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Phytochemical and pharmacological screening of *Zingiber salarkhanii* Rahman et Yusuf roots growing in Bangladesh

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ABSTRACT

The ginger family (Zingiberaceae), one of the most versatile medicinal plants, is also a popular flavoring component in a variety of foods and drinks. The objective of the current study is to examine the phytochemistry and evaluate the biological effects of methanolic extracts from a unique ginger family plant (*Zingiber salarkhanii* Rahman et Yusuf). The analgesic (acetic acid-induced writhing method), CNS anxiolytic (Hole-cross method), cytotoxic (brine shrimp lethality method), and antioxidant (DPPH scavenging method) effects of the plant extract were studied. Then the data were analyzed by one-way ANOVA followed by Dunnett's test using SPSS (version 20). The result obtained assures that *Z. salarkhanii* root extract has a variety of active compounds and showed significant analgesic (58%) and CNS anxiolytic activity (22.7 ± 2.22), as well as this plant, exhibited strong cytotoxic (LC_{50} = 0.784 µg/ml) and free radical scavenging activity (IC_{50} = 1.322 µg/ml).

Keywords: Phytochemical screening, analgesic, anxiolytic, antioxidant, cytotoxic activities.

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INTRODUCTION

Medicinal plants are considered a rich source of ingredients that play a critical role in the development of human cultures across the world. These plants are highly recommended for their different therapeutic values. Medicinal plants hold potential futures as their activities could be decisive in the treatment of present studies and the future. Medicinal plants have shown immense potential and extensive research is possessing great outcomes (Uddin et al., 2014; Hossain et al., 2016; Islam et al., 2019; Rakib et al., 2019). These plants come in handy either as support of official medicine or act synergistically in the living system and also can prevent the reoccurrence of some diseases. Medicinal plants have provided lots of bioactive natural compounds like glycosides, saponins, flavonoids, phenolic compounds, steroids, tannins, gum and amino acids that hold a wide range of therapeutic and pharmacological potentials which are being used as raw materials for new drug discovery and development for different ailments, such as diuretic, anti-rheumatic, anti-inflammatory, anti-carcinogenic, hypoglycemic, antimicrobial, anticonvulsive, relaxation, neuroprotective (Hasan et al., 2017). For example, analgesics relieve pain as a symptom, without affecting its cause. They are used when the noxious stimulus (evoking the pain) cannot be removed or as an adjuvant to a more etiological approach to pain (Ibrahim et al., 2019). Several compounds with analgesic activity have been isolated so far from different plant origins which led the scientists to uncover this therapeutic area with a newer molecule with better pharmacokinetic and pharmacodynamics profile.

Anxiety disorder is increasingly recognized as a highly prevalent and chronic disorder with onset during the teenage years, with an incidence of 18.1% and a lifetime prevalence of 28.8% (Kessler et al., 2005). Pharmaco-therapeutic approaches for the management of anxiety

disorders include psychotropic drugs, but these agents are limited by their side-effect profile, the need for dietary precautions, and drug interactions (Chen et al., 2019). Regular use of benzodiazepines causes deterioration of cognitive functioning, addiction, psychomotor impairment, confusion, aggression, excitement, anterograde amnesia, physical dependence, and tolerance (Kumar and Sharma, 2006). These are some of the factors that aroused interest in many researchers to evaluate new compounds from plant origin in the hope of identifying other anxiolytic drugs with fewer unwanted side effects.

The majority of the diseases or disorders are mainly linked to oxidative stress due to free radicals. Free radicals and related molecules are classified as reactive oxygen species (ROS) for their ability to lead to oxidative changes within the cell. Antioxidants disrupt the process of lipid peroxidation by deactivating free radicals (Schulz et al., 2000; Chowdhury et al., 2017).

However, bioactive compounds are always toxic to the living body at some higher doses and it justifies the statement that 'Pharmacology is simply toxicology at higher doses and toxicology is simply pharmacology at lower doses (Mclaughlin et al., 1998). So bioactive compounds found from plant sources should be screened thoroughly to establish them as a pharmacologic candidate.

Zingiber salarkhanii, a new species (Rahman and Yusuf, 2013) from the Zingiberaceae family is anticipated to bear characteristic properties and pharmacological activities of the well-known Zingiberaceae family.

The family Zingiberaceae is considered one of the well-studied groups of the plant with great beneficial medicinal and nutraceuticals potential benefits to human health (Kumar, Asish, Sabu and Balachandran, 2013). Many species are economically valuable for their spices and perfume. The dried thick rhizome of turmeric (*Curcuma longa*) is commonly ground for culinary, medicinal, and cosmetic uses. The seeds of *Elettaria cardamomum* are the source of cardamom. Ginger is obtained from the fresh or dried rhizomes of *Zingiber officinale*, and a number of ornamental gingers are grown for their attractive flowers and foliage. Several species of shellflower (*Alpinia*) are cultivated as ornamentals.

Ginger lily (*Hedychium*) produces beautiful flowers that are used in garlands and other decorations.

