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Investigation of Anxiolytic, Cytotoxic, and Anthelmintic Activities of the Leaves of *Calamus rotang* L. (Arecaceae)

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Abstract

Nature has always blessed humanity with numerous means of remedy for treating a wide range of diseases. Since different parts of *Calamus rotang* L. have several ethnopharmacological activities, including CNS depressant and anthelmintic action, its crude methanol extract of leaves was used to investigate its anxiolytic, cytotoxic, and anthelmintic activities. The methanol was selected because it could extract a vast range of phytoconstituents. The anti-anxiety activities of CRME were investigated on the Swiss albino mice model through a hole board and elevated plus maze test, while the cytotoxic and anthelmintic activities were screened on brine shrimps and *Pheretima posthuma*, respectively. The current study exhibited that crude methanol extract of *C. rotang* L. leaves had a significant (p>0.001) and dose-dependent effect against anxiety on mice model of hole board and elevated plus maze when compared with that of control. Furthermore, crude methanol extract of *C. rotang* L. leaves showed a concentration-dependent mild anthelmintic effect exhibiting no cytotoxicity (LC_{50} value of 1.02 mg/ml). Thus, the present study revealed the potential of crude methanol extract of *C. rotang* L. leaves as an excellent anxiolytic agent having a mild anthelmintic effect with no cytotoxicity.

Keywords: *Calamus rotang* L. leaves, anxiolytic, anthelmintic, and cytotoxic

Introduction

In the modern world, stress is one of the significant concerns that can lead to life-threatening diseases such as anxiety, depression, epilepsy, schizophrenia, parkinsonism, and restlessness[1]. The drugs that are commonly used to treat these types of diseases often demonstrate serious side effects and can cause physical dependence and tolerance[2]. Herbal medicines can be the best alternative to avoid the side effects of these commercially available synthetic CNS depressant drugs[3].

Helminthiasis is one of the morbid diseases around the world, as well as in Bangladesh, which commonly occurs due to the invasion of the gastrointestinal tract (GIT) by different types of parasitic worms. Other types of helminthic infections in liver, blood, and intestinal tract are common among Bangladeshi people caused by various types of parasites[4]. Several types of anthelmintic drugs are used to treat this kind of helminthic infections which either kill or expel the helminths from the GIT. Although these drugs are highly effective, they can cause adverse effects like gastrointestinal disturbances, nausea, giddiness as well and resistances[5]. So, it dictated the necessity of alternative treatment options to treat helminthiasis, which can possibly be herbal medicines with fewer side effects.

Nature has been gifted with a wide range of medicinal plants, of which few exhibit severe toxicity. Several research studies are being conducted to provide reliable scientific data to evaluate the toxic effects of these plant materials and brine shrimp lethality assay is an excellent method for the experiment of cytotoxicity of

any extract. BSLA is preliminarily conducted to determine the general toxicity of the plant extracts and detect their cytotoxicity as well as anticancer and pesticidal potential[6].

From time immemorial, nature has blessed people with a variety of remedial solutions to critical diseases. The prehistoric literature stated that there were 500 medicinal plants, and the traditional system of medicine used about 800 plants[7]. It has been proved scientifically that different types of plants have a wide range of pharmacological effects and are a source of discovering various synthetic active pharmaceutical ingredients. As the researchers continue their study on medicinal plants, they successfully isolated the medicinal plants' active constituents, and it was a part of their continuous research to isolate quinine from cinchona bark[8]. This effective medicinal agent was proved to add a milestone in the path of alternative systems of medicine, which enthused many of the scientists to invest their time in medicinal plants' research. The use of herbal medicines is also advantageous because of their low cost and fewer side effects, but they possess a wide range of pharmacological effects. Furthermore, they have a better cultural acceptability. Thus, the plant-derived medicinal agents have contributed to commercially manufactured medicines which had been proven to be effective in fighting against cancer, psychotic disorders, algesia, malaria, and so on[9-12].

