PHYTOCHEMICAL INVESTIGATION AND ANTIDEPRESSANT ACTIVITY OF *CELOSIA CRISTATA* LEAVES AND FLOWERS IN EXPERIMENTAL ANIMALS

Naveen Kumar Tripathi[1]*, Neelam Khan[2]

Abstract

Depression is one of the most crucial reasons of disability in adults. The incidence of depression is about 3-10 % in general. Depression is much prolong the patients have chronic diseases. Depression is a neurological disorder that leads to changes in mood, thoughts, behavior and physical health. *Celosia cristata* Linn (Amaranthaceae) is used in traditional medicine for the treatment of headache, sores, ulcers, eye inflammations, skin eruption, painful menstruation and carpal tunnel syndrome. The aim of present study was to investigate the antidepressant activity of petroleum ether and hydroalcoholic extract of leaves and flowers of *Celosia cristata* Linn in albino wistar rats by Forced swimming test. Acute toxicity of the extract (2000 mg/kg) was examined in wistar rats for 14 days. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Phytochemical analysis of hydroalcoholic extract of leaves and flowers extract revealed the presence of carbohydrates, alkaloids, flavonoids, tannins and phenolic compounds, protein and amino acids, glycosides. The total phenolics content of hydroalcoholic extract of leaves and flowers extract of *Celosia cristata* was (79.00 mg/gm and 47.00 mg/gm equivalent to Gallic acid), followed by flavonoids (44.00 mg/gm and 23.00 mg/gm equivalent to Rutin), respectively. Extract up to 2000 mg/kg did not produce any toxic effects. It was observed that there was significant reduction ($p<0.05$) in immobility time in group (CCLHAE 400mg/kg) and group (CCFHAE 400mg/kg) treated group when compared to stress induced showing their potent antidepressant action. Similarly, Imipramine group (30mg/kg) showed significant reduction in immobility time ($p< 0.05$). The present study suggested that *Celosia cristata* possessed potential antidepressant effects which could be of therapeutic interest for using in the treatment of patients with depression.

Keywords: *Celosia cristata* Linn, Antidepressant activity, Phytochemical analysis, Immobility time, Acute toxicity

[1]*Ph.D. Research Scholar, Faculty of Pharmacy, Oriental University, Indore, India.
[2]Faculty of Pharmacy, Oriental University, Indore, India.

***Corresponding Author:** Naveen Kumar Tripathi

*Ph.D. Research Scholar, Faculty of Pharmacy, Oriental University, Indore, India.
Email: tripathi.lahar@gmail.com, Phone: +917828841326

DOI: - 10.31838/ecb/2023.12.si5.035

INTRODUCTION

Depression is a widespread psychiatric ailment[1]. It is already expected to constitute the second largest source of global burden of disease after heart disease in 2020[2]. The monoaminergic hypothesis of depression does not provide a full understanding of the progression, causes and pharmacotherapy of depression[3]. Most accepted hypothesis of depression is postulated and oxidative stress is suggested to be involved in the pathophysiology of depression[4]. According to WHO estimated, 121 million people suffer from clinical depression[5]. It occurs usually in the early adult life of patients with decrease in monoamine neurotransmitters[6]. Medicinal plants therapies may be effective alternatives in the treatment of depression. It possesses least side effects compared to synthetic medicines[7]. It has contributed significantly towards the development of modern medicine. Recently, traditional medicine is being reevaluated by extensive research on different plant species and their active therapeutic principles in worldwide. The rich wealth of plant kingdom can represent a novel source of newer compounds with significant therapeutic activities. The most important merits of herbal medicine seem to be their perceived efficacy, low adverse effects, and low cost[8]. *Celosia cristata* (commonly known as Cockscomb) is an annual herb from Amaranthaceae family. It is locally called Morgaphul and widely seen in Africa, South America, India and some parts of Asia. The plant is applied for the treatment of headache, sores, ulcers, eye inflammations, skin eruption, painful menstruation and carpal tunnel syndrome in ethnomedicine [9-12]. Flower of the plant is used in the treatment of abdominal pain, epistaxis, hemoptysis, hematuria, hematemesis and painful bones [13-15]. Leaves are used in cuts, wounds and body swelling[16, 17]. Seeds are applied in the treatment of mouth sores, inflammation of the ciliary body, cornea and iris and piles[18]. Branches and roots are used in leucorrhea[19]. The plant contains asparagine, asparagine-linked glycon, protein, glycoproteins. Hyaluronic acid (HA) has been found in the plant. The aerial parts of the plant contain cristatein and tlatlancuayin. Seeds of the plant have been reported to contain 5-hydroxy-7-methoxyflavone, 5-methoxy-6,7-methylenedioxyflavone, 5-hydroxy-6,7-dimethoxyflavone 5,7-dimethoxyflavone, cochliophilin A, kaempferol, stigmasterol, β-sitosterol, 4- hydroxyphenethyl alcohol, 2-hydroxyoctadecanoic acid, saponins named celosin A, B, C and D, cristatain and semenoside A. Antiviral glycoproteins named CCP-25 and CCP-27 have been found in leaves. Betanin has been found in the callus line of the plant. The plant has potential pharmacological values screened for its various pharmacological activities, namely, anti-inflammatory, immune-stimulating, anticancer, adipogenesis reduction and acetylcholinesterase, butyrylcholinesterase, tyrosinase enzyme inhibition activity, hepatoprotective, antioxidant, anti-aging, wound healing, antidiabetic, antinociceptive effect, and antibacterial activities which are reported in the extracts of different parts of the plant [20, 21]. Therefore, the present study has been undertaken to investigate the effect of petroleum ether and hydroalcoholic extract of leaves and flowers of *Celosia cristata* on depression in albino wistar rats.

