ejpmr, 2017,4(12), 522-529

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

IN VITRO ANTIOXIDANT, CYTOTOXIC AND *IN VIVO* NEUROPHARMACOLOGICAL EFFECTS OF *IPOMOEA PES-TIGRIDIS*

S. M. Tayabul Hossain[1], Abid Newaz Shihab[1,2*], Fatema Akhter[1], Nazia Hassan[1], Tajmira Zaman[1], Mohammad Moshiur Rahman[3], Rimel Barua[1] and Mir Monir Hossain[1]

[1]Department of Pharmacy, University of Science and Technology Chittagong, Chittagong, Bangladesh.
[2]Drug Discovery, GUSTO A. Research Group, Chittagong, Bangladesh.
[3]Department of Pharmacy, University of Asia Pacific, Dhaka, Bangladesh.

***Corresponding Author: Abid Newaz Shihab**
Department of Pharmacy, University of Science and Technology Chittagong, Chittagong, Bangladesh.
Mail ID: abid762ustc@gmail.com

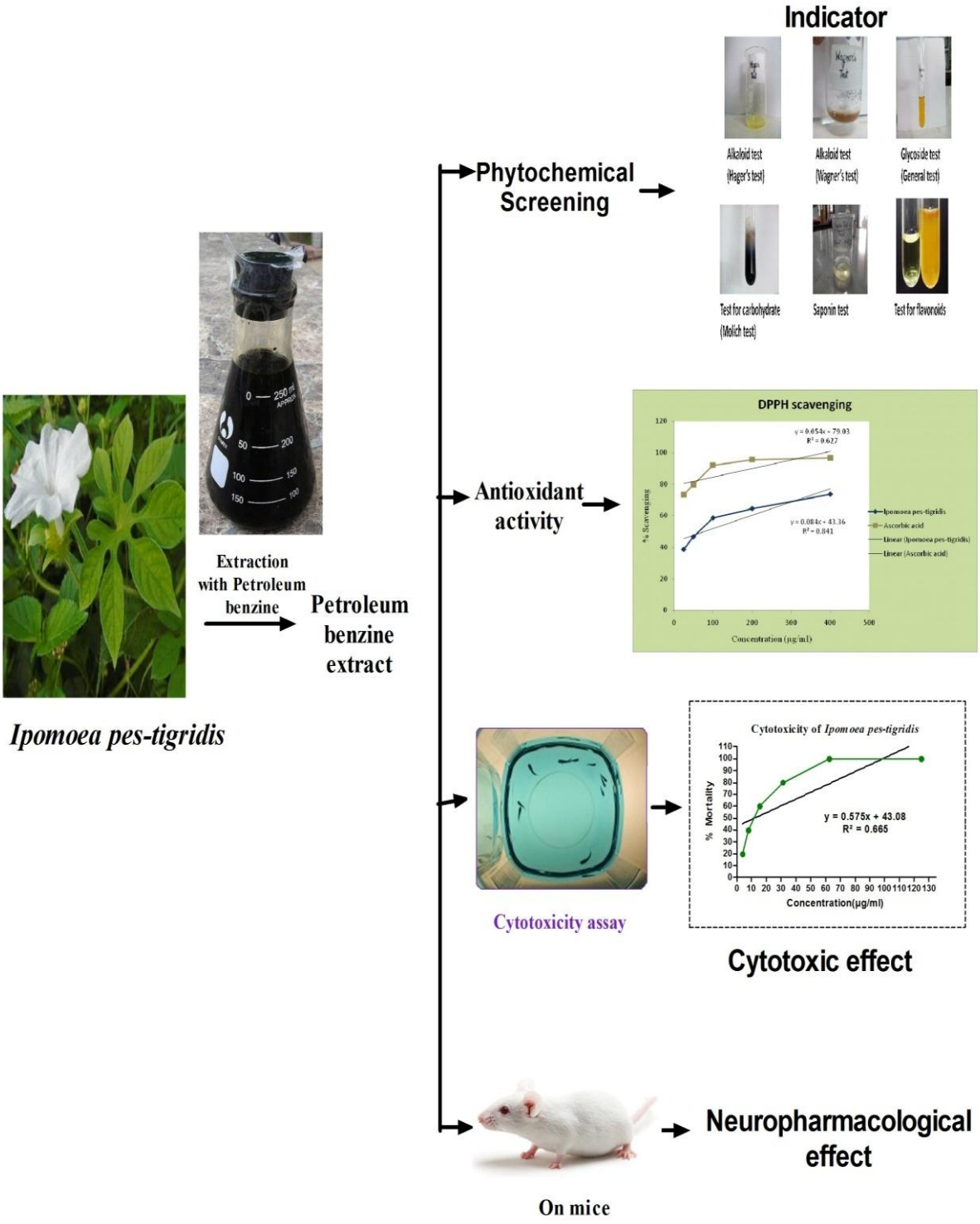
Article Received on 23/10/2017 **Article Revised on 13/11/2017** **Article Accepted on 04/12/2017**

