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Antioxidant and analgesic activity of *Clerodendrum viscosum* leaf

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SUMMARY

The crude ethanolic extract of the leaves of *Clerodendrum viscosum* (Family: Verbenaceae) was evaluated for its antioxidant and analgesic activities to investigate the scientific basis of the traditional uses. The antioxidant property of the extract was assessed by 1, 1-diphenyl -2- picryl hydrazyl (DPPH) free radical scavenging assay. The extract showed prominent antioxidant activity (IC_{50} about ~ 16 μg/ml), which was comparable to standard drug ascorbic acid (IC_{50} about ~15 μg/ml). The extract produced significant ($P < 0.001$) writhing inhibition in acetic acid induced writhing in mice at the dose of 125 mg, 250 mg and 500 mg/kg body weight respectively, which were comparable to the standard drug diclofenac sodium. The results tend to suggest that the crude leaves extract at the above doses have antioxidant and analgesic activities and indicate that it might possess biologically active constituents having free radical scavenging and analgesic activities respectably.

Key words: *Clerodendrum viscosum*; DPPH; Diclofenac Sodium; Antioxidant activity; Analgesic activity

INTRODUCTION

Clerodendrum viscosum (Family: Verbenaceae) locally known as 'Bhant' is small shrub grows commonly in waste places and graveyards in all areas of Bangladesh. Leaf juice is used as strong anthelmintic, emetic, mild laxative and cholagogue. Infusion of leaves is used as bitter tonic and antiperiodic in malaria and in the treatment of chest complaints with coughs and asthma. It is used externally for tumors, skin diseases, snakebite and scorpion-sting. Roots are also used as anthelmintic and antifungal drugs (Gani, 2003). Leaves contain protein, free reducing sugar, a bitter principle clerodin, a sterol, oleic, stearic and lignoceric acids, tannin and gallic acid (Hirandra and Banerjee, 1936; JICS, 1951). The plant also contains saponin, flavonoids, alkaloids, a new glycoside clerodendroside, lupeol, benzoic acid derivatives and beta-sitosterol (Sen and Sing, 1964; Sinha and Dogra, 1985). In our continuous research on randomized screening of traditional medicine, present study was to investigate the antioxidant and analgesic activities of the crude ethanolic extract of *Clerodendrum viscosum* leaves.

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MATERIALS AND METHODS

Plant material and extraction

The leaves of the plant were collected from the campus of the Khulna University, Khulna during the month of January 2005 and were taxonomically identified by Forestry and Wood Technology Discipline, Khulna University, Khulna. The specimen (No. PL-78) sample was preserved in the Phytochemistry

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Laboratory of Khulna University. About 400 g of the leaves were dried for 15 days without the direct contact of sunrays. The dried leaves were finally ground and extracted by maceration over 20 days with 800 ml of 80% ethanol. The solvent was filtered carefully and evaporated under normal environment (room temperature 27.0 ± 2°C, relative humidity 70 ± 5%) by an electric fan to get the dried extract (approx. yield 10%). This extract was used for pharmaco-logical screening. The crude extract was subjected to preliminary phytochemical screening for the detection of major chemical groups (Evans, 1989). The extract showed the presence of alkaloids, tannins, reducing sugars, steroids and flavonoids. In each test 10% (w/v) solution of the extract in solvent was taken unless otherwise mentioned in individual test.

Animals

Young Swiss-albino mice of either sex, weighing 20 - 25 g, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for analgesic activity test. The animals were kept at animal house (Pharmacy discipline, Khulna University, Khulna) for adaptation after their purchase under standard laboratory conditions (relative humidity 55 - 65%, room temperature 25.0 ± 2°C and 12 h light: dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water. The animals were divided in-groups, with each group balanced for sex and body weight.

Acute toxicity test

Test animals were divided into different groups containing six animals in each. The groups received the extract orally at doses of 62.5, 125, 250, 500, 1,000, 2,000 and 4,000 mg/kg body weight whereas the control group received distilled water. General signs and symptoms of toxicity and mortality were recorded for 24 h (Lork, 1983).

Antioxidant activity

Antioxidant activity of the extract was determined on the basis of their scavenging activity of the stable DPPH free radical.

Qualitative assay:

A Suitably diluted stock solution was spotted on pre-coated Silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extract. The plates were dried at room temperature and were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH by the resolved bands was observed for 10 min and the color changes (yellow on purple background) were noted (Sadhu *et al.,* 2003).

Quantitative assay:

Quantitative assay was performed on the basis of the modified method of Gupta *et al*., 2003. Stock solution (10 mg/ml) of the plant extract was prepared in ethanol from which serial dilutions were carried out to obtain concentrations of 1, 5, 10, 50, 100 and 500 mg/ml. Diluted solutions (2 ml) were added to 2 ml of a 0.004% ethanol solution of DPPH, mixed and allowed to stand for 30 min for reaction to occur. The absorbance was determined at 517 nm for each concentration and from these values corresponding percentage of inhibitions were calculated. Then percentage of inhibitions were plotted against respective concentrations used (Fig. 1) and from the graph IC_{50} was calculated. The experiment was performed in duplicate and average absorbance was noted for each concentration. Ascorbic acid was used as positive control.

