

Phytochemical and Pharmacological Evaluation of *Cyperus odoratus* Extract

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(Received: 10 May, 2018; Accepted: 12 July, 2018; Published : 31 July, 2018)

Abstract

Cyperus odoratus (Family: *Cyperaceae*) is a 10 to 50 cm long perennial herb used as traditional medicinal plant in Bangladesh to treat various pathological conditions like piles, tumour and wound. The ethanolic extract of *C. odoratus* (COET) has been evaluated for different bioactivities, for example, antioxidant, analgesic, anthelmintic and cytotoxic activities. Antioxidant activity was investigated by DPPH free radical scavenging assay, analgesic activity was evaluated from 'acetic acid induced writhing inhibition test' in mice, anthelmintic activities was tested by 'time of paralysis and death' assays and cytotoxic activities was observed from the 'brine shrimp lethality bioassay'. Total tannin and phenol contents were also measured by using Folin-Ciocalteu reagent. Phytochemical screening indicated that the presence of alkaloids, tannins, flavonoids, glycosides, proteins, gums, steroids and acidic compounds. In DPPH free radical scavenging assay the extract showed IC₅₀ value of 8.05 µg/ml, whereas the standard ascorbic acid showed 31.40 µg/ml. The total tannin and phenolic contents were found to be 3.35 and 1.68 mg GAE/100 g of dried extract, respectively. In acetic acid induced writhing assay, the extract showed 36.23 and 61.32% writhing inhibition at doses of 250 mg/kg and 500 mg/kg body weight, respectively, whereas standard Diclofenac-Na (25 mg/kg) showed 70.03% writhing inhibition. The plant extract showed anthelmintic activity in a dose dependant manner. COET also showed LD₅₀ against brine shrimp nauplii at 11.344 µg/mL. After observing all preliminary biological data, it has been confirmed that the plant has antioxidant, analgesic, cytotoxic and anthelmintic properties.

Key words *Cyperus odoratus*, antioxidant, analgesic, anthelmintic, cytotoxic.

Introduction

Medicinal plants have long history of use by human being for the cure of various ailments dating back to primitive age, probably when people observed the animal behaviour to utilize certain plant's parts during their sickness (Sofowara, 1982; Hill, 1989). Medicinal plants always have played a major role in human health development and in disease control and cure. In recent times, it has been estimated that at least 80% of world population is reliant on medicinal plants rather natural medicine sources to cure ailments and improve health (Kaur

and Jaggi, 2010). The World Health Organization (WHO) also provided similar data at early 2000s (Alves and Rosa, 2005). Perhaps, it is the fact that natural products have more 'drug like and biological characteristics' than most of the synthetic drugs, with fewer side effects that make them suitable candidate for therapeutic use (Minhajur *et al.*, 2011). Moreover, about 25% pharmaceutically formulated drugs are derived from natural origin (Islam *et al.*, 2016). As expected, researches on natural products are increasing day by day. Bangladesh has wide range of tropical and agro growing conditions that are

advantageous to introduce and domestication of new plant varieties (Hossain *et al.*, 2013). It is a perfect place for *C. odoratus* to grow and they are common in here.

C. odoratus is a perennial grass-like sedge. It is a monocotyledonous graminoid flowering plant which belongs to *Cyperaceae* family. *C. odoratus* has fibrous root along with slender and scaly creeping rhizomes, arises singly from the triquetras tubers with dense tuft of 10 to 60 cm. It has grass-like leaves, having no hair but easily distinguishable from grass. This grass grows in tropical, subtropical and temperate regions. It is prevalent in North America, Japan, Korea and Russia (Maximowicz and Mém, 1859).

