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Assessment of analgesic and neuropharmacological activity of ethanol leaves extract of *Gynura procumbens* (Family: Asteraceae)

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Abstract
Background: *Gynura procumbens* belonging to Asteraceae family is a medicinal plant commonly found in tropical Asian countries such as Bangladesh, India, China, Thailand, Indonesia, Malaysia, and Vietnam. Traditionally, it is widely used in many different countries for the treatment of a wide variety of health ailments such as kidney discomfort, rheumatism, diabetes mellitus, constipation, and hypertension. Based on the traditional uses of *G. procumbens*, it seems to possess high therapeutic potential for treatment of various diseases making it a target for pharmacological studies aiming to validate and provide scientific evidence for the traditional claims of its efficacy. As part of our ongoing research of traditional medicinal plant, this study evaluated the analgesic and neuropharmacological activity of Ethanol Extract of *Gynura procumbens* (EEGP) leaves. Method: The analgesic effect of EEGP was determined by acetic acid induced writhing test, tail immersion test and hot plate test using swiss albino mice as experimental animal. Open field and hole cross test were used to determine the neuropharmacological activity of EEGP. Result: Preliminary phytochemical screening revealed the presence of tannin, flavonoids, steroids, glycoside, alkaloids and terpenoids. The present study demonstrated that EEGP showed significant analgesic (p<0.05) effect at the dose level of 200 and 400 mg/kg body weight in acetic acid induced writhing test, tail immersion test and hot plate test. EEGP decreased the movement of mice significantly at the doses of 200 and 400 mg/kg body weight in open field and hole cross test.
Conclusion: The present study indicates that EEGP possess analgesic and neuropharmacological activity in a dose-dependent manner. Hence, the present work was undertaken to evaluate and co-relate the bioactivity with the traditional uses of *G. procumbens*. So, the plant may be further subjected to chemical investigation to isolate the bioactive compound(s) responsible for its pharmacological activity.

Keywords: *Gynura procumbens*, acetic acid, tail immersion, hot plate, open field, hole cross

Introduction
Gynura procumbens is a well-known traditional herb in South East Asia. The plant belongs to the Asteraceae Family. This plant is about 10-25 cm high and it is presented with succulent, elliptic and glossy purplish leaves [1]. The leaves of *G. procumbens* have been served as food for decades in Malaysia, where it is generally consumed raw as salad. Besides, the plant is widely used to treat inflammation, kidney discomfort, high cholesterol level, diabetic, cancer and high blood pressure. Indeed, *G. procumbens* is used traditionally in South East Asia for its valuable medicinal property. The small molecular weight compounds extracted from *G. procumbens* have been reported to display anti-cancer, anti-oxidant, anti-inflammatory, anti-hyperglycemic and anti-hyperlipidemic activities [2, 3]. Moreover, we have detected the presence of valuable plant defense secondary metabolites from the leaf of *G. procumbens*. We hope that the data obtained will be useful for the future intervention of secondary metabolites-based drug for discovery [3]. As a part of our enduring study about medicinal activity of natural resources [4-11], here we evaluated the analgesic and neuropharmacological activity of ethanol extract of *Gynura procumbens* leaves and saw whether it has analgesic and CNS action.

Materials and Methods
Collection of plant materials
The leaves of *G. procumbens* was collected from Gopalganj, Bangladesh in January, 2019. Later on, the plant was identified and verified by the senior scientific officer of Bangladesh National herbarium, Mirpur, Dhaka and the given accession code was DACB: 48247.

Preparation of plant extract
About 200 gm of the powdered material was taken in a clean and flat-bottomed glass beaker and soaked in 5000 mL methanol (95%) (Merck, Germany) at 25±2 ºC for 15 days accompanying regular shaking and stirring. The solvent mixture was filtrated by a piece of sterile and white cotton material and finally using Whatman No. 1 filter paper. The solvent was totally removed by air drying and obtained 5 gm extract. The prepared extract was used for the phytochemical screening as well as pharmacological studies.

