



EVALUATION OF ANXIOLYTIC AND CNS DEPRESSANT ACTIVITY OF *ALANGIUM SALVIIFOLIUM* WANG FLOWERS

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Article Received on: 14/01/12 Revised on: 09/03/12 Approved for publication: 19/04/12

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ABSTRACT

The present study was designed to investigate *in vivo* anxiolytic and CNS depressant activities of the methanol extract and its chloroform soluble fraction of flowers of *Alangium salviifolium*. Anxiolytic activity was assayed by elevated plus maze (EPM) test where the extract and fraction showed moderate activity in a dose dependent manner. CNS depressant activity was evaluated by using open field and hole cross tests at doses of 50 and 100 mg/kg body weight (methanol extract) and 100 mg/kg body weight of chloroform fraction orally. Locomotor activity and exploratory behavior of rats in hole cross and open field test were decreased in the test group comparing the control group indicating CNS depressant effect of the extract and fraction which was comparable with the standard drug diazepam. The results of the statistical analysis showed that the plant extract had significant (* $p < 0.05$, ** $p < 0.01$) dose dependent anxiolytic and CNS depressant activities. So, the present results suggest that the methanol extract and chloroform fraction of *A. salviifolium* flowers possesses moderate anxiolytic and CNS depressant activities and it is the first report of *Alangium salviifolium* wang flower.

Key words: *Alangium salviifolium*, CNS depressant, anxiolytic activity.

INTRODUCTION

Advance in science and technology has contributed to an enormous improvement in the quality of life of humankind. However, modern life stress, associated trials and tribulation are responsible for the surge in incidence of variety of psychiatric disorders. Path breaking research in psychopharmacology has flooded the market place with drugs for specification. For instance, benzodiazepines (diazepam, nitrazepam, lorazepam and alprazolam etc) are the most frequently prescribed synthetic drugs for variety of condition particularly anxiety, depression, epilepsy and insomnia. But these psychoneural drugs have very serious side effects like chronic use of benzodiazepines causes deterioration of cognitive function, physical dependence and tolerance. Besides addiction liabilities, benzodiazepines adversely affect the respiratory, digestive and immune system of body and the chronic treatment with benzodiazepines often prove more harmful in the longer run¹. Drug acting in the central nervous system were among the first to be discovered by the primitive human and are still the most widely used group of pharmacological agents. The CNS acting drugs are invaluable therapeutically, because they can produce specific physiological and psychological effects from the vast array of material medica of the indigenous system so many plants have been reported to have activity against CNS disorders and thus act as very useful remedies for the alleviation of human suffering².

Alangium salviifolium wang is a deciduous, rambling shrub or a tree belonging to the family Alangiaceae³. The different parts of this plant are used for a wide range of diseases. Root is used in diarrhoea, paralysis, piles, vomiting⁴ and is useful for external application in acute case of rheumatism, leprosy and inflammation⁵. Antibacterial compound was isolated from the flower of *Alangium salviifolium*⁶. The plant has been reported for its anti-tubercular, anti-spasmodic and anti-

cholinesterase activity⁷. Anti-Fertility activity of the stem bark of *Alangium salviifolium* (Linn.F) Wang in Wistar female rats has also been reported⁸. Previous phytochemical investigation revealed that it is a rich source of alkaloids including ipecac alkaloid and benzopyridoquinolizidine alkaloids. It is also known to produce alangiside, a tetrahydroisoquinoline monoterpene glucoside⁹. Recent phytochemical studies of this plant resulted in the isolation of several flavanoid, phenolic compound, irridoid glycosides and oxyglucoside of some alcohol¹⁰. New alkaloid, ankorine was isolated from leaves¹¹. Plant is also rich in tetrahydroisoquinoline monoterpene glycoside. e.g. alangiside-1 or ipecoside- 2 whose structures are closely related to the ipecac alkaloid¹². Two sterol alangol and alengol were isolated from seed kernel¹³.