Calamus rotang L. is a shrub of the Arecaceae family, which is commonly known as Bet in Bangladesh[13]. It is a native plant of the Asian region which has proved to have a content of different types of plant metabolites such as saponin, alkaloids, and flavonoids in different parts of this plant[14,15]. The in vitro and in vivo investigations have already proved its effect against diarrhea, inflammation, pyrexia, hypertension, cough, and bronchitis[16,17]. It has also been proven to be effective as an astringent, antibilious, spasmolytic, alexiteric, diuretic, and wood vermifuge. The ethnic people use its tender shoots as febrifuge and anthelmintic, whereas the sap of the leaf has a remedial effect for eye problems, blood disorders, and biliousness[13,18,19]. The ethnic people also use its roots to treat fever and snakebite[20]. Its leaves sap has phytochemical constituents and ethnomedicinal uses, and different parts have shown numerous effects along with CNS depressant and anthelmintic effects. In addition, we had chosen methanol as a solvent for extraction because of its highly efficient property of solubilizing both polar and nonpolar compounds. It facilitated the extraction of a wide range of phytoconstituents. Also, it could be evaporated quickly to obtain the concentrated leaf extract of the plant[21]. Moreover, most phytochemicals tend to be polar compounds that can be easily extracted using methanol. So, we have focused on the crude methanolic extract of the leaves of *C. rotang* L. (CRME) to investigate whether the leaves have any toxic properties and can act as potential anthelmintic as well as anxiolytic agents.

Materials and methods

Chemicals

Methanol (Merck, Germany), Dimethyl sulfoxide (Merck, Germany), and other laboratory-grade chemicals were used for the extraction, in vitro, and in vivo pharmacological experiments.

Collection and identification of plant materials

The developed leaves of the plant were collected from the Chattogram division of Bangladesh. It was recognized as *C. rotang* L. by Dr. Shaikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong and deposited in the same department under the identification no ra203426.

Extract preparation

The washed and semi-shed dried leaves were cut into small pieces and the chopped pieces of leaves were again dried in an oven at a considerably low temperature followed by crushing into powder by using a mechanical grinder. The powdered materials were soaked in methanol and retained at room temperature with intermittent shaking for 13 days. Then the solution

was filtered through filter cloth and whatman no. 1 filter paper. The solvent from the filtrate was evaporated to acquire the concentrated extract by using a rotary evaporator (Stuart, UK). The percentage of the extract yield was calculated using the following equation [22]:

$$\%\ of\ yield\ of\ extracts = \frac{Weight\ of\ extracted\ material}{Weight\ of\ original\ plant\ material\ used} \times 100$$

Experimental animals

Swiss albino male mice (25-35 gm each) were utilized for the hole board and elevated plus maze experiments. These were obtained from the Bangladesh Council of Scientific and Industrial Research (BCSIR), Chittagong, Bangladesh. They were housed following proper guidelines and provided sufficient diet with drinking water ad libitum during the whole study except 6 hours before and at the time of the experiment. *Pheretima Posthuma* was used for the study of in-vitro anthelmintic tests as it possessed identical physiology and anatomy with human intestinal roundworms.

Study design

In the screening of anxiolytic activities of CRME, four groups of mice (five mice for each group) were used. The treatment group consisted of CRME (200 and 400 mg/kg of body weight of mice), a control (5% DMSO at 10 ml/kg), and a reference standard (Diazepam 1 mg/kg).

In vivo anxiolytic test

Hole-board test

The hole board (40 cm × 40 cm) was made of wood, which was painted white, elevated from the floor, and had sixteen holes, each of 1 cm diameter and 2 cm depth, situated at the same distance from each other. The board was also splitted into four equal sectional squares of 20 cm × 20 cm[23]. Initially, each of the treated mice was kept at the center of the hole board, and it was permitted to move independently on the board for five minutes. Then, the number of head dipping sessions for each mouse was counted at 30, 60, 90, and 120 minutes. The investigation was performed in a silent environment.

Elevated plus maze test

The elevated plus maze was a "+" shaped apparatus, elevated 45 cm from the floor. The maze consisted of two open arms (30 cm × 5 cm × 0.2 cm) and two enclosed arms with high walls (30 cm × 5 cm × 15 cm), each at 90° position relative to the adjacent arms. The arms were extended from a central platform (5 cm × 5 cm). The experiment was done according to a previously described method with minor modifications[24]. After 30 minutes of treatment, each group of mice was put individually on plus-maze fronting toward the open arm. Then, during 5 minutes, the number of entries and the time spent in open and closed arms were counted for each animal.

