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Central nervous system depressant, gastrointestinal motility, and brine shrimp lethality bioassay of the methanolic extract of *Suaeda maritima* in mice model

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ABSTRACT: *Suaeda maritima* (*S. maritima*) is a common yellow-green shrub grows in salt marshes. Present study aims to investigate the *in-vivo* central nervous system depressant, gastrointestinal motility and Brine shrimp lethality bioassay of the methanolic extract of *S. maritima.* Phytochemical screening was performed to observe the main constituents. Central nervous system depressant activity was evaluated through open field and hole cross tests separately. Gastrointestinal motility activity was observed using charcoal meal method. The extract was screened for cytotoxic activity using brine shrimp lethality bioassay. In open field method, the number of squares crossed by mice were 80.80 ± 1.66 and 80.60 ± 1.66 for 250 mg/kg and 500 mg/kg extract respectively at zero time, which reduced gradually to 26.00 ± 1.05 and 20.00 ± 0.95 after 60 minutes. Similar decreasing was found in hole cross method. Locomotor activities and exploratory behavior of mice were found to be decreased after the oral administration of *S. maritima*. On the other hand, *S. maritima* extract inhibit 62.9% and 69.7% gastrointestinal motility for 200 mg/kg and 400 mg/kg body weight of dose. In brine shrimp lethality test, LC_{50} values obtained from the experiment were 0.451 µg/µl and 0.254 µg/µl for *S. maritima* extract and positive control vincristine sulphate respectively. This study confirms the moderate cytotoxic effect of *S. maritima* that implies the presence of potent bioactive compounds. *S. maritima* possessed remarkable central nervous system depressant and gastrointestinal motility activities with moderate cytotoxic activity. Further investigation is warranted to find out the bioactive components with their exact mechanism of actions for drug development program.

KEYWORDS: *Suaeda maritima*; central nervous system depressant; hole cross; gastrointestinal motility; open field; brine shrimp

1. INTRODUCTION

Neurological disorders are currently estimated to affect as many as a billion people worldwide. Moreover, current life stress linked with sufferings and tribulations are accountable for the occurrence of many psychiatric disorders. Drugs acting on central nervous system (CNS) are important pharmacologically as they induce definite physiological and psychological effects. In indigenous procedure, a large number of plants are reported to have their therapeutic effects against CNS disorders and serve as very beneficial remedies for the abatement of human neurological sufferings [1]. Most of the conventional drugs used for the treatment of neurological diseases have side effects, for instance- benzodiazepines cause deterioration of cognitive function, physical dependence, tolerance, respiratory depression etc [2]. However, plant-based drugs gain large popularity due to their fewer side effects, cheap and abundant availability [3].

The brine shrimp lethality bioassay is a simple and prompt method for screening the cytotoxicity of the crude plant extract. This method is a good indicator for screening antitumor, insecticidal and fungicidal efficacies [4, 5]. Moreover, this method has been extensively used for isolating bioactive compounds and toxins [5]. On the other hand, most of the cases antibiotic having antidiarrheal effect show adverse side effects, and microorganisms may develop resistance to antibiotic used for young children who

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are prone to diarrheal diseases [6]. Therefore, it is urgent for searching drugs that might suppress diarrhea with less side effects and as complementary to oral dehydration therapy.

Suaeda maritima L. is a species of flowering plant in the Amaranthaceae family known by the common names herbaceous seepweed and annual sea-blite. It is well-known for having several phytochemicals like-carbohydrates, protein, tannins, alkaloids and flavonoids [7]. The green stem and leaves are used as condiments to impart the salty flavor of the meal. Leaves are used for making juice and curries, cater for livestock. In India, traditionally *S. maritima* leaves are used for the treatment of hepatitis. It also reported for possessing antimicrobial, hepatoprotective and antioxidant activity [8, 9]. However, there is no report available on the pharmacological activities which we studied here. Therefore, our present study has been designed for screening CNS depressant, gastrointestinal (GI) motility, and brine shrimp lethality bioassay of this plant.