ABSTRACT

Background: *Ipomoea pes-tigridis* (Tiger's foot) is an important plant grown in Bangladesh. The local name of tiger's foot is Languli lala. Different parts of this plant used in the treatment of tumors and cancers. Leaf powder is smoked to get relief from bronchial spasm. *Ipomoea pes-tigridis* has been investigated for *in vitro* antioxidant, cytotoxic and *in vivo* neuropharmacological activities. The phytochemical constituents of the dried powdered leaves were extracted using Petroleum Benzine. **Methods:** Primary phytochemical screening was accomplished by using established methods. Antioxidant effect was conducted with DPPH method and Cytotoxicity was studied by brine shrimp lethality test. The *in vivo* action was done using mice of both sexes. Neuropharmacological activity evaluated by open field and hole cross tests. **Results:** Alkaloids, Flavonoids, Glycosidase, Saponins and Carbohydrate groups were present in the extract. The extract showed a dose dependent radical scavenging effect in DPPH assay. IC_{50} for free radicals achieved by the extract is 79.05 µg/ml. Leaf Petroleum Benzine extract of *Ipomoea pes-tigridis* showed lethality in a dose dependent manner. LC_{50} value of leaf extract of *Ipomoea pes-tigridis* was found 12.035 µg/ml. The extract delivered critical sedative impact at the doses of 200 mg/kg and 400 mg/kg (by oral route) treated with reference substance diazepam (DZP) observed in case of mice at both open field and hole cross test. **Conclusion:** The present research suggests that Petroleum Benzine extract of *Ipomoea pes-tigridis* has significant antioxidant, cytotoxic and neuropharmacological activities. Based on the findings of antioxidant, cytotoxic and neuropharmacological activities, we can say that the obtained results support for the uses of this plant as traditional medicine.

KEYWORDS: *Ipomoea pes-tigridis*, Petroleum Benzine, antioxidant, cytotoxic, neuropharmacological.

GRAPHICAL ABSTRACT



INTRODUCTION

Research is a quest for knowledge through diligent search or investigation or experiment aimed at the discovery and interpretation of new knowledge. Scientific method is the systemic body of procedure and techniques applied in carrying out investigation or experimentation targeted at obtaining new knowledge.

Ipomoea pes-tigridis is an annual herbaceous vine, twining, with spreading hispid axial parts. All parts are more or less covered with long, spreading, pale or brownish hairs. It can be found flowering throughout the year when sufficient water is available.[1] It is called "Tiger Foot Morning Glory" in English.[2] Its geographical distribution includes the Sahel zone from Senegal to Niger and North Nigeria, and dispersed across tropical Africa and into Asia and Australasia, Mascarene

Island and Malaysia.[3][4] Tiger's foot is an important plant grown in Bangladesh. The local name of tiger's foot is Languli lala. The area around Dinajpur is the traditional tiger's foot growing area. Tiger's foot is also available in Parbotto Chottogram. The soil and climate is highly favorable for growing tiger's foot here.

Different parts of *Ipomoea pes-tigridis* used ethno botanically for different diseases. Leaves are used to treat poulticing sores and pimples, haemorrhoids, arthritis, rheumatism, dropsy, swellings, oedema, gout, venereal diseases, in boils, carbuncles and dog bites. Petiole is used as diuretics, laxatives and pain killer. Leaf sap is used as antidotes for venomous stings, snake bites, etc. Seeds are used to treat stomach troubles. Stem is used in the treatment of tumours and cancers. Entire creeper is crushed and the juice extracted and taken orally for treatment of or prevention of rabies if bitten by a rabid dog.[5] The plant is used for healing wound and leaf powder is smoked to get relief from bronchial spasm. There were different scientific study done on *Ipomoea pes-tigridis.*[6,7]

So the principal aim of the study was to investigate the scientific basis of the traditional uses of the plant *Ipomoea pes-tigridis* and in the same time find out the chemical groups present in the active parts to get preliminary idea about the active constituent. The main objective of the study was to investigate whether the Petroleum Benzine extract of *Ipomoea pes-tigridis* possess Anti-oxidant activity, Cytotoxic and Neuropharmacological activities or not.