In vitro anthelmintic test

A previously described method was modified slightly and followed to perform the anthelmintic assay[25]. In this experiment, six groups of roundworms (each containing ten worms) were treated with distilled water (control), Albendazole at 10mg/ml (reference standard), and CRME at different concentrations. The anthelmintic activity was determined by counting the 'time of paralysis' and 'time of death' of the worms. The experiment was recapped with *P. posthuma* two times.

In vitro cytotoxic test

Seawater preparation

38 g of iodine-free sea salt was dissolved in one liter of distilled water to prepare a 3.8% NaCl solution, which was used as artificial seawater. After filtration, the pH of the seawater was adjusted to 8.0 by using 1M NaOH solution.

Brine shrimp hatching

Artemia salina, known as brine shrimp eggs, were kept in a covered tank containing two liters of seawater with a constant oxygen supply throughout the 48-hour hatching period. Afterward, the matured phototropic nauplii were collected from the lightened portion of the tank.

Experiment design

Brine shrimp lethality assay was executed using an established experimental method with slight

modifications[26]. 20 mg of the test sample was dissolved in 4 ml of 5% DMSO up to 20 ml with seawater, followed by serial dilution to 500, 250, 125, and 62.5 µg/ml with artificial seawater. 2.5 ml of the plant extract sample was added to 2.5 ml of seawater containing ten nauplii in each petri plate. After 24 hours, the petri plate was inspected to count the number of dead nauplii in each plate. Vincristine sulfate (VS) was used as a reference standard at concentrations of 10, 5, 1, 0.5, and 0.25 µg/ml, whereas 5% DMSO was used as a control. From this data, the percentage (%) of mortality of the brine shrimp nauplii was calculated using the following equation[27]:

$$\%\ of\ Mortality = \frac{Nd}{N} \times 100$$

Here, Nd = Number of dead nauplii**,** N = Number of nauplii taken

Determination of median lethal concentration (LC_{50})

The LC_{50} value was designated as the concentration of the extract, which caused the death of half of the brine shrimp nauplii after a predetermined treatment time. It was measured by the linear regression line, which was obtained by plotting % of mortality of nauplii against the correspondent concentration of the extracts. The concentration-mortality data were converted into a straight line through a trend line fit linear regression analysis using Microsoft Excel 2007. The LC_{50} values were calculated from the best-fit line that was obtained.

Statistical analysis

The calculated data were represented as Mean ± Standard Error of Mean (SEM). One-way ANOVA and post hoc Dunnett's test were utilized to statistically analyze the acquired experimental data. The statistical software named "Statistical Package for Social Science" (SPSS, Version 16.0, IBM Corporation, NY) was used for this purpose. Results below $^{a}p<0.001$ were considered statistically significant as compared to the control.

Results and discussion

Extract yield

The weight of CRME yield was 23.811 g, and the yield percentage was 7.22%.

In vivo anxiolytic test

Hole-board test

In the existing investigation, the hole board created a state of anxiety in which mice did not poke into the holes to avoid height from the floor in an anxious state. When the mice were treated with anxiolytics, there was an increase in their head-dipping behavior in the hole[28,22]. The current study represented that the broad spectrum anxiolytic agent Diazepam exhibited a significant increase in the number of head dipping from 18.60 ± 0.81 to 19.60 ± 0.87 times in the hole during the 120 minutes, whereas the control showed only a small number of head dipping in the hole. CRME at 400 mg/kg demonstrated an increase in the number of head dipping (15.80 ± 0.73 to 17.00 ± 0.83 times) in the hole in comparison with that of the control significantly ($^{a}p<$ 0.001) during the 120 minutes. The result signified its higher potential at higher doses as an anxiolytic agent. The observations on CRME at both doses clearly defined the dose-dependent anxiolytic activity of CRME. The results are represented in Table 1.

Table 1. Anxiolytic activity of crude methanol extracts of *C. rotang* leaves by using the hole-board test

Treatment	Number of Head dipping			
	30 minutes	**60 minutes**	**90 minutes**	**120 minutes**
5% DMSO	7.80 ± 0.37	7.40 ± 0.60	8.00 ± 0.74	7.20 ± 0.58
Diazepam (1 mg/kg)	18.60 ± 0.81^{a}	20.20 ± 1.15^{a}	18.00 ± 1.77^{a}	19.60 ± 0.87^{a}
CRME (200mg/Kg)	12.60 ± 0.81^{a}	12.00 ± 0.83^{a}	11.20 ± 1.02	9.60 ± 0.50
CRME (400mg/Kg)	15.80 ± 0.73^{a}	17.40 ± 0.67^{a}	15.40 ± 0.74^{a}	17.00 ± 0.83^{a}

*DMSO= Dimethyl sulfoxide, CRME= Crude methanol extracts of *C. rotang* leaves; Results were expressed as Mean ± SEM, $^{a}p<$ 0.001 was statistically significant in comparison with the control.