MATERIALS AND METHODS

Plant material

The leaves and flowers of plant *Celosia cristata* were collected from rural area of Gwalior (M.P.) in the month of February, 2021. The sample was identified by Senior Botanist Dr. Arti Garg, Scientist- E and Head of Office, Botanical Survey of India (Ministry of Environment, Forest & Climate Change), Central Regional Centre, 10 Chatham Lines, Allahabad-211002. A herbarium of plants was submitted to the specimen department of Botanical Survey of India, Central Regional Centre, 10 Chatham Lines, Allahabad. And the specimen voucher no. of *Celosia argentea var. cristata* (L.) Kuntze is सं0 भा.व.स./ म.क्षे.के./2020-21 / 429 and accession no. is 105479. The plant material was dried under shade. It was pulverised to coarse powder with the help of hand grinder. The coarse powder was packed into airtight container and stored in cool and dry place. This material was used for the further study.

Fig. : *Celosia cristata* L.

Chemical reagents

Imipramine hydrochloride (Sigma-Aldrich, St Louis, USA) was used in this study. All drugs were dissolved in distilled water and administered either intraperitoneal (i.p.) or orally (p.o.). Distilled water was used as the vehicle and all the other chemicals and reagents were of analytical grade and were purchased from S.D. fine Chemicals Pvt. Ltd., Mumbai, India and SRL Pvt. Ltd. (Mumbai, India).

Soxhlet extraction

Powdered *Celosia cristata* leaves and flowers were placed in a thimble of soxhlet apparatus. The extraction was carried out using organic solvents; Petroleum ether and 70% ethanol for 8-10 hours and 40-60ºC temperature of the heating mantle were adjusted. After the extraction process, the extracts of sample were filtered and concentrated to dryness. Extracts were collected in air tight container [22]. Extraction yield of all extracts were calculated using the following equation below:

Formula of Percentage yield = Actual yield/ Theoretical yield X 100

Qualitative phytochemical screening

Crude extracts were screened to identify the occurrence of primary and secondary metabolites, viz. carbohydrates, alkaloids, glycosides, polyphenols, flavonoids, tannins, saponins, terpenoids, proteins and fixed oils, using standard screening test and phytochemical procedures [23, 24].

Quantitative phytochemical estimation

Spectrophotometric quantification of total phenolic content

The total phenolic content of plant extract was determined using the Folin-ciocalteu assay. The *Celosia cristata* extracts (0.2 ml from stock solution) were mixed with 2.5 ml of Folin-ciocalteu reagent and 2ml of 7.5% sodium carbonate. This mixture was diluted up to 7 ml with distilled water. Then the resulting solutions were allowed to stand at room temperature for 2 hrs before the absorbance was measured spectrophotometrically at 760 nm. Calibration curves were composed using standard solutions of gallic acid equivalent (GAE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/ml of gallic aid was prepared. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically[25, 26].

