Bangladesh Pharmaceutical Journal ***22****(1): 18-26, 2019 (January)*

In vivo Neurological, Analgesic and *In vitro* Antioxidant and Cytotoxic Activities of Ethanolic Extract of Leaf and Stem Bark of *Wedelia chinensis*

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(Received: June 25, 2018; Accepted: August 12, 2018; Published: January 17, 2019)

Abstract

Wedelia chinensis is a widely used anti-inflammatory and hepatoprotective medicinal plant in Bangladesh. In this study, analgesic, neurological, antioxidant and cytotoxic activities of the ethanolic extract of leaf and stem bark of *W. chinensis* were investigated. Oral administration of the ethanolic extract of *W. chinensis* (200- and 300-mg/kg body weight) was investigated on animal model for neurological activity using open field test and hole cross test. Acetic acid induced writhing method was used to assess the analgesic activity. DPPH (1,1-diphenyl, 2-picryl hydrazyl) radical scavenging assay was used for determining the antioxidant activity, while brine shrimp lethality bioassay was used for investigating cytotoxicity. The ethanol extract of the plant produced significant reduction (P<0.05) of locomotion in both doses (200- and 300-mg/kg body weight) indicating pronounced neurological activity. Oral administration of alcoholic leaves and stem extracts significantly ($p < 0.05$) inhibited writhing response in mice. The percentage of scavenging of DPPH free radical was found to be concentration dependent with IC_{50} value of 44.10 ± 0.65 and 38.96 ± 0.50 µg/ml for leaves and stem extracts, respectively. Our findings indicate that *W. chinensis* may be a source of natural antioxidant with potent analgesic, neurological and cytotoxic activities.

Key words: *Wedelia chinensis*, Analgesic, Antioxidant, CNS depressant, Cytotoxic

Introduction

Analgesics can be described as those substances which reduces the sensation of pain by elevating pain threshold to external stimuli (Kumar and Shankar, 2009). Contemporary analgesics like opiates and non-steroidal anti-inflammatory medications might not continually be appropriate for all patients, significantly for those with chronic pain, because of the limitations of efficacy, facet effects and intolerability.

Thus, exploration of analgesics having lower side effects possibly those of natural sources is an urgent need (Sulaiman *et al.,* 2009). CNS depressants, sometimes referred to as sedatives and tranquilizers, are substances that can slow brain activity. This property makes them useful for treating anxiety and sleep disorders (Ramsay *et al.* 1974). Although there are several CNS depressants are available in the market, these are associated with a variety of autonomic, endocrine, allergic, haematopoietic and neurological side effects (Harvey, 1985). Therefore, a large number of complementary and alternative medicines are being used for the treatment of insomnia or anxiety disorder.

Oxidative stress results when there is excess production of free radicals and/or low antioxidant defense that results in chemical alterations of biomolecules inflicting structural and functional modifications (Dewan *et al.,* 2013; Sarwar *et al.,*

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2015). Oxidative damage is a significant modulator of certain diseases like cancer, inflammation, arthritis, diabetes and atherosclerosis (Nahar *et al.*, 2013). Antioxidants are micronutrients that have acquired importance in recent years due to their ability to neutralize free radicals or preventing damage caused by free radicals (Widowati *et al.*, 2014). Currently available synthetic antioxidants are shown to possess low solubility property and also promote negative health and moderate antioxidant activity. So, attention is given on their use and there is a trend to replace these synthetic drugs with naturally available antioxidants (Hossain *et al.*, 2014). The study of bioactive compounds from plant sources and extracts are of great importance because the general bioassay detects a wide spectrum of biological activities and a diversity of chemical structures of the particular plant (Tanaka *et al.*, 2014). One important proposition here is that at a higher dose toxicology is solely pharmacology. Thus, if toxic compounds are found, a lower, non-toxic, dose might reveal a useful pharmacological effect, concerning on a physiologic system (Pozzatti *et al.*, 2014). However, it has been revealed that the simple *in vivo* brine shrimp lethality test might be used as an understandable tool for screening and fractionation of physiologically active plant extract, because of its reasonable correlation with cytotoxic and other biological properties (Tanaka *et al.*, 2014).