MATERIALS AND METHODS

Plant materials

The plant material named as *Ipomoea pes-tigridis* was selected based on its medicinal uses. Using standard taxonomical methods, supplied by the Bangladesh Forest Research Institute (BFRI), Chittagong identified the plant's leaves. The plants were collected from Chittagong University's area. It was then separated and cleaned from impurities.

Preparation of sample

The leaves of the plant were dried without sunlight, through fan for 35 days. The leaves of the plant was cut in to small pieces and ground into fine powder with the help of grinder. Then the powder of the plant stored in air tight container and placed in a cool, dry dark place. 200grams of dried powder was weighed and taken in an aspirator (5L). Before placing powders into the aspirator, the jar was washed properly and then dried. 700 ml of solvent (Petroleum Benzine) was added gradually. The container with its content was sealed and kept for 20 days with occasional shaking & stirring. The major portion of the extractable compounds of the plant materials were dissolved in the solvent. Then whole mixture was filtered through cotton wool and the filtrate was concentrated by evaporation in dry and clean air.

Chemicals and reagents

All chemicals used were of analytical reagent grade. Methanol, Petroleum Benzine was purchased from Merck, Germany. Ascorbic acid and DPPH was purchased from BDH Chemicals (BDH Chemicals Ltd. Poole, England). Diazepam (Eskayef Bangladesh Ltd; Tongi, Bangladesh) and Vincristine sulfate (2mg/vial; Techno Drugs Limited Bangladesh). Dimethylsulfoxide (DMSO) and Tween 80 were from Sigma-Aldrich and rests of the chemicals used were of BDH and Merck.

Phytochemical Screening

The freshly prepared crude methanol extract was qualitatively tested for the presence of secondary metabolites especially Alkaloids, Glycosides, Cardiac glycosides, Steroids, Coumarin, Tannins, Flavonoids, Saponins And Reducing sugar through established methods.[8]

In vitro Antioxidant Activity

DPPH free radical scavenging activity

DPPH scavenging activity was carried out using the method of Braca et al.[9][10] Different concentrations (400, 200, 100, 50, 25 and 12.5 µg/mL) of *I. pes-tigridis* extract were dissolved in methanol and placed 2ml in different test tubes, and 3 mL of a 0.004% w/v methanol solution of DPPH was added to each test tube. Absorbance at 517 nm was determined after 30 min against a blank, and the percent inhibition activity was calculated from $[(A0 – A1) / A0] \times 100$, where A0 is the absorbance of the control and A1 is the absorbance of the sample. Ascorbic acid was used as a reference standard and dissolved in methanol to make the stock solution with the same concentration. The control sample was prepared containing the same volume without any extract or reference drug. Methanol served as a blank. The inhibition curves were prepared and the half maximal inhibitory concentration (IC50) values were calculated using linear regression analysis.[11]

In vitro Cytotoxicity test

General toxicity of the extract was tested by the brine shrimp (*Artemia salina*) lethality assay.[12-14] Artificial sea water was prepared by dissolving 38 g of NaCl (3.8%) in 1000 ml of distilled water and was filtered to obtain a clear solution. The dried cysts of the brine shrimps were hatched in artificial seawater with strong aeration for 48 hours. Petroleum Benzine extract of *Ipomoea pes-tigridis* (PBEIP) was dissolved in sea water with DMSO (not exceed 0.01%) and transferred to test tubes to obtain concentrations of 500µg/ml,250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, 15.625 µg/ml, 7.8125 µg/ml and 3.91 µg/ml in 5 ml artificial sea water with 10 nauplii in each test tube. Standard drug Vincristine sulfate was used as positive control at concentrations of 5, 2.5, 1.25, 0.625, and 0.312 µg/ml. Control test tubes were subjected to DMSO in artificial seawater at the same concentration as it was made for samples. After 24 h incubation at 25-30 °C, the number of viable nauplii was counted using a magnifying glass.

The percent (%) mortality was calculated using the following formula.

$$\% \text{ Mortality} = \frac{Nt}{N0} \text{ X } 100$$

Where, Nt = Number of dead nauplii after 24 hrs of incubation, N_0 = Number of total nauplii transferred (n = 10). The Median lethal concentration (LC_{50}) was then determined.

Neuropharmacological study of *Ipomoea pes-tigridis*

Selection of Animal

The study was conducted on 48 Swiss Albino mice purchased from Jahangirnagar University. They were five to six weeks of age, weighing about 20-30 gm, which were housed in colony cages (six rats per cages) at an ambient temperature of 25-27 Celsius with 12 h light and dark cycles having proper ventilation in the room. The mice were fed normal diets purchased commercially from the vendors and water *ad libitum.* The animals were allowed to acclimatize to the laboratory environment for one week and then randomly divided into groups for experiments.