Spectrophotometric quantification of total flavonoid content

The flavonoid content was determined using Aluminium chloride method. 0.5 ml of *Celosia cristata* extracts solution was mixed with 2 ml of distilled water. Then, 0.15 ml of sodium nitrite (5%) was added and mixed properly. After that, wait for 6minutes before adding 0.15ml Aluminium chloride (10 %) and allowed to stand for 6minutes. Then, 2ml of 4 % sodium hydroxide was added. Then the mixture was diluted up to 5ml with distilled water mixed thoroughly. Absorbance of mixture was estimated at 510nm using UV spectrophotometer. Calibration curves were composed using standard solutions of Rutin Equivalent (RE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/ml of rutin was prepared. Total flavonoid content was determined from the calibration curve and results were indicated as mg rutin equivalent per gram dry extract weight [25, 26].

Animals

Albino wistar rats (190±15gm) were selected and procured from PBRI animal house and were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55-65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee of PBRI, Bhopal, constituted for the purpose of control and supervision of experimental animals. Protocol approval number was PBRI/IAEC/29-03-22/009.

Acute oral toxicity study

The acute toxicity study was performed for *CCLPE, CCFPE, CCFHAE* and *CCLHAE* according to OECD (423) guidelines. The test groups include four treatment groups with dosages at 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg body weight. The test substance was administered in a single dose by gavage using specially designed oral needle or a suitable intubation canula. Animals were fasted 3 hours prior to dosing (only food was withheld for 3 hours but not water). The animals were observed almost constantly for behavioral changes, mortality and appearance during firstly for first 4 hours, periodically during the 24 hours and then every day for a period of two weeks till 14 days [27].

Antidepressant activity

Forced swimming test

The forced swim test (FST) is a rodent behavioral paradigm utilized for assessment of potential antidepressant-like medications. In this model the rat will be placed in a Plexiglas tank. The tank is loaded with water and the behavior of the rat to escape the tank is scored. The rat tries to come out of the water and this behavior is known as "mobility". However, after some time the struggling movements of the rat die down and the rat may surrender and become totally motionless which is known as "immobility". The length of immobility time is scored during the testing period and decrease in the length of time of immobility during this test is termed as antidepressant activity. This method was adapted on the observation of animals exposed to a situation of forced swimming, in which they become passive and immobile after a period of vigorous activity (struggling), producing only the movements required to keep their heads above the water. Each animal made vigorous attempts to get out of water bath during first couple of minutes and thereafter surrendered to experimental conditions and assumed a typical immobile posture with occasional escape attempts [28].

Experiment design

Rats were segregated into six groups of six animals each:

Group I Negative control (untreated stress induced)

Group II Imipramine standard (30 mg/kg, i.p.) for 14 days

Group III CCLPE 400 mg/kg, in dw, p.o. for 14 days

Group IV CCLHAE 400 mg/kg, in dw, p.o. for 14 days

Group V CCFPE 400 mg/kg, in dw, p.o. for 14 days

Group VI CCFHAE 400 mg/kg, in dw, p.o. for 14 days

Treatment groups were administered once daily between 1 and 3 p.m. over a period of 14 days. Rats were placed in an acrylic cylinder (45 cm height = 20 cm diameter) filled with water at 25 ± 1°C to a depth of 15 cm. According to above-mentioned apparatus, a trial session/pre-test session carried out (after 14-day treatment) this allowed the rats to swim for 15 minutes. Twenty-four hours after the pre-test session, the animals were once again exposed to the same conditions for 5 min (test session). Between the pretest session and main session drug solutions were administered orally three times as follows: just after the pre-test session, 5 h before the main test, and 1 h before the main test. A rat was considered to be immobile when it remains floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The FST was performed between 1 and 3 p.m. for 5 min by observers. Duration of immobility was recorded in seconds. Decrease in the duration of immobility during the FST was taken as a measure of antidepressant activity [29, 30].