Collection and maintenance of animals
Swiss-Albino mice of either sex having aged 4-5 weeks, obtained from the animal breeding house of Jahangirnagar University, Savar, Dhaka, Bangladesh were used for the experiment. They were kept in standard environmental condition and fed International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) formulated food and water. As these animals are very sensitive to environmental changes, they are kept before the test for at least 4-5 days in the laboratory. Animals were maintained under standard conditions (temperature: (24.0±1.0 ˚C), relative humidity: 55-65% and 12 hrs light/12 hrs dark cycle) with proper cleaning of husk and excreta.

Drugs and Chemicals
The drugs and chemicals involved in our study are Distilled water, Tween 80, Diclofenac sodium, Acetic acid. All the chemicals and solvent were of analytical grade.

Phytochemical Screening
The crude ethanol extract of *G. procumbens* was qualitatively tested for the detection of different phytochemical groups like alkaloids, glycosides, flavonoids, tannins, reducing sugar, carbohydrates, steroids and saponins following standard procedures [7].

Determination Analgesic activity
The study of analgesic activity of the *Gynura procumbens* was performed in animal models for both central and peripheral mechanism of pain.

Acetic Acid Induced Writhing Test
The antinociceptive activity of the samples was studied using acetic acid-induced writhing model in mice [7]. The animals were divided into control, positive control, and two test groups with five mice in each group. The animals of test groups were given test samples at the doses of 200 and 400 mg/kg body weight. Positive control group was treated standard drug Diclofenac at the dose of 75 mg/kg body weight and control group was treated with distilled water at the dose of 10 mL/kg body weight. After administration of sample, the mice were observed for specific contraction of body referred to as 'writhing' and compared with positive control group [12-13].

Hot- plate test
Hot plate test was used to measure the response latencies based on the procedure describe by Basak, A. *et al*., 2016 [11]. In this experiment hot plate was maintain at 50±5°C. The reaction time was recorded for animals pre-treated with distilled water (10 mL/kg 30 min before orally) as control, extract at the doses of 200 and 400 mg/kg body weight (30 min before), Diclofenac sodium (75 mg/kg body weight intraperitoneally, 15 min before) as positive control group. Animals were placed into the hot plate chamber and the time of latency was defined as the time period between the zero point, when the animal was placed on the hot plate surface and the time when animal licked its back paw or jumped off to avoid thermal pain. The latent period of response was taken as the index of antinociception.

Tail Immersion Test
The procedure is based on the observation that morphine like drugs selectively prolong the reaction time of the typical tail withdrawal reflex in mice. The animals of the positive control, control and test groups were treated with Diclofenac-Na (75 mg/kg body weight), water (10 mL/kg body weight) and test samples at the doses of 200 and 400 mg/kg body weight respectively. 1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55°C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 20 sec was defined as complete analgesia and the measurement was stopped when the latency period exceeded to avoid injury to mice. The latent period of the tail-flick response was taken as the index of antinociception and was determined at 0, 30, 60, 90 and 120 min [14].

Neuropharmacological Activity
The purpose of this study was to examine neuropharmacological effect of ethanol extract of leaves of *Gynura procumbens* on mice in a peripheral model of CNS activity test.

Study Design
Experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III, group-IV consisting of 5 mice in each group. Each group received a particular treatment i.e. control, positive control and the two doses of the extract. Each mouse was weighed properly and the doses of the samples and control materials were adjusted accordingly.

Open-Field Test
The Open Field Test (OFT) is clearly the most frequently used method of all behavioral tests in pharmacology and neuroscience. Despite the simplicity of the apparatus, however, open field behavior is complex. Consequently, it has been used to study a variety of behavioral traits, including general motor function, exploratory activity and anxiety-related behaviors.

Hole Cross Test
The most consistent behavioral change is a hyperemotional response to novel environmental stimuli. The aim of this study was to characterize the emotional behavior of mice using the hole-board test. The number of head-dips in the hole-board test in single-housed mice was significantly greater. Spontaneous movement of the animals through the hole from one chamber to the other was counted for 5 minutes. The observations are made at 0, 30, 60, 90 and 120 minutes.