Open field test

In open field test, the animals were divided into control, positive control and test groups containing five mice each. The test groups received extracts 400 mg/kg body weight orally whereas the control group received vehicle (1% Tween 80 in water). Like hole cross test, animals in positive control group received diazepam (1 mg/kg b.w.). The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the animals was counted for 3 min at 0, 30, 60, 90 and 120 min after oral administration of the test drugs and the standard.[15][16]

Hole cross test

The method was carried out as described by Takagi et al.[17][18] A steel partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the centre of the cage. The animals were divided into control, positive control, and test groups containing five mice each. The test groups received extracts at the doses of 400 mg/kg body weight orally whereas the vehicle control and positive control groups received vehicle (1% Tween 80 in water) and the standard drug diazepam (1 mg/kg b.w.) respectively. The number of passage of a mouse through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of the test drugs and the standard.[31]

Statistical Analysis

All results are expressed as Mean ± Standard error of the mean (SEM). The results were statistically analyzed using repeated measures analysis of variance with Dunnett's multiple comparison when compared against negative control in all model of Sedative activity. $P<0.05$, $P<0.01$ and $P<0.001$ were considered as statistically significant. Statistical programs used were SPSS (Statistical Package for Social Science, version 22.0, IBM Corporation, NY) and for graphical presentation GRAPHPAD PRISM® (version 6.00; GraphPad Software Inc., San Diego, CA, USA) were used.

RESULTS

Results of *In vitro* Phytochemical test

The results of various chemical tests for the detection and identification of chemical constituents are summarized in the Table 1. The present phytochemical study indicates the presence of some chemical constituents in the plant parts which are responsible for various pharmacological activity of the plant.

Table 1: Identification of chemical constituents of *Ipomoea pes-tigridis*.

Chemical Test	Petroleum Benzine Extract
Flavonoids Lead acetate test	+
Glycosidase legal test	+
Steroids Leibermann buchard test	–
Alkaloids Dragendoff's test	+
Saponins Foam test	+
Carbohydrate Molishcs test	+
Proteins Ninhydrin test	-
Tannins Ferric chloride test	_

Where, + = Present and –= Absent.

***In vitro* Antioxidant Activity**

DPPH Radical Scavenging Assay

DPPH antioxidant assay is based on the ability of 1, 1 diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the change in absorbance and % of scavenging activity is calculated. The activity was increased by increasing the concentration of the sample extract. Concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage versus concentration. The results of DPPH radical scavenging assays on plant extract and ascorbic acid are given in Figure 1 and Table 2. And IC_{50} values of the samples are presented in Figure 8. IC_{50} of the standard and samples *Ipomoea pes-tigridis* are 8 µg/ml, 79.05 µg/ml respectively. So, comparison with the ascorbic acid, it is clear that plant extracts possess antiradical activity.

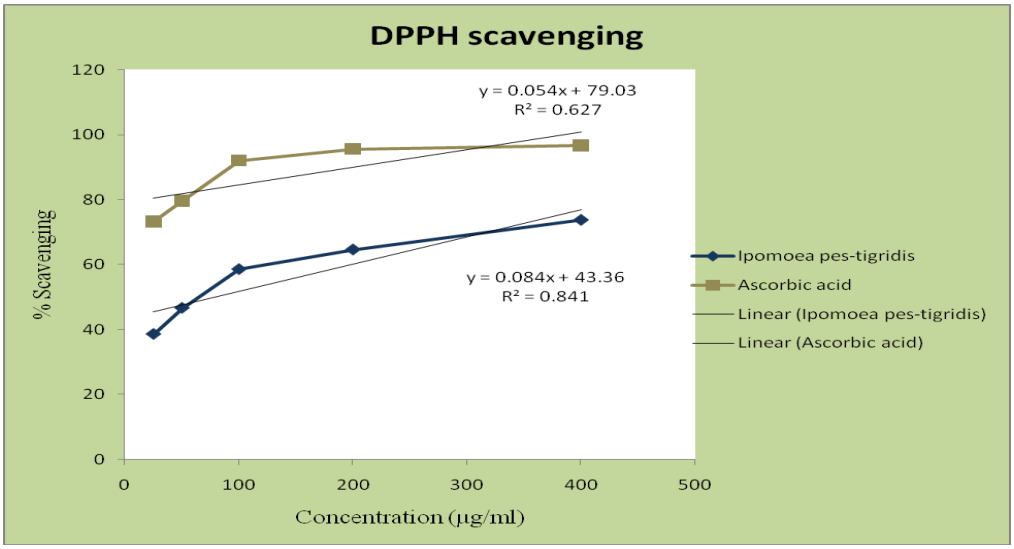


Figure 1: %scavenging activity of ascorbic acid and *Ipomoea pes-tigridis* at different concentration.