Analgesic activity

Analgesic activity of the extract was tested using the model of acetic acid induced writhing in mice (Whitle, 1964; Ahmed *et al.,* 2001). The experimental animals were randomly divided into five groups, each consisting of six animals. Group I was treated

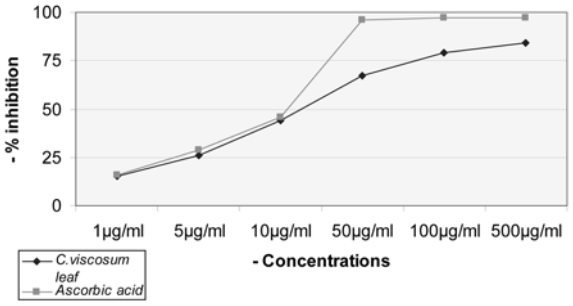

Fig. 1. Graphical representation of % inhibition of DPPH vs. Concentration.

as 'control group' which received 1% (v/v) Tween - 80 solution in water; group II was treated as 'positive control' and was given the standard drug diclofenac sodium at dose of 25 mg/kg of body weight; group III, group IV and group V were test groups and were treated with the extract at the doses of 125 mg, 250 mg and 500 mg per kg of body weight respectively. Control vehicle, standard drug and extracts were administered orally 30 min prior to acetic acid (0.7%) injection, then after interval of 15 min, the numbers of writhes (squirms) were counted for 5 min.

RESULTS

In the acute toxicity test, the animal exhibited decreased mobility but no convulsions or loss of rithing reflex and at highest dose tested 4,000 mg/kg, no mortality was observed in the test animals.

Antioxidant activity of the extract was performed on the basis of the modified method of Gupta *et al*. (2003). The extract showed prominent antioxidant activity (IC_{50} about ~16 µg/ml) against DPPH free radical, which was comparable to that of standard drug, ascorbic acid (IC_{50} about ~15 µg/ml) (Table 2).

Table 2. Antioxidant activity of *Clerodendrum viscosum* leaves

Sample	Concentration (µg/ml)	% inhibition	IC_{50} (µg/ml)
Et. extract of *Clerodendrum viscosum* (leaves)	1	15	~ 16
	5	26	
	10	44	
	50	67	
	100	79	
	500	84	
Ascorbic acid	1	16	~ 15
	5	29	
	10	46	
	50	96	
	100	97	
	500	97	

Values are expressed as mean; Et.= Ethanolic.

Antinociceptive activity of the extract was tested using the acetic acid-induced writhing model in mice. The extract produced about 30.07%, 63.67% and 76.79% writhing inhibition at the doses of 125, 250 and 500 mg per kg body weight respectively, which were comparable to standard drug diclofenac sodium where the inhibition was about 86.89% at the dose of 25 mg per kg body weight (Table 3).

DISCUSSION

Many plants exhibit efficient antioxidant properties owing to their phenolic constituents. Most of tannins and flavonoids are phenolic compounds and may be responsible for antioxidant properties of many plants (Larson, 1988). The role of free radicals in many disease conditions has been well established. Several biochemical reactions in our

Table 1. Results of different groups test of *Clerodendrum viscosum* leaves

Plant Extract	Alkaloids	Reducing Sugars	Taninns	Gums	Flavonoids	Saponins	Steroids
Ethanolic extract of *Clerodendrum viscosum* leaves	+	+	+	-	+	-	+

+: Positive result; - : Negative result.

Table 3. Effect of ethanolic extract of *Clerodendrum viscosum* leaves on acetic acid induced writhing in mice

Animal Group/Treatment	Number of writhes (% writhing) [n]	Inhibition (%)
Control 1% tween-80 solution in water, p.o.	30.5 ± 0.10 (100)	-
Positive control Diclofenac Sodium 25 mg/kg, p.o.	4.0 ± 0.85** (13.11)	86.89
Test group-1 Et. extract 125mg/kg, p.o.	21.33 ± 0.182** (69.93)	30.07
Test group-II Et. extract 250 mg/kg, p.o.	10.08 ± 0.166** (36.33)	63.65
Test group-III Et. extract 500 mg/kg, p.o.	7.08 ± 0.11** (23.21)	76.79

Values are expressed as mean ± S.E.M. (n = 6). n = Number of mice, * $P < 0.01$, ** $P < 0.001$, vs. control, Et.= Ethanolic, % = Percentage, P.O. = Per oral, Students *t*-test.

body generate reactive oxygen species and these are capable of damaging crucial bio-molecules. If they are not effectively scavenged by cellular constituents, they lead to disease conditions (Halliwell and Gutteridge, 1985; Halliwell, 1994). Drugs that act against free radical or scavenge it may be very useful in various free radical-induced debilitating diseases such cancer or carcinoma, diabetes, hypertention, tumour. A number of methods have been developed to evaluate antioxidant activity both in-vitro and in-vivo systems. The DPPH assay is based on the ability of 2,2 - diphenyl -1 picryl- hydrazyl a stable free radical, to decolorize in the presence of antioxidants. The DPPH contains an odd electron which is responsible for absorbance at 517 nm and also for 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 the absorbance. That is why with the increasing concentration of both crude extracts and ascorbic acid it was found that % inhibition of free radical scavenging activity were also increased. As it is externally used for tumours, skin diseases (Gani, 2003). The free radical scavenging property may be one of the mechanisms by which the plants are effective in traditional medicine. Further studies as lipid peroxidation inhibition activity, xanthin oxidase inhibition activity, erythrocytic membrane stability activity etc. necessary to asses the potential clinical uses of this plant or its extracts or its active principles.

Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances (Taesotikul *et al*., 2003). Increased levels of PGE_2 and $PGF_{2\hat{a}}$ in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid (Derardt *et al*., 1980). On the basis of the result of acetic acid induced writhing test, it can be concluded that the extract might possess an analgesic activity and the mode of action might involve a peripheral mechanism.

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