It is well-established that plants and plant products exhibit various medicinal properties. Several herbal plants have been listed in the ancient literatures for their different medicinal values and their formulation has been found to be effective for the treatment of various diseases (Safavi *et al.*, 2014). A number of reports have identified that various species of *Wedelia* consist of different phytochemicals, which are responsible for demonstrating significant biological and pharmacological activities (Liu *et al.*, 2013). *W. chinensis*, locally known as '*Kesraj*' in Bengali, is a widely used plant in folkloric medicine. Various parts of this plant are used in the treatment of various diseases like jaundice, diarrhea, cough, chephalagia, diphtheria and pertussis, etc (Koul *et al.*, 2012). In the quest of searching plants having significant pharmacological and biological activities in Bangladesh, here we tried to investigate the crude ethanolic extract of leaf and stem bark of the plant *W. chinensis* for its analgesic, CNS depressant, antioxidant and cytotoxic activities and reported the results of our preliminary investigation.

Materials and Methods

Drugs and chemicals: Acetic acid (Merck, Germany), indomethacin (Square Pharmaceuticals Ltd.), tween-80 (BDH Chemicals Ltd), normal saline solution (0.9% NaC1) (Beximco Infusion Ltd.), diazepam (Incepta Pharmaceuticals Ltd.), DPPH (1,1-diphenyl, 2-picryl hydrazyl) (Sigma Chemical Co., USA), dimethyl sulfoxide (Merck, Germany) etc. were used for conducting the tests.

Test animals: Healthy albino mice of Swiss strain of either sex were used. They were collected from the animal resource branch of the "International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B)" and were housed in standard conditions of temperature (25 ± 2 °C), 12 hours light per day cycle, relative humidity of 45-55 %. They were fed the ICDDR, B formulated rodent food and water. Animals were kept and all operation on animals was done in aseptic condition. All animals received human care following the guidelines described in 'Guide for the Care and Use of Laboratory Animals', 8th edition, prepared by the National Academy of Sciences and published by the National Institute of Health (US).

Collection and preparation of plant material: The fresh plant parts (leaves and stem) were collected from Bogra, Bangladesh on June 20, 2014.

The plant was identified by the taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh and a voucher specimen was deposited in the herbarium unit (accession number DACB: 36575). The sun dried powdered leaves and stems (250 mg) of *W. chinensis* were macerated in 500 ml of 99.8% ethanol (Merck, Germany) separately. After 3 days of occasional shaking and stirring, the solutions were filtered using filter cloth and

Whatman® filter paper No. 1. The resulting filtrates were then evaporated in water bath maintained at 45 °C to dryness and thus a gummy concentrate of greenish colored extract was found. Finally, about 45.23 g ethanolic extract of leaves of *W. chinensis* named as ELWC and about 30.22 g ethanolic extract of stem of *W. chinensis* named as ESWC were found.

In vivo analgesic activity test: The analgesic activity of the crude ethanolic extracts of leaves and stem barks of *W. chinensis* were studied using acetic acid induced writhing model in mice (Basak *et al.*, 2015; Bhowmick *et al.* 2014). The animals were divided into six groups including control (Group I), positive control (Group II) and four test groups (Group III-VI) of two different doses. The animals of test groups were administered test substance at two different doses of 200 and 300 mg/kg body weight. Positive control group received indomethacin (standard drug) at the dose of 10 mg/kg body weight and vehicle control group was treated with 1% tween 80 in distilled water at the dose of 10 ml/kg body weight. Test samples, standard drugs and control vehicle were administered orally 30 min before intra peritoneal administration of 0.7% acetic acid. After 15 min of time interval, the writhing (constriction of abdomen, turning of trunk and extension of hind legs) was observed on the test animals for 5 min.

In vivo neurological activity by open field test: This experiment was carried out by the method described by Khan (Khan *et al*., 2014). The animals were divided into six different groups namely control, standard and test groups (n = 6 per group). The control group received vehicle 1% tween 80 in water (at the dose of 10 ml/kg body weight) while the test group received the crude extracts (at the doses of 200 and 300 mg/kg body weight) and standard group received diazepam at the dose of 10 mg/kg body weight orally. The animals were placed on the floor of an open field (100 cm×100 cm×40 cm) divided into a series of squares. The number of squares visited by each animal was counted for 3 min, on 0, 30, 60, 90 and 120 min during the study period.