RESULTS AND DISCUSSIONS

The crude extracts so obtained after the soxhlet apparatus, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction Table 1& 2. The results of preliminary phytochemical screening of Pet. ether and hydroalcoholic extract of leaves and flowers of *Celosia cristata* are shown in Table 3. Phytochemical analysis of hydroalcoholic extract of leaves and flowers extract revealed the presence of carbohydrates, alkaloids, flavonoids, tannins and phenolic compounds, protein and amino acids, glycosides and Pet. ether extract show the presence of terpenoids and saponins only. The content of total phenolic compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **$Y = 0.001X+0.089$, $R^2= 0.990$,** where X is the gallic acid equivalent (GAE) and Y is the absorbance. The content of total flavonoid compounds (TFC) content was expressed as mg/100mg of rutin equivalent of dry extract sample using the equation obtained from the calibration curve: **$Y = 0.001X+0.100$, $R^2= 0.994$,** where X is the rutin equivalent (RE) and Y is the absorbance. The total phenolics content of hydroalcoholic extract of leaves and flowers extract of *Celosia cristata* was (79.00, 47.00mg/100mg), followed by flavonoids (44.00, 23.00mg/100mg) respectively Table 4, 5 & Figure 1, 2. There was no sign of any abnormality in the rats. Test samples CCFPE, CCLPE, CCFHAE, CCLHAE at various doses (5, 50, 300 and 2000) did not cause significant changes to the parameters such as urinations, convulsion, tremor, changes in skin colors etc. So, depending on the current observed data of acute toxicity study, the final selected dose for further study were carried out using 1/5th of 2000 mg/kg bw. The extracts of *CCLPE, CCFPE, CCFHAE* and *CCLHAE* showed an antidepressant effect in the FST because it significantly reduced the immobility time compared with the vehicle treated group

(197.80±7.782 sec). The immobility times of the extract of CCLPE, CCFPE at dose of 400 mg/kg/day on the 14th day were found as 132.8±6.585 and 148.2±8.134 sec, and CCFHAE and CCLHAE extract treated groups at dose 400 mg/kg/day were found as 125.7±4.546 and 115.8±7.304 respectively. The extract CCFPE did not reduce immobility time significantly. The group treated with Imipramine showed good activity (90.50±5.891sec). The results of the effect of CCLPE, CCFPE, CCFHAE and CCLHAE extract upon treatment once daily orally for 14 days on the immobility time (seconds) in Forced swim test resulted in decrease in immobility time in rats as shown in Table 6. It was observed that there was significant reduction ($p<0.05$) in immobility time in group (CCLHAE 400mg/kg) and group (CCFHAE 400mg/kg) treated group when compared to Stress induced showing their potent antidepressant action. Similarly, Imipramine group (30mg/kg) showed significant reduction in immobility time ($p< 0.05$).

Table 1: Percentage yield of *Celosia cristata* leaves

S. No.	Solvent	Color of extract	Theoretical weight (gm)	Yield in gms	% Yield
1.	Pet. Ether	Brownish yellow	190.450	0.574	0.301
2.	Hydro-alcoholic	Green	183.05	13.372	7.305

Table 1: Percentage yield of *Celosia cristata* flowers

S. No.	Solvent	Color of extract	Theoretical weight (gm)	Yield in gms	% Yield
1.	Pet. Ether	Yellow	134.17	1.027	0.762
2.	Hydro-alcoholic	Red	130.71	11.914	9.114

Table 3: Qualitative phytochemical analysis of *Celosia cristata* leaves and flowers extracts

S. No.	Experiment	Results			
		Leaves		Flowers	
		Pet. Ether	Hydroalcoholic	Pet. Ether	Hydroalcoholic
Test for Carbohydrates					
1.	Molisch's Test	-	+	-	+
2.	Fehling's Test	-	+	-	+
3.	Benedict's Test	-	+	-	+
4.	Bardford's Test	-	+	-	+
Test for Alkaloids					
1.	Mayer's Test	-	+	-	+
2.	Hager's Test	-	+	-	+
3.	Wagner's Test	-	+	-	+
4.	Dragendroff's Test	-	+	-	+
Test for Terpenoids					
1.	Salkowski Test	+	-	-	-
2.	Libermann-Burchard's Test	+	-	-	-
Test for Flavonoids					
1.	Lead Acetate Test	-	+	-	+
2.	Alkaline Reagent Test	-	+	-	+
3.	Shinoda Test	-	+	-	+
Test for Tannins and Phenolic Compounds					
1.	$FeCl_3$ Test	-	+	-	+
2.	Lead Acetate Test	-	+	-	+
3.	Gelatine Test	-	+	-	+
4.	Dilute Iodine Solution Test	-	+	-	+
Test for Saponins					
1.	Froth Test	+	-	+	-
Test for Protein and Amino acids					
1.	Ninhydrin Test	-	+	-	+
2.	Biuret's Test	-	+	-	+
3.	Million's Test	-	+	-	+
Test for Glycosides					
1.	Legal's Test	-	+	-	+
2.	Keller Killani Test	-	+	-	+
3.	Borntrager's Test	-	+	-	+