The present study dealt with various psychopharmacological effects of the methanol extract and its soluble chloroform fraction from the flowers of *A. salviifolium* on some neuropharmacological experimental models. Previous biological studies have shown that it possessed antioxidant and antitumor activities but still there is no report showing the antianxiety and CNS depressant effect of *A. salviifolium* flowers on an animal behavioral model. In view of that, it was thus necessary to expand the present study for anxiolytic and CNS depressant activity.

MATERIALS AND METHODS

Plant materials

The flowers of the *Alangium salviifolium* were collected from the adjoining area of Rajshahi University Campus, Bangladesh during February 2007 and were identified by Taxonomist, Department of Botany and University of Rajshahi, Bangladesh where a voucher specimen number (Voucher No # 105) has been deposited.

Preparation of extracts

The flower material was shade-dried with occasional shifting and then powdered with a mechanical grinder, passing through sieve #40, and stored in a tight container. The powdered flower (750gm) was taken in large glass bottle and extracted with methanol for 7 days. The procedure was repeated twice using same solvent system for next 3 days. The extract was decanted first through a cotton plug and finally filtered through filter paper to get clear filtrate. The filtrate obtained by repeated maceration was evaporated under reduced pressure at 40°C using Rotary evaporator. The net weight of dry extract was 8.25 gm. The dry plant extract (8.25gm) was suspended in water and fractionated in a conical flask using chloroform solvent. Chloroform fraction further evaporates using Rotary evaporator and then air dried to solid mass 600 mg, respectively.

Animal

Albino mice (25-30g) were used for assessing biological activity. The animals were maintained under standard laboratory conditions and had free access to food and water ad libitum. The animals were allowed to acclimatize to the environment for 7 days prior to experimental session. The animals were divided into different groups, each consisting of six animals which were fasted overnight prior to the experiments. Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethics Committee, Atish Dipankar University of Science & Technology, Dhaka, Bangladesh.

Drugs and chemicals

Diazepam (Merck Limited, Mumbai, India) was used as a standard anxiolytic agent. All other chemicals and reagents used were of highest analytical grade.

Preliminary Phytochemical Investigation

The methanol extract was subjected to qualitative chemical investigation for the identification of different phytoconstituents like sterols, glycosides, saponins, carbohydrates, alkaloids, flavonoids, tannins, proteins and triterpenoids¹⁴.

Acute toxicity

The acute oral toxicity of plant in male Swiss albino mice was studied as per reported method¹⁵.

Experimental method

Elevated plus-maze test (EPM)

The elevated plus-maze is made of wood in the shape of a horizontal cross that consists of two open arms (25×5 cm) and two opposite arms (25×5 cm) enclosed by 20-cm-high walls. The arms are extended from a central platform with a dimension of 5×5 cm. The plus-maze is elevated to a height of 40 cm from the floor. The maze is put inside a box with a dimension of 30×30×50 cm. Mice were treated with methanolic extract (50, 100 mg/kg, p.o) and chloroform fraction (100 mg/kg, p.o) diazepam (1 mg/kg, i.p) and vehicle 30 min before being placed individually in the centre of the EPM, facing a closed arm. The number of arm entries and the time spent in the open and closed arms were counted for 5 min¹⁶. Arm entry was defined as all four feet in the arm. The total number of arm entries provides a measure of general activity. A selective increase in the parameters corresponding to open arm entry reveals an anxiolytic effect¹⁷.

Hole cross test

The method was carried out as described by Takagi¹⁸. A steel partition was fixed in the middle of a cage of 30 × 20 × 14

cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The animals were divided into control, positive control, and test groups containing six mice each. The test groups received methanol extract at doses of 50 and 100 mg/kg and chloroform fraction 100 mg/kg body weight orally whereas the control group received vehicle (1% Tween 80 in water). The number of passages of a mouse through the hole from one chamber to the other was counted for a period of 3 min at 0, 30, 60 and 90 min after oral administration of both doses of the test drug. Diazepam (1 mg/kg, i.p.) was used as the positive control drug.

