Antioxidant, Analgesic and CNS Depressant Activities of *Commelina diffusa* Burm. f.

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ABSTRACT: *Commelina diffusa* Burm. f. is a member of Commelinaceae family, which is widely grown in crop land and treated as a weed. This plant has several important medicinal properties which have not been studied extensively. In this study, the crude powder of *C. diffusa* whole plant was extracted with 95% ethanol and different solvent fractions (*n*-hexane, chloroform and methanol) were prepared from the crude extract by solvent-solvent partitioning. All these plant samples were subjected to bioassays for evaluating the antioxidant, central analgesic, peripheral analgesic and CNS depressant activities. The crude extract and its methanol soluble fraction showed mild free DPPH scavenging property with IC_{50} values of 98.49- and 84.77-µg/ml, respectively as compared to the standard ascorbic acid (IC_{50} = 2.67 µg/ml). In the analgesic activity test, the *n*-hexane fraction of *C. diffusa* at doses of 200- and 400-mg/kg body weight exhibited significant (p<0.05) central analgesic activity (tail flick test) in mice. Similarly, all the test samples showed statistically significant (p<0.05) reduction in abdominal writhing induced by acetic acid. *C. diffusa* showed significant CNS depressant activity which was measured by 'open field test' and 'hole cross test'. Considering the potential bioactivities, the plant materials can be further studied elaborately to explore the activities of the purified compounds to aid in future drug development.

Key words: Analgesic, antioxidant, CNS activity, *Commelina diffusa*, phytochemicals.

INTRODUCTION

Bangladesh is a tropical country with a variety of medicinal plants, which are used as traditional medicines. Medicinal plants have been providing the human beings with the basic requirements of life from the very beginning of their existence. A wide variety of secondary metabolites isolated from medicinal plants can serve as lead for the development of new pharmaceuticals.[1,2] The 'Materia Medica' of the great Greek physician Dioscorides consists of some 657 medicinal plants which included opium, mint, onion, sage and coriander.[3] Different isolating techniques of modern science yields many compounds having excellent pharmacological action, for example, two anticancer drugs vincristine and vinblastine have been isolated from a periwinkle plant *Catharanthus roseus* possess antimitotic properties.[4,5]

The plant *Commelina diffusa* is under the plant family Commelinaceae which includes total 731 species divided in 41 genera.[6] Plants belonging to Commelinaceae are both perennials and annuals. There are three sepals in each flower, which may be of same size or different size.[7] In Bangladesh, there are also a wide number of species of this family.[8] The plant is widely distributed in the tropical regions of the Africa, America and Asia (including Bangladesh). Moreover, it is also found in some subtropical regions. The leaves of the plant are used

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as diuretic and febrifuge. It is also used as a remedy for irregular menstruation. The crushed plant is also used in the treatment of frequent urination, diarrhea, vomiting, laryngo-pharyngitis, tonsillitis and common colds. The fresh juice is also used for the treatment of poisonous snake bites.[9] In Chinese traditional medicine, the plant is used for the reduction of swelling, in the treatment of infection occurred urinary and respiratory tract, and other diseases like diarrhea, enteritis and hemorrhoids. The plant leaf is employed for the treatment of boil, abscesses, and wounds and joint pains. Moreover, the plant is also used in dermatitis, burns, as well as in snake bite and insect bite. Additionally, the plant is used in the treatment of tuberculosis, venereal disease, otitis media, malaria, leprosy, dysentery and various heart problems.[10]

The different parts of the plant are used as a remedy for irregular menstruation, frequent urination, diarrhea, vomiting, laryngo-pharyngitis, tonsillitis, headache, fever and common colds.[11,12] A significant antioxidant activity was found from the crude extract of *Commelina diffusa* compared to the standard substance ascorbic acid. The plant also showed positive hepato-protective and nephro-protective activities in leaf extract treated rats.[13,14] Sultana *et al.* also reported CNS depressant activity of methanolic extract of *C. diffusa*.[15] The antimicrobial activity of the plant was also established with a strong positive result.[16] The present research work has been undertaken with a keen interest to investigate the antioxidant, antimicrobial, analgesic and CNS depressant activities of the plant *Commelina diffusa* Burm. f.

MATERIALS AND METHODS

Collection and Identification of the plant. *Commelina diffusa* was collected from Sitakunda, Chattogram during the month of March, 2019. The plant was identified by the taxonomist of Bangladesh National Herbarium and a voucher specimen of this plant was deposited (Accession no. DACB 55436).

