INTERNATIONAL RESEARCH JOURNAL OF PHARMACY **ISSN 2230 – 8407**
Available online http://www.irjponline.com
Research Article

CYTOTOXICITY, ANTIMICROBIAL AND NEUROPHARMACOLOGICAL EVALUATION OF ETHANOLIC EXTRACT OF *PISTIA STRATIOTES* L.

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Article Received on: 12/12/10 Revised on: 26/01/11 Approved for publication: 16/02/11

ABSTRACT
In phytochemical screening of the ethanolic extract of *Pistia stratiotes* L. aerial part anthraquinone glycosides, cardiac glycosides, cyanogenetic glycosides, flavonoids, steroids, tannins, carbohydrates were found to be present. The LC_{50} and LC_{90} were 1.8µg/ml & 2.07µg/ml respectively in brine shrimp lethality assay. It was to have produced significant zones of inhibition against gram positive *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, gram negative *Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis* and yeast *Candida albicans* and *Rhodotorula rubra*, which were comparable with standard antimicrobial drugs tetracycline, vancomicin and nystatin. In acute-toxicity test in mice LD_{50} was 850mg/kg body weight (i.p). In CNS depressant tests; hole cross, open field, beam walking and thiopental sodium induced sedative test in mice it significantly ($p<0.005$, $p<0.001$) decreased the locomotor activity in mice. The extract showed significant ($p<0.005$, $p<0.001$) antinociceptive activity when subjected to hot plate, tail immersion and acetic acid-induced writhing tests in mice.

KEYWORDS: Acute toxicity, Antimicrobial, Antinociceptive, CNS Depressant, Lethality, *Pistia stratiotes* L.

INTRODUCTION

Pistia is a genus of aquatic plant in the family Araceae, comprising a single species. *Pistia stratiotes* L. is also called water cabbage or water lettuce. *P. stratiotes* L. leaves are used in traditional medicine for the treatment of ringworm infection of the scalp, syphilitic eruptions, skin infections, boils, wounds. Moreover the oil extract of it is used in the treatment of worm infestations, tuberculosis, asthma and dysentery. It is topically used to treat skin diseases, inflammation, piles, ulcers syphilitic infections and burns[1].

Water lettuce is a seriously nausiating widely distributed floating aquatic plant in Asia and Africa[2]. From previous research it was found that *P. stratiotes* L. contains large amounts of two di-C-glycosylflavones of the vicenin and lucenin and lesser amounts of the anthocyanin cyanidin-3-glucoside and a luteolin-7-glycoside, and traces of the mono-C-glycosylflavones, vitexin and orientin[3]. Three sitosterol acylglycosides have been isolated from the ethereal extract of the plant[4]. A stigmastane, 11α-hydroxy-24*S*-ethyl-5α-cholest-22-en-3, 6-dione also has been isolated from the plant[5].

The traditional uses claim that *P. stratiotes* L. is potential folk medicine but very few phytochemical and biological works of medicinal interest have been carried out so far on this plant. The present study was

designed to evaluate the pharmacological basis for the use of the plant in folk medicine by using established scientific methods.

MATERIALS AND METHODS

Plant material: The aerial part of *Pistia stratiotes* L. was collected from Narail in July 2009 and was identified by the expert Mohammed Mohiuddin, Bangladesh Forest Research Institute Herbarium, Chittagong (Accession No. 090725). Then it was dried at room temperature in a dry tidy room for 15 days.

Extraction: The dried plant material was finely powdered and extracted with 98% ethanol in a glass container keeping it at normal room temperature and repetitive stirring per day. Then the extract was filtered and the chlorophyll was removed. Then the solvent was evaporated at room temperature and the dried crude extract was used for investigation. Percent yield of the extract was 19.4%.

Phytochemical group tests: The ethanol extract was screened by using standard methods as outlined by Trease *et al*[6]. The extract was screened for the presence of alkaloids, anthraquinone glycosides, cardiac glycosides, cyanogenetic glycosides, flavonoids, steroids or terpenoids, tannins, resins, carbohydrates and proteins. The reagents were first tested by using standard drugs of corresponding groups available in market.