Statistical analysis
Results were expressed as mean±S.E.M. Variance was analyzed using One-way Analysis Of Variance (ANOVA), followed by Newman-Keul's multiple comparisons test. $P<0.05$ was considered to be statistically significant.

Results

Preliminary qualitative phytochemical analysis

In the preliminary phytochemical screening the extract showed the presence of tannin, flavonoids, steroids, glycosides, alkaloids and terpenoids.

Table 1: Result of Phytochemical group test

Constituents	Result
Reducing sugar	-
Tannins	+
Flavonoids	+
Terpenoids	+
Saponin	-
Steroids	+
Alkaloids	+
Gums	-
Glycosides	+
Protein	-

Here, + = Presence, - = Absence

Acetic acid induced writhing test

Table 2 shows the analgesic effect of *Gynura procumbens* by acetic acid-induced writhing in mice. The extract significantly inhibited writhing response induced by acetic acid in a dose-dependent manner. The result was statistically significant and was comparable to the reference drug Diclofenac sodium.

Table 2: Analgesic effect of *Gynura procumbens* by acetic acid-induced writhing in mice

Treatment	Dose	Mean of writhing ± SEM	% of writhing	% of Inhibition
Control	0.1 mL/Mouse	36±8.112	100	0.00
Positive control	75 mg/kg	7.1±3.201	28.7	71.3
Group-I	200 mg/kg	23.2±0.55	63.5	36.5
Group-II	400 mg/kg	13.8±7.2	40.4	59.6

Hot- plate test

The analgesic effects of the ethanol extract of *Gynura procumbens* at two different doses on the experimental mice evaluated by Hot plate method. EEGP showed comparable analgesic effect as shown in Table 3.

Table 3: Analgesic effect of *Gynura procumbens* by acetic hot plate method in mice

Test Group	Dose	0 min	30 min	60 min	90 min	120 min
Control	0.1 mL/kg	3.19±0.69	4.19±0.98	5.19±2.25	6.24±1.01	6.29±0.367
Positive Control	75 mg/kg	1.23±0.07	4.54±1.25	8.21±2.92	9.37±1.193	5.31±0.605
Group-I	200 mg/kg	2.12±0.46	7.15±1.12	6.23±0.36	7.32±1.494	5.11±0.82
Group-II	400 mg/kg	3.81±0.31	4.03±1.12	4.23±0.45	5.11±0.650	3.19±0.95

Tail Immersion Test

Table 4 shows the analgesic activity test that were carried out by tail-flick method. Time interval for the test was 30 minutes. The tail withdrawal reflex time after administration of the *Gynura procumbens* was comparable to standard.

Table 4: Analgesic effect of *Gynura procumbens* by tail withdrawal reflex in Mice

Treatment	Dose	Response Times (in seconds)				
		0 min	30 min	60 min	90 min	120 min
Control	0.1 mL/ mice	1.31±0.75	2.03±0.21	2.21±0.12	2.15±0.98	1.59±0.19
Diclofenac-Na	75 mg/kg	1.85±0.73	2.38±0.33	2.55±0.41	2.45±0.29	1.71±0.171
Group-I	200 mg/kg	2.12±0.13	3.68±0.19	2.35±***0.***23	2.81±0.36	1.54±0.159
Group-II	400 mg/kg	1.61±0.21	2.19±0.21	2.15±***0.***19	2.24±***0***.17	2.56±0.325

Open-Field Test

The neuropharmacological activity of EEGP by open field test is shown in table 5. The number of squares traveled by the mice was suppressed significantly by EEGP from its initial score at the doses of 200 and 400 mg/kg body weight which is comparable to the reference drug, Diazepam

Table 5: Neuropharmacological effect of *Gynura procumbens* by open field test

Group	Dose (mg/kg b.w.)	Observation				
		0 min	30 min	60 min	90 min	120 min
Control	0.1 mL/mouse	66.4±5.11	51.3±3.15	44.2±3.15	34.1±1.2	20.6±4.14
Positive Control	1	84.6±8.20	3.65±1.12	32.91±2.34	55.8±0.34	22.5±1.4
Group-1	200	52.2±12.32	51.8±4.758	31.6±6.81	18.4±6.2	17.4±1.88
Group-2	400	91.8±9.06	43.2±4.032	31.1±4.79	13.6±2.18	12.2±2.24

Hole Cross Test

The sample significantly decreased the number of movement of mice compared to control group at the doses of 200 and 400 mg/kg body weight in hole cross experiment (Table 6).

