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PHYTOCHEMICAL INVESTIGATION AND NEUROPHARMACOLOGICAL ACTIVITY OF STEM EXTRACTS OF *Gynocardia odorata R.Br.*

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ABSTRACT

From the stem part of *Gynocardia odorata R.Br.* the presence of steroids, alkaloids, saponins, tannins, flavonoids etc. has been indicated through phytochemical screening. The crude extract was prepared with absolute methanol and later on pet ether, chloroform, n-hexane and carbon tetrachloride were used as solvents to prepare soluble fractions. Neurophamacological effect was investigated by open field, hole cross, hole board and elevated plus maze test for screening CNS depressant and anxiogenic effect on Swiss albino mice and compared with Diazepam as a reference standard. The hole board method showed dose-dependent significant decrease in the number of head dipping ($P < 0.001$). Similarly, the open field, hole cross and elevated plus maze methods also showed dose-dependent anxiogenic effects which were statistically significant ($p < 0.05$). In conclusion, the present data provide evidence for an important role of extracts of *G. odorata* stem in the CNS depression process.

Keywords: Neuropharmacological activity, *Gynocardia odorata R.Br.* stem, Phytochemical investigation.

INTRODUCTION

The Genus Gynocardia (Achariaceae) is a flowering plant which includes various species around the world (Rao, 2005). *Gynocardia odorata*, primitively identified as an Indian habitat (also grows in the hilly areas of Bangladesh with rain), has long been known as Chaulmoogra. Its traditional name is Chhalmoogra (chhal means bark and *mogra* is a generic name for jasmine) (Sundar *et al.*, 2018).

In this study, we have investigated neuropharmacological (CNS depressant) effects of the stem of the plant which had not been performed on any part of this plant so far. We have also run phytochemical screening tests on the stem extract and found many pharmacologically active constituents.

Over 20% of the adult populations suffer from anxiety and depression at some time during their lives (Buller, 2001; Yadav *et al.*, 2008; Titov *et al.*, 2010). It has become an important

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area of research interest in psychopharmacology during this decade (Woode *et al.*, 2011; Proma *et al.*, 2018). The traditional uses of the leaves of *G. odorata showed* a good possibility of its CNS depressant activity. In the present study, the stem extract of *Gynocardia odorata* was evaluated for its central nervous system (CNS) depressant effect using mice (Swiss albino) and standard tests were performed such as hole board, hole cross, open field and elevated plus maze method. As stems of *G. odorata* have potential phytochemical and pharmacological properties, this research has got a good rationale.

MATERIALS AND METHODS

Solvents and Chemicals:

Analytical and laboratory grade (e.g. SIGMA, E. Merck or BDH) solvents and chemicals were used in most of the experiments. Analytical and laboratory grade absolute Methanol, n-Hexane, Carbon tetrachloride, Chloroform, Petroleum Ether and others were used in extraction and in soluble fraction of Methanolic extract. All other reagents like Mayer's Reagent, Wagner's Reagent, Copper sulphate, Sulfuric acid, Potassium tartarate etc. used for phytochemical screening were of analytical grade.

Plant Collection and Authentication:

The plant material, *G. odorata (stem)*, was collected from Hajarikhil Wildlife Sanctuary of Chittagong, Bangladesh. The plant was identified and authenticated by Dr. Shaikh Bokhtear Uddin, Taxonomist and Associate Professor of the Department of Botany, University of Chittagong, Bangladesh.

Preparation and Isolation of Plant Extract:

After washing, the plant samples were first sun dried for several weeks, cut into small pieces and dried again. Then the samples were crushed and ground into coarse powder in the Phytochemical Research Laboratory, Department of Pharmacy, University of Chittagong using high capacity grinding machine. About 800gm of the powdered material was taken in a clean, round bottomed flask (5 liters) and soaked in 3.5 liters of methanol for 21 days. The extract was filtered through Whatman filter paper number 1 and concentrated on a rotary evaporator (RE 200, Bibby Sterling Ltd., England) at 45°C under reduced pressure. Then solvent-solvent partitioning of crude methanolic extract was done by 4 different solvents (Petroleum ether, Carbon tetrachloride, Chloroform, n-Hexane) using the protocol designed by Kupchan (Beckett and Stenlake, 1986) and modified by Van Wagenen.

Phytochemical Analysis:

Qualitative phytochemical tests for the identification of alkaloids, carbohydrates, flavonoids, phytosterols, glycosides, proteins and amino acids, lipids, volatile oil, terpenes, fixed oils and fats, saponins, phenols and tannins were carried out for the methanolic extract by the method described earlier (Harborne, 1998; Siddique *et al.*, 2009).

Experimental Animals:

Swiss-albino mice of either sex, aged 4-5 weeks, weighing 20-25 gm each obtained from the BCSIR laboratories, Chittagong were used for the experiment.

Acute Toxicity Studies: Acute toxicity study was conducted and the LD_{50} for each of the extract was determined and one tenth of the extract dose (LD_{50}) was selected as maximum dose for the evaluation of neuropharmacological activity.