Elevated plus maze test

The elevated plus maze test used natural stimuli like the fear of moving in open space and the height from the floor, which could induce anxiety in humans. In this study, the rise in the time spent and several entries in the open arm without a changed locomotor activity suggested the anxiolytic effect of investigated medicinal plants[22,29,30]. The existing study showed that the time spent and the number of entries in the open arm was increased in CRME (200 mg/kg) treated mice non-significantly compared to the control. In addition, those indices were significantly decreased in closed arms in CRME (200 mg/kg) treated mice. However, those indices increased in the open arm in Diazepam (reference standard), and CRME (400 mg/kg) treated mice and decreased in the closed arm when compared to the control significantly ($^{a}p<0.001$). These observations indicated that CRME at 400 mg/kg doses possessed significant anxiolytic activity. Compared to Diazepam, CRME showed an effective anxiolytic activity in a dose-dependent manner. Since the anxiolytic effect of CRME was comparable to Diazepam, it might act via a GABA receptor complex like Diazepam[31]. The result of the investigation of the anxiolytic property of CRME using an elevated plus maze is represented in Table 2.

In vitro anthelmintic test

The study demonstrated that the anthelmintic activity was determined by the time CRME took for paralysis and death of the earthworms. The more time taken for paralysis and death of the earthworms, the less anthelmintic activity of CRME was. Previous research reported 120 mins to be the maximum paralysis as well as death time for *P. posthuma*[32,33]. No death or paralysis was observed when the earthworms were treated with the control. Since Albendazole was a highly effective anthelmintic agent, it was used at a deficient 10 mg/ml concentration. It decreased microtubules in the intestinal cells, absorptive function, and glucose uptake of the parasites by binding with colchicine sensitive site of β tubulin[34]. It took 32.54 ± 1.33 and 53.89 ± 2.60 minutes to cause paralysis and death of the earthworms, respectively. The paralysis time for CRME-treated (from higher to lower concentration) earthworms was from 56.50 ± 4.44 to 123.37 ± 1.69 minutes, and death time from 53.89 ± 2.60 to 169.80 ± 2.14 minutes, and the results were significant ($^{a}p<0.001$, $^{b}p<0.01$) while comparing with the reference standard. It was evident that CRME revealed mild and concentration-dependent anthelmintic activity. The result of the anthelmintic study of CRME is shown in Table 3.

Table 2. Anxiolytic activity of crude methanol extracts of *C. rotang* leaves by using elevated plus maze test

Treatment	Time spent (in sec)		Number of entries	
	Open Arm	Closed Arm	Open Arm	Closed Arm
5% DMSO	9.50 ± 0.15	287.50 ± 1.21	1.80 ± 0.37	16.80 ± 1.74
Diazepam (1 mg/kg)	47.15 ± 2.76^{a}	241.40 ± 2.32^{a}	10.40 ± 0.60^{a}	6.40 ± 0.60^{a}
CRME (200mg/kg)	19.80 ± 2.87	269.70 ± 3.33^{a}	4.00 ± 0.31	9.40 ± 0.50^{a}
CRME (400mg/kg)	32.20 ± 1.65^{a}	251.10 ± 0.67^{a}	5.4 ± 0.50^{a}	6.60 ± 0.81^{a}

*DMSO= Dimethyl sulfoxide, CRME= Crude methanol extracts of *C. rotang* leaves; Results were expressed as Mean ± SEM, $^{a}p< 0.001$ was statistically significant in comparison with control

Table 3. Anthelmintic activity of crude methanol extracts of *C. rotang* leaves

Group	Concentration (mg/ml)	Time taken for paralysis (P) (min)	Time taken for death (D) (min)
		Pheretima posthuma	
		P	**D**
Control	-	-	-
Albendazole	10	32.54 ± 1.33	53.89 ± 2.60
CRME	100	56.50 ± 4.44^{b}	98.00 ± 1.53^{b}
	50	89.75 ± 0.64^{a}	117.61 ± 1.37^{b}
	35	106.56 ± 1.78^{a}	145.58 ± 2.87^{a}
	25	123.37 ± 1.69^{a}	169.80 ± 2.14^{a}

