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Assessment of Analgesic, Cytotoxic and Antioxidant activities of *Vallaris solanacea* (Roth) Kuntze

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Original Research Article

ABSTRACT

The ethanolic extract of leaves and stem of *Vallaris solanacea* (Roth) Kuntze (Family: Apocynaceae) was screened for its analgesic, cytotoxic and antioxidant activities. Phytochemical analysis of the extract indicated the presence of Reducing Sugar, Tannins, Saponins, Gums, Steroids, Alkaloids, and Glycosides. The ethanolic extract showed statistically significant analgesic activity ($p<0.005$) in acetic acid induced writhing inhibition in mice at the dose of 500mg/kg body weight and also showed mild effect at the doses of 250mg/kg body weight. In the brine shrimp lethality test, the extract showed cytotoxicity with LC_{50} 80 μg/ml and LC_{90} 320 μg/ml. In the qualitative antioxidant assay using DPPH (1, 1-diphenyl-2-picryl hydrazyl) the extract showed free radical scavenging properties. These primary findings suggest that the extract might possess some chemical constituents that are responsible for analgesic, cytotoxic and antioxidant activities.

Key words: *Vallaris solanacea* (Roth) Kuntze, phytochemical study, analgesic activity, cytotoxic activity, antioxidant activity.

INTRODUCTION

Vallaris solanacea (Roth) Kuntze (Family: Apocynaceae), local name: Agarmoni, Bread flower, is a tall climbing shrub which is locally used as medicinal plant. It is native in India and Burma and also found in Sylhlet in Bangladesh. It is traditionally used for sores and wounds. Barks are chewed for fixing teeth. (Vohra *et al,* 1966) Previous research revealed that it contains a mixture of glycosides vallarisoside and a known one, 3β-O-(α-acofriosyl) along with a new glycoside, benzyl 2-O-β-apiofuranosyl-(1→2)-β-D-glucopyranosyl-2 (Ahmed *et al*, 2010) and also O- acetyl-solanoside (O-acetyl-acofreosyl digitoxigenin) (Vohra *et al*, 1966). In this work, an attempt was made to justify the traditional uses as per scientific experiments. Moreover by using various standard qualitative chemical tests the presence of reported compounds were detected. Upon sufficient literature survey it is found that a little work has been performed to evaluate the rationale for the use of this plant in traditional medicine of Bangladesh. In the present study, we therefore aim to evaluate the analgesic, cytotoxic and antioxidant activity of the ethanolic extract of leaves and stem of *V. solanacea* (Roth) Kuntze.

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MATERIALS AND METHODS

Sample collection and extraction

The plant was collected from Bagerhat, Bangladesh on March, 2009 and identified by the experts at Bangladesh National Herbarium, Mirpur, Dhaka (Accession No. DACB- 34410). After complete shade drying, the sample was cut into small pieces and then slashed to coarse powder with the help of mechanical grinder and the powder was stored in a suitable container. About 500 mg of powder was extracted by

maceration over 20 days with 1200 ml of 80% ethanol. The extract was filtered off. The solvent was evaporated at room temperature with an electric fan to get the dried extract (approx. yield value 13.4%).

Animals

Swiss-Albino mice of either sex (20-25 gm body weight) were collected from animal research branch of the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) and were used for the experiments. The animal were kept in the standard polypropylene cages and provided with standard diets (ICDDR, B formulated). The animals were acclimatized in animal house, Pharmacy Discipline, Khulna University, Khulna under standard Laboratory conditions (relative humidity 55-60%, room temperature 25±2^0 C and 12 hours light: dark cycle) for period of 14 days prior to performing the experiments.

Drugs

DPPH from Sigma Aldrich, USA, Diclofenac sodium (Beximco Pharmaceuticals Ltd., Dhaka, Bangladesh) and Chloramphenicol (Beximco Pharmaceuticals Ltd., Dhaka, Bangladesh).

Phytochemical tests

The crude extract was subjected to preliminary phytochemical screening for the detection of major groups (Evans, 1989). The extract showed the presence of Reducing Sugar, Tannins, Saponins, Gums, Steroids, Alkaloids, and Glycosides. In each test 10% (w/v) solution of the extract in solvent was taken. Then, the extract was used for pharmacological screening.

Determination of analgesic activity

The analgesic activity of the sample was studied using acetic acid induced writhing model in mice. Experimental animals were randomly selected and divided into four groups denoted as Control group, Positive control group and Test group I and Test group II consisting 05 mice in each group. Control group received orally 1% Tween-80 at the dose of 10 mg/kg body weight and Positive control group received orally diclofenac sodium at the dose of 25 mg/kg body weight. Test group I and Test group II were treated with test sample orally at the dose of 250 and 500 gm/kg body weight. A thirty minutes interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%) was administered intra-peritoneally to each of the animals of a group. After an interval of 5 minutes was given for absorption of acetic acid and number writhing was counted for 15 minutes. The animals do not always perform full writhing. The incomplete writhing was taken as half-writhing, so two half-writhing were taken as one full writhing. This is why total writhing was halved to convert all writhing to full writhing or real writhing (Whittle, 1964; Ahmed *et al.*, 2004).