To elucidate the pharmacological activities of *C. odoratus*, three studies were commissioned recently. Three compounds, 3,4-dimethoxy benzoic acid (1), 4-hydroxybenzoic acid (2) and piceatannol (3) were isolated from the active ethyl acetate soluble fraction of *C. odoratus* and was investigated for antioxidant property using DPPH scavenging assay. (Lee *et al.*, 2008). *C. odoratus* showed declined proliferation of human hepatocellular carcinoma (cell line used: Hep3B) by inducing apoptosis and arresting the G0/G1 phase of cell cycle (Pham *et al.*, 2016). *C. odoratus* also gave 57.3% *in-vitro* pancreatic lipase inhibitory activity (Sharma *et al.*, 2005). Traditionally, this grass is used to treat wounds, piles and tumours in Bangladesh by traditional medicinal practitioners (Rahmatullah *et al.*, 2009).

Since, there is no reported data on analgesic, anthelmintic, cytotoxic activity, and antioxidant properties and total phenolic and tannin content of *C. odoratus*, the present study was undertaken to elucidate and evaluate these bioactivities to justify its medicinal use.

Materials and Methods

Plant collection and extraction: *C. odoratus* plant was procured from Sataish area of Gazipur district, Bangladesh in June, 2017 and experts at Bangladesh National Herbarium, Mirpur, Dhaka has identified it (voucher specimen no.: 45931). The

whole plant was taken except the root and properly washed with fresh water to remove dirt, was shade dried for few days. The plant was then crushed into coarse powder with grinding machine. The powdered plant materials were stored at room temperature for further use. Five hundred gram of the dried-crushed materials were then soaked with about 1500 ml ethanol in an amber glass container. This mixture was occasionally shaken and stirred for 7 days and eventually was filtered with a piece of clean white cotton. Then it was again put through filtration using Whatman No. 1 filter paper. The filtrate was evaporated and dried at room temperature with continuous air flow. Total yield of COET was 2.05%.

Chemicals: Sodium carbonate and Folin-Ciocalteus's reagent were obtained from Merck (Damstadt, Germany), potassium dichromate, gallic acid (GA), ascorbic acid (AA), AlCl₃, H₂SO₄, Nitric acid, NaOH, DMSO, EDTA, Diclofenac-Na, sodium nitrite, tween-80 and FeCl₃ were purchased from the Sigma Chemical Co.

Phytochemical screening: Standard chemical reagents were used to identify and categorize the chemical constituents of COET using some positive control processes, for example, reducing sugar with Fehling's Solution and Benedict's reagent, combined reducing sugar with Fehling's Solution, glycosides with NaOH solution, Protein-Xanthoprotein with HNO₃, flavonoids with concentrated H₂SO₄, dilute HCl and dilute NaOH, Terpenoid with H₂SO₄, acidic compound with NaHCO₃ solution, alkaloid with Mayer's and Dragendroff's reagent, saponins with distilled water and tannin with FeCl₃ and K₂Cr₂O₇ solution (Ghani, 1995).

DPPH free radical scavenging assay: Quantitative antioxidant activity of COET was estimated by DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay (Choi *et al.*, 2000). In this method, 0.004 % (w/v) DPPH solution was prepared in ethanol and 3 ml of it was mixed with 1 mL ethanolic crude extract at a different concentration. Subsequently, the mixture was vigorously shaken and kept in a dark place at room temperature for about 30 minutes. UV absorbance

was measured at 517 nm. Percentage of scavenging activity was measured by following equation:

$$\% \text{ DPPH free radical scavenging activity} = [(A_0 - A_1)/A_0] \times 100$$

Here, A_0 is the absorbance of control, and A_1 is the absorbance of *C. odoratus*/positive control. The % scavenging activity was then plotted against log concentration and a graph for IC_{50} was calculated.

Estimation of total phenolics: Total phenolic content of COET was determined by Folin-Ciocalteu technique (Borah et al. 2011). 2 ml Folin-Ciocalteu reagent was diluted 10 times with 2 ml Na_2CO_3 (75 g/l) and water (1:10 v/v) and an aliquot of COET or positive control was mixed with this. To develop colour, the tubes were shaken for 15 seconds and then the mixtures were allowed to stand 20 minutes at room temperature. UV absorbance was measured at 750 nm. COET or positive control was evaluated at 0.1 mg/ml concentration. Gallic acid equivalent, GAE (mg of GA/g of dried plant extract) was used to express total phenolic content. Then a gallic acid standard calibration curve was developed with a positive control curve equation of $y = 0.062x - 0.0153$, $R^2 = 0.982$.