Extraction and preparation of plant materials. Following identification, the plant was washed with water to remove mud and other dust particles. It was first dried at room temperature and then in an oven at around 40 ^{o}C. The dried plants were ground to powder by a grinder. The powder (2.1 kg) was kept in an air tight bag and this was used throughout the investigations.

Extraction and solvent-solvent partitioning of the crude extract. The crude extract of the plant was obtained by cold extraction process. For this, about 800 g of the powdered material was taken in a clean, round bottom flask and soaked in 2.5 liter of 95% ethanol (5% water) solution. The flask was tightly closed by foil papers and kept for a period of 15 days with occasional shaking and stirring. After that the whole mixture was filtered using cotton plugs and the filtrate was concentrated with a rotary evaporator. The above process was repeated three times with fresh solvent to increase the amount of extract. Finally, all the filtrates were mixed together to get the crude ethanol extract. The crude ethanol extract was partitioned using different solvents (*n*-hexane, chloroform and methanol) and respective extracts were obtained.

Phytochemical screening. The crude extract and its different solvent fractions were analyzed for qualitative evaluation of phytochemicals using standard procedure. [17]

Antioxidant activity. The *in vitro* free radical scavenging activity of *C. diffusa* Burm. f. (whole plant) was evaluated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) by the method described by Brand-Williams *et al*.[18] A methanol solution (2.0 ml) of the plant samples at different concentration were added to 3.0 ml of a DPPH methanol solution (20 µg/ml). The antioxidant activity was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extract as compared to that of standard ascorbic acid by UV spectrophotometer.

Central analgesic activity. Evaluation of central analgesic activity of the plant *Commelina diffusa* was performed by tail flick method. The changes in sensitivity of test animals as an effect of analgesic activity of test samples were measured following the method described by Aydin *et al*.[19] A constant heat

stress was applied to the mouse tail which produced a pain sensation in the tail of mouse that caused the mouse to withdraw its tail from the hot object. In such tests, compounds having analgesic effect are expected to elongate the withdrawal time from the hot water. After administering the samples to the respective groups, about 3 cm of the tail of mice were immersed in hot water (maintained at 55ºC). The time required for withdrawing tail from the water was observed at 0, 30, 60, 90 and 120 min.

Peripheral analgesic activity. Acetic acid induced writhing method was used to evaluate the peripheral analgesic activity of the plant where acetic acid (1%) was administered intraperitoneally in the test animal to produce pain sensation.[20] In such tests, the number of writhing is counted and considered as indication of presence of pain sensation. Diclofenac sodium was used as the positive control. All mice of each group were observed individually to count the number of writhing they produced in 10 minutes. The % inhibition of writhing was calculated as following equation:

$$\% \text{ Inhibition} = \frac{\text{Average writhing of control} - \text{Average writhing of sample}}{\text{Average writhing of control}} \times 100\%$$

Central nervous system (CNS) activity. The central nervous system (CNS) activity of a test compound can be evaluated by a number of experimental models including open field test, hole cross test, thiopental sodium-induced sleeping test, forced swimming test, tail suspension test etc. In this present study, the open field [21] and hole cross test [22] were performed to evaluate potential CNS depressant activity of the extract *C. diffusa* plant. Here, diazepam at 1 mg/kg body weight was used as the standard.

Open field test. A specially designed open field was used for evaluating the locomotor activity of the test animals. The field was a combination of several alternate black and white squares separated by black borders. The field was surrounded by a wall of 40 cm height. The animals were divided into control (distilled water, 10 ml/kg, p.o.) standard (diazepam, 1 mg/kg, i.p.) and eight test groups (ME 200, ME 400, NHF 200, NHF 400, MF 200, MF 400, CF 200 and CF 400) containing five mice each. Following the intraperitoneal administration of the test samples, mice were kept in the field. About 30 min after each treatment, the experimental mice of the control as well as the treated groups were placed individually in the center of the open field and the number of the fields crossed were measured.

Hole cross test. A specially designed case (30 × 20 × 14 cm) with a wooden partition in the middle of the case, was used for this test. There was a hole in the partition at 7.5 cm height having a diameter of 3 cm. After administering each treatment (two doses of four fractions and standard), the animals were kept in one chamber of the cage. The mice were passed through the hole from one chamber to another and the number of passages was counted for 3 min at 0, 30-, 60-, 90-, and 120-min intervals.