*CRME= Crude methanolic extracts of *C. rotang* leaves, values were expressed as Mean ± SEM, $^{a}p<0.001$, $^{b}p<0.01$ were significant when compared with the corresponding value of the reference standard

In vitro cytotoxic test

BSLA was performed to assess the cytotoxicity and the anticancer potential of CRME. The level of toxicity against brine shrimp was discerned as cytotoxic when the LC_{50} value was less than 1000 µg/ml and non-cytotoxic when this value was more significant than 1000 µg/ml[35,36]. In this regard, no brine shrimp mortality was observed from the control, which discerned its non-cytotoxic profile. In addition, CRME was non-cytotoxic, showing an LC_{50} value of 1021.24 µg/ml, whereas the reference standard VS showed an LC_{50} value of 1.63 µg/ml. The LC_{50} value of CRME was much higher than that of VS, proving CRME to be non-cytotoxic and incompatible with further research as an antineoplastic drug. The cytotoxicity profile also showed that the highest concentration of CRME showed the highest mortality, whereas the lower concentration showed no mortality at all. However, the non-cytotoxic profile of CRME has opened a wide array of investigations to find other safe and valuable pharmacological activities at a lower concentration. The presence of flavonoids in CRME might contribute to its pharmacological effect [33, 37-39]. The results are shown in Figure 1, Figure 2, and Table 4.

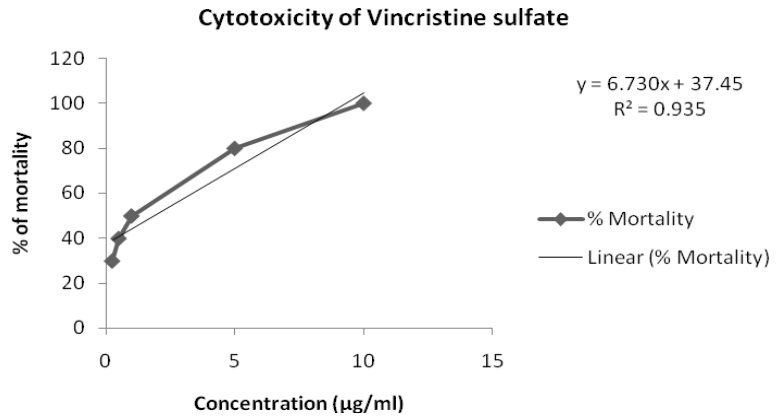

Figure 1. The concentration versus percentage (%) of the mortality curve obtained by Vincristine sulfate against brine shrimp

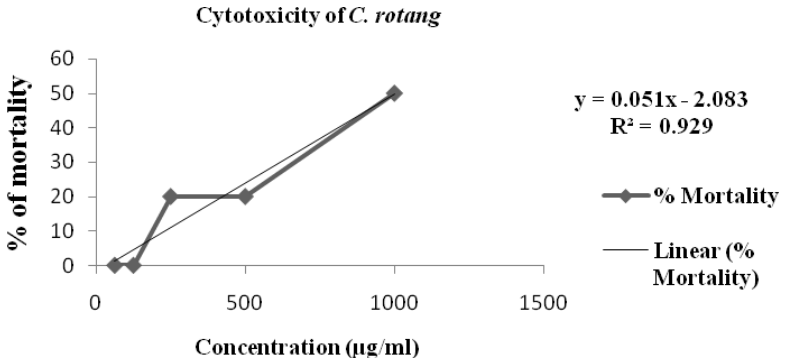

Figure 2. The concentration of crude methanol extracts of *C. rotang* leaves versus the percentage (%) of the mortality curve of brine shrimp

Table 4. Cytotoxic activity of crude methanolic extracts of *C. rotang* leaves

Group	LC_{50} (µg/ml)	Regression equation	R^2
VS	1.86	y = 6.730x + 37.45	R^2 = 0.94
CRME	1021.24	y = 0.05x - 2.08	R^2 = 0.93

*VS= Vincristine sulfate, CRME= Crude methanolic extracts of *C. rotang* leaves

Conclusion

The existing study illustrated CRME's anxiolytic and anthelmintic potential, which was proved to be non-cytotoxic. Furthermore, since it possesses different types of pharmacological activities, advanced research can be conducted to isolate and identify its active phytochemical compounds to correlate with its pharmacological properties.