Open field test

In the open field test, the animals were divided into control, positive control, and test groups containing six mice each. The test groups received methanol extract and chloroform fraction of the flowers of *A. salviifolium* at doses of 50, 100 and 100 mg/kg body weight orally whereas the control group received vehicle (1% Tween 80 in water). The floor of a half square meter open field^{19, 20} was divided into a series of squares each alternatively colored black and white. The apparatus had a 40 cm height wall. The number of squares visited by the animals was counted for 3 min at 0, 30, 60 and 90 min after oral administration of both doses of the test drug. Diazepam (1 mg/kg, i.p.) was used as the positive control drug.

Statistical Analysis

Statistical evaluation was done using one-way analysis of variance (ANOVA) followed by Dunnett's test by using the statistical package of social sciences (SPSS) 15.00 for windows. The significant level was set at *p<0.05, **p<0.01.

RESULTS

Phytochemical Screening

The phytoconstituents were identified by various chemical tests which showed the presence of alkaloids, tannins, phenolic and flavonoid compounds and steroid in crude extract of *Alangium salviifolium* flower (table 1).

Acute Toxicity Studies

The acute toxicity studies mainly aims at establishing the therapeutic index, i.e., the ratio between the pharmacologically effective dose and the lethal dose on the same strain and species. The extract of *A. salviifolium* was safe up to a dose of 1000 mg/kg (p.o.) body weight. Behavior of the animals was closely observed for the first 3h then at an interval of every 4h during the next 48h. The extract did not cause mortality in mice and rats during 48h observation but little behavioral changes, locomotor ataxia and diarrhea and weight loss were observed. Food and water intake had no significant difference among the group studied.

EPM test

The methanol extract of *A. salviifolium* at the dose of 50 and 100 mg/kg and chloroform fraction at the dose of 100 mg/kg body weight, significantly increased the percentage of entries (Table 2) of mice into the open arms, and the percentage of time spent (Table 2) in the open arms of the EPM.

Hole cross test

In the hole cross test, the extracts and fraction also showed a decrease in locomotion in the test animals from the second observation period at both dose levels of methanolic extract (50 and 100 mg/kg body weight) and chloroform fraction at the dose level of 100 mg/kg body weight. The results were dose dependent and statistically significant (Fig 1).

Open field test

In the open field test, the methanol extracts showed a noticeable decrease in locomotion in the test animals from the second observation period at both dose levels (50 and 100 mg/kg body weight) and chloroform fraction (100 mg/kg body weight). The depressant actions were increased with dose dependent manner and statistically significant. Fig 2 represents the result of open field activity.

DISCUSSION

The present study evaluated the effects of methanol extract and chloroform fraction of the *A. salviifolium* flower on experimentally induced antianxiety and depression. Elevated plus-maze test is used to evaluate psychomotor performance and emotional aspects of rodents²¹. The results showed that *A. salviifolium* wang flower significantly increased the time spent on the open arms and decreased the number of entries into closed arms. This type of effect is observed with the drugs that act on GABA/ benzodiazepine receptor complex as well with drugs that stimulate glucocorticoid production and release in the adrenal cortex²², after administration of 5-HT1B receptor antagonists and 5-HT1A agonists²³. Therefore, with the present data, it is difficult to predict the precise mechanism for the anxiolytic activity of the *A. salviifolium* wang flower.

Locomotor activity refers to an increase in alertness and decrease in locomotor activity considered as sedative effect. The major inhibitory neurotransmitter in the central nervous system is Gamma-amino-butyric acid (GABA). Different types of anxiolytic, muscle relaxant, sedative-hypnotic drugs are shown their action through GABAA, that's why the extract and fraction of *A. salviifolium* may acts by membrane hyperpolarization which potentiating GABA-ergic inhibition in the CNS that leads to either decrease in the firing rate of critical neurons in the brain or direct activation of GABA receptor by the extracts²⁴. The result indicated that the extract significantly decreased locomotor activity which indicates it has CNS depressant activity.