Determination of total tannins: Total tannin content of COET was determined by using Folin-Ciocalteu (FC) reagent (Marinova et al., 2005). To begin the process, 0.1 ml COET (positive control) was added to 0.5 ml FC reagent and 7.5 ml distilled water. This preparation was shaken and kept for 5 minutes. Then 1 ml 35% Na_2CO_3 solution was added by following a dilution with 10 ml distilled water. This preparation was then kept at room temperature for 30 minutes. UV absorbance was measured at 725 nm against blank after 30 minutes. Gallic acid equivalent, GAE (mg of GA/g of dried plant extract) was used to express total tannin content. Then gallic acid standard calibration curve was developed and positive control curve equation was $y = 0.0209x - 0.0214$, $R^2 = 0.904$.

Acetic acid induced writhing inhibition test: To assess the analgesic activity of COET, acetic acid induced writhing method was rendered on mice (Ahmed et al., 2004). Swiss-albino mice of any sex

(n=5) weighing 25-40 g were taken as study subjects. All subjects were unfed for 2 hrs prior to commencing the experiment. Subjects were divided into four groups. 1% tween-80 solution in water (10 ml/kg) was given to Group-I as the control, Group-II received Diclofenac-Na as positive control at 25 mg/kg body weight dose. The other two groups, Groups-III and -IV were treated with COET dose of 250 and 500 mg/kg body weight, respectively. 30 minutes later, 0.7% acetic acid was intra-peritoneally injected into subject animals. 5 minutes later writhing number was counted for the period of 15 minutes.

Evaluation of anthelmintic activity: Anthelmintic activity of COET was investigated on live adult earth worm *Pheretima posthuma* (Ghosh et al., 2005). Four (4) different groups were made having four (4) parasites in each of them. COET concentrations of 25 and 50 mg/ml and standard solution (i.e., standard Albendazole) of 15 mg/ml were prepared in PBS. They were then transferred to petri dishes. 0.1% tween-80 in PBS was used to treat the control group. Every petri dish had four (4) parasites and their activities were observed. The paralysis time (i.e., when the parasites had no movement without robust shaking) and the death time (i.e., when the parasites did not show any movement or response upon vigorous shaking, treatment with warm water (50°C) or external stimuli) were recorded. As compared to control, the time required for paralysis and death of parasites were used to assess the anthelmintic activity of COET.

Brine shrimp lethality bioassay: Brine shrimp lethality bioassay was employed to evaluate the cytotoxic activity of COET (McLaughlin et al., 1998). *Artemia* was chosen to convey this test. Artificial sea water was produced by using 3.8% NaCl solution and the brine shrimp eggs were hatched for 1 day (24 hrs) to mature the shrimp (i.e., nauplii). Stock solution of 5 ml was serially diluted to make concentrations 320, 160, 80, 40, 20, 10 and 5 µg/mL. 5 ml stock solution of vincristine sulphate (positive control) was serially diluted to make concentrations 5, 2.5, 1.25, 0.625 and 0.325 µg/ml. Ten (10) different test tubes were taken and ten (10)

nauplii were taken in each of them with volume adjusted to 10 ml with sea water. The concentration of DMSO was kept at 10 μ l/ml. After 1 day (24 hrs), the test tubes were checked against light to count the number of alive shrimps. Lastly, the LC₅₀ values were measured from Probit Analysis Chart. Probit Analysis Chart was done with "LdP line software" to measure the LC₅₀ values (Bauer et al., 1966).

Statistical analysis: All analyses were double checked and passed in two replications. Mean \pm SEM was used to present data. All experimental parameters were evaluated for their significance level by correlation and regression analysis, the t-tests (P<0.05) was used. Microsoft Excel 2016 was used

for both statistical analysis and graphical presentation.

Results

Phytochemical screening: Phytochemical screening of *C. odoratus* plant extract (COET) showed the presence of alkaloids, tannins, flavonoids, glycosides, proteins, gums, steroids and acidic compounds, which are summarized in Table 1.