The resulting data is summarized in the table 1.

Determination of antibacterial activity by the disc diffusion method: The extract was tested for antibacterial activity by the disc diffusion method as depicted by Latha *et al*[7]. Here 100µl of suspension of the tested microorganisms; containing 2.0×10^{6} CFU/ml for bacteria and 2.0×10^{5} CFU/ml spore for yeast strains. Mueller–Hinton agar and Sabouraud dextrose agar were sterilized in a flask and cooled to 45–50°C and then taken in sterilized Petri dishes with a diameter of 9cm (15ml). The filter paper discs (6mm in diameter) were individually impregnated with 10 and 20µl/disc of the crude extract and then placed onto the agar plates previously inoculated with the tested microorganisms. The Petri dishes were kept at 4°C for 2 hours. The plates were incubated at 37°C for 24 hours for bacteria and at 30°C for 48 hours for the yeast. The diameters of the zones of inhibition were measured in millimeters. All the tests were repeated triplicate. Vancomycin, tetracycline and nystatin were used as standards at the dose of 30µg/disc in positive control.

The resulting data is summarized in the table 2.

Determination of cytotoxic activity by Brine shrimp lethality bioassay: *In vitro* lethality bioassay of the ethanol extract of *P. stratiotes* L. was exploited to detect cytoxicity following the method described by Meyer *et al*[8]. Brine shrimp eggs were placed in seawater (3.8% w/v sea salt in distilled water) and incubated at 24-28°C in front of a lamp. Eggs were hatched within 48 hours providing large number of larvae (nauplii). A solution of 50µg/µl of the extract was prepared by using Dimethyl sulfoxide. 21 clean test tubes were taken. 14 of these were used for the samples at seven concentrations (duplicate of each concentration) and 7 for control test. Then 10ml of seawater was given to each of the test tubes. Afterwards, with the help of micropipette specific volume (4, 8, 12, 16, 20, 24, 28µl) of samples were transferred from the stock solutions to the test tube to get final sample concentrations of 20, 40, 60, 80, 100, 120 and 140µg/ml respectively. In the test tubes taken for the control same volumes of DMSO (as in the sample test tubes) were taken. With the help of a Pasteur pipette 10 living nauplii were kept to each of the test tubes. Alive nauplii were counted after 16 hours and the lethal concentrations (LC_{50} and LC_{90}) were calculated. The plot of percent mortality versus log concentration of the extract produced an approximate linear correlation between them graphically. From the graph (Fig 1) the concentration at which 50% & 90% mortality (LC_{50}) & LC_{90}) of brine shrimp nauplii occurred were obtained. All other data were expressed as mean ± SD.

The resulting data and statistical analysis are summarized in table 3.

Determination of neuropharmacological activity: For the experiments male Swiss albino mice of 3-4 weeks of age, weighing between 20-25gm were collected from the Pharmacology laboratory of Bangladesh Center for Scientific and Industrial Research (BCSIR). Animals were maintained under standard environmental conditions (temperature: (23.0 ± 2.0º), relative humidity: 55-65% and 12h light/12h dark cycle) and had free access to feed and water. The animals were acclimatized to laboratory condition for one week prior to experiments.

Acute Toxicity Studies: This experiment was conducted according to Lorke *et al*[9]. The acute toxicity (LD_{50}) of the extract in mice was determined by intraperitoneal injection (i.p.). The study was divided two phases. In the initial phase, the range of doses producing the toxic effects was established. Four groups of 3 mice each were selected. The first group received extract at a dose of 10mg/kg while the second, third and fourth groups received 100, 500 and 1000mg/kg body weight respectively. The animals were observed for signs of toxicity and death within 24 hours. In the second phase, four groups of 1 mouse each were used. Specific doses were administered which depended on the result of the first phase. The final LD_{50} values were calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose, i.e. the geometric mean of consecutive doses for which 0 and 100% survival rates were recorded.