Table 2: IC_{50} values of *Ipomoea pes-tigridis* and ascorbic acid (standard).

Sample/Standard	IC_{50} (µg/ml)
Ipomoea pes-tigridis	79.05
Ascorbic acid	8

Cytotoxic activity of *Ipomoea pes-tigridis*

In brine shrimp lethality bioassay, the Petroleum Benzine extract of *Ipomoea pes-tigridis* leaves showed optimistic result in comparison with the positive control Vincristine Sulphate. By plotting concentration versus percent (%) of mortality for all test samples showed an approximate linear correlation. From the graph, the median lethal concentration (LC_{50}) Cytotoxic effect of the extract is summarized in the Table 3 and Figure 2. The LC_{50} for Petroleum Benzine extract of *Ipomoea pes-tigridis* leaf were found to be 12.035µg/ml respectively, and that of Vincristine Sulphate was 0.74µg/ml. DMSO was used as negative control to validate the test method. So, it is evident that this extract has potential cytotoxic effect.

Table 3: Effect of Petroleum Benzine extract of *Ipomoea pes-tigridis* on shrimp nauplii.

Conc.(µg/ml)	No. of nauplii taken	No. of nauplii dead	%mortality	LC_{50} (µg/ml)
3.91	10	2	20	
7.8125	10	4	40	
15.625	10	6	60	
31.25	10	8	80	12.035
62.5	10	10	100	
125	10	10	100	

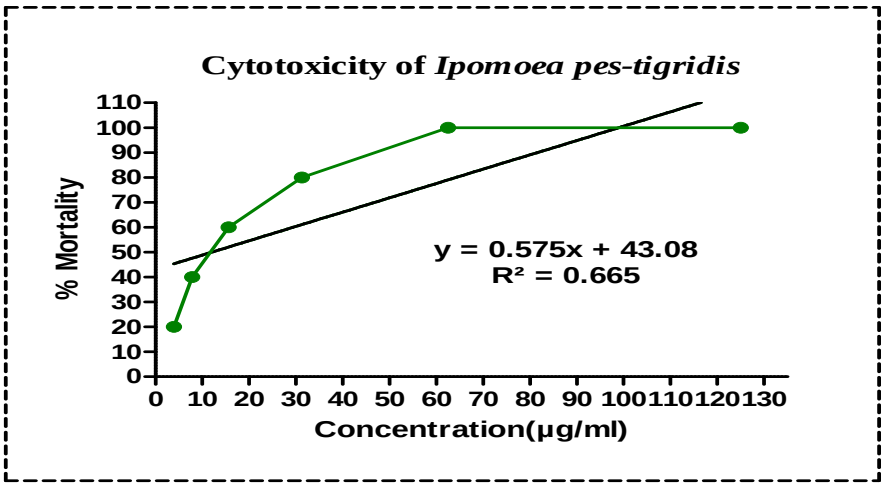


Figure 2: Effects of various concentrations of Petroleum Benzine extract of *Ipomoea pes-tigridis* leaves on the viability of brine shrimp nauplii after 24 hrs incubation.

Neuropharmacological Activity

Open field test

Open field test of *Ipomoea pes-tigridis* treated groups (200 and 400 mg/ kg body weight) showed significant and dose-dependent reduction of movement from its initial value at 0 to 120 min (Figure 3). The number of squares traveled by the mice was decreased significantly from its initial value at 0 to 90 min at the dose level of 400 mg/kg body weight (P<0.01) of the Petroleum Benzine extract from the leaves of *Ipomoea pes-tigridis* (Figure 3).