Total phenolic and tannin content: Gallic acid equivalent was used to determine and express total phenolic and tannin contents of COET (Figure 2). Total phenolic and total tannin content were 1.68 g GAE/100 g and 3.35 g GAE/100 g, respectively of *C. odoratus* plant extract as represented in Table 2.

Table 1. Result of phytochemical screening of ethanol extract of *C. odoratus*.

Phytochemical Group	Result
Reducing sugar	-
Combined reducing sugar	-
Tannins	+
Flavonoids	+
Saponin	-
Gums	+
Steroids	+
Alkaloids	+
Glycoside	+
Proteins	+
Acidic compounds	+

(+) = present; (-) = absent

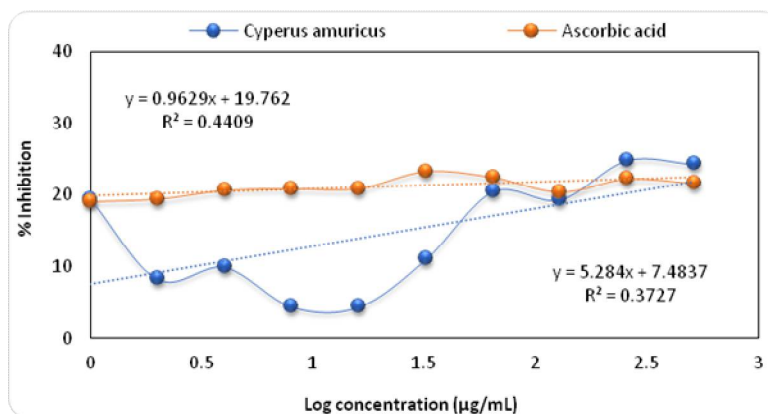


Figure 1. Comparison of % inhibition vs. log concentration graph for standard (ascorbic acid) vs. *Cyperus odoratus* ethanolic extract

Analgesic activity: COET exhibits a significant inhibition of writhing reflex as signified by the result of analgesic activity. COET reduced writhing by 36.23% and 61.32% at 250 mg/kg and 500 mg/kg doses, respectively. The positive control (Diclofenac-Na) showed 70.03% writhing inhibition at a dose of 25 mg/kg body weight (Table 3).

DPPH free radical scavenging activity: Quantitative antioxidant activity of COET was performed by DPPH free radical scavenging assay, where the IC₅₀ value for scavenging free radicals of the extract was 8.05 µg/ml, whereas standard ascorbic acid showed the IC₅₀ value of 31.40 µg/ml (Figure 1).

Table 2. Phenolic and tannin content of ethanol extract of *C. odoratus*.

Polyphenolic compounds	Content
Total phenol	1.68± 0.001
Total tannin	3.35 ± 0.001

Here, each value represents the average of two analysis ± standard error of mean expressed in terms of gm GAE /100 gm dried plant extract.

Anthelmintic activity: The anthelmintic activity of COET and standard Albendazole are shown in Table 4 and Figure 3. COET caused paralysis at approximately 70 minute and 44 minute at concentrations of 25 and 50 mg/ml, whereas the approximate death times were observed at 72 minute and 46 minute at respective concentration. Standard Albendazole also showed paralysis at around 18 min and death at 26 minute for 15 mg/ml concentration. Extract showed more effectiveness at higher concentration (50 mg/ml) against *Pheretima posthuma*.

Brine Shrimp Lethality Bioassay: The percent mortality of the nauplii caused by both the plant extract and the positive control (Vincristine Sulphate) is expressed in Figure 4. The LD₅₀ of sample as well as positive control was calculated by Probit analysis software (LdP Line software, USA). The LD₅₀ for COET was found to be 11.344 µg/ml whereas the positive control (Vincristine Sulphate) showed LD₅₀ at 0.7158 µg/ml (Table 4).