2. RESULTS

2.1. Phytochemical screening

The preliminary phytochemical screening of the crude methanolic extract of *S. maritima* was performed to get the information about main constituents. Detailed phytochemical procedures are described in the materials and methods section. We found the presence of alkaloids, flavonoids, saponins and tannins in the extract (Table 1).

2.2. Evaluation of CNS depressant activity

2.2.1. Open Field Test

In open field test (OFT), 81.20 ± 1.39 square crossing was found for diazepam treated mice (Group-II) at zero time, which reduced gradually and reached at 13.00 ± 0.45 after 60 minutes. The number of movements of mice were 80.80 ± 1.66 and 80.60 ± 1.66 at zero time, which reduced remarkably and after 60 minutes it reached at 26.00 ± 1.05 for the mice in Group-III (250 mg/kg dose) and 20.00 ± 0.95 for Group-IV mice (500 mg/kg dose). It was noticed that there was a significant decline in the number of movements with time when compared with control (Group-I). The depression actions were more for the dose 500 mg/kg than 250 mg/kg which confirmed dose-dependent response. The observations were shown in (Table 2).

2.2.2. Hole Cross Test

A similar experiment was conducted using hole cross test (HCT) method to confirm CNS depressant activity, which was shown in (Table 3). At zero-time, average number of hole crossing was 18.80 ± 0.37 and 19.20 ± 0.58 for Group-III and Group-IV respectively, which were reduced gradually with time. After 60 minutes, these numbers were 2.20 ± 0.20 and 0.40 ± 0.24 respectively. It was also noticed that the depression action was highly noticeable at 5th observation (60 min) than at 4th observation (45 min). This experiment also confirmed the dose dependent depressant response of *S. maritima*. Diazepam was used as standard (Group-II) and Group-I was considered as control.

Table 1. Phytochemical screening of the methanolic extract of *S. maritima.*

Phytochemical	Name of the Test	Observation
Alkaloid	Meyer Test	+
	Wagner Test	++
Phenolic	Ferric Chloride Test	-
Tanins	Ferric Chloride Test	+
	Potassium Dicromate Test	+
Saponin	Froth's Test	++
Glycoside	Keller Kilani Test	-
Protein	Xanthroprotic Test	-
Reducing Sugar	Fehling's Test	-
Phytosterol	Liebermann-Burchard's Test	-
Flavonoids	Alkaline Reagent Test	+

++ = Strong intensity reaction, + = Medium intensity reaction, - = Absent/ No reaction

Table 2 CNS depressant activity testing of the methanolic extract of *S. maritima* through OFT.

Treatment Groups	Number of movement (square crossed) of mice				
	0 min	15 min	30 min	45 min	60 min
G-I (Vehicle: 10 ml/kg)	80.60 ± 1.54	74.60 ± 1.54	65.20 ± 1.77	54.20 ± 3.02	48.60 ± 2.01
G-II (Diazepam: 1 mg/kg)	81.20 ± 1.39	34.00 ± 0.84**	27.20 ± 0.86**	21.60 ± 0.51**	13.00 ± 0.45**
G-III (*S. maritima:* 250 mg/kg)	80.80 ± 1.66	49.80 ± 0.80**	47.80 ± 3.65	37.60 ± 2.73	26.00 ± 1.05**
G-IV (*S. maritima:* 500 mg/kg)	80.60 ± 1.66	44.00 ± 1.52**	34.40 ± 1.03**	26.20 ± 0.37*	20.00 ± 0.95**

Values are presented as mean ± SEM; Data was analyzed using one way ANOVA followed by Dunnet's t-test.
All the groups were compared with control; n=5; *p<0.05, **p<0.001

Table 3. CNS depressant activity testing of the methanolic extract of *S. maritima* through HCT.