Plants of the Zingiberaceae family consist of essential oils with distinctive aromatic profiles in all parts, mainly in the rhizomes and leaves. Their rhizomes are utilized as spices and as parts of traditional medicine. Economic importance includes the source of important spice plants, e.g., *Curcuma* spp., including *C*. *domestica* (turmeric), *Elettaria cardamomum* (cardamom), and *Zingiber* spp., including *Z*. *officinale* (ginger); some species are grown as cultivated ornamentals,e.g., *Alpinia* and *Hedychium*. Larsen et al. (1998) and Kress et al. (2002) show recent treatments of the family. A good depth finding of some of its actions and phytochemical analysis is reviewed below.

METHODS AND MATERIALS

Plant collection and preparation

Fresh rhizomes of *Zingiber salarkhanii* were collected from hilly areas of Chittagong like Khagrachari, cantonment and Sitakundu during monsoon (Sept. to Oct. 2019). Then the collection was identified by Dr. M Atiqur Rahman, Professor, University of Chittagong. As identified (Accession no: 03-2019), a large amount of the selected plant was collected for investigation purposes. They were washed with tap water, and then with distilled water, cut into small pieces, shade dried and ground to fine powder, about 420 g of the powder was soaked in 1800 ml of methanol for 15 days and then filtered and the crude methanolic extract was further freeze-dried and preserved at +4°C for further analysis.

Test animals

Swiss-albino mice of 4 to 5 weeks of age and weighing 25 to 30 g of female sexes each obtained from the Animal House of BCSIR, Chittagong were used for the experiment. They were kept in clean and only dry polypropylene cages with a 12-hour light-dark cycle at 25 ± 2°C and 45 to 55% relative humidity in the Animal House of the Food and Nutrition Department of the University of Chittagong. The mice were given a laboratory diet and water. Food was withdrawn 12 hours before and during the experiment. As these animals are very sensitive to environmental changes, they are kept before the test for at least 3 to 4 days in the environment where the experiment will take place.

Phytochemical screening

Different phytochemical Testing was carried out to determine the presence of different chemical groups in the extract, to represent the preliminary phytochemical studies. A small quantity of freshly prepared methanolic extract of roots of *Z. salarkhanii* was subjected to preliminary quantitative phytochemical investigation for detection of phytochemicals such as alkaloids, carbohydrates, glycosides, flavonoids, tannins, saponins, phenols, terpenes using the standard methods such as Keller-Killiani test for Glycosides, Molisch's test for Carbohydrates, Wagner's test for Alkaloids, Biuret test for proteins and amino acids (Roopashree et al., 2008).

Analgesic activity

Acetic acid-induced writhing test

The analgesic activity of the extract was conducted by using the acetic acid-induced writhing method (Suresha et al., 2014; Le Bars et al., 2001) in Swiss-albino mice

(*Mus musculus*) of the female sex. Twenty experimental animals were randomly selected and divided into four groups denoted Group-I, group-II, group-III and group-IV consisting of 5 mice in each group. Each group received a particular treatment (i.e. control, standard and different dose) of the methanolic extract. Group I mice were given saline water 10 ml/kg/Body weight, i.p.; group II mice were given 100 mg/kg/Body weight acetylsalicylic acid subcutaneously (s.c.) while group III and IV i.p. was given 200mg/kg/Body weight and 400 mg/kg/Body weight of the crude extract. After 1 hour of administration of the drug and extract, 0.6% glacial acetic acid (10 ml/kg/Body weight) was given i.p. to all the mice to induce pain (Ezeja et al., 2011). The number of writhes (visualized by contraction of the abdominal musculature and extension of the hind limbs) was then counted at 5 min intervals for 15 min. The percentage of protection against abdominal writhing was used to assess the degree of analgesia and was calculated by using the formula given below (Akindele et al., 2012). For the acetic acid-induced writhing test:

% inhibition = {(No. of writhes in the control group - No. of writhes in the test group) × 100} / No. of writhes in the control group.

Anxiolytic activity

Hole board test

The hole-board consists of a wooden box (40 × 40 × 25 cm) with 16 holes (each of diameter 3 cm) evenly distributed on the floor. The apparatus was 35 cm in height. Similarly, 20 Swiss albino mice (25 to 30 g) were selected randomly and kept on fast overnight and again separated into four groups of five animals denoted as Group I (negative control group), Group II (positive control group), and Group III and IV (test sample groups). These four groups of animals were treated by i.p. route with saline water (10 ml/kg/Body weight), diazepam (1 mg/kg/Body weight), and plant extract (200 and 400 mg/kg/Body weight) respectively. After one hour each mouse was placed in turn at one corner of the board and then renders the animal to move and dip its head into the holes. The number of head dips during a 5 min period was recorded for individual mice (Kessler et al., 2005; Dhara et al., 2002).

Cytotoxicity activity

Brine shrimp lethality bioassay

Brine shrimp lethality bioassay (Otang et al., 2013) was performed to see the cytotoxic effect of the root extract of *Z. salarkhanii* on brine shrimp nauplii. Brine shrimp eggs were hatched in the simulated seawater to get nauplii. 38 g of sea salt (pure NaCl) was weighed, dissolved in one liter of distilled water and then filtered off to get a clear solution of seawater. *Artemia salina* leach (brine shrimp eggs) was collected from pet shops and it was used as the test organism. Seawater was poured into the small chamber and brine shrimp eggs were added to the chamber. Two days were permitted to hatch the shrimp and to be matured as nauplii. Constant oxygen supply and light were carried out through the hatching time and they were taken for assay.