Hole cross test: The test procedure was conducted according to the method described by Ali (Ali *et al.*, 2013). The animals were divided into six different groups as control, standard and test groups (n = 6 per group). The control group received vehicle (1% tween 80 in water at the dose of 10 ml/kg body weight) whereas the test group received ELWC and ESWC extracts (at the doses of 100 and 200 mg/kg body weight) and standard group received diazepam at the dose of 10 mg/kg body weight orally. Each animal was then placed on one side of the chamber and the number of passages of each animal through the hole from one chamber to the other was recorded for 3 min on 0, 30, 60, 90 and 120 min during the study period.

In vitro antioxidant activity: The ability of ethanolic extracts of leaves and stem bark of *W. chinensis* to scavenge 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals was estimated as previously described by Dewan (Dewan *et al.* 2013; Hasanuzzaman *et al.*, 2013). *W. chinensis* extracts (2 ml) with six different concentrations (ranging from 500 to 5 µg/ml) were mixed with 3 ml of a 0.1 M of DPPH solution. Here, ethanol was used as the vehicle. The absorbance was measured by a spectrophotometer at 517 nm at 30 min intervals against a blank. The percentage of radical scavenging activity was calculated by the following equation:

$$\text{Radical scavenging (\%)} = [(A_0 - A_1 / A_0) \times 100]$$

Where, A_0 = Absorbance of the control and A_1 = Absorbance of the sample extracts.

Lower absorbance values show higher free radical scavenging activity. Ascorbic acid was used as a reference standard in different concentrations (ranging from 500 to 5 µg/ml). The 50% inhibitory concentration value (IC_{50}) is indicated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radicals.

Cytotoxic activity: The cytotoxicity was conducted using brine shrimp lethality test following the method of Khatun (Khatun *et al.*, 2014). The brine shrimp eggs were placed in 1 liter of sea water, aerated and hatched for 48 hrs at 37°C to become nauplii. After 48 hours, ten brine shrimp nauplii were placed in a small container filled with seawater. Ethanolic extracts of *W. chinensis* leaves and stem

bark, serially diluted with 1% DMSO (Dimethyl sulfoxide), were then added to the container. The mortality of brine shrimp was observed after 24 hrs of treatment was given. An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality was plotted and the values of LC50 were calculated by probit analysis using SPSS (version 16.0). Vincristine sulphate was used as positive control.

Statistical analysis: Data were processed and analyzed using both MS Excel version 2007® and SPSS (version 16.0,

IBM Corporation, NY, USA). The results obtained were expressed as mean ± SEM (Standard error of mean) of six animals. For statistical analysis, ANOVA was followed by post hoc Dunnett's test for multiple comparisons. Effects were considered to be significant at the $p < 0.05$ level. The lethal concentrations of plant extracts, resulting in 50% mortality of the brine shrimp (LC_{50}) from the 24 hours counts and the dose-response data were transformed into a straight line by means of a trend line fit linear regression analysis.

Results and Discussion

Analgesic activity: The results showed that the pain relief was achieved in a significant ($p < 0.05$) dose dependent manner, at all test doses (200 and 300 mg/kg body weight) as shown in Table 1. Maximum writhing inhibition (80.93%) was observed at 300 mg/kg dose of ESWC while for ELWC, 300 mg/kg dose exhibited 76.20% inhibition. The inhibitory effect of indomethacin (58.50%) was lower than that of the highest dose of ELWC and ESWC.

Table 1. Effect of ethanolic extracts of leaves and stem barks of *W. chinensis* on acetic acid induced writhing in mice.

Groups	Treatment	Dose	No. of writhing (Mean ± SEM)	% Writhing inhibition
Group-I (Control)	1% Tween 80 in water	10 ml/kg body weight	24.50 ± 1.90	--
Group-II (Standard)	Indomethacin	10 mg/kg body weight	10.17 ± 1.50	58.50*
Group-III	ELWC	200 mg/kg body weight	7.67 ± 0.85	68.69*
Group-IV	ELWC	300 mg/kg body weight	5.83 ± 1.01	76.20**
Group-V	ESWC	200 mg/kg body weight	7.83 ± 1.11	68.04*
Group-VI	ESWC	300 mg/kg body weight	4.67 ± 0.51	80.93***

Here, n = 6, SEM = standard error of mean, ELWC = Ethanolic leaves extract of *W. chinensis*, ESWC = Ethanolic stem bark extract of *W. chinensis*. *P < 0.05, **P < 0.01, ***P < 0.001, treated animals compared with control animals.

