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Evaluation of Antioxidant and Antinociceptive Properties of Methanolic Extract of *Clerodendrum viscosum* Vent.

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Original Research Article

ABSTRACT

The methanolic extract of *Clerodendrum viscosum* Vent. (Verbenaceae) was evaluated for *in vitro* antioxidant activity by determination of total antioxidant capacity, assay of nitric oxide scavenging activity and reducing power test and *in vivo* antinociceptive effect in acetic acid induced writhing model in swiss albino mice. The results revealed the presence of pronounced antioxidant property as compared with ascorbic acid used as standard and a dose-dependent (250 and 500 mg/kg) analgesic effect in *Clerodendrum viscosum*. The antioxidant and antinociceptive activities obtained seem to be in good accordance with the traditional uses of *Clerodendrum viscosum*.

***Key words*:** *Clerodendrum viscosum* , Verbenaceae, Antioxidant, Ascorbic acid, Antinociceptive, Swiss albino mice.

INTRODUCTION

The use of herbal medicine has become increasingly popular worldwide and medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Estakhr *et al.*, 2011). Plant secondary metabolites play an important role in health care for about 80% of the world's population (Wohlmuth *et al.*, 2002). Approximately, half of the world's 25 best-selling pharmaceutical agents are derived from natural products (Baker *et al.*, 1995). Thus, emphasis is now given on the standardization of herbal medication by screening of biological activities of medicinal plants and isolating active principles from them (Abelson, 1990).

Clerodendrum viscosum Vent. (Bengali name-Bhat, Ghetu; Family-Verbenaceae) is a small shrub, about 4 feet high, with broadly ovate leathery leaves, pinkish white flowers and small fruits enclosed in red bracts, grows commonly in waste places and graveyards in all areas of the country The plant contains saponin, flavonoids, alkaloids, a new glycoside, cleodendroside, lupeol, benzoic acid derivatives and beta-sitosterol. Roots contain the antifungal flavonoids, cabruvin and quercetin. The seeds contain a fatty oil in which the major fatty acids are palmitic, oleic and linoleic acids. Clerodendroside has hypotensive property. Leaf juice is used as strong anthelmentic, emetic, mild laxative and cholagogue. It is externally used for tumors, skin diseases, snake bite and scorpion-sting (Ghani, 2003). In this paper, the antioxidant and the antinociceptive activities of the methanolic extract are being reported to validate the traditional use of the crude drug through *in vivo* and *in vitro* evaluation.

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EXPERIMENTAL

Plant materials

Clerodendrum viscosum was collected from Gazipur in July 2008 and was identified by

Bangladesh National Herbarium, Mirpur, Dhaka, where a voucher specimen (DCAB accession no: 33.112) has been deposited. The leaves of the plant were first sun dried and then ground into coarse powder.

Extraction of plant materials

About 100 gm of powered material was taken in a clean, flat-bottomed glass container and soaked in 500 ml of 90% methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. The filtrate (methanol extract) obtained was evaporated under ceiling fan and in a water- bath until dried. It rendered a gummy concentrate of blackish color. The gummy concentrate was designated as crude extract of methanol.

To get preliminary idea about the active constituents present in the plant (Leaves) extracts different chemical tests were performed and showed the presence of alkaloid, flavonoids and tannins (Evans, 1989).

Antioxidant properties

Qualitative assay

A suitably diluted stock solutions (sample solutions) were spotted on pre-coated Silica gel TLC (Thin layer chromatography) 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 and to choose the solvent system in which stock solutions run well. 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 minutes and the color changes (yellow on purple background) were noted (Sadhu *et al.*, 2003).

Quantitative assay

Free radical scavenging activity of the methanol extract was evaluated by determination of total antioxidant capacity, assay of nitric oxide scavenging activity and reducing power test. In all methods ascorbic acid is used as standard.