The desired different concentration of the test sample is prepared with the calculated amount of Dimethyl sulphoxide (DMSO). By visual inspection, the number of nauplii was calculated and taken in vials containing 5 ml of simulated seawater (Shaala et al., 2017). Then samples of different concentrations were added to the premarked vials through a micropipette. The vials are left for 24 h and survivors were calculated after 24 h.

In the present experiment, Vincristine sulfate was used as a positive control. The measured amount of the Vincristine sulfate was dissolved in DMSO to get a primary concentration of 500 µg/ml from which serial dilutions are made by using DMSO to get 250, 125, 100, 50 and 25 µg/ml. The positive control solutions were added to the premarked vials containing ten living brine shrimp nauplii in 5 ml simulated seawater to gain positive control groups.

The median lethal concentration (LC_{50}) of all test samples was calculated from the straight-line equation of the graph.

% of mortality can be calculated by using the following equation:

% Mortality = Nt/ No × 100 (Sathasivam and Lakshmi, 2017).

Where Nt = Number of killed nauplii after 24 h of incubation

No = Number of total nauplii transferred i.e., 10.

Antioxidant activity

DPPH assay

The free radical scavenging activities (antioxidant capacity) of the plant extracts on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the described method (Braca et al., 2001).

2.0 ml of methanol solution of the extract at different concentrations was mixed with 3.0 ml of a DPPH methanol solution (0.004%). The antioxidant potential was assayed from the bleaching of a purple-colored methanol solution of DPPH radical by the plant extract as compared to that of ascorbic acid by UV spectrophotometer (Dewan et al., 2013).

2.0 ml of a methanol solution of the sample (Control / extractives) at different concentration such as 25, 50, 100, 200, 300, 400 and 500 µg/ml were mixed with 3.0 ml

of a DPPH methanol solution (0.004%). After 30 min reaction period at room temperature in a dark place, the absorbance was measured at 517 nm against methanol as blank by a UV spectrophotometer.

Inhibition of free radical DPPH in percent (I %) was calculated as follows:

$$(I\ \%) = ((A_{blank} - A_{sample})/A_{blank}) \times 100$$

Where $\mathbf{A_{blank}}$ is the absorbance of the control reaction (containing all reagents except the test material).

Statistical analysis

All the results were expressed as mean ± SEM. P-value was calculated by one-way ANOVA using SPSS software version 20.0 (IBM Corporation, New York, NY, U.S.A.). Where, *p < 0.05, **p <0.01, ***p < 0.001, stands for significant, more significant and most significant respectively.

RESULTS

The present study was an attempt to determine the phytochemical properties and analgesic, anxiolytic, cytotoxic and antioxidant activities of methanolic extract of *Zingiber salarkhanii* and the results have been illustrated with graphs in Tables 1, 2, 3, 4 and 5, respectively.

Table 1. Phytochemical screening of methanolic extract of roots of *Z. salarkhanii.*

Test name	Test method	Methanolic extract
Glycosides	Keller-Killiani test	+
Carbohydrates	Molisch's test	+
Alkaloids	Wagner's test	-
Flavonoids	Shinoda's test	+
Terpenes	Salkowski reaction test	-
Phenol	Ferric chloride test	+
Tannins	General test	+
Saponin	Shake or foam test	-
Proteins and amino acids	Biuret test	-

Note: Here (+) = presence of constituents; (−) = absence of constituents.

Table 2. Peripheral analgesic activity of methanolic extract of *Z. salarkhanii* by acetic acid induced writhing test.

Animal group	Number of writhing (Mean ± SEM)	% of Inhibition of writhing
Control	36.8 ± 3.44093	0
Standard	13 ± 1.37840***	65.00
Methanolic extract (200 mg/kg/Body weight)	28.4 ± 1.20830*	23.00
Methanolic extract (400 mg/kg/Body weight)	15.6 ± 1.94936***	58.00

Note: Each value represents mean ± SEM (n= 5). One-way ANOVA was followed by Dunnett's t-test. ***P < 0.001, **P < 0.01, *P < 0.05 compared with the control.

Table 3. Effect of methanolic extract of *Zingiber salarkhanii* roots by hole board test in mice.

Animal group	Frequency of dipping					Mean ± SEM
	M1	M2	M3	M4	M5	
Control	24	16	15	16	14	17 ± 1.79
Standard	31	14	24.5	27	28.5	25 ± 2.95***
Methanolic extract (200 mg/kg/Body weight)	33.6	5.8	12.2	11.4	19	16.4 ± 4.78*
Methanolic extract (400 mg/kg/Body weight)	23.4	29.8	17	19	24	22.7 ± 2.22***

Note: Each value represents mean ± SEM (n= 5). One-way ANOVA was followed by Dunnett's t-test. ***P < 0.001, **P < 0.01, *P < 0.05 compared with the control. *Indicates the significance of the result.