Test animals. Swiss-albino mice of both sexes, aged 4-5 weeks weighing 25-30 g were procured from the International Center for Diarrheal Diseases and Research, Bangladesh (icddr,b), Dhaka, Bangladesh. The mice were kept for 7 days under standard housing conditions (25±1 °C temperature and 12:12 h day/night cycle) in animal house of Institute of Nutrition and Food Science, University of Dhaka. The icddr,b formulated rodent pellet food were fed to the animals. They also had free access to the tap water. The mice were kept on a polyvinyl cage with a layer of soft husk.

Statistical analysis. Where applicable, the data were expressed as mean ± SEM. The level of significance was verified by using Student's t-test and one-way ANOVA followed by Dunnett's multiple test, as required and $p < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Phytochemicals present in the plant. Various phytochemical screening processes indicated the presence of several compounds in the plant. These compounds are summarized in the table 1. Notably, flavonoids, phlobatannin and steroid were detected in all the fractions. Except the *n*-hexane fraction, glycosides were present in the three other factions. The presence of all these phytochemicals prompted us to investigate the biological activities for such type of compounds in the following sections.

Table 1. Presence of different phytochemicals in the plant extract of *C. diffusa.*

Phytoconstituents	Solvent fractions			
	n-Hexane	Chloroform	Methanol	Aqueous
Alkaloid	-	-	+	-
Anthraquinones	-	-	-	-
Flavonoid	+	+	+	+
Glycoside	-	+	+	+
Phlobatannin	+	+	+	-
Saponin	+	+	-	-
Steroid	+	+	+	+
Tannin	-	+	+	+

Antioxidant evaluation by DPPH method. This experiment showed that methanol fraction and crude extract of *C. diffusa* have greater antioxidant properties than other extracts which is presented as median inhibitory concentration (IC_{50}). Therefore, the response obtained in this experiment suggests that these extracts may contain compounds having free radical scavenging property. However, this cannot be confirmed without further confirmatory (total phenolic content test) and specific tests. The results of different extracts of the plant along with the standard are presented in Table 2**.** Mensah *et al.* (2014) previously reported the antioxidant activity of the ethanolic extract of the plant (IC_{50} = 11.35 µg/ml). In our study, different fractions exhibited varying degree of DPPH scavenging activity with varying IC_{50} values. Since we used slightly different method and since the experiment was done in different setting using different instruments, the observed IC_{50} values were a bit higher than that reported by Mensah *et al.* (2014).

Central analgesic activity. The *n*-hexane, chloroform and methanol fractions along with the crude extract of *C. diffusa* were used for evaluating central analgesic activity by tail flick method in mice. The experiment revealed that the *n*-hexane fraction (NHF) of the crude extract of the plant *C. diffusa* has better central analgesic activity than other fractions (Figure 1). This is evident from figure 1 that the NHF at the doses of 200- and 400 mg/kg significantly ($p<0.05$) prolong the tail withdrawal time after 0 min (NHF 200 = 2.05 sec and NHF 400 = 1.78 sec), 30 min (NHF 200 = 11.21 sec and NHF 400 = 19.43 sec), 60 min (NHF 200 = 20.12 sec and NHF 400 = 38.32 sec), 90 min (NHF 200 = 24.56 sec and NHF 400 = 42.32 sec) and 120 min (NHF 200 = 27.31 sec and NHF 400 = 36.23 sec) as compared the standard (after 0 min = 1.56 sec, after 30 min = 15.67 sec, after 60 min = 37.45 sec, after 90 min = 43.45 sec and after 120 min = 46.67 sec).

Table 2. **IC_{50} value of ascorbic acid and different extractives of *C. diffusa***

Extracts	IC_{50} (µg/ml)
Ascorbic acid	2.67
Crude methanol extract	98.49
n-Hexane fraction	432.53
Chloroform fraction	291.41
Aq soluble fraction	84.77

Peripheral analgesic activity. In the acetic acid induced writhing method, the intraperitoneal

injection of acetic acid (1%) produced strong contortions in all groups, which were counteracted by various fractions of the plant extract at varying levels. As compared to the standard (diclofenac), the strongest writhing inhibition were observed (Figure 2) for the aqueous soluble fractions at both doses, *i.e.* 200 mg/kg (72%) and 400 mg/kg (80%). The chloroform fraction (CF) also produced comparable writhing inhibition, 73% and 61%, at 200 mg/kg and 400 mg/kg, respectively. The other two fractions (NHF and ME) were next to follow the CF fraction, as is evident in figure 2. Overall, the various fractions of the plant exhibited strong peripheral analgesic effect.