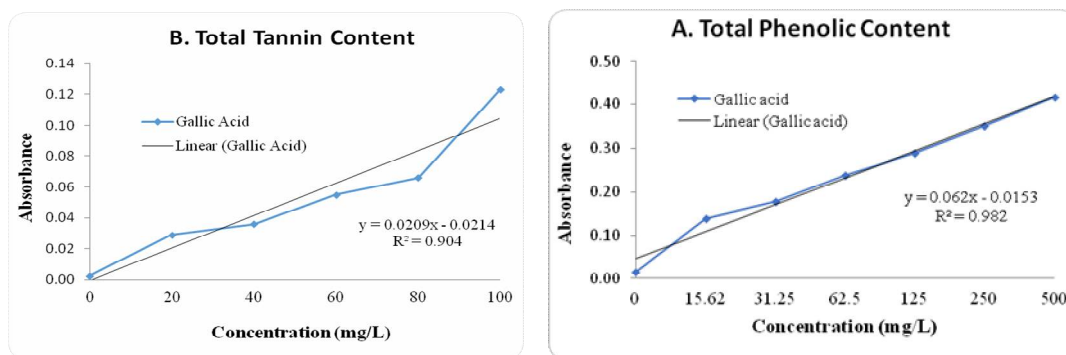


Figure 2. Determination of total phenolic (A) and total tannin (B) contents.

Table 3. Analgesic activity of COET in acetic acid induced pain in mice model.

Treatment (n=5)	Dose (mg/kg)	Mean writhes (no)	% Inhibition
Control	1% Tween-80	23.4 ± 3.14	---
Diclofenac-Na	25	7 ± 1.04*	70.03
Extract 250	250	16.6 ± 1.4**	36.23
Extract 500	500	11.6 ± 0.7***	61.32

Values are expressed as mean ± SEM, SEM= Standard error for mean. n = number of mice (5), *P = 0.0011, **P = 0.0844 and ***P = 0.0064 vs. control, Student's t-test.

Table 4. Anthelmintic activity of COET.

Treatments Concentration (mg/ml)	Worm No.	Time taken for paralysis (min)	Mean time of paralysis (min \pm SD)	Time taken for death (min)	Mean time of death (min. \pm SD)
Control 0.1% Tween-80 in PBS	C1	-			
	C2	-			
	C3	-	-	-	-
	C4	-			
Standard Albendazole 15	S1	15.25		23.32	
	S2	18.45		27.55	
	S3	21.11	18.01 \pm 2.45	28.22	26.12 \pm 2.22
	S4	17.21		25.39	
Extract 25	T1	65.4		67.26	
	T2	68.48		71.19	
	T3	73.19	70.19 \pm 3.55	74.21	72.38 \pm 3.60
	T4	72.08		75.29	
Extract 50	T1	30.21		31.42	
	T2	44.47		47.49	
	T3	46.28	44.04 \pm 9.77	49.45	46.40 \pm 10.84
	T4	53.56		57.25	

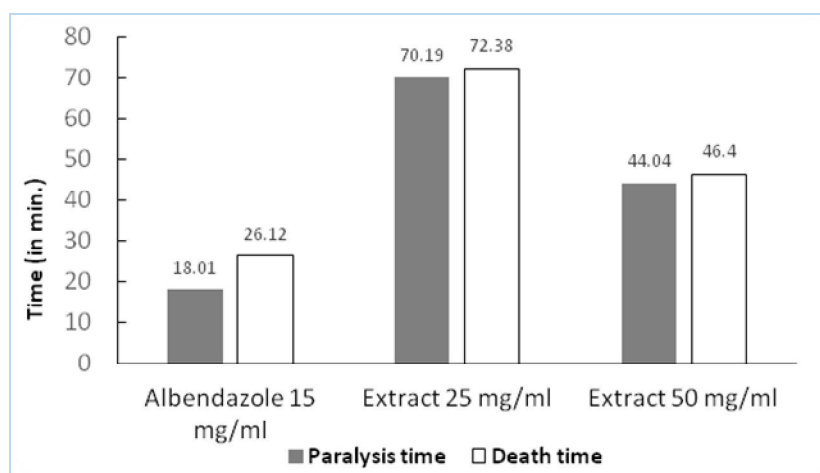


Figure 3. Determination of anthelmintic activity of COET through paralysis time and death time.

