



Assessment of membrane stability, central nervous system depressant, and gut motility effects of *Lablab purpureus* seeds

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ABSTRACT

Introduction: *Lablab purpureus*, under the family of Fabaceae, is a plant with various pharmacological activities. The present study was aimed to investigate the phytoconstituents, membrane stabilizing activity, central nervous system (CNS) depressant potential, and gastrointestinal (GI) motility of the methanol extract of *L. purpureus* seeds (MELPS).

Methods: The methanol plant extract was screened for different phytochemical groups. Mice were classified into four groups for in vivo activities. Group-I was designated as negative control and received distilled water (10 mL/kg body weight); group-II served as positive control and received diazepam (1 mg/kg body weight). Group-III and group-IV both were experimental groups and received plant extract at 200 and 400 mg/kg body weight, respectively.

Results: Alkaloids, carbohydrates, saponins, glycosides, tannins, phenols, flavonoids, and proteins were found after phytochemical analysis. On hypotonic solution-induced hemolysis of erythrocyte membrane, MELPS9 (9 mg/mL) resulted in the highest percentage of inhibition (60.51 ± 0.889), and on heat-induced hemolysis, MELPS9 (9 mg/mL) resulted in the highest percentage of inhibition (33.97 ± 0.21). In the case of the CNS depressant potential experiment, mice that received a sample at a dose of 400 mg/kg body weight showed the highest result (54.40 ± 4.51) compared with the positive control (14.2 ± 3.70) ($P < 0.001$). Similarly, 400 mg/kg dose sample exhibited the highest percentage of inhibition (60.51 ± 0.889) of hemolysis and GI motility (22.26%).

Conclusion: It can be concluded that the MELPS has potential membrane stability, CNS depressant, and antimotility effects.

Implication for health policy/practice/research/medical education:

Lablab purpureus methanol seeds extract showed significant membrane stability, CNS depressant, and antimotility effects. The isolation of compounds of this plant might help in drug development programs against inflammation, depression, and diarrhea.

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Introduction

The demand for herbal medicines increases daily due to their high potential to treat numerous diseases (1-3). Many current pharmaceuticals are derived from natural sources, such as plants, which have traditionally been utilized for a variety of chronic and severe illnesses, as well as for indigenous communities' basic healthcare. Most of these natural medications possess significant pharmacological activities, greater safety margins, and lower toxicities,

making them popular in developed and developing countries (4-6). The World Health Organization (WHO) reported that approximately 70%–80% of the world's population depends on traditional therapies primarily from natural sources (7). Herbal medicine's extensive application is due to the vast natural source, easy accessibility, and healing potential, which is also proven by numerous investigations (8). Plant species are becoming more important in the pharmaceutical sector as a result

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of biodiversity and certain biological functions on the human body and animals (9,10). These various medicinal properties are attributed to active constituents that differ from plant to plant. More than 300 medicinal and aromatic plants and their secondary metabolites provide a vital contribution to the foods, pharmaceuticals, perfume, and cosmetics industries (11).

Lablab purpureus is derived from the Fabaceae family. This plant is renowned among general people for its edible fruits and seeds, commonly known as the Hyacinth bean. Besides Bangladesh, this herb is also found in many tropical and subtropical regions and many African countries. It is traditionally planted for fodder and cover crops in this region. Studies found that the leaves and seeds of *L. purpureus* contain around 20-28% proteins with a well-balanced composition of amino acids (12). Besides, this plant constitutes a wide range of chemical components such as sugar, essential oils (13,14). *L. purpureus* has been used for its numerous healing potentials, including anti-inflammatory, anti-microbial, anti-oxidant, anti-diabetic, hypolipidemic, analgesic, anti-spasmodic, cytotoxic, hepatoprotective, and insecticidal effects. It is also used for the treatment of anemia caused by iron deficiency (14).