Treatment Groups	Number of holes crossed by mice				
	0 min	15 min	30 min	45 min	60 min
G-I (Vehicle: 10 ml/kg)	22.60 ± 0.40	21.00 ± 0.32	22.40 ± 0.87	22.40 ± 0.81	21.40 ± 0.75
G-II (Diazepam: 1 mg/kg)	18.40 ± 1.44	6.40 ± 0.24**	1.40 ± 2.44**	0.60 ± 0.24**	0.00 ± 0.00**
G-III (*S. maritima:* 250 mg/kg)	18.80 ± 0.37	13.00 ± 0.55**	5.60 ± 0.51**	3.40 ± 0.24**	2.20 ± 0.20**
G-IV (*S. maritima:* 500 mg/kg)	19.20 ± 0.58	6.60 ± 0.24**	3.40 ± 0.24**	1.40 ± 0.40**	0.40 ± 0.24**

Values are presented as mean ± SEM; Data was analyzed using one way ANOVA followed by Dunnet's t-test.
All the groups were compared with control; n=5; *p<0.05, **p<0.001

2.3. Evaluation of GI motility activity

In GI motility test, the methanolic extract was given to the Group-III and Group-IV at a dose of 200 mg/kg and 400 mg/kg body weight respectively. It was found that distances traveled of charcoal meal after 30 minutes, were reduced significantly (18.98±2.18 cm and 16.41±1.41 cm respectively) when compared to control. The percentage inhibition of the gastrointestinal motility was more (69.7±2.77 %) for Group-IV than Group-III (62.90±1.13 %), which indicated dose dependent activity of the extract. Loperamide (standard) reduce the GI motility upto 72.78±1.57 % (Group-II). The observations were presented in (Table 4), (Figure 1).

2.4. Evaluation of the brine shrimp lethality bioassay

Results of the brine shrimp cytotoxic bioassay are shown in Figure 2. It was found both the methanolic extract of *S. matima* and positive control vincristine sulphate showed linear dose dependent linearity when plotted log concentration vs. percent of mortality (R^2 is 0.97 for the extract, and 0.92 for vincristine sulphate) (Figure 2). The LC_{50} values obtained from the experiment were 0.451 µg/µl and 0.254 µg/µl for *S. maritima* extract and vincristine sulphate respectively (Figure 2(C)). Compared to positive control, *S. maritima* extract showed a significant cytotoxic property, which implies the presence of potent bioactive compounds.

Table 4. Effect of the methanolic extract of *S. maritima* on GI motility of mice.

Treatment Group	Dose	Mean length of intestine (cm)	Mean length traveled by charcoal meal (cm)	% Inhibition of motility	P-value
G-I (Control)	10 ml/kg	53.83	42.25±3.33	21.52±1.71	-
G-II (Loperamide)	25 mg/kg	54.17	14.74±1.43	72.78±1.57**	0.000
G-III (Extract)	200 mg/kg	51.17	18.98±2.18	62.90±1.13**	0.001
G-IV (Extract)	400 mg/kg	54.17	16.41±1.41	69.7±2.77**	0.003

Values are presented as mean ± SEM; Data was analyzed using one way ANOVA followed by Dunnet's t-test.
All the groups were compared with control; n=5; *p<0.05, **p<0.001

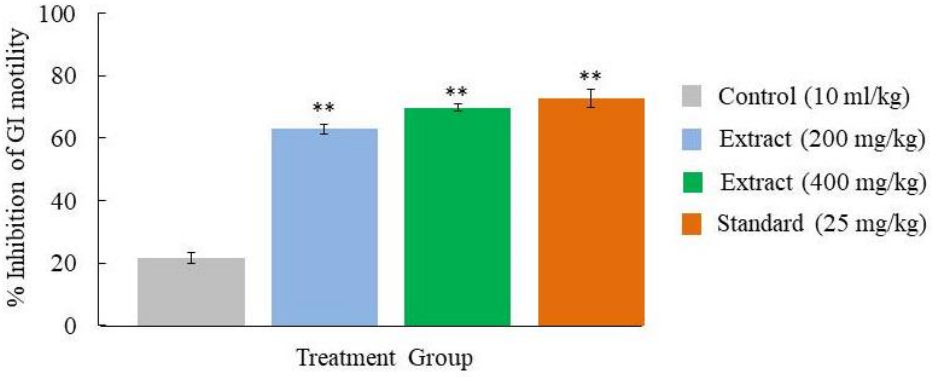

Figure 1. Effect of *S. maritima* extract on GI motility activity of mice.