Determination of CNS depressant activity

Hole cross test: The experiment was performed according to the methodology depicted by Takagi *et al*[10].In this experiment, animals were divided into four groups (control, positive control and test I and test II group) with 5 mice in each group. A steel partition was fixed in the middle of a cage having a size of 30 × 20 × 14cm. A hole of 3cm diameter was made at a height of 7.5cm in the centre of the cage. The number of passage of a mouse through the hole from one chamber to the other was counted for a period of 5min started at 0, 30, 60, 90 and 120th min after oral administration of the extract. Diazepam was used in positive control as reference standard at the dose of 1 mg/kg body weight.

The resulting data is summarized in the table 4.

Open field test: This test was designed following method as outlined by Gupta *et al*[11]. The animals were divided into four groups (control, positive control and test I and test II group) with 5 mice in each group. The test group received the extract at the doses of 250 and 500mg/kg body weight per orally whereas the control group received vehicle (1% Tween 80 in water). In a plain board an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40cm height. The number of squares passed anyway by the animals was counted for 3min started at 0, 30, 60, 90 and 120th min after oral administration of the test drugs. Diazepam was used in positive control as reference standard at the dose of 1mg/kg body weight.

The resulting data is summarized in the table 5.

Beam walking test: We adopted a method to perform this test as outlined by Stanley *et al*[12]. Mice were selected randomly and well trained to walk from a start platform along a ruler (100cm long, 3cm wide) elevated 30cm above the bench by metal supports to a goal box. The successful mice were divided into four groups (control, positive control and test I and test II group) with 5 mice in each group. The mice were treated intrperitoneally with the extract at the doses of 250 and 500mg/kg body weight, vehicle (1% Tween 80 in water) or diazepam (1mg/kg body weight). Thirty minutes after the treatment; each mouse was placed on the beam at one end and allowed to walk to the goal box. Mice that fell were returned to the position they fell from, with a maximum time of 60sec allowed on beam. The number of foot slips (one or both hind limb slipped from the beam) was recorded. The number of foot slip depicts the insufficiency in motor coordination.

The resulting data is summarized in the table 6.

Thiopental sodium-induced sedative test: This experiment was conducted following the method described by Ferrini *et al*[13]. The animals were divided into four groups (control, positive control and test I and test II group) with 5 mice in each group. Control group animals received thiopental sodium (i.p.); positive control group animals received thiopental sodium (i.p.) plus diazepam (i.p.) and test I and test II

were treated with thiopental sodium (i.p.) plus (p.o.) ethanolic extract of *P. stratiotes* L. respectively. All groups of mice (n=5) were injected with thiopental sodium (10mg/kg i.p) 15min after administration of either diazepam (1mg/kg body weight) or the extract (250mg/kg and 500mg/kg body weight), the time interval between losing and regaining of righting reflex was measured as sleeping time.
The resulting data is summarized in the table 7.

Determination of Antinociceptive activity

Hot plate test: This method was adopted from Toma *et al*[14]**.** The animals were divided into four groups (control, positive control and test I and test II group) with 5 mice in each group. Control group animals received vehicle (1% Tween 80 in water, 10ml/kg body weight), positive control group animals received nalbuphine at 10mg/kg body weight while test I and test II were treated with the extract at the doses of 250 and 500mg/kg body weight (p.o.) respectively. The animals were placed on Eddy's hot plate kept at a temperature of 55 ± 0.5°C. A cut off period of 15sec, was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60 and 90th min after oral administration of the extract.
The resulting data is summarized in the table 8.