CNS depressant activity by hole cross test: After statistical analysis of the experimental data (Table 2), it was observed that both the extracts of *W. chinensis* at 200- and 300-mg/kg dose gave rapid onset of action and produced sleeping which may be attributed to an action on the cerebral mechanism involved in the regulation of the sleep. The experiment proved that the stem bark extract of *W. chinensis* at the dose of 300 mg/kg body weight showed better CNS depressant activity in comparison to the standard drug diazepam, whereas the leaf extracts of *W. chinensis* at both the doses of 200- and 300-mg/kg showed lower CNS depressant activity than the standard drug.

CNS depressant activity by open field test: It was observed from the statistical analysis of the experimental data (Table 3) obtained in the open field test, that 300 mg/kg body weight dose of ELWC showed significant CNS depressant effect as it decreased the movement of the test animals when compared to the standard drug diazepam.

It was also observed from the study that ELWC at the dose of 200 mg/kg and ESWC at the dose of 300 mg/kg produced almost similar type of effect. However, the activity of ELWC at the dose of 200

mg/kg was lower than the standard drug. Thus the experiment proved that the leaves extract of *W. chinensis* has neurological activity.

Antioxidant activity: The 50% inhibitory concentration (IC50) of ethanolic extract of stem barks of *W. chinensis* (IC_{50} 38.96 ± 0.50 µg/ml) was significantly ($p < 0.05$) lower than that of the leaf extract (IC_{50} 44.10 ± 0.65 µg/ml) (Table 4). Both of these *W. chinensis* extracts had a lower scavenging activity than the standard ascorbic acid (IC_{50} = 35.03 ± 0.21 µg/ml), which was used as standard. These data revealed that, the percentage of free radical inhibition increased with the increasing of concentration of both the extracts. However, the percentage of free radical inhibition is higher in ESWC than that of ELWC.

Table 2. Effect of ethanolic extracts of leaves and stem barks of *W. chinensis* on hole cross test in mice.

Group	Treatment	Dose	Number of movements (Mean ± SEM)				
			0 min	30 min	60 min	90 min	120 min
Group-I (Control)	1% Tween 80 in water	10 ml/kg	17.00 ± 1.95	16.00 ± 1.20	14.33 ± 1.40	13.5 ± 0.95	12.17 ± 0.91
Group-II (Standard)	Diazepam	1 mg/kg	15.50 ± 1.13	6.67 ± 0.32**	4.17 ± 0.50**	2.33 ± 0.47***	1.17 ± 0.30***
Group-III	ELWC	200 mg/kg	13.60 ± 0.43*	6.50 ± 0.50**	4.20 ± 0.60**	2.33 ± 0.65***	1.83 ± .55***
Group-IV	ELWC	300 mg/kg	12.20 ± 0.75*	5.20 ± 1.10**	4.00 ± 0.50 ***	2.67 ± 0.95***	1.67 ± 0.65***
Group-V	ESWC	200 mg/kg	15.30 ± 1.79*	3.67 ± 0.47***	2.83 ± 0.58***	1.33 ± 0.15***	1.17 ± 0.15***
Group-VI	ESWC	300 mg/kg	14.50 ± 0.50	5.83 ± 0.95**	4.33 ± 0.38**	2.50 ± 0.50***	1.33 ± 0.29***

Here, n = 6, SEM = standard error of mean, ELWC = Ethanolic leaves extract of *W. chinensis*, ESWC = Ethanolic stem bark extract of *W. chinensis*. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, treated animals compared with control animals.

Table 3. Effect of ethanolic extracts of leaves and stem barks of *W. chinensis* on open field test in mice.