Table 4: Total phenolic content in *Celosia cristata* leaves and flowers extracts

	Total phenolic content (mg/gm equivalent to Gallic acid)

Extracts	Leaves extract	Flower extract
Absorbance Mean±SD	0.168±0.004	0.136±0.005
TPC	79.00	47.00

Table 5: Total flavonoid content in *Celosia cristata* leaves and flowers extracts

	Total flavonoid content (mg/gm equivalent to rutin)	
Extracts	Leaves extract	Flower extract
Absorbance Mean±SD	0.144±0.004	0.123±0.005
TFC	44.00	23.00

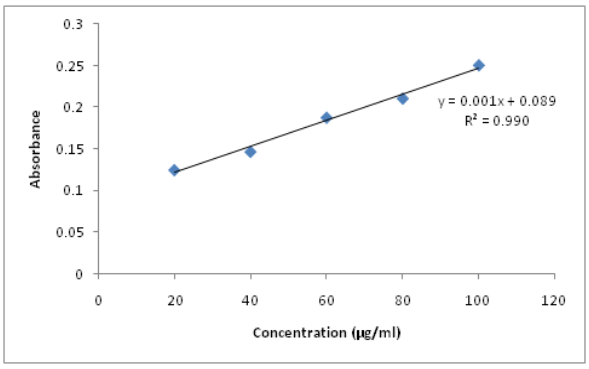


Figure 1: Represent standard curve of Gallic acid

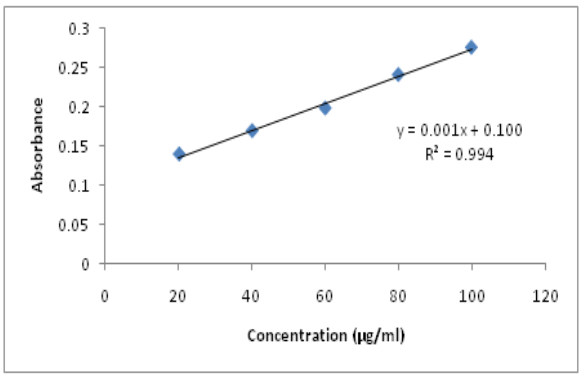
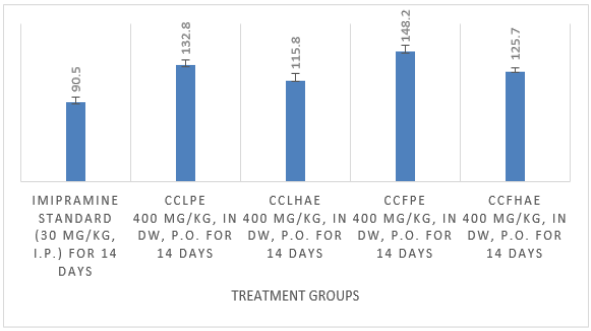


Figure 2: Represent standard curve of Rutin

Table 6: Effect of *Celosia cristata* extract on immobility time using Forced swim test

Groups	Treatment	Immobility time (sec)
I	Negative control (untreated stress induced)	197.80±7.782
II	Imipramine standard (30 mg/kg, i.p.) for 14 days	90.50±5.891**
III	CCLPE 400 mg/kg, in dw, p.o. for 14 days	132.8±6.585**
IV	CCLHAE 400 mg/kg, in dw, p.o. for 14 days	115.8±7.304**
V	CCFPE 400 mg/kg, in dw, p.o. for 14 days	148.2±8.134**
VI	CCFHAE 400 mg/kg, in dw, p.o. for 14 days	125.7±4.546**



Values are expressed as Mean ± SD (no=6); **p< 0.05 statistically significant as compared to Stress induced group by One Way ANOVA followed by Bonferroni's Test. P>0.05 was considered as non-significant (NS) v/s Stress induced.