Tail immersion test: The experiment was performed according to the methodology depicted by Toma *et al*[14] .This procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice. The animals were divided into four groups (control, positive control and test I and test II group) with 5 mice in each group. Control group animals received vehicle (1% Tween 80 in water, 10ml/kg body weight), positive control group animals received nalbuphine at 10mg/kg body weight while test I and test II were treated with the extract at the doses of 250 and 500mg/kg body weight (p.o.) respectively. The animals were placed on Eddy's hot plate kept at a temperature of 55 ± 0.5°C. A latency period of 15sec was defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The latent period of the tail-flick response was determined before and 0, 30, 60 and 90th min after the administration of drugs.
The resulting data is summarized in the table 9.

Acetic acid-induced writhing test: This method was adopted from Ahmed *et al*[15].Test animals were randomly selected and divided into four groups; control, positive control, test I, test II consisting of 5 mice in each group. In the animals of positive control group diclofenac sodium was used at the dose of 25mg/kg body weight. To induce pain in the test animals 0.7% acetic acid solution was used. Group test I and test II were treated with the sample at a dose of 250mg/kg and 500mg/kg body weight respectively by oral rout. Thirty minutes were given to ensure proper absorption of the administered substances. After an interval of 5 minutes number of writhing was counted for 30min. The incomplete writhing was taken as half writhing and two halves were counted as one full writhing.
A statistical evaluation of the results of analgesic activity is given in table 10.

STATISTICAL ANALYSIS

Statistical analysis was carried out using one-way ANOVA followed by Dunnett's multiple comparisons for analgesic screening tests and Tuckey's multiple comparisons for neuropharmacological tests. The results obtained were compared with the control group. $p<0.05$, $p<0.001$ were considered to be statistically significant.

RESULTS

Phytochemical group tests: In phytochemical screening the ethanolic extract of *Pistia stratiotes* L. was found to contain anthraquinone glycosides, cardiac glycosides, cyanogenetic glycosides, flavonoids, steroids, tannins, carbohydrates (Table 1).

Determination of antibacterial activity by the disc diffusion method: The extract showed significant zones of inhibition 14mm and 23mm with gram positive *Bacillus cereus* at the doses of 10µl/disc and 20µl/disc respectively, whereas the standard drugs Vancomicin and Tetracycline showed zone of inhibition 13mm and 27mm respectively at the dose of 30µg/disc. In case of the bacteria *B. subtilis* and

Staphylococcus aureus extract produced zone of inhibition 16mm (10µl/disc), 21mm (20µl/disc) and 25mm (10µl/disc), & 34mm (20µl/disc) respectively. On the other hand the standard drugs Vancomicin and Tetracycline produced 13mm and 27mm zones of inhibition respectively at the dose of 30µg/disc. Similarly with the gram negative bacterial strain, zones of inhibition produced by the extract were (16mm & 24mm) for *Escherichia* coli, (9mm & 19mm) for *Salmonella typhi* and (11mm & 13mm) for *Proteus mirabilis* at the dose of 10µl/disc and 20µl/disc. In comparison, the standard drugs Vancomicin and Tetracycline produced that of *Escherichia* coli (21mm & 25mm), *Salmonella typhi* (18mm & 23mm) and *Proteus mirabilis* (22 mm by the both drug). When the yeast *Candida albicans* and *Rhodotorula rubra* were treated with the extract zones of inhibition found to be 18mm and 28mm with the dose 10µl/disc and 20µl/disc respectively. The standard drugs Nystatin (30µg/disc) produced that of *Candida albicans* and *Rhodotorula rubra* 23mm and 26mm respectively (Table 4).

Determination of cytotoxic activity by Brine shrimp lethality bioassay: The % mortality of larvae (nauplii) was found similar with both doses (80µg/ml and 100µg/ml) of the extract. The mean ± SD was found to be 62.9 ± 31.5. The LD_{50} & LD_{90} were 1.8µgm/ml & 2.07µgm/ml respectively (Table 3). And the result was statically significant.

Determination of CNS depressant activity

To determine the CNS depressant activity first the extract was subjected to acute toxicity study for the test animals, mice. Subsequent experiments viz. hole cross test, open field test, beam walking test and thiopental sodium induced sedative tests were performed.