Determination of Cytotoxic Activity

Brine shrimp

The investigation was done on *Artemia salina* (Brine shrimp). One spoon of cyst were hatched for 48 h in saline water, prepared by dissolving 30mg pure NaCl and 53mg table salt into 1.5 litre water. The cyst become nauplii (Meyer *et al.*, 1982).

Lethality bioassay

Solution of different concentrations was prepared with the extract by using dimethyl sulfoxide (DMSO) as solvent. Eight test tubes were used, in each test tube 10 shrimps were taken and solution of different concentration applied on it. Finally volume of liquid was adjusted by saline water. The test tubes were kept for 24 hours. For blank control, a test tube with saline water was kept for observation with 10 shrimps under the same condition with the test sample. For positive control, in another test tube 10 shrimps were taken with saline water. A known drug chloramphenicol as standard was introduced in the test tube with a concentration of 200 μg/ml. The percent of mortality of the brine shrimp nauplii was calculated for every concentration to determine LC_{50} (lethal concentration).

Determination of Antioxidant Activity

Antioxidant activity was determined on the basis of their scavenging activity of the stable DPPH free radical (Sadhu *et al.*, 2003). Commercially prepared TLC plate was used. The sample and ascorbic acid were spotted. Here ascorbic

Table 1. Effect of *Vallaris solanacea on* acetic acid induced writhing in mice.

Animal Group	Treatment	Writhing Count (%Writhing)	%Writhing Inhibition
Control (n=5)	1% tween-80 solution in water	16 ± 1.415 (100)	0
Positive Control (n=5)	Diclofenac sodium (25mg/kg)	4.4 ± 0.51* (27.5)	72.5
Test group I (n=5)	Et. Extract (250mg/kg)	12.4 ± 3.015** (77.5)	22.5
Test group I (n=5)	Et. Extract (500mg/kg)	7.4 ± 1.355*** (46.25)	53.75

Values are expressed as mean ± SEM, SEM=Standard error of Mean, n=No. of mice, Et.= Ethanolic
*: $P < 0.001$; **: $P < 0.25$; $P < 0.005$ vs. control

Table 2. Result of Brine Shrimp lethality bioassay of ethanolic extract of *Vallaris solanacea* (Roth) Kuntze (Leaves and Stem).

Sample	Conc. of extract (µg/ml)	Number of Shrimp taken	Number of Shrimp alive	Number of Shrimp died	% Mortality
Ethanolic extract of *Vallaris solanacea* (Roth) Kuntze (Leaves and Stem)	5	10	10	00	0
	10	10	10	00	0
	20	10	09	01	10
	40	10	08	02	20
	80	10	05	05	50
	160	10	03	07	70
	320	10	01	09	90

acid was used as standard. The chromatogram was developed by ascending technique using two types of solvent systems i.e. medium polar solvent system ($CHCl_3$: CH_3OH = 5:1) and polar solvent system ($CHCl_3$: CH_3OH: H_2O = 40:10:1). The solvent system was allowed to move upto a previously marked line. The plates were then dried naturally. The plates were viewed under UV detector both in short (254 nm) and long (360 nm) wavelength. DPPH (1, 1-diphenyl-2-picryl hydrazyl) forms deep pink color when it dissolved in ethanol. When it is sprayed on the chromatogram of the extract, it forms pale yellow or yellow color which indicates the presence of antioxidants. Two spotted TLC plates were again subjected to universal spry reagent i.e. 10% H_2SO_4 and then heated on hot plate which indicates dark spot.

Statistical analysis

Student's t-test was used to determine significant differences between the control group and test group.

RESULTS

The ethanolic extract was subjected to different qualitative phytochemical tests for detection of different biologically active chemical groups. Analgesic activity of the ethanolic extract was tested by acetic acid induced writhing model in mice. The extract produced 53.75% ($p<0.005$) acetic acid induced writhing inhibition in mice at the dose of 500 mg/kg body weight, which is comparable to diclofenac sodium 72.5% ($p<0.001$) at the dose of 25 mg/kg body weight (Table 1).

Brine shrimp lethality bioassay indicates cytotoxicity of extract. The extract was found to show lethal activity against brine shrimp nauplii and LC_{50} was found at 80 µg/ml and the LC_{90} value was 320 µg/ml respectively (Table 2).

Antioxidant activity was assayed using DPPH and ascorbic acid. Results are represented in the Figure 1, Figure 2, Figure 3 and Figure 4 for the medium polar and polar solvent system and showed antioxidant activity.

DISCUSSION

To get preliminary idea about the active constituents present in the plant (Leaves and stem) extracts different chemical tests were performed and found the presence of Reducing sugar, Tannins, Saponins, Gums, Steroids, Alkaloid, Glycosides.