Group	Treatment	Dose	Number of movements (Mean ± SEM)				
			0 min	30 min	60 min	90 min	120 min
Group-I (Control)	1% Tween 80 in water	10 ml/kg	151.80 ± 1.93	150.5 ± 1.28	149.80 ± 1.65	147.70 ± 1.35	145.60 ± 1.41
Group-II (Standard)	Diazepam	1 mg/kg	161.20 ± 2.02*	86.20 ± 1.25**	64.30 ± 0.82**	43.80 ± 1.00*	12.30 ± 0.35
Group-III	ELWC	200 mg/kg	167.50 ± 2.64***	122.80 ± 2.00***	89.70 ± 2.05***	61.80 ± 1.23***	11.60 ± 0.46***
Group-IV	ELWC	300 mg/kg	161.30 ± 1.10***	105.80 ± 1.25***	77.80 ± 0.50 ***	54.20 ± 0.92***	10.80 ± 0.70***
Group-V	ESWC	200 mg/kg	158.50 ± 3.23***	82.70 ± 0.80***	65.20 ± 0.56***	44.50 ± 0.75***	15.30 ± 0.85***
Group-VI	ESWC	300 mg/kg	153.70 ± 1.40***	80.50 ± 0.95***	43.20 ± 0.69***	38.30 ± 0.85***	11.70 ± 1.04***

Here, n = 6, SEM = standard error of mean, ELWC = Ethanolic leaves extract of *W. chinensis*, ESWC = Ethanolic stem bark extract of *W. chinensis*. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, treated animals compared with control animals.

Cytotoxic activity: The lethal concentration (LC50) of the test samples after 24 hours was determined by a plot of percentage of the shrimps died against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the curve data by means of

regression analysis. The lethality of the extracts to brine shrimps was determined and the results are given in table 5. Vincristine sulphate (VS) was used as positive control and the LC_{50} value was found 8.907 µg/ml. The LC_{50} value of ELWC (8.118 µg/ml) was found better when compared with ESWC (LC_{50} = 9.267 µg/ml) and standard vincristine sulphate.

Table 4. Antioxidant activity of ethanolic extracts of leaves and stem barks of *W. chinensis.*

Sample	IC_{50} (µg/ml)
Ethanol (vehicle)	3.001 ± 0.07
DPPH (Standard)	35.030 ± 0.21*
ELWC	44.100 ± 0.65**
ESWC	38.960 ± 0.50*

Data represents mean ± standard deviation (n = 3) of duplicate analysis. Data are found to be significant by testing through one way ANOVA at 5% level of significance (*p < 0.05, **p < 0.01) compared to negative control.

Table 5. Cytotoxic potential of ethanolic extracts of leaves and stem barks of *W. chinensis.*

Sample	LC_{50} (µg/ml) ± SEM	Regression equation	R^2
DMSO (vehicle)	16.004± 0.11	y = 3.040x + 1.347	0.684
Vincristine sulphate	8.907 ± 0.03*	y = 49.977x + 1.6744	0.982
ELWC	8.118 ± 0.63*	y = 51.131x + 2.954	0.969
ESWC	9.267 ± 0.19*	y = 49.977x + 1.6744	0.982

ELWC = Ethanolic leaves extract of *W. chinensis*, ESWC = Ethanolic stem bark extract of *W. chinensis*, LC_{50} = Lethal concentration at which 50% test animals are dead. Here, level of significance *p < 0.05 LC_{50} was calculated relative to vehicle, DMSO.

The present study was conducted to elucidate diverse pharmacological activities of ethanolic leaves and stem bark extracts of *W. chinensis*. We observed that both the ethanolic leaf and stem bark extracts of *W. chinensis* has significant analgesic, CNS depressant, antioxidant, and cytotoxic activities with a reasonable safety profile. In our study, the analgesic activity was assessed by acetic acid induced writhing model. Acetic acid-induced writhing model can be described as a well suggested protocol in estimating the medicated agents for their analgesic property. Pain is generally induced by the liberation of endogenous substances and also because of some other pain mediators like as arachidonic acid via cyclooxygenase and prostaglandin biosynthesis (Khan *et al*., 2010). The acetic acid-induced writhing model is extensively used for the determination of analgesic activity because of its sensitivity and response to the molecules at a dose that is not effective in other models (Muhammad *et al.*, 2012). In this model, pain is produced by acetic acid through local inflammatory response by the release of free arachidonic acid from tissue phospholipids via cyclo-oxygenase (COX) and producing prostaglandin specifically PGE_2 and $PGF_{2\alpha}$. It is also seen that the level of lipoxygenas enzyme in peritoneal fluids is also increased by acetic acid (Jiang *et al.*, 2014). Substances that prohibit writhings must have significant analgesic activity which may be attributed by the inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Muhammad *et al.*, 2012). Several phytochemicals such as flavonoids, tannins, alkaloids etc. have been reported to possess analgesic activity (Zulfiker *et al.*, 2010). The preliminary study on *W. chinensis* revealed that the plant is abundant of a wide spectrum of phytochemicals like tannin, carotene, phytosterol, resin, gum, isoflavonoids, alkaloids and saponins (Koul *et al.*, 2012). These compounds may attribute to the potent analgesic activity of the ethanolic extracts of the plant.