Free radicals cause oxidative damage and subsequent inflammation in the cells and tissues of the body. That is why a stabilized membrane is required. The erythrocyte membrane stability test is a common and popular method for defining the anti-inflammatory activity of natural and synthetic agents by exposing the erythrocyte membrane to a hypotonic solution in the presence of high temperature or heat (15,16).

For decades, the diarrheal disease has been a major global healthcare burden that leads to patient mortality and morbidity. The prevalence of diarrhea is high in most developing countries (17). Microbial agents like *Salmonella typhi*, *Shigella flexneri*, *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* are typical and common causative agents of diarrhea in the human body (18). For the prevention and management of diarrheal disorders, a broad range of treatment options is available. Therefore, WHO always encourages conducting natural plant-based research to prevent and treat diarrhea based on traditional therapeutic practices (19).

To date, the *L. purpureus* plant has been explored for different chemical and biological studies; however, there is not enough scientific evidence of its membrane stability, gastrointestinal (GI) motility, and CNS depressant activities. Thus, the present study was commenced to elucidate the phytochemical constituents, *in vivo* membrane stabilizing activity, CNS depressant activities, and GI motility of the methanolic extract of *L. purpureus* seeds.

Materials and Methods

Sample collection and preparation

The seeds of *L. purpureus* were collected from Noakhali,

Bangladesh, and identified by Professor Dr. Shaikh Bokhtiar Uddin, Department of Botany, University of Chittagong. The fresh plant seeds were then sun-dried, and the dried seeds were ground into powders. A total of 500 g of crushed powder was soaked with 1.5 L of methanol and stored for 15 days in the bio-safety cabinet. Afterward, the solution was filtered initially using a clean cloth and then the Whatman filter paper. A rotary evaporator was used to sieve the derived filtrate to make brownish-colored gummy extract labeled as crude methanol extract (20).

Phytochemical screening

The extracted crude sample was investigated for the presence of alkaloids, phenols, flavonoids, carbohydrates, tannins, glycosides, saponins, phytosterols, proteins, steroids, and terpenes. The existence of these phytochemicals was verified using established phytochemical screening methods (21,22).

Membrane stabilizing activity

In vitro membrane stabilizing activity was performed for methanol extract of *L. purpureus*. Membrane stabilizing *L. purpureus* seeds' activity to inhibit hypotonic and heat-induced hemolysis of human erythrocytes was evaluated by following the method of previously published articles (23,24).

Central nervous system (CNS) depressant activity

Two methods (open field and hole board test method) were applied (25). Mice were classified into four groups and denoted as group-I (negative control), group-II (positive control), group-III, and group-IV (experimental). Each group contained an equal number of mice (n = 5). Each of the mice of groups I, II, III, and IV received distilled water (10 mL/kg), diazepam (1 mg/kg), and plant extract of 200 and 400 mg/kg body weight, respectively.

Open field method

In the open field method, mice received distilled water, diazepam, or plant extracts. They were analyzed at a time interval of three minutes by observing the number of square areas crossed. The mean number of square fields crossed by each mice group was compared with the negative controls to detect the CNS depressant behavior (18,20). In this experiment, group-I received distilled water (10 mL/kg), group-II received diazepam (1 mg/kg), group-III and group-IV received seed extracts at 200 mg/kg and 400 mg/kg body weight, respectively.

Hole board method

In this method, the head dipping of mice was observed in a hole at least up to eye level. An elevated number of head-dipping compared to negative control suggests anxiolytic activity. On the other hand, sedative activity refers to a reduced number of head-dipping compared to control. The hole board equipment comprises 16 holes with a

wooden chamber (40 × 40 × 25 cm³); each hole has a 3 cm diameter. The vertical extension of this equipment is 25 cm so that the movement of mice can be viewed through the holes in a furtive manner. After 60 minutes, each of the mice group received either water, diazepam, or plant extract. Each mouse from different groups was held off to one side of the screen, recording the number of head dips for 5 minutes (21,26).

