57.42	68.03
Group III	200	3.43 ± 0.31	3.08 ± 0.35	2.98 ± 0.39	2.68 ± 0.35[a]	11.04	20.65	23.23	27.21
Group IV	400	3.23 ± 0.5[b]	3.03 ± 0.39	2.87 ± 0.45[a]	2.45 ± 0.36	16.23	21.94	25.81	33.33

All values are means ± SEM (n = 5).
[a]$P < 0.05$. Probability values (calculated compared with control using one-way ANOVA followed by Dunnett's *t* test): Group I animals received vehicle (1% Tween 80 in water), group II received ibuprofen (10 mg/kg body weight), and groups III and IV were treated with, respectively, 200 and 400 mg/kg methanolic acetate fraction of *P. stratiotes*.

TABLE 4 Effect of methanol extract of *P. stratiotes* on hole cross test in mice

		Number of movements				
Group	**Dose (mg/kg)**	**0 min**	**30 min**	**60 min**	**90 min**	**120 min**
Group I	Vehicle	6.75 ± 1.31	5.50 ± 1.38	4.25 ± 1.23	4.25 ± 0.98	3.50 ± 0.76
Group II	10	6.25 ± 0.98	5.00 ± 0.90	4.00 ± 0.90	3.75 ± 0.71[b]	3.25 ± 1.30
Group III	200	7.75 ± 1.42	3.50 ± 1.14	3.25 ± 0.98	2.50 ± 0.76	2.75 ± 0.98
Group IV	400	6.00 ± 1.0	3.00 ± 1.08	3.00 ± 0.00	1.75 ± 1.12[a]	1.75 ± 1.12

Values are means ± SEM (n = 5).
[a]$P < 0.05$, [c]$P < 0.01$. Dunnett's *t* test compared to vehicle control. Group I animals received vehicle (1% Tween 80 in water), group II received diazepam (1 mg/kg body weight), and groups III and IV were treated with, respectively, 200 and 400 mg/kg of methanolic acetate fraction of *P. stratiotes*.

TABLE 5 Effect of methanolic acetate fraction of *P. stratiotes* on open field test in mice

		Number of movements				
Group	**Dose (mg/kg)**	**0 min**	**30 min**	**60 min**	**90 min**	**120 min**
Group I	Vehicle	182.5 ± 7.16	150 ± 6.56	123.75 ± 6.73	80.5 ± 3.63	93 ± 6.41
Group II	10	90 ± 1.72[a]	87.2 ± 1.44	70 ± 2.06	66 ± 1.61	52 ± 1.78
Group III	200	197.50 ± 6.21	125 ± 4.56	99.7 ± 2.79	87.2 ± 3.54	70 ± 4.27
Group IV	400	142.50 ± 5.46	93 ± 2.50[b]	70 ± 4.61	65 ± 4.97	53.7 ± 4.24

Dunnett's *t* test compared to control. Group I animals received vehicle (1% Tween 80 in water), group II received diazepam (1 mg/kg body weight), and groups III and IV were treated with, respectively, 200 and 400 mg/kg methanolic acetate fraction of *P. stratiotes*.
Values are means ± SEM (n = 5).
[a]$P < 0.05$, [b]$P < 0.01$.

endogenous mediators. Non-steroidal anti-inflammatory drugs act by blocking this stimulation of the sensory neurons in response to inflammatory mediators.[21] The formalin test is another well-used method of assessing analgesic activity associated with clinical pain.[22,23] This methodology differentiates between central and peripheral activities. Formalin-induced nociception is biphasic; the initial part involves direct stimulation of nerve fibers representing neuropathic pain, and the second part involves inflammatory pain mediated by autacoids, serotonin, histamine, bradykinin, and cytokines such as IL-1β, IL-6, TNF-α, eicosanoids, and NO.[24-26] In the present study, in both tests the methanolic acetate fraction of *P. stratiotes* showed a dose-dependent significant analgesic action compared to that of the reference drug, indomethacin. The results suggest that the extract exerts its action by pathological modification of the peripheral mechanisms of the physiological condition, and this conclusion is supported by some other previous findings.[22-28]

Carrageenan-induced paw puffiness has been most notably used in experimental animal models for the assessment of acute inflammation and is considered to be biphasic, with the early phase (1-2 hours) of the carrageenan model being principally mediated by histamine, serotonin, and enhanced synthesis of autacoids in injured tissue, while the late phase is mediated by bradykinin, leukotrienes, polymorphonuclear cells, and prostaglandins made by tissue macrophages.[29,30] In our study, the crude methanolic acetate fraction of *P. stratoites* produced significant inhibition of paw puffiness from 0 minutes to 3 hours, which was comparable with the effect of standard ibuprofen. A possible mechanism for the extract's observed anti-inflammatory activity might be its ability to reduce the release of histamine, serotonin or kinin-like substances or biosynthesis of prostaglandins.[29,30]

Locomotor activity is thought to act on the CNS by raising alertness and thus reducing motion activity could induce a sedative action.[31] Gamma-aminobutyric acid (GABA) is the major depressing neurochemical within the central system. Our present methanolic acetate fraction of *P. stratiotes* showed potent CNS depressant activity in both study tests. So this fraction of *P. stratoites* could act by potentiating GABAergic inhibition in the CNS system via membrane hyperpolarization, which results in a decrease in the firing rate of important neurons within the brain, or it could also act by directly activating GABA receptors.[32] Several analyses have suggested that plants containing flavonoids, saponins and tannins may have an effect on multiple CNS disorders.[33,34] Earlier investigations of phytoconstituents and plant extracts suggest that many flavonoids and neuroactive steroids are ligands for the GABA receptors in the central nervous system; which suggests that they can act as benzodiazepine-like molecules.[34,35] Our methanolic acetate fraction of *P. stratoites* may contain all or any of these phytocontituents, and thus have potential benzodiazepine-like antidepressant activity.

5 | CONCLUSION

Our analysis reveals that the methanolic acetate fraction of *P. stratiotes* has considerable medicinal value, via its analgesic, anti-inflammatory and CNS depressant properties, all of which were measured with reference to a standard drug. Based on these preliminary results, further investigation is needed to characterize the active compounds in the extract and develop formulation studies and possible dose types.

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AVAILABILITY OF DATA AND MATERIALS

Data and materials are available in the Department of Pharmacy, Atish Dipankar University of Science and Technology.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

MSH, MI, MNA and MTA were directly involved in conducting this research work. MSH, AK, SAS, MGU, and MMH also contributed during data generation, manuscript preparation and collection of the plant material. All those who qualify for authorship are listed as authors of this research work.

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