Determination of total antioxidant capacity

The antioxidant activity of the extract was evaluated by the phosphomolybdenum method according to the procedure of Prieto and colleagues (Prieto *et al.*, 1999). The assay is based on the reduction of Mo(VI) – Mo(V) by the extract and subsequent formation of a green phosphate / Mo(V) complex at acid pH. 0.3 ml extract was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer (Hach, DR-4000U) against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

Assay of Nitric oxide scavenging activity

The procedure is based on the method, where sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10 mM) in phosphate buffered saline was mixed with different concentrations of methanolic extract of *Clerodendrum viscosum* dissolved in methanol and incubated at room temperature for 150 min. The same reaction mixture without the methanol extract but the equivalent amount of methanol served as the control. After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% N-(1- naphthyl) ethylenediamine dihydrochloride was added. The absorbance of the chromophore formed was read at 546 nm. (Sreejayan and Rao, 1997).

Reducing power

The reducing power of methanolic extract was determined according to the method of Oyaizu (Oyaizu 1986). Different amounts of methanolic extracts (50 – 250 mg) in 1 ml of methanol were mixed with phosphate buffer (2.5 ml, 0.2 mol/l, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$]

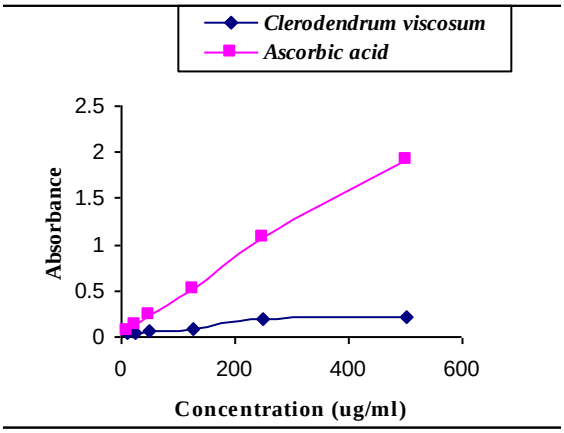

Figure 1. Total antioxidant capacity of methanolic extract of *Clerodendrum viscosum* Vs Ascorbic acid.

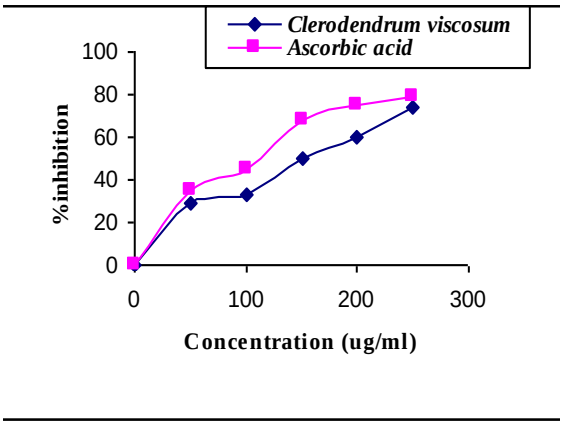

Figure 2. Nitric oxide scavenging activity of methanolic extract of *Clerodendrum viscosum.*

(2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged (650 x g at room temperature) for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Antinociceptive properties

Evaluation of antinociceptive property was performed by acetic acid induced writhing model in mice (Whittle 1964). The acetic acid induced writhing method is an analgesic behavioral observation assessment method that demonstrates a noxious stimulation in mice. The test consists of injecting the 0.7% acetic acid solution intraperitoneally and then observing the animal for specific contraction of body referred as 'writhing'. A comparison of writhing was made between positive control (Diclofenac - Na). Control and test sample are given orally 30 minutes prior to acetic acid injection. If the sample possesses analgesic activity, the animal that received the sample will give lower number of writhing than the control, i.e. the sample having analgesic activity will inhibit writhing.

Table 1. Total antioxidant capacity of methanolic extract of *Clerodendrum viscosum.*

Materials	Concentration (µg/mL)	Equivalent to ascorbic acid
Methanol extract of *Clerodendrum viscosum*	10	0.067±0.11
	25	0.122±0.06
	50	0.239±o.13
	125	0.526±0.15
	250	1.074±0.09
	500	1.921±0.13

RESULTS

Antioxidant properties

Qualitative assay: The color changes (yellow on purple background) on the TLC plates were observed due to the bleaching of DPPH by the resolved bands.