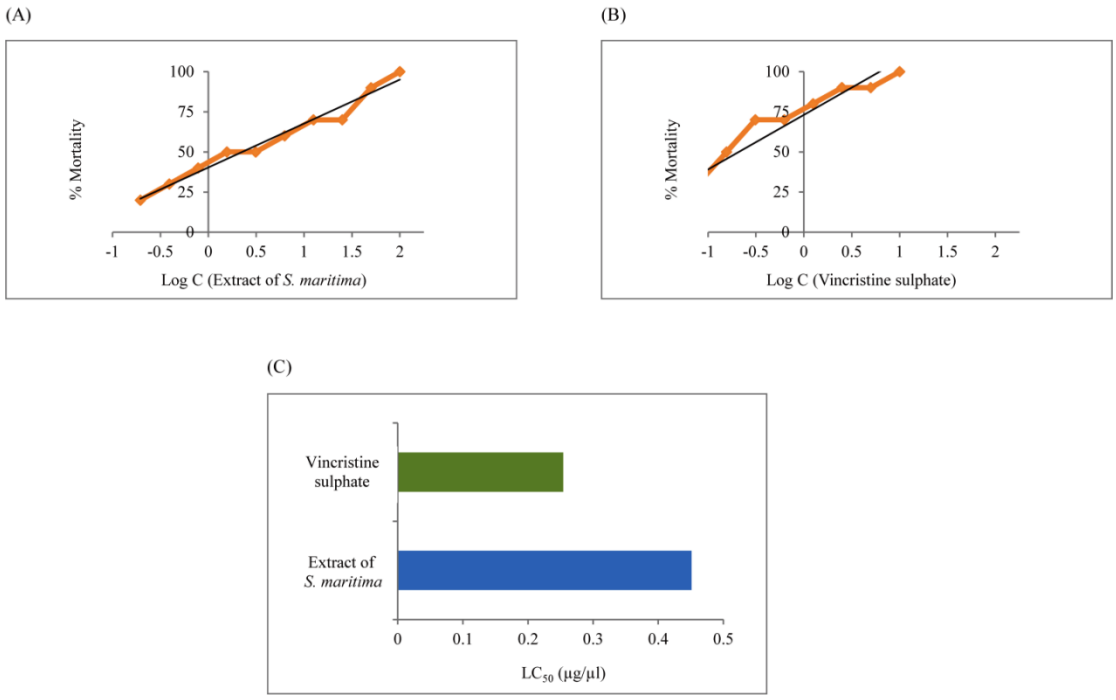

Figure 2. Plot of log concentration versus percentage of mortality in brine shrimp lethality test after 24 h of exposure to (A) *S. maritima* methanolic extract (B) Vincristine sulfate (C) Comparison of LC_{50} of *S. maritima* extract and vincristine sulfate.

3. DISCUSSION

The most popular method for evaluating drug's action on CNS is to conduct behavioral study on experimental animals [10] along with observing the effect on locomotor activity. This activity is a measure of CNS excitability; hence, a decrease of this activity may have a close relationship with sedation-like results for the depression of CNS [11]. Our result revealed that *S. maritima* possesses strong CNS depressant activity. Phytochemical screening studied here confirmed the possession of alkaloids, flavonoids, saponins, tannins etc. phytoconstituents in *S. maritima* extract (Table 1). Many researchers [12-15] showed that plants containing flavonoids, saponins, phenols, and tannins are useful for combating many CNS disorders. It has been reported that phytochemicals flavonoids, and neuroactive steroids act as ligand for the GABA-A receptors in the central nervous system by functioning as benzodiazepine (GABA agonist)-like molecules [13]. Moreover, polyphenolic compounds (gallic acid, vanillic acid, ellagic acid, etc.) have been claimed to exert their anxiolytic activities by slowing amyloid beta-induced glutamate release and the production of ROS which results in the lessening of apoptotic death of the cortical neurons [14]. Perhaps, *S. maritima* decreased locomotor activities, which indicates its CNS-depressant potential. GABA is a major inhibitory neurotransmitter in CNS. Many anxiolytic, muscle relaxant, sedative-hypnotic drugs exert their