Acute Toxicity Studies: Signs and symptoms observed in test animals injected with the extracts included piloerection, reduced mobility and respiratory embarrassment including gasping with eventual immobility, unconsciousness and death. The LD_{50} values were calculated and found to be 850mg/kg body weight i.p. This helped to determine the maximum dose indicating the optimal dose range that can be used for the mice.

Hole cross test: There is negligible variation in number of hole crossed from one chamber to another by mice of the control group from 30 to 120min. The groups Test I and Test II treated with the extract at the doses of 250 and 500mg/kg body weight showed significant decrease of movement from its initial value at 0 to 120min which was comparable with that of the group treated with standard diazepam (Table 4). The result was found to be statistically significant ($p<0.001$).

Open field test: In the open field test, test I and test II groups treated with the extract at the doses of 250 and 500mg/kg body weight showed significant ($p<0.001$) dose dependent decrease of movement from its initial value at 0 to 120min (Table 5).

Beam walking test: In the beam walking test, test II group treated with the extract significantly ($p<0.05$, $p<0.001$) induce motor coordination deficit in mice at the dose of 500 mg/kg body weight. But the extract at the dose of 250mg/kg test I group did not significantly induce motor coordination deficit in mice (Table 6).

Thiopental sodium induced sedative test: In thiopental sodium induced sleeping time test, test I and test II groups treated with the extract both the doses 250 and 500mg/kg showed significant ($p<0.05$, $p<0.001$) decrease in onset of action and increased the duration of sleep (Table 7) which was comparable with the positive control group.

Determination of Antinociceptive activity

To determine the antinociceptive activity hot plate test, tail immersion test and acetic acid induced writhing test were performed.

Hot plate test: As shown by the Table 8, in hot plate test both Test I and Test II groups treated with the extract produced a dose dependent increase in latency time relative to the control group ($p<0.05$, $p<0.001$).

Tail immersion test: The tail withdrawal reflex time following administration of the extract was found to increase in dose dependent manner. The result was statistically significant ($p<0.05$, $p<0.001$) and was comparable to the reference drug nalbuphine (Table 9).

Acetic acid-induced writhing test: From the table 10 it is evident that test I and test II groups treated with the extract significantly ($p<0.001$) inhibited writhing response induced by acetic acid in a dose dependent manner. The percent protection was increased with the increase in dose (Table 10).

DISCUSSION

Cytotoxicity test depicts that the extract may contain antitumor, antibacterial or pesticidal compounds. However, this cannot be confirmed without further higher and specific tests.

Antimicrobial assessment demonstrates whether any species under investigation possess inhibitory activity agianst microbial species. In this experiment the ethanolic crude extract was found to have prominent inhibitory property against several pathogenic microbial species relative to standard drug vancomycin and tetracycline. This property certainly indicates the existence of one or several chemical moieties contained in the crude extract having antimicrobial potential. These compounds may have therapeutic potentials against infectious ailments caused by the microbial strains used in this experiment. However, further research on this plant extract is crucial to identify and isolate the responsible compounds for discovery of each of their specific action mechanism.

The results from the CNS depressant tests indicated that it significantly decreased the locomotor activity as shown by the results of the open field and hole cross tests. The locomotor activity is a measure of the level of excitability of the CNS[16]. And decrease of this activity may be closely related to sedation resulting from depression of the central nervous system[17]. In case of the thiopental sodium induced sedative test both the doses of the extract produced a significant increase in the hypnotic effect in a dose dependent manner, thus suggesting a profound sedative activity. This method is a very sensitive way to detect agents with CNS depressant activity[18]. The sedative effect recorded here may be related to an interaction with benzodiazepines and related compounds that bind to receptors in the CNS and have already been identified in certain plant extracts.

In hot plate test and tail immersion test, the extract increased mean basal latency which indicates that it may act via centrally mediated analgesic mechanism as because the hotplate method and tail immersion test are considered to be selective to examine compounds acting through opioid receptor[19, 20].