Table 6: Neuropharmacological effect of *Gynura procumbens* by Hole Cross Test

Group	Dose (mg/kg b.w.)	Observation				
		0 min	30 min	60 min	90 min	120 min
Control	0.1 mL/mouse	13.2±0.44	10.8±0.583	9±0.547	3.2±0.316	2.7±0.41
Positive Control	1	4.6±1.32	0.6±1.913	0.4±1.32	2±0.509	3.2±0.214
Group-1	200	13.6±1.4	12.4±0.66	8.2±0.583	5±0.387	0.6±0.374
Group-2	400	9.8±0.41	9.2±0.92	7.52±0.2	9.17±0.32	7.2±0.583

Discussion

Phytochemical screening was performed to get preliminary idea about the phytochemicals present in the extract which revealed the presence of tannin, flavonoids, steroids, glycosides, alkaloids and terpenoids.

From the sensitization of pain receptors by prostaglandins release analgesic activity of EEGP was assessed by acetic acid-induced writhing method [7]. The extract displayed significant analgesic activity exhibited by inhibition of writhing as compared to control group. The active principle responsible for the analgesic activity of extract may be terpenoids and flavonoids [14-16]. The probable mechanism may be the inhibition of prostaglandins (PGE2 and PGE 2α) and bradykinin synthesis or anatomization the action of these substances [17].

A key step in assessing drug action on CNS is to observe its effect on locomotors activity of the animal. The activity is a measure of the level of excitability of the CNS and this decrease may be closely associated to sedation causing from depression of the central nervous system [18]. We used open field and hole cross tests as the methods of assessing sedative activity. The extract significantly decreased the locomotor activity as shown by the results in a dose-dependent manner. The locomotor activity lowering effect was marked at the 2nd observation (30 min) and continued up to 4th observation period (90 min). Moreover, the rationalization of sedation was carried out by measuring external signs through hole-cross test. Open field test showed that the depressing action of the extract was also evident from the second observation period in the test animals at the doses of 200 and 400 mg/kg body weight (Table 5). Our rudimentary screening for phytochemicals suggests the availability of alkaloids, glycosides, flavonoids; tannins may be responsible for neuropharmacological activity. According to hole cross test, the behavioral state of mice reduced the number of hole cross from second to 4th observation that confirms the sedative activity of EEGP. The probable mechanism may be through the interaction with GABA-benzodiazepine receptor.

Conclusion

The ethanol extract of *Gynura procumbens* has significant analgesic and CNS effect. The effect is dose-dependent and statistically significant. However, further studies are needed to isolate and purify the pharmacologically active compound(s) responsible for these biological activities.

Declarations

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Ethics Approval and Consent to Participate

All the experimental mice were treated following the Ethical Principles and Guidelines for Scientific Experiments on Animals (1995) postulated by the Swiss Academy of Medical Sciences and the Swiss Academy of Sciences. The Institutional Animal Ethical Committee (SUB/TAEC/11.01) of Stamford University Bangladesh allowed all experimental rules.

Competing Interests

The authors declare that they have no conflict of interests.