CONCLUSION

Since ancient times, people have been using plants in various ways as a source of medicine. From the above preclinical study, we can conclude that hydroalcoholic leaves and flowers extracts of *Celosia cristata* show a significant antidepressant activity as compared to pet. ether extract. Phytochemical analysis of hydroalcoholic extract of leaves and flowers extract revealed the presence of carbohydrates, alkaloids, flavonoids, tannins and phenolic compounds, protein and amino acids, glycosides. We believe that *Celosia cristata* has the potential to be used as an adjuvant in the treatment of depression and other mood disorders. Further research is required to gain closer insights into the exact mechanism of its action.

ACKNOWLEDGEMENT

The authors express special thanks to Dr. M.A. Khan and all supporting staff of Pinnacle Biomedical Research Institute (PBRI), Bhopal for providing lab facilities & their kind support.

AUTHORS CONTRIBUTION STATEMENT

Naveen Kumar Tripathi conceptualized, design and perform the research work. Neelam Khan supervised in research work.

CONFLICT OF INTEREST

Conflict of interest declared none.

ABBREVIATIONS

CCLPE- *Celosia cristata* leaves petroleum ether
CCLHAE- *Celosia cristata* leaves hydroalcoholic extract
CCFPE- *Celosia cristata* flower petroleum ether
CCFHAE- *Celosia cristata* flower hydroalcoholic extract

REFERENCES

1. Ferrari AJ, Charlson FJ, Norman RN, Patten SB, Freedman G, CGL M, Vos T, Whiteford HA. Burden of depressive disorders by country, sex, age, and year: findings from the global burden of disease study 2010. PLoS Med. 2013; 10(11):1-12.
2. Smith AJ, Sketris I, Cooke C, Gardner D, Kisely S, Tett SE. A comparison of antidepressant use in Nova Scotia, Canada and Australia. Pharmacoepidemiol Drug Saf. 2008; 17(7):697-706.
3. Schildkraut JJ, Gordon EK, Durell J. Catecholamine metabolism in affective disorders. I. Normetanephrine and VMA excretion in depressed patients treated with imipramine. J Psychiatr Res. 1965; 3(4):213-28.
4. Michel TM, Frangou S, Thiemeyer D, Camara S, Jecel J, Nara K, Brunklaus A, Zoechling R, Riederer P. Evidence for oxidative stress in the frontal cortex in patients with recurrent depressive disorder-a postmortem study. Psychiatry Res. 2007; 151(1-2):145–50.
5. Cryan JF, Lucki I. Antidepressant like behavioral effects mediated by hydroxy tryptamine receptors. The Journal of Pharmacology experimental Therapeutics. 2000; 295:1120-6.
6. Dhingra D, Sharma A. Review on antidepressant plants. Natural Products Radiance. 2005:144-52.
7. Zhang J, Wu J, Fujita Y, Yao W, Ren Q, Yang C, Li S, Shirayama Y, Hashimoto K. Antidepressant effects of TrkB ligands on depression-like behaviour and dendritic changes in mice after inflammation. Int J Neuropsychopharmacol. 2015:1-12.
8. Ghani A. Medicinal plants of Bangladesh. The Asiatic Society of Bangladesh: Dhaka; 1998.
9. Navarra T. The encyclopedia of vitamins, minerals, and supplements. New York: Facts on File, Inc.; 2004.
10. Li TS. Taiwanese native medicinal plants: phytopharmacology and therapeutic values. London: Taylor and Francis; 2006.
11. Rahman AM, Gulshana MIA. Taxonomy and medicinal uses on amaranthaceae family of Rajshahi, Bangladesh. App Ecol Environ Sci. 2014; 2:54–9.
12. Song MJ, Kim H, Lee BY, Brian H. Analysis of traditional knowledge of medicinal plants from residents in Gayasan National Park (Korea). J Ethnobiol Ethnomed. 2014; 10:1–47.
13. Anderson EF. Ethnobotany of hill tribes of northern Thailand. I. Medicinal plants of Akha. Econ Bot. 1986; 40:38–53.
14. Foster S, Chongxi Y. Herbal emissaries: bringing Chinese herbs to the West: a guide to gardening, herbal wisdom and well-being. Vermont: Healing Arts Press; 1992.
15. Motaleb MA, Abdullah-Al-Mamun MM, Hossain MK, Alam MK, Sultana M. Herbal healing: an old practice for healthy living among Khumi, Marma and Tripura communities of Thanchi upazila, Bangladesh. European J Med Plants. 2015; 5:23–52.
16. Yusuf M, Wahab MA, Yousuf MD, Chowdhury JU, Begum J. Some tribal medicinal plants of Chittagong hill tracts, Bangladesh. Bangladesh J Plant Taxonomy. 2007; 14: 117–28.
17. Kichu M, Malewska T, Akter K, Imchen I, Harrington D, Kohen J, et al. An ethnobotanical study of medicinal plants of Chungtia village, Nagaland, India. J Ethnopharmacol. 2015; 166:5–17.
18. Halim MA, Chowdhury MSH, Wadud AI, Uddin MS, Sarker SK, Uddin MB. The use of plants in traditional health care practice of the "Shaiji" community in southwestern Bangladesh. J Trop For Sci. 2007;19:168–75.
19. Afroz SS, Sen US, Islam MJ, Morshed MT, Bhuiyan MSA, Ahmed I, et al. Ethnomedicinal plants of various tribal and folk medicinal practitioners of six localities of Rangamati and Khagrachari districts in Bangladesh. Am-Eurasian J Sustain Agric. 2013; 7:262–71.
20. Tripathi NK, Khan N. Phytochemical and pharmacological overview on celosia cristata linn. Journal of Advanced Scientific Research. 2021;12(03):46-51.
21. Islam S, Shajib MS, Ahmed T. Antinociceptive effect of methanol extract of Celosia cristata Linn. in mice. BMC complementary and alternative medicine. 2016; 16(1):1-4.
22. Alara OR, Abdurahman NH, Ukaegbu CI, Kabbashi NA. Extraction and characterization of bioactive compounds in Vernonia amygdalina leaf ethanolic extract comparing Soxhlet and microwave-assisted extraction techniques. Journal of Taibah University for Science. 2019;13(1):414-22.