The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics. When acetic acid is induced, pain sensation is produced by triggering localized inflammatory response. This pain stimulus leads to the release of free arachidonic acid from cell phospholipids[21]. And the response is thought to be mediated by peritoneal mast cells[22], acid sensing ion channels[23] and the prostaglandin pathways[24]. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain[19, 20]. The extract was active in both cases i.e. inhibited both mechanisms of pain. It depicts that the plant extract may be employed as a narcotic analgesic. Such a mode of action is proposed for opioid analgesic such as morphine. This suggests that the extract may contain morphine like compounds. It is also reported that the inhibition of pain could arise not only from the presence of opioids and/or opiodiomimetics but could also arise from the presence of phenolic constituents[25] and also steroidal constituents[26]. Hence similar type of constituents may present in the extract. There are also reports on the role of flavonoid in analgesic activity primarily by targeting prostaglandins[27, 28]. Tannins are also claimed to possess analgesic activity[29].

However, further investigation is underway to determine the exact phytoconsituents that are responsible for the biological activities of the ethanol extract of *P. stratiotes* L.

CONCLUSION

This study results show that ethanolic extract of *Pistia stratiotes* L. possesses cytotoxic, antimicrobial, CNS depressant and antinociceptive activity and lend credence to the traditional use of the plant. The phytochemical assay provides a preliminary idea about the chemical constituents in the plant and further isolation and structure determination is required to get conspicuous idea about which compounds are responsible for those activities individually.

ACKNOWLEDGEMENT

The authors are grateful to the management of Bangladesh Council of Scientific and Industrial Research for the help providing the necessary facilities to conduct this study. We thank the staffs at the pharmacology laboratory of BCSIR who cordially helped in the experiments. We also like to show our gratitude to Mohammed Mohiuddin who helped us in taxonomical identification of the plant.

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Table 1: Result of phytochemical screening of the ethanolic extract of *Pistia stratiotes* L.

Phytochemical constituents	Inference
Alkaloids	-
Anthraquinone glycosides	+
Cardiac glycosides	+
Cyanogenetic glycosides	+
Flavonoids	+
Steroids/terpenoids	+
Tannins	+
Resins	-
Carbohydrates	+
Proteins	-

+ → Presence, - → Absence

Table 2: Result of antimicrobial activity of the ethanolic extract of *Pistia stratiotes* L. by the disc diffusion method

Microorganisms	Crude extract zone inhibition (mm)		Antimicrobial agents zone inhibition (mm)		
	10 µl/disc	20 µl/disc	Vancomicin 30 µg/disc	Tetracycline 30 µg/disc	Nystatin 30 µg/disc
Gram positive					
Bacillus cereus	14	23	13	27	nd
Bacillus subtilis	16	21	16	24	nd
Staphylococcus aureus	25	34	16	28	nd
Gram negative					
Escherichia coli	16	24	21	25	nd
Salmonella typhi	9	19	18	23	nd
Proteus mirabilis	11	13	22	22	nd
		Yeast			
Candida albicans	21	28	nd	nd	23
Rhodotorula rubra	18	19	nd	nd	26

nd = Not Detected

Table 3: Result of brine shrimp lethality bioassay of the ethanolic extract of *Pistia stratiotes* L.

Test sample	Conc. (µg/ml)	Log Conc.	Alive shrimp No.	Mortality (%)	Mean	Standard deviation (SD)	Mean ± SD	LC_{50} (µg/ml)	LC_{90} (µg/ml)
Extract	20	1.3	8	20	62.9	31.5	62.9 ± 31.5	1.8	2.07
	40	1.6	7	30					
	60	1.8	5	50					
	80	1.9	3	70					
	100	2.0	3	70					
	120	2.07	1	90					
	140	2.14	0	100					

Table 4: Effect of the ethanolic extract of *P. stratiotes* L. in hole cross test on mice