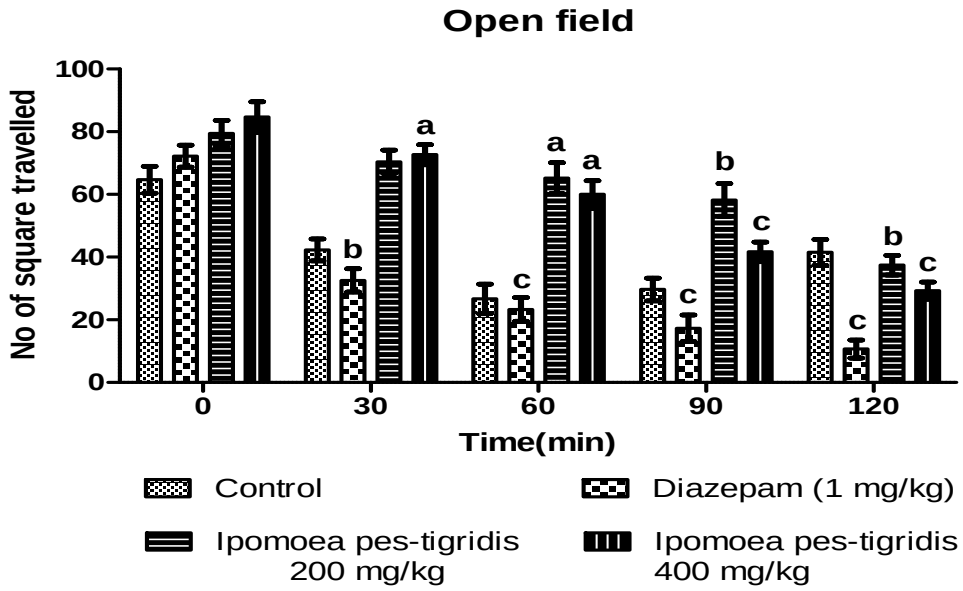


Figure 3: Effect of Petroleum Benzine extract of *Ipomoea pes-tigridis* on exploratory behavior open field test in mice. Values are mean±SEM; [a]P< 0.05, [b]P< 0.01 and [c]P< 0.001, Dunnett's test as compared to control.

6.5.2 Hole cross test

The number of hole crossed from one chamber to another by mice of the control group was similar from 30 to 120 min (Figure 4). Hole cross test of *Ipomoea pes-tigridis* treated groups showed decrease of movement from its initial value at 0 to 90 min. But, at doses of 400 mg/kg, maximum suppression of locomotor activity was displayed which was comparable to the reference drug diazepam (Figure 4).

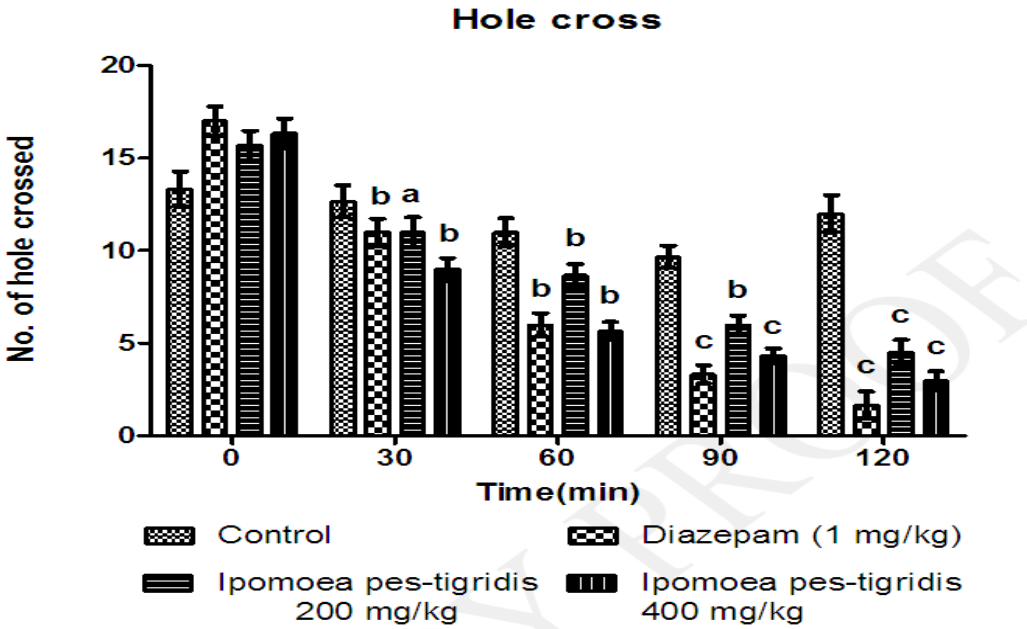


Figure 4: Effect of Petroleum Benzine extract of *Ipomoea pes-tigridis* on exploratory behavior hole cross test in mice. Values are mean±SEM; [a]P< 0.05, [b]P< 0.01 and [c]P< 0.001, Dunnett's test as compared to control.

DISCUSSION

The present study has been approached to demonstrate the phytochemical investigation, Antioxidant, Cytotoxic and Neuropharmacological effects of Petroleum Benzine extract of the leaves of *Ipomoea pes-tigridis*. The leaves of the plant were extracted by cold extraction process using Petroleum Benzine as solvent. By phytochemical screening we can find out the chemical constituents of *Ipomoea pes-tigridis*. *Ipomoea pes-tigridis* contain flavanoids both root and leaf part, glycosides in leaf part, alkaloids both root and leaf part, saponins both root and leaf part, carbohydrates both root leaf part, proteins only root part, tannins only root part. The present phytochemical study indicates the presence of some chemical constituents in the plant parts which are responsible for various pharmacological activity of the plant.