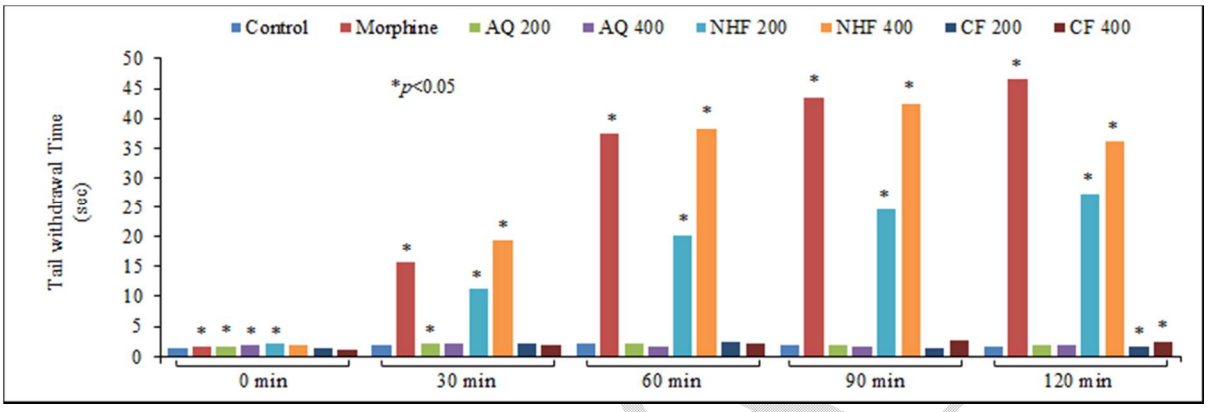

Figure 1. Tail withdrawal time of mice after administering different fraction of *C. diffusa*. Here, ME= mother extract, NHF= n-hexane fraction, CF= chloroform fraction and AQ= aqueous soluble fraction of the plant *C. diffusa*

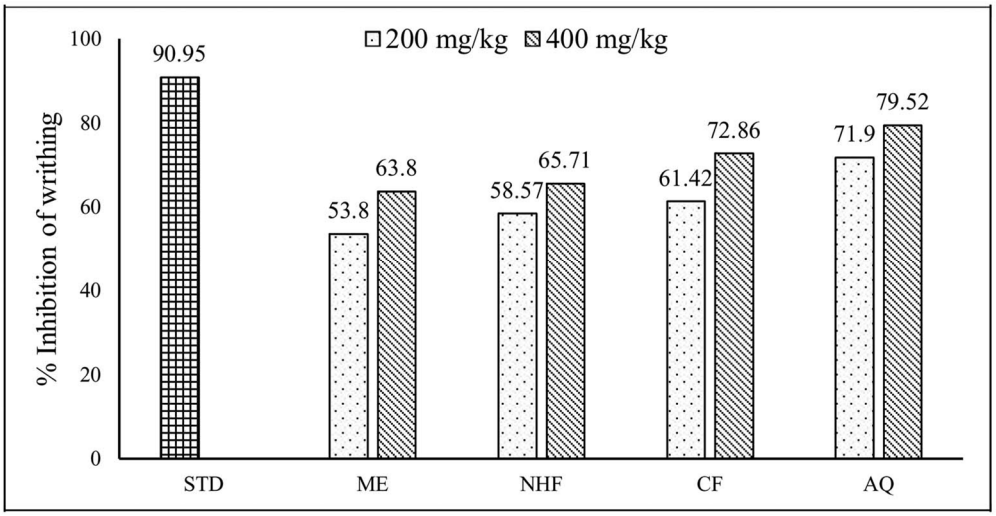

Figure 2. Comparison of % inhibition of writhing response among different test samples. Here, STD= standard drug (diclofenac sodium), ME= mother extract, NHF= *n*-hexane fraction, CF= chloroform fraction and AQ= aqueous soluble fraction of the plant *C. diffusa*.