23. Dutta R, Sharma MK, Khan A, Jha M. Phytochemical and in vitro antioxidant assay of Fumaria officinalis leaf extract. Journal of Advanced Scientific Research. 2020; 11(03):176-82.
24. Dutta R, Sharma MK, Jha M. Pharmacological evaluation of antiasthmatic activity of fumaria officinalis extracts. Plant Archives. 2020; 20(2):4308-15.
25. Joshi S, Parkhe G, Aqueel N, Dixit N, Jain DK. Estimation of total phenolic, total flavonoids and total protein content of hydroalcoholic extract of Anacyclus pyrethrum. Pharmacol-ogyonline. 2019; 1:27-33.
26. Nayak A, Garg M, Jain S, Khan MA, Jain DK, Khan N. Anti-Urolithiatic and invitro-invivo anti-oxidant effects of methanolic extract of thunbergia laurifolia on ethylene glycol-induced kidney calculi in rats. Sch Acad J Pharm. 2019; 8(3):94-104.
27. Guideline Document on Acute oral Toxicity Testing, Series on Testing and Assessment No. 423. Paris: Organization for Economic Co-Operation and Development, OECD Environment, Health and Safety Publications; 1996. Available from: http//www.oecd.org/ehs.
28. Porsolt RD, Bertin A, Blavet N, Deniel M, Jalfre M. Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity. European journal of pharmacology. 1979; 57(2-3):201-10.
29. Sakakibara H, Ishida K, Grundmann O, Nakajima JI, Seo S, Butterweck V, Minami Y, Saito S, Kawai Y, Nakaya Y, Terao J. Antidepressant effect of extracts from Ginkgo biloba leaves in behavioral models. Biological and Pharmaceutical Bulletin. 2006; 29(8):1767-70.
30. Hsu LC, Ko YJ, Cheng HY, Chang CW, Lin YC, Cheng YH, Hsieh MT, Peng WH. Antidepressant-like activity of the ethanolic extract from Uncaria lanosa Wallich var. appendiculata Ridsd in the forced swimming test and in the tail suspension test in mice. Evidence-Based Complementary and Alternative Medicine. 2012.