pharmacological actions through GABA; therefore, it is predictable that the phytochemicals present in *S. maritima* may act by enhancing the GABAergic inhibition in CNS via membrane hyperpolarization, either by a decrease in firing rate of critical neurons in the brain or direct activation of GABA receptor [16].

Previous investigations reported that plant's phytoconstituents like- tannins, alkaloids, saponins, flavonoids, sterol, triterpenes etc. might have antidysentric properties. Moreover, these constituents also increase intestinal absorption of Na^+ and water [17]. Sarkar et al.(2016) claimed that *M. paniculata* steam bark showed significant inhibition (with dose-dependent consequence) of diarrhea in mice model due to the presence of saponins, terpinoids, flavonoids, steroidsetc. phytoconstituents in its methanolic extract[18]. The inhibitory activity of flavonoids on intestinal motility is a dose-dependent manner, which was reported earlier; and tannins are responsible for the denaturation of proteins of intestinal mucosa by composing protein tannates, thereafter decrease secretion [19, 20]. The phytoconstituents present in *S. maritima* might be responsible for inhibiting GI motility of charcoal meal in dose dependent manner by increasing intestinal reabsorption of Na^+ and water, and ultimately reduce the motility of GI tract.

In-vivo lethality study is a simple zoological model that can be used to predict the presence of bioactive compounds in plants [21]. In addition, Meyer et al., 1982 reported that brine shrimp lethality is a good test for the initial detection of antitumor compounds in plant extracts [22]. Our study revealed that, methanolic extract of *S. maritima* possesses moderate cytotoxic properties when compared to control, vincristine sulphate. The effect of this cytotoxicity follows dose-dependent manner. The presence of the secondary metabolites like alkaloid, flavonoid, phenolicetc has been reported to be responsible for the cytotoxic activity of the plant extract [23,24]. In our study, we found that *S. maritima* possesses high number of alkaloids, flavonoids etc. compounds (Table 1). Hence, it is desirable to observe the cytotoxic effect of the extract due to presence of the above compounds. Some researchers suggested that diterpenoid alkaloids can damage the tumor cells as well as normal cells by interfering of DNA synthesis pathway during the cell divisions [25].

4. CONCLUSION

Based on our results and discussion, it can conclude that *S. maritima* (methanolic extract) possesses significant CNS depressant and GI motility retardation effects. In addition, this extract also shows moderate cytotoxic effects. The activities are due to the presence of bioactive compounds in plant extract. Further studies should be conducted for isolating and characterizing of bioactive compounds, and *in-vivo* testing of individual phytoconstituents for exploiting the possibility of pharmaceutical use is recommended.

5. MATERIALS AND METHODS

5.1. Collection of materials

Plant sample was collected from Sonadia Deep, Moheshkhali, Cox's Bazar, Bangladesh on May 7, 2016. Collected plant sample was identified and authenticated by the National Harberium, Mirpur, Dhaka. The voucher specimen no. was (Accession No DACB 41628). Diazepam and loperamide were kind gifts from Square Pharmaceutical Limited, Bangladesh. Other reagents used in this study were analytical grades.