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Note: Each value represents mean ± SEM (n= 5). One-way ANOVA was followed by Dunnett's t-test. ***P < 0.001, **P < 0.01, *P < 0.05 compared with the control. *Indicates the significance of the result.

Table 4. Cytotoxic activity of methanolic extract of *Z. salarkhanii* roots by brine shrimp lethality bioassay.

Concentration (µg/ml)	log C (µg/ml)	% Mortality	LC_{50} (µg/ml) based on log C
500	2.69	100 ± 0.12%***	0.784
250	2.39	100 ± 0.12%***	
125	2.09	90 ± 0.19%**	
100	2.00	80 ± 0.06%	
50	1.69	70 ± 0.02%*	
25	1.39	70 ± 0.01%*	

*Note: LC means lethal concentration; Each value represents mean ± SEM (n = 5). One-way ANOVA was followed by Dunnett's t-test. ***P < 0.001, **P < 0.01, *P < 0.05 compared with the control. *Indicates the significance of the result.*

Table 5. Superoxide scavenging (%) activity of methanolic extract of *Z. salarkhanii* roots by DPPH radical scavenging assay.

Absorbance of blank	Conc. (µg/ml)	Absorbance of extract	Inhibition (%) Mean ± SEM	IC_{50} (µg/ml)
0.755	500	0.029	96 ± 0.31%***	1.322
	400	0.040	94 ± 0.21%***	
	300	0.095	87 ± 0.15%**	
	200	0.135	82 ± 0.15%**	
	100	0.192	74 ± 0.06%	
	50	0.282	62 ± 0.005%	
	25	0.356	52 ± 0.001%*	

Note: IC means Inhibitory Concentration; Each value represents mean ± SEM (n = 5). One-way ANOVA was followed by Dunnett's t-test. ***P < 0.001, **P < 0.01, *P < 0.05 compared with the control. *Indicates the significance of the result.

Phytochemical properties

The results of the phytochemical group test revealed that carbohydrates, glycosides, phenol, tannins, and flavonoids were present in the root extract (Table 1).

Analgesic activity

Table 2 shows that there was a significant increase in the average number of writhes in both extract groups in a dose-dependent manner and standard in comparison with the control group. The crude methanolic extract at the afore-mentioned two doses (200 and 400 mg/kg/Body weight) was significantly susceptible to the inhibition of pain respectively by 23 and 58%. These results were statistically most significant (*p < 0.05, ***p < 0.001) when compared to control. Standard anti-nociceptive drug when administered at the dose of 100 mg/kg/Body weight, exhibited 65% of pain inhibition, which was also found statistically significant (***p < 0.001) in contrast with control. Table 2 and Figure 1 represent the results of the acetic acid-induced writhing test for analgesic activity.

Anxiolytic activity

The results of the Hole board test are manifested in Table 3 and Figure 2 to represent the anxiolytic effect of the crude methanolic extract of *Zingiber salarkhanii*. The extract doses (400 mg/kg) and standard diazepam

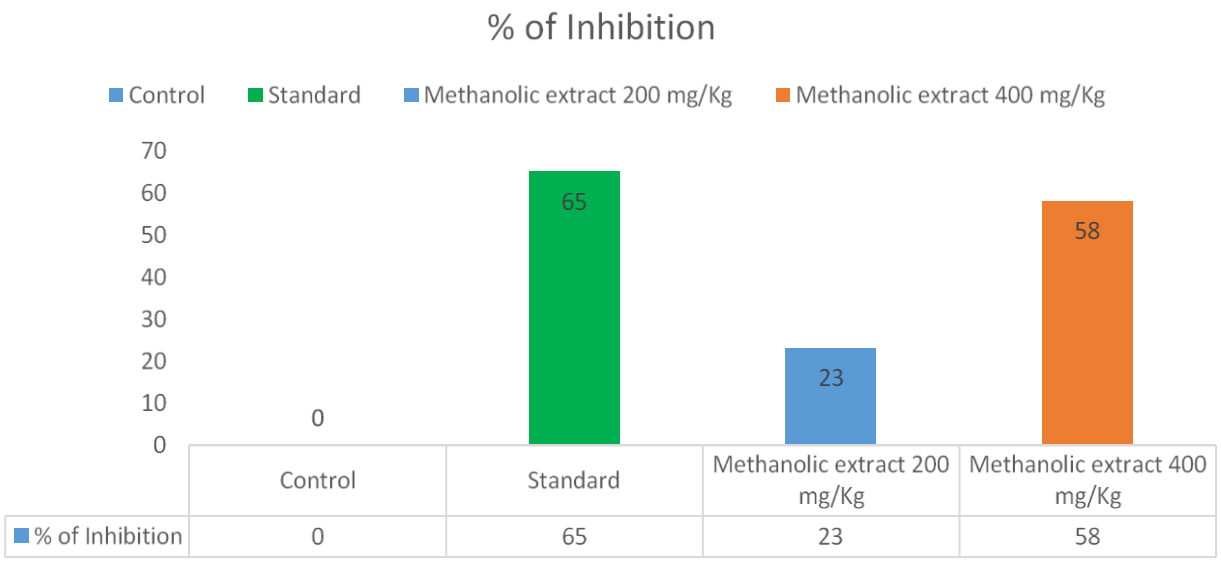

Figure 1. Analgesic effects of *Zingiber salarkhanii* methanolic extract in mice.