Table 5. LD₅₀ for extract and standard with lower and upper limit.

Treatment	Conc. (μ g/ml)	Lower limit (μ g/ml)	Upper limit (μ g/ml)
Extract	11.344	6.1642	16.7322
Standard	0.7158	0.4123	1.0293

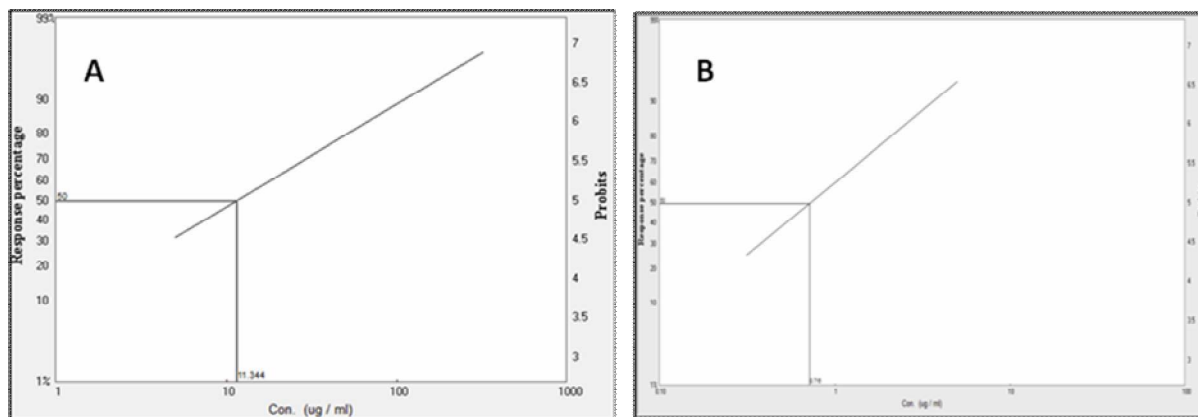


Figure 4. Graphical representation of brine shrimp lethality bioassay and LD₅₀ for COET (A) and positive control (*i.e.*, Vincristine sulphate) (B) by LdP line software.

Discussion

Antioxidant activity is one of the most important pharmacological properties of plants. To test the free radical scavenging activity or the antioxidant activity of plant extracts and foods a stable free radical, DPPH is widely used (Porto *et al.*, 2000; Soares *et al.*, 1997), which is readily scavenged by antioxidants (Lu and Yeap Foo, 2001). The scavenging ability of the extract was found to be concentration-dependent and expressed as IC₅₀ (*i.e.*, sample concentration required to decrease the initial concentration of DPPH by 50%) values. A higher antioxidant activity is indicated by a lower IC₅₀ value. In this study, the ethanol extract of *C. odoratus* showed an acceptable IC₅₀ value, which is compared to standard ascorbic acid (Figure 1) and it indicates the presence antioxidant components in the plant extract. The result also suggests that the plant contains phytochemical constituents that are capable of donating hydrogen in order to protect the cell from potential damage.

Plant phenolic compounds such as flavonoids, phenolic acids, and tannins present in the fruits and vegetables have potential biological activities including antiatherosclerotic, anticancer and anti-inflammatory activities and such activities might be associated with their antioxidant properties (Chung *et al.*, 1998). Plant phenolic compounds containing hydroxyl groups have a good scavenging capacity (Kahkonen *et al.*, 1999; Naczka and Shahidi, 2004).

The result (1.68 g GAE/100g of dried extract) proved that the extract possesses phenolic compounds containing hydroxyl groups.