Open field test. The open field test was performed to evaluate the CNS depressant potential of the crude extract and various fractions of *C. diffusa*. In this test, diazepam was used as the standard drug, which is reported to produce CNS depression and thereby causing a marked reduction of locomotion.[23] As expected, more CNS depression was produced in the standard group, causing the animal to slow down their movement. The number of visited squares by the test animals were recorded to measure such CNS depression potential for the standard as well as the crude extract and various fraction of *C. diffusa* (Table 3). Notably, the *n*-hexane fraction (NHF) and the chloroform fraction

(CF), exhibited dose-dependent activity as revealed from the comparatively less number of square crossed. Specifically, all the fractions including the crude extract exhibited pronounced inhibition while crossing the squares at the higher dose used (i.e. 400 mg/kg), as compared to the standard drug, diazepam (92% inhibition). Among all the fractions, the *n*-hexane fraction (NHF 400) produced the greatest inhibition (approx. 74%) with a remarkably reduced number of the fields crossed (15.4±1.15). This may be due to the presence of compounds in the NHF that might be responsible for alteration of locomotor function in the CNS.

Table 3. Central nervous system (CNS) activity by open field test and hole cross test.

Treatment	Dose	Open Field Test				Hole Cross Test			
		No. of squares crossed			% Inhibition of square crossed	No. of holes crossed			% Inhibition of hole crossed
		Avg	±	SEM		Avg	±	SEM	
Control*	10 ml/kg, p.o.	58.2	±	3.61	-	18.4	±	1.21	-
Diazepam	1 mg.kg, i.p.	4.8	±	0.77	91.75	1.2	±	0.18	93.48
ME 200	200 mg/kg, p.o.	42.1	±	1.79	27.66	12.8	±	1.24	30.43
ME 400	400 mg/kg, p.o.	32.0	±	1.00	45.02	11.2	±	0.58	39.13
NHF 200	200 mg/kg, p.o.	35.8	±	1.32	38.49	13.8	±	1.16	25.00
NHF 400	400 mg/kg, p.o.	15.4	±	1.15	73.54	11.4	±	0.46	38.04
CF 200	200 mg/kg, p.o.	32.7	±	0.91	43.81	12.6	±	0.67	31.52
CF 400	400 mg/kg, p.o.	25.5	±	1.00	56.19	11.6	±	0.96	36.96

*Normal saline

Hole cross test. Like the open field test, the hole cross test is another proven assay for measuring the CNS depressant activity. In the hole cross test, as described in the method section, the experimental mice receive the treatment and are allowed to pass through the holes in the specially designed case. The smaller number of passages through the holes, the more prominent the CNS depression activity produced. Accordingly, for our plant extracts and different fractions, the numbers of passages through the hole were counted and recorded. As demonstrated in the table 3, the observed data clearly indicates that the plant *C. diffusa* has moderately strong CNS depressant activity. Here, the methanol fraction (MF) produced the greatest CNS depressant activity (, followed by the crude extract (ME), the *n*-hexane fraction (NHF)and chloroform fraction (CF), with a range of 36-50% inhibition and in a dose-dependent manner.

The mechanism by which the plant extracts might involve interaction with the endogenous mediators within the body.[24] Some authors also suggested that the interaction with the serotonergic pathway might be responsible for producing such activities.[25] In short, *C. diffusa* has a potentially good CNS depressant activity. The compounds related to such bioactivity can be identified and used for developing drugs in future.

CONCLUSIONS

The qualitative phytochemical screenings of the plant extract revealed the presence of several important secondary metabolites including alkaloids, flavonoids, glycosides, phlobatannins, saponins, sterols and tannins. The crude extract of the plant and its different fractions (*n*-hexane, chloroform and aqueous) were subjected to a number of biological tests and their results were compared. The crude ethanolic extract as well as its mentioned fractions

revealed mild to moderate antioxidant and activities in the DPPH free radical scavenging assay. The *n*-hexane fraction of the crude extract showed highly significant central analgesic activity when tested by tail flick method using morphine as a standard. The peripheral analgesic activity was highly significant for every fractions of the plant along with the mother extract. Most of the fractions along with the crude extracts also exhibited remarkable CNS depressant activities.-Taken together, the plant materials needs to be studied further to determine the bioactivity of the purified compounds for their unexplored efficacy and to rationalize their uses as traditional medicines.

CONFLICT OF INTEREST

The authors declare 'no conflict of interest'.

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AUTHORS' CONTRIBUTION

This work was carried out in collaboration between all authors. Author MSA designed and wrote the protocol for the studies. Author MMI performed the different test and analyzed the data with the active co-operation of MA and MR. Author MMI drafted the manuscript and MA helped him with technical advises and critical review and editing of the manuscript. Author MSA has taken care of the whole work. All authors meticulously read and approved the final manuscript.

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