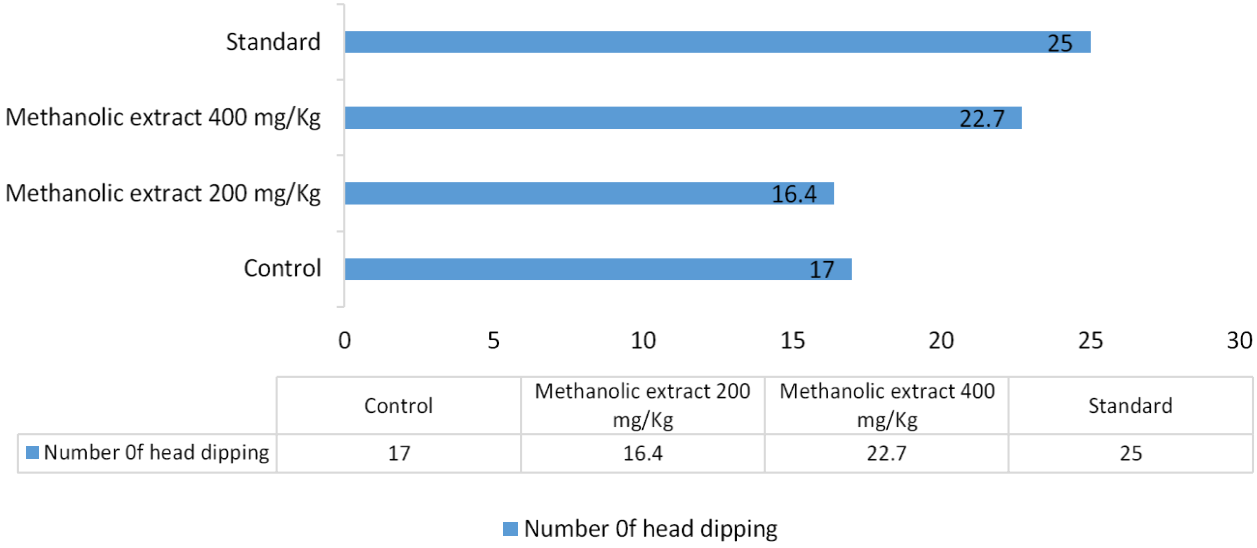

Figure 2. Anxiolytic activity of *Zingiber salarkhanii* extract on hole-board test method in mice.

(1 mg/kg) most significantly (***p < 0.001) ameliorated the number of head dipping when compared to control. On average 22.7 ± 2.22*** and 25.0 ± 2.95*** head dips were performed by group II and IV mice, respectively. However, no significant increases were observed in the number of head dipping in the case of mice treated with 200 mg/kg/Body weight of the extract.

Cytotoxic activity

The crude extract showed lethality indicating the presence of cytotoxic compounds in these extracts (Table 4). A completely randomized design was followed during this bioassay. The mortality rate of brine shrimp was increased according to the increasing concentration of the root extract (Figure 3). The concentrations at which 50 % mortality (LC_{50}) of brine shrimp nauplii was found to be 0.784 µg/ml represented in Figure 3 with a linear relationship.

Antioxidant activity

In the DPPH radical scavenging assay, the methanolic extract of *Z. salarkhanii* roots has shown potent free radical scavenging activity compared to standard Ascorbic acid. As shown in Table 5 and Figure 4, the

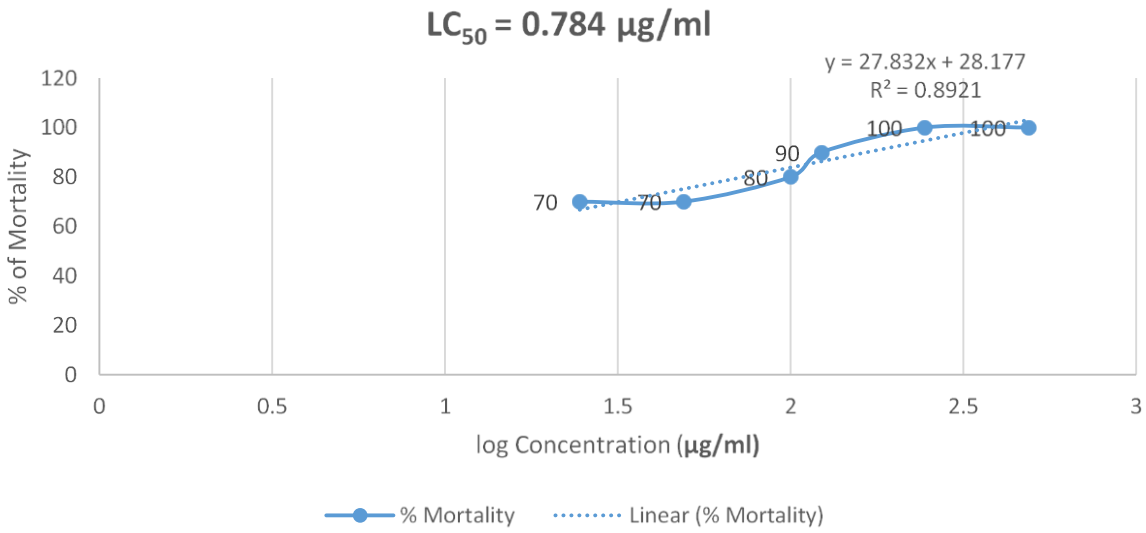

Figure 3. Cytotoxic activity of *Zingiber salarkhanii* methanolic extract.