Bitter plants contain polyphenols such as tannins, which have astringent properties and thus they can bind to protein molecules to precipitate or shrink them. Tannins contain sufficient hydroxyl groups and other free radicals for instance carboxyl and they bind proteins and other macromolecules. These free radicals protect cell damage (AfifyAel-M. *et al.*, 2012). Some studies specify that tannins are effective against ulcerated or inflamed tissues and they also possess a good anticancer activity (Ruch *et al.*, 1989; Motar *et al.*, 1985). Our studied result suggests that the extract contains considerable amount of tannins that may be a source of free radicals to protect the cell from death and satisfy the possible mechanism of plants' free radical scavenging properties.

Pathological condition such as tissue injury induced pain results in the local release of prostaglandins, cytokinins, leukotrienes etc. that act on the nerve terminals in both activating them directly and enhancing their sensitivity to other stimulations (Kanodia and Das, 2008; Goldstein *et al.*, 1970). Intraperitoneal administration of acetic acid produces pain by consequent abdominal writhing due to the release of mediators like prostaglandin E2 and other lipooxygenase products (Sulaiman *et al.*, 2008). There are mainly two

prostaglandins, prostacyclines (PGI₂) and prostaglandin E (PG-E), which are responsible for pain sensation due to the excitation of A δ -nerve fibers (Lourens *et al.*, 2004). Thus, ethanol plant extract of *C. odoratus* may produce analgesic activity due to the inhibition of prostaglandin synthesis by blocking of lipooxygenase and cyclooxygenase activities. In this study, extract of *C. odoratus* showed a comparable writhing inhibition to Diclofenac-Na, standard analgesic drug. The extract showed analgesic activity in a dose dependent manner.

The extracts showed anthelmintic activity in a dose-dependent manner. Evaluation of anthelmintic activity was compared with reference standard Albendazole. Phytochemical screening revealed that the presence of tannins along with other chemical constituents contained within them. Tannins have been reported to be responsible for anthelmintic activities (Niezen *et al.*, 1995; Shrestha *et al.*, 2009), as they can cause the death of parasites upon binding to free proteins in the gastrointestinal tract of host animal (Athanasiadou *et al.*, 2001) or glycoprotein on the cuticle of the parasite (Thompson *et al.*, 1995).

The brine shrimp lethality bio assay is a rapid, inexpensive and simple bioassay for testing plant extract's bioactivity, which in most cases correlates reasonably well with cytotoxic and anti-tumour properties (McLaughlin *et al.*, 1993). This assay was proposed by Michael *et al.* in 1956 and developed by Vanhaecke and co-workers in 1981 and improved by Sleet and Brendel in 1983. The brine shrimp lethality bioassay has been widely used in the primary screening of the crude extracts and the isolated compounds to assess the toxicity towards brine shrimps (Meyer *et al.*, 1982). This assay is recommended for the detection of antitumour and pesticidal compounds due to its simplicity and low cost (Mazid *et al.*, 2008). The assay is a useful tool for preliminary assessment of toxicity. Fungal toxins, plant extract toxicity, heavy metals, pesticides and cytotoxicity can be detected with the help of this assay (Harwig and Scott, 1971; McLaughlin *et al.*, 1991; Pelka *et al.*, 2000). Bioactive compounds can

be isolated from plant extracts using brine shrimp assay (Sam, 1993). The significant lethality of brine shrimp due to extracts of *Cyperus odoratus* is an indicative of the presence of potent cytotoxic components.

Conclusion

The present study reveals that the ethanol extract of *C. odoratus* plant possess antioxidant activity and significant analgesic activity as well as cytotoxic and anthelmintic activity which justifies its uses in folk medicine. These preliminary studies do not describe the actual mechanism for various pharmacological actions reported in this manuscript. However, more extensive studies (including isolation and identification of active compounds and in-depth pharmacological mechanistic assays) are required to determine the exact mechanism of action of the extract and its active compound(s) to authenticate it as a potent antioxidant, analgesic, cytotoxic and anthelmintic agent.

Declaration of interest

The authors declare no conflict of interest.

Acknowledgements

The authors are grateful to Department of Pharmacy, ASA University Bangladesh and Dhaka International University for providing necessary instrumental facilities and others supports. We are also grateful to the Director of Bangladesh National Herbarium, Dr. Hosne Ara, for her kind assistance in deposition of voucher specimens.

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