5.2. Experimental animals

For this study, Swiss albino mice of both sexes (25-35 g) of either sex, aged 3-4 weeks, were purchased from the animal house of Jahangirnagar University (Savar, Dhaka, Bangladesh). Animals were kept in animal house at ambient temperature 25°C and 45-60% humidity and allowed to adapt to the laboratory environment for 7 days prior to experimental session. They were allowed to take standard pellets as basal food and water *ad libitum*. Ethical approval was taken from the Institutional Ethical Committee for conducting this study (NSTU/SCI/EC/2022/92).

5.3. Preparation of *S. maritima* extract

After collection, plants were cleaned and sundried for 10 days. The dried plants were ground into coarse powder by using grinding machine. Then the powder was macerated for 15 days by using 95% methanol with occasional shaking. The whole mixture was filtrated by cotton bed forwarded by Whatman filter paper. After drying at room temperature, we got methanolic fraction of *S. maritima*. This gummy extract was stored in refrigerator until further study.

5.4. Phytochemical screening of *S. maritima* methanolic extract

The qualitative phytochemical screening of the methanolic extract of *S. maritima* was performed to find out the main phytoconstituents including alkaloids, phenolics, tannins, saponins, glyosides, protein, reducing sugar, phytosterols, flavonoids etc. using the standard procedures [26-29]. Mayer test and Wagner test were performed to assess the presence of alkaloid [26-28]. In Mayer' s test, 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Later, we added 1 ml of Mayer' s reagent into the tube. Yellow colour precipitation was found which indicated as the evidence of the presence of alkaloids. On the other hand, in Wagner's test, 1 ml of iodine solution (Wagner' s reagent) was added into the tube containing extract solution and hydrochloric acid described previously. Redish brown precipitate was regarded as the confirmation of the presence of alkaloids. For phenol test, 50 mg extract was dissolved in distilled water (5 ml). Few drops of 5% ferric chloride solution were added into the mixture [26]. We didn't find any changes of the color, which indicated the absence of the significant phenolic compounds (dark green color is the indicator of the presence of phenol). Potassium dichromate test and ferric chloride test were done to observe the presence of tannins [28]. Briefly, a small amount of crude extract was taken in a test tube with water, and then heated on a water-bath which was allowed to react with 1 ml $FeCl_3$ solution (5%). The color of the resultant mixer turned into dark blue-green that indicated the presence of tannin compounds. For potassium dichromate test, we had added 1 ml of 10% of aqueous potassium dichromate solution in the above-mentioned test tube instead of $FeCl_3$. Yellowish brown precipitation suggested the presence of tannins. For the detection of glycosides in crude extract, Keller-Killani test was done [27]. Extract solution (5 ml) was mixed with 2 ml of glacial acetic acid and 1 drop of $FeCl_3$ solution. Concentrated H_2SO_4 (1 ml) was added into the resultant mixture. We didn't notice the brown ring at the inference, which indicated the absence of glycosides in the extract. Crude methanolic extract of *S. maritima* (0.5 mg) were taken in 20 ml of water, followed by vigorous shaking for 15 minutes. Many small froths were observed which endorsed the existence of saponin compounds [28]. For protein test, few drops of HNO_3 were added into a test tube containing 1 ml extract. Yellow color was not formed, which indicated the absence of protein (i.e.- xanthoprotein) [29]. Presence of reducing sugar was confirmed through Fehling's test [28]. Briefly, 5 ml of extract containing solution was mixed well with 5 ml of Fehling's solution (equal volume of Fehling reagent A and B) and then boiled gently. As we know that formation of brick red colored precipitate is the indicator of the presence of reducing sugars; in our experiment we didn't notice similar type of the changes of color. Libermann-Burchard test was performed for the assessment of phytosteroids [27, 29]. In brief, 1 ml *S. maritima* extract containg solution was mixed with 2 ml of acetic anhydride afterward 2 ml of H_2SO_4. Change in color into blue or green not found which indicated the absence of phytosteroids. Formation of the intense yellow color due to addition of NaOH (few drops) to the *S. maritima* extract containing solution, and later it turned into colorless solution after adding diluted acid indicated the presence of flavonoids [26].