Assessment of In Vivo Neuropharmacological Activity:

Hole Board Test: The hole-board was used to assess emotionality, anxiety and responses to stress in animals as described previously (Echandia *et al.,* 1987). Treatments were administered in a volume of 10 ml/kg of mice body weight (5 mice in each group/test). After treatment the mice were placed in the center of the hole board and allowed to freely explore the apparatus for 5 min. The number of times an animal dipped its head into the holes in a period of 30 minutes was manually counted and recorded (Wolfman *et al.,* 1994).

Open-field Test: The experiment was carried out according to the methods described by Gupta *et al.* (Archer, 1973). The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The number of squares visited by the animals was counted. It was counted for 3 min at 0, 30, 60, 90 and 120 min after oral administration of the test extract and the standard.

Hole Cross Test: The method was carried out as described by Takagi *et al.,* (Takagi *et al.,* 1971). The number of passages of a mouse through the hole from one chamber to other was counted. It was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of the test extract and the standard.

Elevated Plus Maze Method: Sixty minutes after the administration of the test extract and standards, each animal was placed at the center of the maze facing one of the enclosed arms. During the 5 min test period, the number of enclosed and open arms entries, plus the time spent in open and enclosed arms were recorded.

Statistical Analysis: Results of the study were represented as mean ± SEM (Standard Error Mean). Data were analyzed by one-way ANOVA followed by Dunnett's *t* test and P values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical analysis:

Testing of different chemical groups present in the extract, represent the preliminary phytochemical studies. Small quantity of freshly prepared methanolic extracts of stem of *G. odorata R.Br.* were subjected to preliminary quantitative phytochemical investigation for detection of phytochemicals such as alkaloids, carbohydrates, glycosides, phytosterols, proteins, flavonoids, tannins, saponins, phenols, terpenes, fats & fixed oils using the

standard methods (Harborne, 1993; Akinmoladun *et al.*, 2007; Khandelwal, 2007; Roopashree *et al.*, 2008) (Table 1).

TABLE 1: PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACT OF *G.ODORATA* STEM.

Chemical Constituents	Results
Alkaloids	+++
Carbohydrates	+++
Glycosides	+++
Saponins	+++
Tannins	++
Steroid	+++
Flavonoids	++
Reducing sugar	++
Phytosterol	+
Dierpenes	+
Coumarin	-
Gums and Mucilages	-

+ and – signs indicate the presence and absence of the constituent respectively.

Alkaloids, Carbohydrates, Saponins and Steroids were present in high concentration, whereas Tannins, Reducing sugar and Flavonoids were found in low concentration. On the other hand, Coumarin, Gum and Mucilages were absent in the investigated plant part. Different chemical groups are responsible for exhibiting different pharmacological effect. Identification for different chemical group reveals the possible pharmacological effect of crude extract.

In vivo* neuropharmacological activity of *Gynocardia odorata R.Br.

Hole Board Test

The test was performed by taking methanolic extract and its partitioning fractions at doses of 200 mg/kg and 400 mg/kg body weight. The result was found statistically significant (Table 2). The methanolic extract of *G. odorata R.Br.* dose dependently induced a significant ($P<0.001$) decrease in the number of head dipping, 24±1.48 and 21±2.12 at the dose of 200 and 400 mg/kg body weight, respectively when compared to the control untreated group which was comparable to that of the standard drug diazepam (18.20±1.28, $P<0.001$).

TABLE 2: CNS DEPRESSANT ACTIVITY OF METHANOLIC EXTRACT AND ITS DIFFERENT FRACTIONS OF *G. ODORATA R.Br.*

Animal Group	Number of Head Dipping (Mean±SEM)
Control	38.8± 3.15
Standard	18.20±1.28***
NHF 200	31.80±1.66
NHF 400	29.20±0.80*
CTF 200	30.80±2.71*
CTF 400	23.20±2.78***
ME 200	24±1.48***
ME 400	21±2.12***

Note: Each value represents the mean ± SEM. (n= 5). One-way ANOVA followed by Dunnett's *t* test. ***P <0.001, *P <0.05 compared with control. * indicates significance i.e. considerable CNS Depressant activity. NHF=n-Hexane Fraction, CTF = Carbon tetrachloride Fraction, ME = Methanolic Extract.

Open Field Test

The extract showed a noticeable decrease in movement in the test animals from the second observation period to last study period at both dose levels (200 and 400 mg/kg body weight). Test animals showed significant decrease in number of movements in the dosages of 200 and 400 mg/kg (11.4± 2.01, 7.2± 1.36, respectively, as compared to 36.6± 3.23 in the control group at 120 min of administration of the extract. The depressant action increased in a dose dependent manner (Table 3).

TABLE 3: EFFECT OF *G. ODORATA* ON CNS DEPRESSANT ACTIVITY IN OPEN FIELD TEST.