Gastrointestinal (GI) motility activity

This study followed standard laboratory protocol to evaluate this function (27). Here, applied castor oil for the GI transition. In this experiment, mice were distributed into four main groups. Each group contained an equal number of mice (n=4). Mice were fasted for 18 hours and received free access to water. Then, mice were treated with distilled water, standard drugs (loperamide), and plant extracts. After thirty minutes, each mouse of all four groups was orally treated with 1 mL of marker (charcoal suspension of 10% in 5% gum acacia). Each mouse was sacrificed fifteen minutes later, and the dissected small intestine was put on a clean field. The distance traversed by charcoal was carefully examined and determined by a measuring scale. The pylorus' space to the caecum flowing through the charcoal plug was represented as a percentage of the small intestine (28,29). The inhibition percentage was determined by using the following equation (29).

$$PI\% = \left(\frac{LM}{LSI} \right) \times 100$$

$$\% \text{ Inhibition} = \frac{\{IP\%(\text{Control}) - IP\%(\text{treatment})\}}{IP\%(\text{Control})}$$

Here, PI = Peristaltic index; LM = Length of charcoal meal; LSI = Length of the small intestine.

Statistical analysis

All the results, including *in vitro* and *in vivo* analysis, are expressed as mean ± SD. *P* values were analyzed statistically by One-way ANOVA with post-hoc Dunnett's multiple comparisons test, and *P* < 0.05 was considered statistically significant. Statistical analysis was performed using the statistical software package for social science (SPSS, version 23, IBM Corporation).

Results

Phytochemical screening

The phytochemical screening of methanolic extract of *L. purpureus* seeds showed the presence of alkaloids, glycosides, carbohydrates, phenols, saponins, flavonoids, tannins, and proteins (Table 1). However, phytosterols, terpenes, and steroids were absent. Tests were repeated three times to avoid error.

From the analysis of different concentrations of methanol extract of *L. purpureus* seeds (MELPS) on

hypotonic solution-induced hemolysis of the erythrocyte membrane, it was observed that the higher concentration of MELPS (9 mg/mL) resulted in the highest percentage of inhibition (60.51 ± 0.889) of hemolysis of RBC. On the other hand, the standard acetylsalicylic acid (ASA) showed 70.04 ± 0.132% inhibition of hemolysis (Table 2). Again, on heat-induced hemolysis of the erythrocyte membrane, it was observed that the higher concentration of MELPS (9 mg/mL) resulted in the highest percentage of inhibition (33.97 ± 0.21) of hemolysis of RBC. At the same time, ASA showed 58.48 ± 0.24% inhibition of hemolysis (Table 3).

CNS depressant activity

Open field test

In the case of CNS depressant activity in the open field method, both doses of plant extracts produced decreased movement compared to the control group at 0-60 minutes in a dose-dependent manner. The mice that were received

Table 1. Preliminary phytochemical screening of the methanolic extracts of *L. purpureus* Membrane stabilizing activity

Phytochemicals	Methanolic extract
Alkaloids	+
Carbohydrates	+
Glycosides	+
Saponins	+
Phytosterols	-
Phenols	+
Tannins	+
Flavonoids	+
Proteins	+
Terpenes	-
Steroid	-

"+" indicates presence and "-" indicates the absence of phytochemicals

Table 2. The effect of different concentrations of methanolic extract of *L. purpureus* seeds on hypotonic solution-induced hemolysis of erythrocyte membrane

Treatment	Dose	Optical density of samples in hypotonic solution (Mean ± SD)	% Inhibition of hemolysis
Control	-	1.57 ± 0.010	-
MELPS1	1 mg/mL	1.213 ± 0.015	22.72 ± 0.749***
MELPS3	3 mg/mL	0.998 ± 0.002	36.43 ± 0.482***
MELPS5	5 mg/mL	0.893 ± 0.006	43.10 ± 0.707***
MELPS7	7 mg/mL	0.840 ± 0.006	46.28 ± 0.500***
MELPS9	9 mg/mL	0.620 ± 0.010	60.51 ± 0.889***
ASA	0.10 mg/mL	0.470 ± 0.001	70.04 ± 0.132***

Abbreviations: MELPS, methanol extract of *L. purpureus* seeds; ASA, acetylsalicylic acid.