5.5. CNS depressant activity test

5.5.1. Open field method

Open field test (OFT) was performed according to Sharmin et al. (2018) with slight modification [30]. Mice were grouped randomly in four groups. Mice of Group-I were given 1% tween 80 containing distilled water orally (10 ml/kg), which served as a negative control; whereas standard drug diazepam (1 mg/kg body weight) was administered in Group-II mice. The experimental groups, Group-III and Group-IV were treated *S. maritima* methanolic extract orally at the dose of 250 mg/kg and 500 mg/kg body weight respectively. After administration, each mouse was placed in the OFT instrument and number of square crossings was recorded at 0, 15, 30, 45 and 60 minutes (for a period of 3 minutes). For each group, the mean number of squares in open fields apparatus crossed by the mice were compared with standard group to evaluate the CNS depression activity.

5.5.2. Hole cross method

The hole cross test (HCT) method as described by Mishra et al. (2011) was adopted with some modifications for the screening of CNS depressant activity [31]. A wood partition was fixed in the middle of a box having a size of (30×20 ×14) cm^3 with six holes. The diameter of each hole was 3 cm which was made at the height of 7 cm from horizontal board. After the oral treatment with extract at the dose of 250 and 500 mg/kg body weight, the number of passages of mice through the hole from chamber to chamber were counted for 3 min period at the time of 0-, 15-, 30-, 45- and 60-min. Mice of Group-I were given 1% tween 80

containing distilled water orally (10 ml/kg) as vehicle, which served as control. Diazepam is used in-serviced as standard in this experiment (1 mg/kg body weight of mice).

5.6. Gastrointestinal motility test

Gastrointestinal (GI) motility study was performed by following the method of Tafesse and Mekonnen(2012) with slight modification [32]. Mice of Group-I treated with vehicle at a dose 10 ml/kg; whereas Group-II was given standard drug loperamide at a dose of 25 mg/kg body weight orally. The methanolic extract of *S. maritima* was administered at a dose of 200 mg/kg and 400 mg/kg body weight orally to the experimental groups (Group-III and Group-IV respectively). After 30 minutes of treatment, 1 ml of charcoal meal was given for each mouse orally. Then all the animals were dissected after 30 minutes of charcoal meal treatment and the intestine was rapidly dissected out from pylorus to the caecum and the distance traveled by the charcoal meal from the pylorus to caecum was measured and the percentage of inhibition of motility was calculated as follows

$$\% \text{ of Inhibition} = \frac{MTLI - MDCC}{MTLI} \times 100\%$$

MTLI = Mean total length of the intestine; MDCC = Mean distance covered by the charcoal

5.7. Brine shrimp lethality bioassay

Brine shrimp lethality bioassay was used to anticipate the cytotoxic activity of the *S. maritima* methanolic extract. Seawater, test samples, and control were prepared as described by Mayer et al, 1982 [22]. Four (4) mg extract was dissolved in dimethylsulfoxide (DMSO), and various concentrations of the solution (20, 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078, 0.039 µg/µl) were obtained through serial dilutions using the prepared seawater. The solutions were added up to the vials where live *Artemia salina* nauplii (n = 10) in 5 ml water were retained already. Vincristine sulfate and DMSO were used as the positive control and negative control respectively. Survived nauplii were counted after 24 h, and the median lethal concentrations (LC_{50}) of the crude extract were determined by plotting % of the deceased shrimps against the logarithm of the sample concentrations.

5.8. Statistical analysis

All values were expressed as Mean ± SEM and were analyzed statically by using one way ANOVA followed by Dunnet's t-test. All the groups were compared with control. $p<0.05$ was considered to be statistically significant and $p<0.001$ was considered to be highly significant.

List of abbreviations: OFT: Open Field Test; HCT: Hole Cross Test; *S. maritima*: *Suaeda maritima*; GABA: Gamma Amino Butyric Acid.

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Conflict of interest statement: Authors have no competing interests to declare.

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