Quantitative assay

Total antioxidant capacity

Total antioxidant capacity exerted by the extract is concentration dependent (**Figure 1**). It is observed that the extract is likely to have the capacity of reduction of Mo (VI) to Mo (V) by the antioxidant principle and the formation of a green phosphate / Mo (V) complex with a maximal absorption at 695 nm. The antioxidant activity is expressed as the number of equivalents of ascorbic acid (**Table 1**).

Nitric oxide scavenging activity

From **Figure 2**, it is observed that the extract is likely to have concentration dependent nitric oxide scavenging activity. The leaves may have the property to counteract the effect of NO formation and in turn may be of considerable

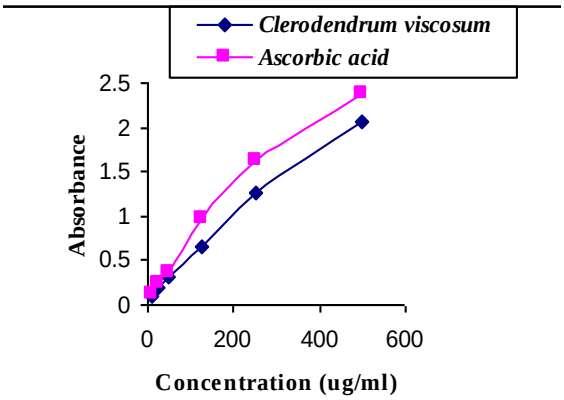

Figure 3. The reducing power of methanolic extract of *Clerodendrum viscosum.*

interest in preventing the ill effects of excessive NO generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to the human health. Nitric oxide is also implicated for inflammation, cancer and other pathological conditions (Moncada *et al.*, 1991).

Reducing power

Reduction ability of the extract has been investigated from the Fe^{+++} – Fe^{++} transformation using the method followed by Oyaizu (Oyaizu 1986). Earlier authors (Duh 1998; Tanaka *et al.*, 1988) have observed a direct correlation between antioxidant activity and reducing power of certain plant extracts. The reducing properties are generally associated with the presence of reductones (Duh 1998) which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordon 1990). Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. **Figure 3,** demonstrates the reduction ability of *Clerodendrum viscosum.*

Antinociceptive properties

The methanolic extract of plant leaves produced 83.57% and 73.91% writhing inhibition at the doses of 500 mg/kg and 250 mg/ kg body weight respectively, in acetic acid induced mice which are comparable to Diclofenac sodium (67.65% at the dose of 25 mg/kg). **Table 2,** represents the antinociceptive activity of *Clerodendrum viscosum*.

Table 2. Antinociceptive property of methanolic extract of *Clerodendrum viscosum* on acetic acid induced writhing on mice.

Animal group/ Treatment	Number of Writhings (% writhing)	Inhibition (%)
Control (1% Tween-80 solution in water) 10 ml/kg, p.o. n=4	34.5±1.01[a] (100)	-
Positive control Diclofenac sodium 25 mg/kg, p.o. n=4	11±1.7[a] (32.35)	67.65
Test group I Methanolic extract 250 mg/kg, p.o. n=4	9±0.21[a] (26.09)	73.91
Test group II Methanolic Extract 500 mg/kg, p.o. n=4	5.67±0.15[a] (16.43)	83.57

Values are expressed as mean± S.E.M.; a indicates P<0.001 vs. control; %: percentage. p.o.: per oral.

DISCUSSION

The present study shows that the plant extract exhibits moderate antioxidant property while, it has potent antinociceptive property. Antioxidant has been suggested to possess both anti-inflammatory and analgesic activity in humans (Edmonds *et al.*, 1997). A combination of antioxidants with analgesics normalized the oxidative stress which, suggest that the administration of antioxidants in pain treatment may be employed to decrease the doses of analgesics (Rokyta1 *et al.*, 2003). Antioxidant-based pain killers may one day become a viable alternative to addictive medications such as morphine. Therefore, medicinal plants like *Clerodendrum viscosum which* possesses both antioxidant and antinociceptive properties can be used in the treatment of pain stimulated oxidative stress condition through their pharmacological validation.

CONCLUSION

The present report demonstrates the antioxidant and antinociceptive properties of *Clerodendrum*

viscosum and validates its use in traditional medicine.

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