Each value represents the mean ± SD (n=3). *** *P* < 0.001 compared to control in one-way ANOVA with post hoc Dunnett's *t* test.

Table 3. The effect of different concentrations of methanolic extract of *L. purpureus* seeds on heat-induced hemolysis of erythrocyte membrane

Treatment	Dose	Optical density of samples		% Inhibition of hemolysis
		Heated solution	Unheated solution	
Control	-	0.374 ± 0.002	-	-
MELPS1	1 mg/mL	0.174 ± 0.008	0.100 ± 0.01	26.92 ± 0.21***
MELPS3	3 mg/mL	0.163 ± 0.007	0.080 ± 0.01	28.37 ± 0.41***
MELPS5	5 mg/mL	0.196 ± 0.002	0.117 ± 0.002	30.90 ± 0.20***
MELPS7	7 mg/mL	0.201 ± 0.003	0.123 ± 0.002	31.25 ± 0.27***
MELPS9	9 mg/mL	0.213 ± 0.002	0.130 ± 0.002	33.97 ± 0.21***
ASA	0.10 mg/mL	0.241 ± 0.003	0.053 ± 0.006	58.48 ± 0.24***

Abbreviations: MELPS, methanol extract of *L. purpureus* seeds; ASA, acetylsalicylic acid.

Each value represents the mean ± SD (n=3). ***P<0.001 compared to control in one-way ANOVA with post hoc Dunnett's *t* test.

a sample at a dose of 200 mg/kg body weight showed the highest activity of 28.2 ± 3.1 at 60 minutes. The mice who received a sample at a dose of 400 mg/kg body weight showed the most increased activity of 24.40 ± 3.58 at 60 minutes. But, the standard drug diazepam showed the highest activity of 11.20 ± 1.30 at 60 minutes. The results of the CNS depressant experiments are shown in Figure 1.

Hole board test

Mice also showed potential CNS depressant activity in the hole board test. A dose of 400 mg/kg body weight showed the most prominent result with the highest frequency of head dipping (54.40 ± 4.51) compared with the positive control (14.2 ± 3.70; *P*<0.001). The results are presented in Figure 2.

GI motility test

For the GI motility test, 400 mg/kg body weight dose showed the maximum percent inhibition (22.26%), while the standard loperamide showed the maximum percent inhibition of 48.86% (Table 4).

Discussion

In most plants throughout nature, some common compounds, including alkaloids, carbohydrates, glycosides, tannins, saponins, resins, phenolic compounds, proteins, flavonoids, and steroids, are present in the cell wall (30). In our investigation, the phytochemical screening of methanolic extract of *L. purpureus* seed revealed alkaloids, glycosides, carbohydrates, phenols, saponins, tannins, flavonoids, and proteins. Our findings are in line with prior research that looked at the presence of several phytochemicals (12,31). However, we did not find some constituents, including phytosterols, terpenes, and steroids.

Different phytochemical studies clarified the role of *L. purpureus* as a potent anti-inflammatory agent. In the current study, different concentrations of *L. purpureus* methanolic seed extracts showed potential membrane stabilizing activities on hypotonic solution-induced

hemolysis. The higher concentration (9 mg/mL) showed the highest red blood cell hemolysis (60.51 ± 0.889). On the other hand, when we applied the standard ASA for the comparison, it demonstrated about 70.04 ± 0.132% inhibition of hemolysis. The present findings are