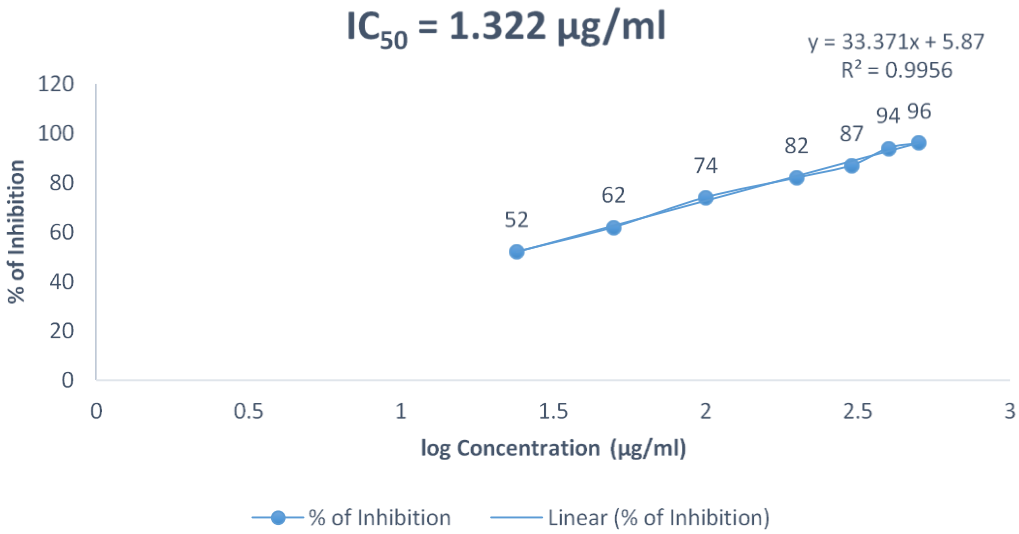

Figure 4. Antioxidant activity of *Zingiber salarkhanii* methanolic extract.

activity of the extract was found to be concentration-dependent, that is, the DPPH scavenging activity increased with increasing concentration of the extract. This assay was carried out on the ability of DPPH, a stable free radical molecule along with a standard antioxidant Ascorbic acid, and the result was evaluated using the parameter IC_{50}. Here, IC_{50} is the concentration of the antioxidant required for 50% scavenging of DPPH radicals in a specified time. The IC_{50} value of the methanolic extract of *Z. salarkhanii* roots is 1.322 µg/ml.

DISCUSSION

The above study was carried out to assess phytochemical screening, in vivo analgesic and anxiolytic effects and in vitro cytotoxic and antioxidant activity of crude methanolic extract of *Zingiber salarkhanii*. The phytochemical analysis conducted on the roots of *Zingiber salarkhanii* extract revealed the presence of carbohydrates, phenol, tannins glycosides, and flavonoids (Table 1). All of them are known to possess medicinal activity as well as physiological activity. These compounds may open the field of further analysis of their local usage for the management of different ailments.

From the results, it might be concluded that the plant extract possesses significant inhibition of pain and abatement of anxiety. Acetic acid-induced twisting of abdominal muscle constrictions (Raju et al., 2014), followed by the measurement of several writhing is one of

the most important methods for the determination of both central and peripheral analgesia, as had been done in the present study. In this test, pain sensation is elicited by instigating a localized inflammatory response which is associated with the release of free arachidonic acid from tissue phospholipid via cyclooxygenase (COX), and prostaglandin (PG) biosynthesis (Suleiman et al., 2014). In a nutshell, acetic acid administration increases the levels of PGE2 and PGF2α and also lipoxygenase products in the peritoneal cavity. These excess amounts of prostaglandins within the peritoneal cavity then accelerate inflammatory pain by facilitating capillary permeability (Sen et al., 2018). From our investigation, it was observed that methanolic extract of *Zingiber salarkhanii* at two different doses (200 and 400 mg/kg/Body weight) demonstrated a significant reduction of writhes in mice in a dose-dependent manner as compared to control (saline water). So it can be assumed that its cyclooxygenase (COX) inhibitory activity may reduce the production of free arachidonic acid from phospholipid or may inhibit the enzyme system, which is responsible for the synthesis of prostaglandins and ultimately relieve the pain sensation.