Groups	Dose (mg/kg body weight)	Movements on open field before and after drug administration (mean ± SEM)			
		0 Min	30 min	60 min	90 min
Control	1% Tween in water (p.o.)	15.4 ± 2.2	16.5 ± 1.9	15.3 ± 1.2	13.6 ± 0.7
Diazepam	1 (i.p.)	18.3 ± 2.7	12.1 ± 1.6**	6.4 ± 1.2**	1.3 ± 1.3**
Test I	250 (p.o.)	17.2 ± 1.9	13.7 ± 2.8**	9.5 ± 1.2**	5.6 ± 2.4**
Test II	500 (p.o.)	18.9 ± 2.3**	11.2 ± 1.3**	7.7 ± 1.4**	2.3 ± 1.9**

(n = 5); ** $p < 0.001$

Table 5: Effect of the ethanolic extract of *P. stratiotes* L. in open field test on mice

Groups	Dose (mg/kg body weight)	Movements on open field before and after drug administration (mean ± SEM)			
		0 Min	30 min	60 min	90 min
Control	1% Tween in water (p.o.)	126.23± 6.5	109.2 ± 3.2	95.9 ± 5.1	84.4 ± 2.3
Diazepam	1 (i.p.)	78.8 ± 5.1	23.5 ± 3.1**	14.6 ± 2.6**	6.8 ± 1.3**
Test I	250 (p.o.)	89.4 ± 4.7	57.3 ± 3.6**	44.4 ± 2.9**	27.8 ± 2.7**
Test II	500 (p.o.)	89.8 ± 4.8	42.8 ± 3.4**	26.2 ± 2.6**	15.1 ± 1.7**

(n = 5); ** p < 0.001

Table 6: Effect of the ethanolic extract of *P. stratiotes* L. in beam walking test on mice

Groups	Dose (mg/kg body weight)	Mean time (min) to complete the task (mean ± SEM)	Mean number of foot slips (mean ± SEM)
Control	1% Tween in water (p.o.)	8.7 ± 1.2	5.3 ± 0.4
Diazepam	1 (i.p.)	48.6 ± 4.9**	7.1 ± 1.3**
Test I	250 (p.o.)	11.6 ± 2.7**	6.2 ± 0.7*
Test II	500 (p.o.)	32.4 ± 2.9*	26.6 ± 1.3**

(n=5); * p < 0.05, **P <0.001

Table 7: Effects of the ethanol extract of *P. stratiotes* L. in thiopental sodium-induced sedative test on mice

Groups	Dose (mg/kg body weight)	Onset of sleep in min. (mean ± SEM)	Duration of sleep in min. (mean ± SEM)
Control	10	13.2 ± 2.312	67.6 ± 4.325
Diazepam	10 + 5	9.4 ± 1.312*	102.8 ± 5.648**
Test I	10 + 250	12.5 ± 2.513	79.3 ± 5.214**
Test II	10 + 500	10.2 ± 1.268*	95.8 ± 7.362**

(n = 5); * p < 0.05, ** p < 0.001

Table 8: Effect of the ethanolic extract of *P. stratiotes* L. in hot plate test on mice

Groups	Dose (mg/kg body weight)	Mean latency (s) before and after drug administration (mean ± SEM)			
		0 min	30 min	60 min	90 min
Control	1% Tween in water (p.o.)	2.6±0.3	2.9±0.2	2.2±0.7	2.1±0.2
Nalbuphine	10 (i.p.)	2.4±0.08	4.9±0.4**	8.1±0.9**	10.7±1.1**
Test I	250 (p.o.)	2.1±0.07	4.1±0.6*	4.7±0.4**	5.3±0.3*
Test II	500 (p.o.)	2.7±0.21	3.7±0.8**	4.9±0.5**	5.5±0.6*

(n = 5); * p < 0.05, ** p < 0.001

Table 9: Effects of the ethanolic extract of *P. stratiotes* L. in tail withdrawal reflex test on mice