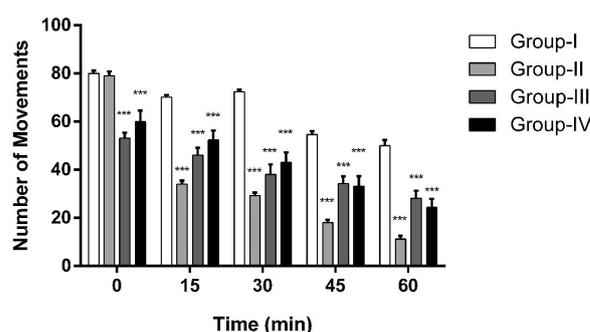


Figure 1. Effect of methanolic extract of *L. purpureus* on the open field test. Each value represents the mean ± SD (n=5). ****P*<0.001 compared to the negative control (group I) in one way ANOVA with post hoc Dunnett's *t* test.

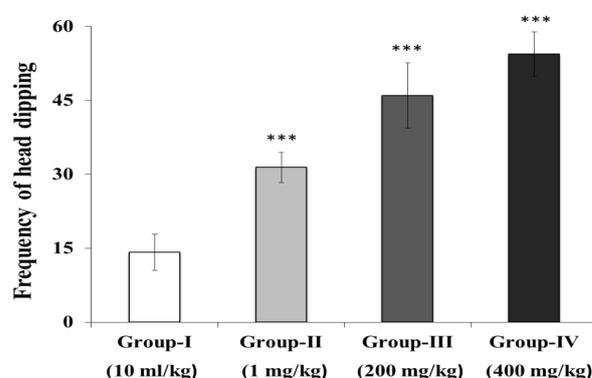


Figure 2. Effect of methanolic extracts of *L. purpureus* on hole board test. Each value represents the mean ± SD (n=5). ****P*<0.001 compared to control in one way ANOVA with post hoc Dunnett's *t* test.

Table 4. GI motility profile of methanol extract in a dose-dependent manner of charcoal meals through small intestine of mice

Group	Treatment	Dose (mg/kg)	MLTI	MDTC	% Motility	% Of inhibition
Control	Distilled water	0.2 mL/10 g	46.25 ± 1.93	36.5 ± 1.85	78.85 ± 1.08	--
Standard	Loperamide	5 mg/kg	46.75 ± 1.25	18.88 ± 0.97	40.32 ± 1.27	48.86
Group III	ME 200	200 mg/kg	45.50 ± 1.76	30.25 ± 0.75	66.59 ± 1.02	15.54
Group IV	ME 400	400 mg/kg	46.13 ± 1.48	28.25 ± 0.88	61.30 ± 5.14	22.26

Abbreviations: MLTI, mean length travelled by intestine; MDTC, mean distance travelled by charcoal. Each value represents the mean ± SD (n=5).

comparable to standard anti-inflammatory drug aspirin, reported in both *in vitro* and *in vivo* experiments where flavonoids showed significant stabilizing potential on lysosomes (12,32,33). In contrast, both tannins and saponins can bind to cations that stabilize red blood cells (12). Our current investigation provides evidence that the membrane stabilizing activity of *L. purpureus* plays a profound membrane stabilizing activity because of its flavonoid or tannin contents.

Anxiety and depression-related disorders are increasing day by day around the globe. To investigate the role of *L. purpureus*, we used the methanolic seeds extract that indicated the suppression of the locomotor's activity. An increase in locomotor activity is probably attributed to a rise in alertness. So, we applied two methods, including open field test and whole board test for CNS depressant potential experiment where those mice that received a dose of 400 mg/kg body weight showed the highest result (54.40 ± 4.51) compared to the positive control (14.2 ± 3.70) depicting the amplitude of movements compared to the standard drug diazepam. Because the locomotor activity measures the CNS excitability, a decrease in it may indicate the extract's depressing or sedative properties (34-36).