Gamma-aminobutyric acid (GABA) is an important inhibitory neurotransmitter found in the central nervous system. It has been reported that the GABAergic system substantiates a promising destination for new pharmacological techniques for the treatment of anxiety (Domschke and Zwanzger, 2008). The Hole board test is founded on a supposition that the head-dipping of animals is inversely proportional to their anxiety state in a moderately aversive environment (Sen et al., 2018). Therefore, the increased number of head dips into the holes on the board means a downfallen anxiety state. Our study revealed that methanolic extract of *Zingiber salarkhanii* 400 mg/kg, that is, increased head-dip counts without changing locomotion in the hole-board test most significantly (***$p < 0.001$) as compared with the control group. Flavonoids and terpenoids have been reported to be responsible for anxiolytic and sedative effects observed in different plant extracts (Carlini, 2003; Dhawan et al., 2001).

The brine shrimp lethality bioassay is comprehensively used as a basic screening method for testing the cytotoxic activity of plant materials (Bajracharya and Tuladhar, 2011; Rajabi et al., 2015). In the brine shrimp lethality bioassay, methanolic crude extract has shown potent cytotoxic activity compared to standard Vincristine sulfate and was found to be concentration dependent in each case, that is the % mortality was increased with increasing concentration of each extract.

Oxidative stress describes a condition in which cellular antioxidant defenses are insufficient to keep the levels of reactive oxygen species (ROS) below a toxic threshold (Kumar and Sharma, 2006). ROS are regulated by endogenous superoxide dismutase, glutathione peroxidase, and catalase but due to over-production of reactive species, induced by exposure to external oxidant substances or a failure in the defense mechanisms, damage to cell structures, DNA, lipids, and proteins (Valko et al., 2007) occur which increases the risk of more than 30 different disease processes (Adnan et al., 2019). Antioxidants that scavenge free radicals are known to possess an important role in preventing these free radical induced-diseases. The free radical scavenging activity of methanolic root extract of *Zingiber salarkhanii* is found highly significant as discussed above.

Conclusion

On the above analysis, it can be asserted that the methanolic extract of *Zingiber salarkhanii* is a promising source of important phytochemicals having the potential to reduce pain and anxiety as well as have the ability to suppress cell growth and antioxidant properties. Therefore, it may suggest further investigations to disclose the underlying causes of analgesic, anxiolytic, cytotoxic and antioxidant actions scientifically.

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Ethical clearance

This study was performed in strict accordance with the Institutional Ethical Guidelines of the Department of Pharmacy, University of Chittagong (Ref. No. Pharmacol/DPH/UC/03, 2018).

REFERENCES

Adnan MT, Amin MN, Uddin MG, Hussain MS, Sarwar MS, Hossain MK, Uddin SN, Islam MS, **2019**. Increased concentration of serum MDA, decreased antioxidants and altered trace elements and macro-minerals are linked to obesity among Bangladeshi population. Diabetes & Metabolic Syndrome: Clin Res Rev, 13(2): 933-938.

Akindele AJ, Ibe IF, Adeyemi OO, **2012**. Analgesic and antipyretic activities of Drymaria cordata (Linn.) Willd (Caryophyllaceae) extract. Afr J Tradit Complement Altern Med, 9(1): 25-35.

Bajracharya GB, **Tuladhar** SM, **2011**. Brine-shrimp bioassay for assessment of anticancer property of essential oils from spices. Nepal J Sci Technol, 12: 163-70.

Braca A, De Tommasi N, Di Bari L, Pizza C, Politi M, Morelli I, **2001**. Antioxidant principles from bauhinia t arapotensis. J Nat Prod, 64(7): 892-5.

Carlini EA, **2003**. Plants and the central nervous system. Pharmacol Biochem Behav, 75(3): 501-512.

Chen U, Hussain MS, Mazumder T, Uddin SN, Banik S, **2019**.