Literature review of the plant reveals that *Alangium salviifolium* contains Flavonoids & Tannin²⁵. Different types of flavonoids and neuroactive steroids were found to be ligands for the GABAA receptors in the central nervous system; which indicate that they act as benzodiazepine-like molecules²⁴.

In conclusion, it could be suggested that the crude methanolic extract and its chloroform soluble fraction of *Alangium salviifolium* surely possess central nervous system depressant activities. However, further studies are needed to isolate the active principles responsible for the observed activity.

ACKNOWLEDGMENT

The authors are thankful to the Department of Pharmacy, Atish Dipankar University of Science & Technology, Banani, Dhaka-1213, Bangladesh, for providing laboratory facilities to carry out this research work.

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Table 1. Result of chemical group tests of the Methanol extract of *Alangium salviifolium* flower.

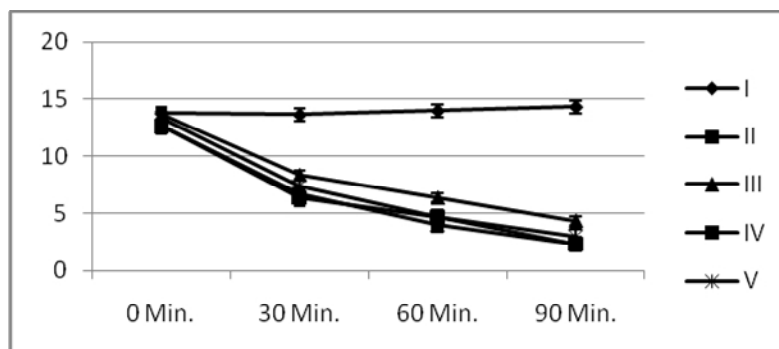
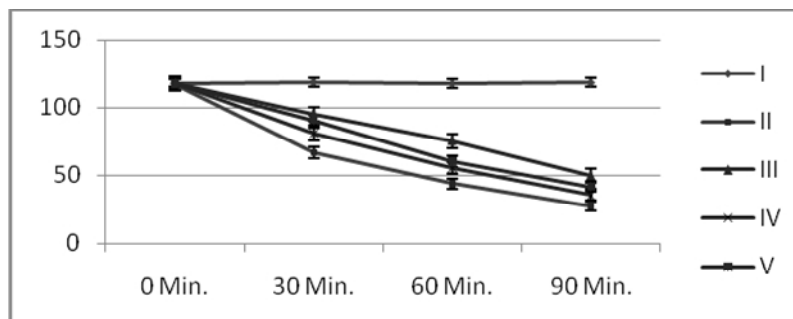
Extract	Carbohydrate	Tannin	Flavonoid	Saponin	Phenol	Steroid	Alkaloid
<i>Alangium salviifolium</i>	-	++	++	-	++	+	+++

ME: Methanolic extract; (+): Present; (-): Absent; (+++): Reaction intensity is high; (++): Reaction intensity is medium; (+): Reaction intensity is normal;

Table 2. Effect of methanol extract and chloroform fraction from flowers of *A. salviifolium* on EPM test in mice.

Group	Dose (mg/kg)	% No. of entry into Open arm	% time (Seconds) spent into open arm
I (Vehicle 1% tween 80)		45.66 ± 2.88	41.39 ± 2.15
II (Diazepam)	1	83.55 ± 4.52**	75.73 ± 3.33**
III (Methanol extract)	50	60.06 ± 2.15*	54.45 ± 2.53*
IV (Methanol extract)	100	76.81 ± 4.88**	69.62 ± 5.33**
V (chloroform fraction)	100	79.16 ± 3.15**	71.75 ± 4.57**

Values are mean ± S.E.M., ($n = 6$); * $p < 0.05$, ** $p < 0.01$. Dunnett's test as compared to control (Vehicle = 0.5 mL/mouse).

**Fig1: Effect of methanol extracts and chloroform fraction from flowers of *A. salviifolium* on hole cross test in mice.****Fig 2: Effect of methanol extracts and chloroform fraction from flowers of *A. salviifolium* on open field test in mice.**

Source of support: Nil, Conflict of interest: None Declared