Animal Group	Treatment	Dose	Route	Number of movements (Mean±SEM)				
				0 min	30 min	60 min	90 min	120 min
Control	1% Tween 80 in normal saline	10 ml/kg	Oral	56.4±2.54	50.6± 5.35	52.4± 2.80	53.8± 4.12	36.6± 3.23
Standard	Diazepam	1 mg/kg	i.p	55±7.66	46.2± 5.29	24± 5.15***	15± 4.64***	9.6± 2.44***
Test	Methanolic Extract	200 mg/kg	Oral	40.2± 1.77*	30.6± 2.58*	20.6± 3.71***	15.8± 3.56***	11.4± 2.01***
Test	Methanolic Extract	400 mg/kg	Oral	34± 3.08***	20± 2.92***	16.4± 2.91***	12± 1.95***	7.2± 1.36***

Note: Each value represents the mean ± SEM. (n= 5). One-way ANOVA followed by Dunnett's *t* test. Significances ***P <0.001, *P <0.05 compared with control.

Hole Cross Test

At 400 mg/kg dose, number of hole crossed (1± 0.45) was comparable with that of standard drug diazepam (1.4 ± 0.68). Diazepam was used as the standard drug in the experimental animals to evaluate the CNS depressant effect of the plant extract (Table 4).

TABLE 4: EFFECT OF *G. ODORATA* ON CNS DEPRESSANT ACTIVITY IN HOLE CROSS TEST.

Animal Group	Treatment	Dose	Route	Number of hole crossed (Mean±SEM)				
				0 min	30 min	60 min	90 min	120 min
Control	1% Tween 80 in saline water	10 ml/kg	Oral	9± 1.41	7.6± 0.97	5.2± 0.66	3.6± 1.03	3± 0.84
Standard	Diazepam	1 mg/kg	i.p	6.8± 0.86	4± 0.71*	2.4±0.75*	2.6±0.6*	1.4± 0.68**
Test	Methanolic Extract	200 mg/kg	Oral	7.2± 1.16	6± 1.14	4.2± 1.16	2.4± 0.81	1.6± 1.52*
Test	Methanolic Extract	400 mg/kg	Oral	6.4± 0.53	4.6± 0.68	3±0.45*	1.6±0.68*	1± 0.45**

Note: Each value represents the mean ± SEM. (n= 5). One- way ANOVA followed by Dunnett's *t* test. Significances **P <0.01, *P <0.05 compared with control.

Elevated plus maze (EPM) test

The percentage of time spent into closed arms also increased at the 200 mg/kg doses (235 ± 12.54) as compared to the control group (240 ± 17.37) (Table 5). There was also a significant increase in duration of time into closed arm for this dose, which was statistically significant as compared to standard group (175.5 ± 60.10). So, the methanolic extract showed dose dependent anxiogenic effect which were statistically significant (p <0.05) (Table 5 & 6).

TABLE 5: EFFECT OF PET ETHER FRACTION OF *G. ODORATA* ON OPEN ARM ENTRIES IN ELEVATED PLUS MAZE (EPM) TEST

Group	Treatment and dose	No. of open arm entries	Duration (sec)	% of time spent into open arm
Control	1% Tween 80 in saline water (10 ml/kg)	2.5 ± 0.90	27 ± 2.1	10.21 ± 1.57
Standard	Diazepam (1 mg/kg)	2 ± 1.41	35.5 ± 3.54	17.36 ± 3.57
Test (200)	Pet ether fraction (200 mg/kg)	3 ± 1.67	15 ± 6.7	9.18 ±2.8
Test (400)	Pet ether fraction (400 mg/kg)	2.5 ± 0.32	10 ± 3.73	7 ± 0.56

PEF= Pet ether fraction

TABLE 6: EFFECT OF PET ETHER FRACTION *G. ODORATA* ON CLOSED ARM ENTRIES IN ELEVATED PLUS MAZE (EPM) TEST

Group	Treatment and dose	No. of closed arm entries	Duration (sec)	% of time spent into closed arm
Control	1% Tween 80 in saline water (10 ml/kg)	6 ± 1.12	240 ± 17.37	90.11 ± 1.25
Standard	Diazepam (1 mg/kg)	3 ± 1.11	175.5 ± 60.10**	82.64 ± 3.56**
Test (200)	Methanolic Extract (200 mg/kg)	6 ± 5.87	235 ± 12.54**	89.24 ±4.14**
Test (400)	Methanolic Extract (400 mg/kg)	6 ± 4.68	227.5 ± 14.76**	87 ± 2.16**

ME = Methanolic Extract * indicates considerable CNS Depressant activity.

CONCLUSION

This study has explored the various phytochemicals, including alkaloids, steroids, saponins, glycosides, phytosterols, tannins, flavonoids etc. present in the stems of *G. odorata* for the first time. The results of this study also demonstrate that the extracts of *G. odorata* stem have significant CNS depressant activity and they show significant decrease in locomotor activity in mice in all the four testing methods applied. Present work was a preliminary effort which will require further detailed investigation including characterization of active compounds and requires pre-formulation studies for development of a potential dosage form.

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