Consent for Publication

Not applicable

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References

1. Rahman A, Asad M. Chemical and biological investigations of the leaves of *Gynura procumbens*. Int. J. Biosci. 2013; 3:36-43. 10.12692/ijb/3.4.36-43.
2. Tan HL, Chan KG, Pusparajah P, Lee LH, Goh BH. *Gynura procumbens*: An Overview of the Biological Activities, Front Pharmacol. 2016; 7:52.
3. Hew CS, Khoo BY, Gam LH. The Anti-Cancer Property of Proteins Extracted from Gynura procumbens (Lour.) Merr, PLoS ONE, 2013; 8(7):doi.org/10.1371/journal.pone.0068524.
4. Aliza, Sultana S, Hossain ML. *In vivo* Assessment of Neuropharmacological Activity of Methanol Leaves Extract of *Callicarpa arborea* (Family: Verbenaceae) in Swiss albino Mice. Sch Int J Tradit Complement Med. 2019; 2(6):85-89.
5. Smrity KK, Sultana S, Hassan MA, Hossain ML. Medicinal activity of *Vitex negundo* L. (Family: Lamiaceae) leaves extract: Assessment of phytochemical and pharmacological properties. J. Pharmacogn. Phytochem. 2019; 8(3):3571-3575.
6. Hossain ML, Sultana S. *In-vivo* Assessment of Neuropharmacological Activity of Methanol Bark Extract of *Mimosa pudica* in Mice. Adv. Pharmacol. Pharm. 2019; 7(2):33-37.
7. Karmakar UK, Sultana S, Nishi S, Biswas NN, Hossain L, Sheikh S. Antioxidant, Analgesic, Antimicrobial, and Anthelmintic Activity of the Dried Seeds of *Bixa orellana* (L). Int. J. Pharm. 2018; 8(1):150-163.
8. Khushi S, Hasan MM, Monjur-Al-Hossain ASM, Hossain ML, Sadhu SK. Medicinal Activity of *Avicennia officinalis*: Evaluation of Phytochemical and Pharmacological Properties. Saudi J Med. Pharm. Sci. 2016; 2(9):250-255.
9. Fahad SM, Islam AFM, Ahmed M, Uddin N, Alam MR, Khalik MF *et al*. Determination of elemental composition of Malabar spinach, lettuce, spinach, hyacinth bean, and cauliflower vegetables using proton induced X-ray emission technique at Savar sub district in Bangladesh. BioMed. Res. Int. 2015; 2015:1-11.
10. Hossain ML, Monjur-Al-Hossain ASM, Sadhu SK. HPLC Profiling and Evaluation of *In vitro* Antioxidant Activity of Cirsium arvense L. (Family: Asteraceae). J. Pharmacogn. Phytochem. 2016; 5(1):272-277.
11. Basak A, Hossain ML, Parvin MN. Evaluation of phytochemical and pharmacological activities of *Bacopa monnieri* (L.). Int. J. Sci. Rep. 2016; 2(10):242-247.
12. Algariria K, Kuong Y, Asmawia MZ, Sadikuna A, Murugaiyaha V, Ismail N. Hypoglycemic and anti–hyperglycemic study of *Gynura procumbens* leaf extracts, Asian Pacific Journal of Tropical Biomedicine, 2013; 3(5):358-366.
13. Keng CL, Yee LS, Lai PLP. Micropropagation of *Gynura procumbens* (Lour.) Merr. an important medicinal plant, Journal of Medicinal Plants Research. 2009; 3(3):105-111.
14. Ahmadiani A, Hosseiny J, Semnanian S, Javan M, Saeedi F, Kamalinejad M *et al*. Antinociceptive and anti-

inflammatory effects of *Elaeagnus angustifolia* fruit extract. Journal of Ethnopharmacology. 2000; 72:287-292.
15. Rajnarayana K, Reddy MS, Chaluvadi MR. Bioflavanoids Classification, Pharmacological, Biochemical effects and Therapeutic potential. Indian Journal of Pharmacology. 2001; 33: 2-16.
16. Choi J, Jung H, Lee K, Park H. Antinociceptive and Antiinflammatory effects of saponin and sapogenin obtained from the stem of *Akebia quinata*. Journal of Medicinal Food. 2005; 8(1):78-85.
17. Hossain ML, Monjur-Al-Hossain ASM, Sarkar KK, Hossin A, Rahman MA. Phytochemical screening and the Evaluation of the Antioxidant, Total Phenolic Content and Analgesic properties of the plant *Pandanus foetidus*. Internation Research Journal of Pharmacy. 2013; 4(2):170-172.
18. Ali MS, Dash PR, Nasrin M. Study of sedative activity of different extracts of *Kaempferia galanga* in Swiss albino mice, BMC Complementary Altern. Med. 2015; 15:158, DOI 10.1186/s12906-015-0670-z; 2015.