Antimotility drugs act as the mainstay agents for the treatment of diarrhea. Many natural plants showed their antimotility potential on the intestine (37). We have also investigated the antimotility efficacy of *L. purpureus*. For the GI motility test, our results showed that a dose of 400 mg/kg body weight has the maximum % of inhibition (22.26%) compared with standard drug loperamide (48.86%), proving its antimotility activity.

Conclusion

In conclusion, *L. purpureus* seeds could be a source of potent membrane stabilizing agents, anti-CNS depression-related disorders, and antimotility agents that can fulfill current therapeutic needs. Further pharmacological studies are suggested to understand the in-depth mechanism of action and precise biological applications.

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Authors' contributions

JR designed the study and performed the experiments. Data analysis and interpretation were performed by SB, MGU, and MNH. MGU, AAM, SZ, and MAA performed the literature review and wrote the manuscript. MKH, MNH, SB, and MFH made the necessary corrections and critically revised the manuscript. MSI supervised this research work. All the authors gave consent to submit the final version.

Conflict of interests

The authors declare no conflict of interests.

Ethical considerations

All authors hereby certify that they followed the "Principle of laboratory animal care" (NIH publication No. 85-23, revised 1985), as well as any applicable country laws. The ethical committee of Noakhali Science and Technology University has reviewed and approved all proposed study protocols (ID-10/2018).

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References

1. Sen S, Chakraborty R, De B, Devanna N. An ethnobotanical survey of medicinal plants used by ethnic people in West and South district of Tripura, India. *J For Res.* 2011;22(3):417. doi: 10.1007/s11676-011-0184-6.
2. Stickel F, Schuppan D. Herbal medicine in the treatment of liver diseases. *Dig Liver Dis.* 2007;39(4):293-304. doi: 10.1016/j.dld.2006.11.004.
3. Hosseinzadeh S, Jafarikukhdan A, Hosseini A, Armand R. The application of medicinal plants in traditional and modern medicine: a review of *Thymus vulgaris*. *Int J Clin Med.* 2015;06(09):635-42. doi: 10.4236/ijcm.2015.69084.
4. Tagboto S, Townson S. Antiparasitic properties of medicinal plants and other naturally occurring products.