The results of DPPH radical scavenging assays on plant extract and ascorbic acid are calculated and IC_{50} of the standard and samples *Ipomoea pes-tigridis* are 8 µg/ml, 79.05 µg/ml respectively. So, comparison with the ascorbic acid, it is clear that plant extracts possess antiradical activity.

In brine shrimp lethality bioassay, the Petroleum Benzine extract of *Ipomoea pes-tigridis* (PBEIP) leaves showed optimistic result in comparison with the positive control Vincristine Sulphate. By plotting concentration versus percent (%) of mortality for all test samples showed an approximate linear correlation. From the graph, the median lethal concentration (LC_{50}) Cytotoxic effect of the extract were found to be 12.035 µg/ml respectively, and that of Vincristine Sulphate was 0.74µg/ml. DMSO was used as negative control to validate the test method.

The behavioral effects of *Ipomoea pes-tigridis* was examined by using well-validated animal models of CNS activity, namely open field and hole cross tests. The hole cross and open field test demonstrates that the extract depresses CNS activity as the locomotor activity of mice was decreased with time in our observation period.

Diazepam is central sensory system depressant utilized as a part of the administration of rest issue, for example, a sleeping disorder; these mixes have a coupling site on GABA receptor sort an ionophore complex GABA. It decreases activity, moderates excitement, and calms the recipient. Substances like diazepam (which has been picked as the standard reference drug in this study) diminish onset of and expand length of time of barbiturate-actuated rest and decrease exploratory action having possibilities as soothing. Diazepam is a very well-known anxiolytic benzodiazepine (BDS) which produces not only anxiolytic-like effect but also important sedative action. In this respect, *F. cunia* extract produced to allow hyperpolarizing the membrane, leading to CNS depression and resulting in sedative and hypnosis activity. Glutamate and GABA are quantitatively the most important excitatory and inhibitory neurotransmitters, respectively, in the mammalian brain. Thus, receptors for these two neurotransmitters are regarded as important targets for psychotropic drugs. In the test of thiopental-induced sleep in mice, the potentiated effect of lavender extract in mice was represented. It not only prolonged the sleeping time but also decreased the latency of falling asleep and increases the mice of sleep onset. The PBEIP extract has produced hypnosis at high doses' that is, 200 and 400 mg/kg. Since the effect of thiopental on the CNS involves the activation of the inhibition GABAergic system.

CONCLUSIONS

The overall results of the study indicated significant Antioxidant, Cytotoxic and *in vivo* Neuropharmacological activities of Petroleum Benzine extract of leaves of *Ipomoea pes-tigridis*. The results of DPPH radical scavenging assays on plant extract and ascorbic acid are calculated and IC_{50} of the standard and samples *Ipomoea pes-tigridis* are 8 µg/ml, 79.05 µg/ml respectively. So, comparison with the ascorbic acid, it is clear that plant extracts possess antiradical activity. In this investigation we used brine shrimp lethality method to investigate cytotoxicity of Petroleum Benzine extract of *Ipomoea pes-tigridis* leaves. Shrimp can survive at low concentration but at high concentration extract no shrimp can survive. From this study, it may be concluded that the extract of the leaves of *Ipomoea pes-tigridis* has cytotoxic property. Petroleum Benzine extract of *Ipomoea pes-tigridis* showed *In vitro* cytotoxic activity. However, *In vivo* cytotoxic property yet to be discovered. And, it will also use in the treatment of CNS complication. But further studies are also required to identify the phytoconstituents responsible for these bioactivities and to establish the mechanism of action of such activities.

ACKNOWLEDGMENT

The authors are greatly to the authority of the Department of Pharmacy, University of Science and Technology Chittagong (USTC), Bangladesh for providing all the laboratory facilities. The authors are also thankful to Mr. Mohammad Shah Hafez Kabir, CEO, GUSTO A Research Group for his support and help in the statistical analysis and manuscript preparation.

Competing interests

The authors declare that they have no competing interests.

Employment or leadership: None declared.

Honorarium: None declared.