Neuropharmacological evaluation of methanolic extract of Costus speciosus Linn. rhizome in Swiss albino mice. Asian Pacific Journal of Tropical Biomed, 9(5): 217.
Chowdhury MI, Hasan M, Islam MS, Sarwar MS, Amin MN, Uddin SN, Rahaman MZ, Banik S, Hussain MS, Yokota K, Hasnat A, **2017**. Elevated serum MDA and depleted non-enzymatic antioxidants, macro-minerals and trace elements are associated with bipolar disorder. J Trace Elem Med Biol, 39: 162-8.
Dewan SM, Amin MN, Adnan T, Uddin SN, Shahid-Ud-Daula AF, Sarwar G, Hossain MS, **2013**. Investigation of analgesic potential and in vitro antioxidant activity of two plants of Asteraceae family growing in Bangladesh. J Pharm Res, 6(6): 599-603.
Dhara AK, Pal S, Nag Chaudhuri AK, **2002**. Psychopharmacological studies on Tragia involucrata root extract. Phytother Res, 16(4): 326-30.
Dhawan K, Kumar S, Sharma A, **2001**. Anti-anxiety studies on extracts of Passiflora incarnata Linneaus. Journal of ethnopharmacology, 78(2-3): 165-70.
Domschke K, **Zwanzger** P, **2008**. GABAergic and endocannabinoid dysfunction in anxiety-future therapeutic targets?. Curr Pharm Des, 14(33): 3508-17.
Ezeja MI, Omeh YS, Ezeigbo II, Ekechukwu A, **2011**. Evaluation of the analgesic activity of the methanolic stem bark extract of Dialium guineense (Wild). Ann Med Health Sci Res, 1(1):55-62.
Hasan MM, Hossain A, Shamim A, Rahman MM, **2017**. Phytochemical and pharmacological evaluation of ethanolic extract of Lepisanthes rubiginosa L. leaves. BMC Complementary Altern Med, 17(1): 496.
Hossain MF, Talukder B, Rana MN, Tasnim R, Nipun TS, Uddin SMN, Hossen SM, **2016**. In vivo sedative activity of methanolic extract of Stericulia villosa Roxb. leaves. BMC Complementary Altern Med, 16(1): 398.
Ibrahim M, Hussain MS, Sultana F, Abhi N, Ahmed T, Uddin SMN, Rahaman A, Azmerin M, Aka TD, Mosharraf S, Uddin AHMM, **2019**. Therapeutic Potentiality of Diospyros blancoi Linn. Seeds against Pain, Thrombus and Inflammation: An in vivo and in vitro Study. Asian J Biol Sci, 12: 36-41.
Islam MR, Naima J, Proma NM, Hussain MS, Uddin SN, Hossain MK, **2019**. In-vivo and in-vitro evaluation of pharmacological activities of Ardisia solanacea leaf extract. Clin Phytosci, 5(1): 32.
Kessler RC, Chiu WT, Demler O, Walters EE, **2005**. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry, 62(6): 617-27.
Kumar S, **Sharma** A, **2006**. Apigenin: The Anxiolytic Constituent of Turnera aphrodisiaca. Pharm Biol, 44(2): 84-90.
Le Bars D. Gozariu M, Cadden SW, **2001**. Animal model of nociception. Pharmacol Rev, 53: 597-652.
Mclaughlin JL, Rogers LL, Anderson JE, **1998**. The use of biological assays to evaluate botanicals. Drug Inf J, 32(2): 513-24.
Otang WM, Grierson DS, Ndip N, **2013**. Assessment of potential toxicity of three South African medicinal plants using the brine shrimp (Artemia salina) assay. Afr J Pharm Pharmacol, 7(20):1272-1279.
Rahman MA, **Yusuf** M, **2013**. Zingiber salarkhanii (Zingiberaceae), a new species from Bangladesh. Bangladesh J Plant Taxon, 20(2): 239-42.
Rajabi S, Ramazani A, Hamidi M, Naji T, **2015**. Artemia salina as a model organism in toxicity assessment of nanoparticles. DARU J Pharm Sci, 23(1): 20.
Raju GS, Rahman Moghal MM, Hossain MS, Hassan MM, Billah MM, Ahamed SK, Rana SM, **2014**. Assessment of pharmacological activities of two medicinal plant of Bangladesh: Launaea sarmentosa and Aegialitis rotundifolia roxb in the management of pain, pyrexia and inflammation. Biol Res, 47(1):55.
Rakib Ahmed S, Islam A, Haye A, Uddin SMN, Uddin MMN, Hossain MK, Paul A, Emran TB, **2019**. Antipyretic and hepatoprotective potential of Tinospora crispa and investigation of possible lead compounds through in silico approaches. Food Sci Nutr, 00: 1–10.
Roopashree TS, Dang R, Rani SR, Narendra C, **2008**. Antibacterial activity of antipsoriatic herbs: Cassia tora, Momordica charantia and Calendula officinalis. Int J Appl Res Nat Prod, 1(3): 20-8.
Sathasivam P, **Lakshmi** T, **2017**. Brine shrimp lethality assay of Sesbania grandiflora ethanolic extract–in vitro study. J Adv Pharm Edu Res, 7(1).
Schulz JB, Lindenau J, Seyfried J, Dichgans J, **2000**. Glutathione, oxidative stress and neurodegeneration. Eur J Biochem, 267(16): 4904-11.
Sen N, Bulbul L, Hussain MS, Banik S, Choudhuri MS, **2018**. An in vivo study regarding analgesic and anxiolytic activity of methanolic extract of Typha elephantina Roxb. Clin Phytosci, 4(1): 2.
Shaala NM, Zulkifli SZ, Ismail A, Azmai MN, Mohamat-Yusuff F, Omar H, **2017**. Effects of tributyltin chloride (TBTCl) antifouliong biocide on adult males and females of brine shrimp (Artemia salina). Int J Biol Res, 5(1): 30-35.
Suleiman M, Mamman M, Igomu E, Muhammad YU, Abdullahi A, Talba A, **2014**. Evaluation of analgesic and anti-inflammatory effects of the crude methanol extract of the stem-bark of Annona senegalensis Pers. International Journal of Medicinal and Aromatic Plants. 2014; 4(2):88-96.
Suresha RN, Amoghimath S, Vaibhavi PS, Shruthi SL, Jayanthi MK, Kalabharathi HL, **2014**. Evaluation of analgesic activity of perindopril in albino mice. J Adv Pharm Technol Res, 5(3): 129.
Uddin SMN, Amin MN, Shahid-Ud-Daula AF, Hossain H, Haque MM, Rahman MS, Kader MA, **2014**. Phytochemical screening and study of antioxidant and analgesic potentials of ethanolic extract of Stephania japonica Linn. J Med Plants Res, 8(37): 1127-33.
Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J, **2007**. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol, 39(1): 44-84.

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