Figure 1. Medium polar solvent applied and DPPH treated Ascorbic Acid and *Vallaris solanacea* (Roth) Kuntze.

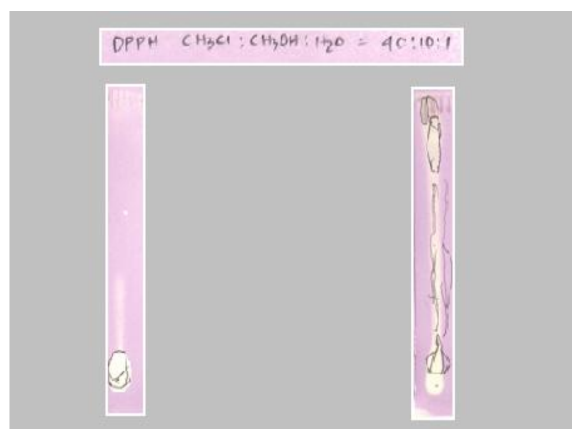

Figure 2. Polar solvent applied and DPPH treated Ascorbic Acid and *Vallaris solanacea* (Roth) Kuntze.

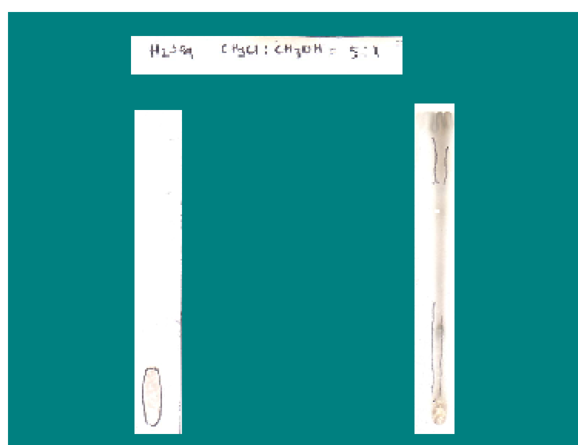

Figure 3. Medium polar solvent applied and 10% H_2SO_4 treated Ascorbic Acid and *Vallaris solanacea* (Roth) Kuntze.

Figure 4. Polar solvent applied and 10% H_2SO_4 treated Ascorbic Acid and *Vallaris solanacea* (Roth) Kuntze.

Analgesic activity of the ethanol extract of *V. solanacea* (Roth) Kuntze was tested by acetic acid induced writhing model in mice. Acetic acid induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which in turn excite the pain nerve endings (Taesotikul *et al.*, 2003). Increased levels of PGE_2 and $PGF_{2\alpha}$ in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid (Derardt *et al.*, 1980). The ethanolic extract of *V. solanacea* (Roth) Kuntze produced significant writhing inhibition comparable to the standard drug diclofenac sodium. On the basis of this result it can be concluded that the ethanol extract of *V. solanacea* (Roth) Kuntze possesses analgesic activity.

Preliminary phytochemical screening showed the presence of various classes of constituents, such as alkaloids, glycoside, tannins, saponins and flavonoids. Since several flavonoids and tannins isolated from medicinal plants have been discovered for their significant antinociceptive and/or anti-inflammatory activity (Pathak *et al.*, 1991; Bittar *et al.*, 2000; Duke, 1992), it is, therefore, possible that the antinociceptive effects observed with this extract in the present study may be attributing to its and tannins component.

Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharma-

cological activities such as antimicrobial, pesticidal, antitumor, etc. of the compound (Meyer *et al*., 1982; McLaughlin *et al*., 1988). The ethanolic extract of the leaves and stem of *Vallaris solanacea* (Roth) Kuntze was found to show significant activity against the brine shrimp nauplii; LC_{50} was found at 80μg/ml and LC_{90} was found 460μg/ml. However, further investigations using carcinoma cell line are necessary to isolate the active compound(s) responsible for the activity.

Antioxidant activity of the ethanolic extract was determined on the basis of their scavenging activity of the stable DPPH free radical and 10% H_2SO_4. Only qualitative test was performed. Drugs with antioxidant activity are useful in free radical induced different types of diseases. The result might partially support the traditional uses of it for different tumors. Further studies as lipid per-oxidation inhibition, xanthin oxidase inhibition, erythrocyte membrane stability and other studies are essential to characterize them as biological antioxidants.

CONCLUSION

According to above discussion *Vallaris solanacea* (Roth) Kuntze contains important chemical constituents that confer upon it as a medicinal agent. It was revealed that the plant extract contains Reducing Sugar, Tannins, Saponins, Gums, Steroids, Alkaloids, and Glycosides which have potential role in its Analgesic, Cytotoxic and Antioxidant activity. This could provide a rationale for traditional uses of this plant and suggests for further investigation and isolation of biologically active constituents responsible for the activity.

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