Two different neuropharmacological models were used to study the CNS depressant activity of the ethanolic extracts of leaves and stem barks of *W. chinensis.* The results of the study provided evidence that extracts of *W. chinensis* leaves and stem barks induced sedative-hyponotic activity in test animals confirming their CNS depressant activity. The major inhibitory neurotransmitter in the central nervous system is the gamma-aminobutyric acid (GABA). Several anxiolytic, muscle relaxant and sedative-hypnotic drugs exhibit their action via GABA. Thus it may be assumed that the leaf and stem bark

extracts of *W. chinensis* may act by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization that lead to a reduction in the firing rate of critical neurons in the brain or the extracts may simply activate the GABA receptors directly (Riaz *et al.*, 2014). Again, research has shown that plants containing flavonoids, saponins and tannins are useful for the treatment of many CNS disorders as they reduce the locomotor activity of the CNS (Azanchi *et al.*, 2014). Earlier investigation of the phytoconstituents of *W. chinensis* proved the presence of these phytochemicals (Koul *et al.* 2012) which may also be responsible for the CNS depressant activity of the plant extracts though the key compound for producing such effect is yet to be discovered.

The DPPH assay is one of the most common and relatively quick methods used for testing radical scavenging activity of various plant extracts (Elmastas *et al.*, 2007). In our present study, the antioxidant activity was determined using this method. Results of this study indicated that, the IC_{50} in ethanolic extract of *W. chinensis* leaves is significantly lower than the IC_{50} of the alcoholic extract of stem barks. It is reported that several active compounds such as anthocyanins, saponins, tannins, flavones and polyphenols etc. are responsible for demonstrating antioxidant activity of plant extracts (Firdaus *et al.*, 2013). *W. chinensis* is proved to be a potent source of flavonoid, saponins and tannins (Koul *et al.*, 2012). Therefore, it may be said that these compounds may play the significant role in revealing the antioxidant property of the plant extracts.

We tried to evaluate the cytotoxic activity of *W. chinensis* using brine shrimp lethality bioassay method. The findings of the study showed that the ethanolic extract of leaves of *W. chinensis* possesses better cytotoxic activity in comparison to the stem bark extract. Previous studies have proved that several bioactive compounds like glycosides, alkaloids, flavonoids and saponis show cytotoxic activities due to their diverse chemical compounds (Vital and Rivera, 2011). Some of these are present in the plant extract which may be accountable for the cytotoxicity of the plant extracts. However, this type of cytotoxicity is non-specific. Thus, further studies including animal model should be conducted to test the possible antitumor or anti-carcinogenic activity of the plant extracts.

Conclusion

Our preliminary pharmacological studies on the ethanolic extract of *W. chinensis* leaves and stem barks provide scientific support for the use of this species in traditional medicine, particularly in various ailments related to pain and CNS disorders. However, the plant has also showed to have good antioxidant and cytotoxic properties. Our findings indicate that *W. chinensis* may be a source of natural antioxidant with potent analgesic, neurological, and cytotoxic activity. Therefore, further pharmacological investigations are required to understand its underlying mechanism of pain inhibition, and mode of action on the CNS. In addition, future bioactivity-guided phytochemical work should be carried out to identify any active constituent(s).

Author's contributions

SK carried out the collection of plants, extraction process and conducted the experiments. SK and MSS carried out conception and design of the study, analysis and interpretation of data. MSS wrote the manuscript, and done the statistical analysis. MSI revised the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors would like to thank Animal Research Division of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) for supplying mice and the National Herbarium of Bangladesh for identifying the plant sample.

Conflict of interest

Authors have no conflict of interest.

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