REFERENCES

1. Available at: https://florafaunaweb.nparks.gov.sg/special-pages/plant-detail.aspx?id=6472
2. Available at: http://www.flowersofindia.net/catalog/slides/Tiger%20Foot%20Morning%20Glory.html
3. Bhati P, Sen D. Temperature Responses of Seeds in Ipomoea pes-tigridis L. Biologia Plantarum, 1978; 20(3): 221-4.
4. Available at: http://www.stuartxchange.org/Malasandia.html
5. Available at: http://tropical.theferns.info/viewtropical.php?id=Ipomoea+pes-tigridis
6. Begum MSS, Aruna A, Sivakumar T, Premanand C, Sribhuaneswari MC. Invitro Cytotoxic Activity On Ethanolic Extracts Of Leaves Of Ipomoea Pes-Tigridis (Convolulaceae) Against Liver Hepg2 Cellline, 2015.
7. Chowdhury MRH, Saha R, Islam KMM, Fatema K, Afrin F, Hossain MM, et al. ANALGESIC AND NEUROPHARMACOLOGICAL EFFECT ON ETHYL ACETATE EXTRACT OF IPOMOEA PES-TIGRIDIS IN ALBINO MICE. European Scientific Journal, ESJ, 2014; 10(27).
8. Sofowora A. Medicinal plants and traditional medicine in Africa: John Wiley and sons LTD, 1982.
9. Braca A, Tommasi ND, Bari LD. Antioxidant principles from Bauhinia terapotensis. J Nat Prod, 2001; 64: 892-895.
10. Kabir MSH, Hossain MM, Kabir MI, Ahmad S, Chakrabarty N, Rahman MA, et al. Antioxidant, antidiarrheal, hypoglycemic and thrombolytic activities of organic and aqueous extracts of Hopea odorata leaves and in silico PASS prediction of its isolated compounds. BMC Complementary and Alternative Medicine, 2016; 16(1): 474.
11. Tanvir Ahmad Chowdhury, Mohammad Shah Hafez Kabir, Syed Md. Abdul Kader, Mahmudul Hasan, Md. Ariful Islam, Md. Rakibul Hasan, Md. Nazmul Huda, Shefatun Nur, Joy Chakraborty, Abul Hasanat, Md. Mominur Rahman. Total phenol, total flavonoid content and antioxidant activity of methanol extract of Lygodium palmatum (Bernh.) Sw. leaves. International Journal of Pharmacy, 2016; 6(2): 277-282.
12. Meyer B, Ferrigni N, Putnam J, Jacobsen L, Nichols Dj, McLaughlin J. Brine shrimp: a convenient general bioassay for active plant constituents. Planta medica, 1982; 45: 31-4.
13. Kabir MSH, Mahamoud M, Chakrabarty N, Ahmad S, Masum M, Al A, et al. Antithrombotic and cytotoxic activities of four Bangladeshi plants and PASS prediction of their isolated compounds. Journal of basic and clinical physiology and pharmacology, 2016; 27(6): 659-66.
14. Kabir H, Shah M, Hossain MM, Kabir M, Rahman M, Hasanat A, et al. Phytochemical screening, Antioxidant, Thrombolytic, α-amylase inhibition and cytotoxic activities of ethanol extract of Steudnera colocasiifolia K. Koch leaves. Journal of Young Pharmacists, 2016; 8(4).
15. Kulkarni S, Reddy DS. Animal behavioral models for testing antianxiety agents. Methods and findings in experimental and clinical pharmacology, 1996; 18(3): 219-30.
16. Mahmudul Hasan, Mohammad Shah Hafez Kabir, Md. Abu Aiube Ansary, Md. Rakibul Hasan, Md. Nazmul Huda, Shefatun Nur, Sagar Shil, Akter Hossen, Joy Chakraborty, Abul Hasanat, Md. Rabiul Islam. Sedative and anxiolytic activities of methanol extract of Lygodium palmatum (bernh.) Sw. Leaves. World Journal of Pharmaceutical Research, 2016; 5(5): 323-333.
17. Takagi K, WATANABE M, SAITO H. Studies of the spontaneous movement of animals by the hole cross test; effect of 2-dimethyl-aminoethanol and its acyl esters on the central nervous system. The Japanese Journal of Pharmacology, 1971; 21(6): 797-810.
18. Syed Md. Abdul Kader, Md. Abdullah Al Masum, Mohammad Shah Hafez Kabir, Md. Saif Uddin, Mohammad Nazmul Hoque Jony, Mohammed Sohel Chowdhury, Imran Bin Habib, Arkajyoti Paul, Shefatun Nur, Joy Chakraborty, Abul Hasanat, Md. Rafikul Islam. Sedative and Anxiolytic activities of methanol extract of Boehmeria platyphylla D Don Leaves. International Journal of Pharmacy, 2016; 6(2): 292-298.