- Adv Parasitol. 2001;50:199-295. doi: 10.1016/s0065-308x(01)50032-9.
5. Yu Y, Shen M, Song Q, Xie J. Biological activities and pharmaceutical applications of polysaccharide from natural resources: a review. *Carbohydr Polym.* 2018;183:91-101. doi: 10.1016/j.carbpol.2017.12.009.
 6. Hussain MS, Ibrahim M, Hasan MM, Aziz MT, Suchi SA, Uddin MG. An in vivo study of the pharmacological activities of a methanolic acetate fraction of *Pistia stratiotes* L.: an ethno-medicinal plant used in Bangladesh. *Animal Model Exp Med.* 2018;1(3):221-7. doi: 10.1002/ame2.12037.
 7. Wise J. Herbal products are often contaminated, study finds. *BMJ.* 2013;347:f6138. doi: 10.1136/bmj.f6138.
 8. Panda AK. Evidence based Ayurveda Practice. *J Homeop Ayurv Med.* 2012;1(4):1-3. doi: 10.4172/2167-1206.1000e108.
 9. Dhakad AK, Pandey VV, Beg S, Rawat JM, Singh A. Biological, medicinal and toxicological significance of *Eucalyptus* leaf essential oil: a review. *J Sci Food Agric.* 2018;98(3):833-48. doi: 10.1002/jsfa.8600.
 10. Liu Q, Luo L, Zheng L. Lignins: biosynthesis and biological functions in plants. *Int J Mol Sci.* 2018;19(2):335. doi: 10.3390/ijms19020335.
 11. Farnsworth NR. Ethnopharmacology and future drug development: the North American experience. *J Ethnopharmacol.* 1993;38(2-3):145-52. doi: 10.1016/0378-8741(93)90009-t.
 12. Rahman MS, Uddin MG, Alam MB, Yoo JC. Simplified the screening and in vitro appraisal of antioxidant, cytotoxic, thrombolytic, antimicrobial and membrane stabilizing activities of *Lablab purpureus* at a time. *J Chosun Nat Sci.* 2014;7(3):173-82. doi: 10.13160/ricns.2014.7.3.173.
 13. Al-Snafi AE. The pharmacology and medical importance of *Dolichos lablab* (*Lablab purpureus*)-a review. *IOSR J Pharm.* 2017;7(2):22-30. doi: 10.9790/3013-0702012230.
 14. Parvin D, Nasir Uddin MM, Islam MS, Parvin S, Shahriar M. Phytochemical screenings, thrombolytic activity and antimicrobial properties of the leaf extracts of *Lablab purpureus*. *Am J Res Commun.* 2013;1(2):49-55.
 15. Islam T, Das A, Shill KB, Karmakar P, Islam MS, Sattar MM. Evaluation of membrane stabilizing, anthelmintic, antioxidant activity with phytochemical screening of methanolic extract of *Neolamarckia cadamba* fruits. *J Med Plants Res.* 2015;9(5):151-8. doi: 10.5897/jmpr2014.5720.
 16. Longhi-Balbinot DT, Lanznaster D, Baggio CH, Silva MD, Cabrera CH, Facundo VA, et al. Anti-inflammatory effect of triterpene 3 β , 6 β , 16 β -trihydroxylup-20(29)-ene obtained from *Combretum leprosum* Mart & Eich in mice. *J Ethnopharmacol.* 2012;142(1):59-64. doi: 10.1016/j.jep.2012.04.013.
 17. Shoba FG, Thomas M. Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhoea. *J Ethnopharmacol.* 2001;76(1):73-6. doi: 10.1016/s0378-8741(00)00379-2.
 18. Hossain MS, Akter S, Das A, Sarwar MS. CNS depressant, antidiarrheal and antipyretic activities of ethanolic leaf extract of *Phyllanthus acidus* L. on Swiss albino mice. *Br J Pharm Res.* 2016;10(5):1-9. doi: 10.9734/bjpr/2016/24790.
 19. Umer S, Tekewe A, Kebede N. Antidiarrhoeal and antimicrobial activity of *Calpurnia aurea* leaf extract. *BMC Complement Altern Med.* 2013;13:21. doi: 10.1186/1472-6882-13-21.
 20. Ruhul Amin KM, Or Rashid MM, Uddin MG, Sharmin T. New insight in neuropharmacological activities of *Dioscorea alata*. *Discov Phytomedicine.* 2018;5(1):1-6. doi: 10.15562/phytomedicine.2018.52.
 21. Hasan MS, Uddin MG, Shoibe M, Al Mahmud A, Banik S. Evaluation of anxiolytic and hypoglycemic potential of *Cissus adnata* Roxb. in animal model. *J Complement Integr Med.* 2019;17(2). doi: 10.1515/jcim-2018-0130.
 22. Sarker MA, Banik S, Hussain MS, Ghosh A, Hossain MS. In-vitro and in-vivo pharmacological activities with phytochemical evaluation of methanolic extract of *Microcos paniculata* stem barks. *Curr Drug ther.* 2016;11(2):142-9. doi: 10.2174/1574885511666160520154529.
 23. Rahman MA, Hussain MS, Millat MS, Ray MC, Amin MT, Moghal MM. Screenings of in-vitro antimicrobial, cytotoxic and anti-inflammatory activity of crude methanolic extracts of *Crinum latifolium* (Leaves). *J Med Plants Res.* 2016;10(37):649-55. doi: 10.5897/jmpr2016.6204.
 24. Moghal MM, Millat MS, Hussain MS, Islam MR. Thrombolytic and membrane stabilizing activities of *Launaea sarmentosa*. *Int J Pharmacogn.* 2016;3(8):354-8. doi: 10.13040/ijpsr.0975-8232.ijp.3(8).354-58.
 25. Gupta BD, Dandiya PC, Gupta ML. A psychopharmacological analysis of behaviour in rats. *Jpn J Pharmacol.* 1971;21(3):293-8. doi: 10.1254/jjp.21.293.
 26. Shahed-Al-Mahmud M, Lina SM. Evaluation of sedative and anxiolytic activities of methanol extract of leaves of *Persicaria hydropiper* in mice. *Clinical Phytoscience.* 2017;3(1):20. doi: 10.1186/s40816-017-0056-5.
 27. Bakare RI, Magbagbeola OA, Akinwande AI, Okunowo OW, Green M. Antidiarrhoeal activity of aqueous leaf extract of *Momordica charantia* in rats. *Journal of Pharmacognosy and Phytotherapy.* 2011;3(1):1-7.
 28. Rani S, Ahamed N, Rajaram S, Saluja R, Thenmozhi S, Murugesan T. Anti-diarrhoeal evaluation of *Clerodendrum phlomidis* Linn. leaf extract in rats. *J Ethnopharmacol.* 1999;68(1-3):315-9. doi: 10.1016/s0378-8741(99)00103-8.
 29. Abedin MF, Hussain MS, Islam A, Sen N, Das A, Kar A, et al. Thrombolytic, CNS depressant and anti-diarrhoeal activities of ethanolic extract of bark of *Syzygium cumini* L. skeels: an in-vivo and in-vitro study. *J Pharm Nutr Sci.* 2018;8(3):129-36. doi: 10.6000/1927-5951.2018.08.03.7.
 30. khatoon M, Islam E, Islam R, Rahman AA, Alam AH, Khondkar P, et al. Estimation of total phenol and in vitro antioxidant activity of *Albizia procera* leaves. *BMC Res Notes.* 2013;6:121. doi: 10.1186/1756-0500-6-121.
 31. Soetan KO. Comparative evaluation of phytochemicals in the raw and aqueous crude extracts from seeds of three *Lablab purpureus* varieties. *Afr J Plant Sci.* 2012;6(15):410-5. doi: 10.5897/ajps12.059.
 32. Middleton E Jr. Biological properties of plant flavonoids: an overview. *Int J Pharmacogn.* 1996;34(5):344-8. doi: 10.1076/phbi.34.5.344.13245.