Groups	Dose (mg/kg body weight)	Mean reaction time (s) before and after drug administration (mean ± SEM)			
		0 min	30 min	60 min	90 min
Control	1% Tween in water (p.o.)	2.1 ± 0.4	2.50 ± 0.8	2.5 ± 0.9	2.32 ± 0.6
Nalbuphine	10 (i.p.)	1.6 ± 0.7	5.1 ± 0.7*	11.0 ± 1.3*	11.3 ± 1.9*
Test I	250 (p.o.)	1.3 ± 0.5*	5.4 ± 0.4*	5.8 ± 1.7**	5.4 ± 0.5**
Test II	500 (p.o.)	1.8 ± 0.3**	4.7 ± 0.9*	6.7 ± 0.9**	5.2 ± 0.8*

(n = 5); * $p < 0.05$, ** $p < 0.001$

Table 10: Effects of the ethanolic extract of *P. stratiotes* L. in acetic acid-induced writhing test on mice

Groups	Dose (mg/kg body weight)	No. of writhing (mean ± SEM)	% protection
Control	1% Tween in water (p.o.)	67.2 ± 3.2(100)	-
Diclofenac-Na	10 (i.p.)	9.1 ± 0.9** (30.9)	69.1
Test I	250 (p.o.)	25.0 ± 0.8** (45.8)	54.2
Test II	500 (p.o.)	12.1 ± 0.6** (27.1)	72.9

(n = 5); ** $p < 0.001$

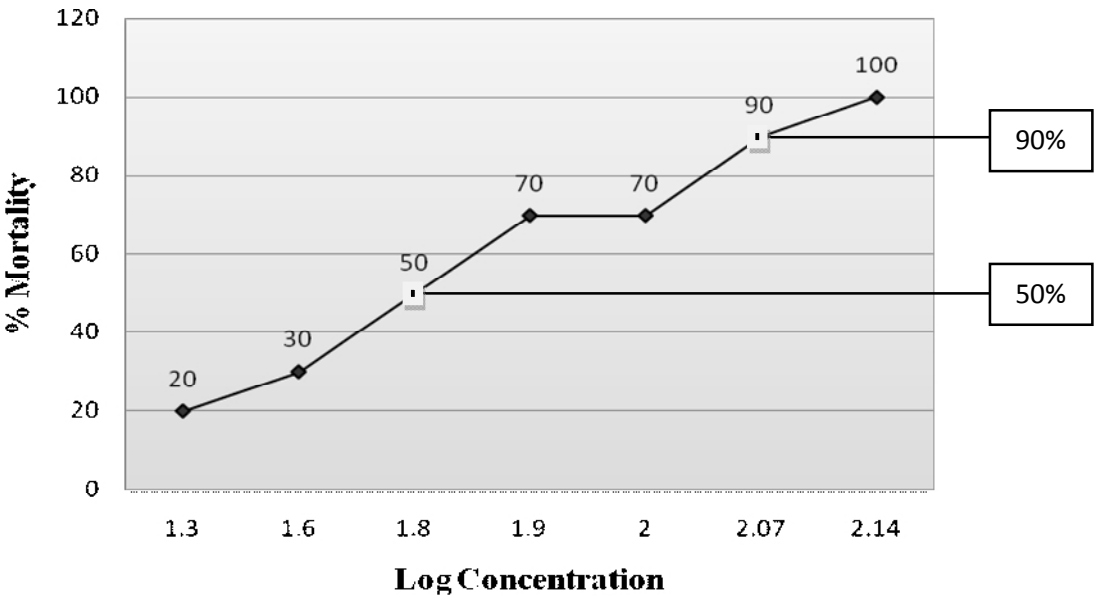

Fig 1: Graphical presentation of lc_{50} and lc_{90} of the ethanolic extract of *Pistia stratiotes* L. in brine shrimp lethality bioassay

Source of support: Nil, Conflict of interest: None Declared