References

1. Sheik, H.S., Vedhaiyan, N., and Singaravel, S. 2015. Evaluation of Central Nervous System Activities of Cyperus rotundus L. Extract on Rodents. Curr Res Neurosci. **5**:10-19.
2. Sheik, H.S., Vedhaiyan, N., and Singaravel, S. 2014. Evaluation of central nervous system activities of Citrus maxima leaf extract on rodents. J App Pharm Sci. **4**(9):77.
3. Carlini, E. 2003. Plants and the central nervous system. Pharmacol Biochem Behav. **75**(3):501-512.
4. Braseth, A.L., Elliott, D.E., and Ince, M.N. 2021. Parasitic infections of the gastrointestinal tract and liver. Gastroenterol Clin. **50**(2):361-381.
5. Taman, A., and Azab, M. 2014. Present-day anthelmintics and perspectives on future new targets. Parasitol res. **113**:2425-2433.
6. Olowa, L.F., and Nuñeza, O.M. 2013. Brine shrimp lethality assay of the ethanolic extracts of three selected species of medicinal plants from Iligan City, Philippines. Mortal. **2**(11): 74-77.
7. Chopra, R.N., Nayar, S.L., and Chopra, I.C. 1956. Glossary of Indian medicinal plants. **1**: Council of Scientific & Industrial Research New Delhi.
8. Phillipson, J.D. 2001. Phytochemistry and medicinal plants. Phytochem. **56**(3):237-243.
9. Cragg, G.M., and Newman, D.J. 2005. Plants as a source of anti-cancer agents. J ethnopharmacol. **100**(1-2):72-79.
10. Cott, J., and Misra, R. 2013. Medicinal plants: a potential source of new psychotherapeutic drugs. Herbal Medicines for Neuropsychiatric Diseases: Curr Develop Res. 51.
11. Rates, S.M.K. 2001. Plants as source of drugs. Toxicon. **39**(5):603-613.
12. Queiroz, E.F., Wolfender, J-L., and Hostettmann, K. 2009. Modern approaches in the search for new lead antiparasitic compounds from higher plants. Curr drug targets. **10**(3):202-211.
13. Basumatary, S., Ahmed, M., and Deka, S. 2004. Some medicinal plant leaves used by Boro (tribal) people of Goalpara district, Assam.
14. Morris R. 2004. Plants For A Future: Database Search Results: Creative Commons License, England.
15. Ripa, F.A., Dash, P.R., and Faruk, O. 2015. CNS depressant, analgesic and anti-inflammatory activities of methanolic seed extract of Calamus rotang Linn. fruits in rat. J Pharmacog Phytochem. **3**:121-125.
16. Khare, C.P. 2008. Indian medicinal plants: an illustrated dictionary: Springer Science & Business Media.
17. Ganesan, K., and Xu, B. 2017. Ethnobotanical studies on folkloric medicinal plants in Nainamalai, Namakkal District, Tamil Nadu, India. Trends Phytochem Res. **1**(3):153-168.
18. Rethy, P., Singh, B., Kagyung, R., and Gajurel, P. 2010. Ethnobotanical studies of Dehang–Debang Biosphere Reserve of Arunachal Prad esh with special reference to Memba tribe.
19. Sukumaran, S., Brintha, T., Subitha, P., Sheebha, Y.A., and Jeeva, S. 2014. Usage of medicinal plants by two cultural communities of Kanyakumari district, Tamil Nadu, South India. J Chem Pharm Res. **6**:67-79.
20. De Sarker, D, Saha, M.R., and Saha, S. 2015. Perspective of dietetic and antioxidant medicinal plants: Notion Press.
21. Truong, D.H., Nguyen, D.H., Ta, N.T.A., Bui, A.V., Do, T.H., and Nguyen, H.C. 2019. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of Severinia buxifolia. J food qual. **2019**(1):8178294
22. Islam, M.R., Naima, J., Proma, N.M., Hussain, M.S., Uddin, S.N., and Hossain, M.K. 2019. In-vivo and in-vitro evaluation of pharmacological activities of Ardisia solanacea leaf extract. Clin Phytosci. **5**(1):32.
23. Ajao, M.Y., and Akindele, A.J. 2013. Anxiolytic and sedative properties of hydroethanolic extract of Telfairia occidentalis leaves in mice. Rev Bras Farmacogn. **23**(2):301-309.
24. Herbst, L., Gaigher, T., Siqueira, A., Joca, S., Sampaio, K., and Beijamini, V. 2019. New evidence for refinement of anesthetic choice in procedures preceding the forced swimming test and the elevated plus-maze. Behav brain res. **368**:111897.