33. Sourì E, Amin G, Farsam H, Barazandeh Tehrani M. Screening of antioxidant activity and phenolic content of 24 medicinal plant extracts. *DARU J Pharm Sci.* 2008;16(2):83-7.
34. Verma A, Jana GK, Sen S, Chakraborty R, Sachan S, Mishra A. Pharmacological evaluation of *Saraca indica* leaves for central nervous system depressant activity in mice. *J Pharm Sci Res.* 2010;2(6):338-43.
35. Rakotonirina VS, Bum EN, Rakotonirina A, Bopelet M. Sedative properties of the decoction of the rhizome of *Cyperus articulatus*. *Fitoterapia.* 2001;72(1):22-9. doi: 10.1016/s0367-326x(00)00243-4.
36. Ibrahim M, Amin MN, Millat MS, Raju JA, Hussain MS, Sultana F, et al. Methanolic extract of peel of citrus maxima fruits exhibit analgesic, CNS depressant and anti-inflammatory activities in Swiss albino mice. *BEMS Rep.* 2018;4(1):7-11. doi: 10.5530/bems.4.1.3.
37. Ezeja IM, Ezeigbo, II, Madubuike KG, Udeh NE, Ukwèni IA, Akomas SC, et al. Antidiarrheal activity of *Pterocarpus erinaceus* methanol leaf extract in experimentally-induced diarrhea. *Asian Pac J Trop Med.* 2012;5(2):147-50. doi: 10.1016/s1995-7645(12)60014-5.