25. Saddiqe, Z., Khalid, S., and Maimoona, A. 2019. In vitro anthelmintic activity of extracts of withania somnifera. J Nat App Sci Pak. **1**(1):89-97.

26. Sharma, S.C.D, Shovon, M.S., Jahan, M., et al. 2019. Antibacterial and cytotoxic activity of Bacillus methylotrophicus-SCS2012 isolated from soil. J Microbiol, Biotech Food Sci. **2019**:2293-2307.

27. Sathasivam, P., and Lakshmi, T. 2017. Brine shrimp lethality assay of Sesbania grandiflora ethanolic extract–in vitro study. J Adv Pharm Edu Res. Jan-Mar. **7**(1).

28. Diniz, T.C., de Oliveira Júnior, R.G., Medeiros, M.A.M.B., et al. 2019. Anticonvulsant, sedative, anxiolytic and antidepressant activities of the essential oil of Annona vepretorum in mice: involvement of GABAergic and serotonergic systems. Biomed Pharmacotherapy. **111**:1074-1087.

29. Proma, N.M., Naima, J., Islam, M.R., Afrin. S.R., and Hossain, M.K. 2019. Phytochemical investigation and Neuropharmacological activity of stem extracts of Gynocardia odorota R.BR. Chittagong Univ J Biolog Sci. **9**(1&2):49-56

30. Sahoo, S., and Brijesh, S. 2020. Anxiolytic activity of Coriandrum sativum seeds aqueous extract on chronic restraint stressed mice and effect on brain neurotransmitters. J Funct Foods. **68**:103884.

31. Masiulis, S., Desai, R., Uchański, T., et al. 2019. GABA A receptor signalling mechanisms revealed by structural pharmacology. Nat. **565**(7740):454-459.

32. Das, S., Dey, M., and Ghosh, A. 2011. Determination of anthelmintic activity of the leaf and bark extract of Tamarindus indica Linn. Indian j pharmaceut sci. **73**(1):104.

33. Islam, M.R., Proma, N.M., Naima, J., Uddin, M.G., Afrin, S. R., and Hossain, M. K. 2020. Assessment of the Pharmacological Activities of Ardisia solanacea Roxb: An Ethnomedicinal Plant used in Bangladesh. J Pharm Nutri Sci. **10**(5), 302-314.

34. Adate, P.S., Parmesawaran, S., and Chauhan, Y. 2012. In vitro anthelmintic activity of stem extracts of Piper betle Linn against Pheritima posthuma. Pharmacog J. **4**(29):61-65.

35. Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, Dj., and McLaughlin, J.L. 1982. Brine shrimp: a convenient general bioassay for active plant constituents. Planta med. **45**(05):31-34.

36. Afrin, S. R., Islam, M. R., Khanam, B. H., Proma, N. M., Didari, S. S., Jannat, S. W., and Hossain, M. K. 2021. Phytochemical and pharmacological investigations of different extracts of leaves and stem barks of Macropanax dispermus (Araliaceae): a promising ethnomedicinal plant. Fut J Pharm Sci.**7**, 1-13.

37. Aguirre-Hernández, E., González-Trujano, M.E., Terrazas, T., Herrera Santoyo, J., and Guevara-Fefer, P. 2016. Anxiolytic and sedative-like effects of flavonoids from Tilia americana var. mexicana: GABAergic and serotonergic participation. Salud mental. **39**(1):37-46.

38. Sreejith, M., Kannappan, N., Santhiagu, A., and Mathew, A.P. 2013. Phytochemical, anti-oxidant and anthelmintic activities of various leaf extracts of Flacourtia sepiaria Roxb. Asian Pac j trop biomed. **3**(12):947-953.

39. Matsuo, M., Sasaki, N., Saga, K., and Kaneko, T. 2005. Cytotoxicity of flavonoids toward cultured normal human cells. Biol Pharm Bull. **28**(2):253-259.