

Evaluation of Analgesic and Antioxidant Properties in the Ethanolic Root Extract of *Clerodendrum viscosum* Vent.

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Abstract

Purpose: To investigate potential analgesic and antioxidant properties in the ethanolic root extract of *Clerodendrum viscosum* Vent. **Methods:** Phytochemical analysis of the ethanolic root extract was evaluated using standard procedure. Analgesic activity test was carried out by acetic acid induced writhing in mice model. The antioxidant property of the plant extract was assessed by DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging activity. **Results:** Phytochemical analysis displayed that the plant extract contains carbohydrates, glycosides, tannins, saponins, flavonoids and steroids types of compounds. The extract exhibited dose-dependent and statistically significant ($P < 0.01$) writhing inhibition in acetic acid induced mice. The extract produced about 38.59 % & 59.07% writhing inhibition at the doses of 250 mg/kg & 500 mg/kg respectively, which was comparable to the standard drug diclofenac sodium (writhing inhibition was about 77.78% at the dose of 25 mg/kg). In DPPH scavenging assay, the IC_{50} value was found 32.5 μ g/ml which was comparable to that of a standard antioxidant ascorbic acid (IC_{50} : 7 μ g/ml). **Conclusion:** Our findings provide a support for the use of this plant in traditional medicine as an analgesic and antioxidant as well as its further phytochemical and pharmacological investigation to identify and isolate the actual molecule(s) which exhibited that analgesic and antioxidant activities.

Keywords: *Clerodendrum viscosum* Vent, phytochemical, analgesic, antioxidant

Introduction

Clerodendrum viscosum Vent is a perennial shrub of the family Verbenaceae. It is commonly known as hill glory bower or Ghentu in Bangladesh. It is also available in the tropical regions of Asia including India, Myanmar, Pakistan, Thailand and Sri Lanka. It is one of the most well-known natural health remedies in traditional practices and siddha medicine (Das *et al* 2010).

It contains saponin, alkaloids, flavonoid, clerodendroside, lupeol, benzoic acid derivatives and β -sitosterol. It also comprises clerosterol, clerodone, and clerodolone. Leaves of the plants contain protein, free reducing sugar, tannin, glucuronide, oleic, stearic, lignoceric acids, and gallic acid. Lupeol, β -sitosterol, antifungal flavonoids, cabrubin and quercetin have been found in the roots of the plants. The plant seeds possess fatty oil, in which the major fatty acids are palmitic, oleic and linoleic acids. Clerodin and hentriacontane have been isolated from flowers (Ghani 2003).

The plant has been known as tonic, antipyretic and anthelmintic. The leaf and root have been widely used as asthma, tumors, antidandruff, antipyretic, ascaricide, laxative, vermifuge, and in treatments of convulsion, diabetes, gravel, malaria, scabies, skin diseases, sore, spasm, scorpion sting, snake bite and tumor. Infusion of leaves is used as bitter tonic and antiperiodic in malaria. Leaves are also used in chest complaint with cough and difficult expectoration. In Rangamati, Bangladesh, leaf-boiled water has been used as a bath in jaundice by the tribal; Marmas take bath for scabies. Root juice is warmed and rubbed on the penis to treat impotency. Root juice along with ginger is given to relieve colic pain by the Garo in Madhupur, Bangladesh. In Thai medicine the leaves and root are known to be diuretic; and used for treatment of intestinal infections and kidney disfunction. In many traditional practices the leaves and root are widely used as antihyperglycemic.

Since Bangladesh is a country of low economic growth, scientific exploration and standardization of potential crude drugs is an urgent need to revolutionize our drug sector. The presence of diverse bioactive metabolites like alkaloids, glycosides, flavonoids, tannins etc. in plants has formed the therapeutic basis of herbal medications. Thus emphasis is given on the biological and phytochemical screening of medicinal plants for further exploration of their active constituents. Besides, Bangladesh imports a large quantity of pharmaceutical

raw materials including medicinal plants and semi processed plant products to produce drugs and medicines. This huge foreign exchange can be saved if the manufactures utilize the indigenous medicinal plants or their semi-processed products to satisfy their needs. From the literature survey we found no scientifically elegant studies supporting traditional uses of this plant has yet been reported. Therefore, to judge the traditional use of this medicinal plant, this study was investigated to evaluate the anti-oxidant and analgesic effects of *Clerodendrum viscosum* Vent. Our results suggest that the plant possesses significant analgesic and antioxidant properties which support the traditional uses of the plants.

Materials and methods

Plant material collection and identification

The plant *Clerodendrum viscosum* Vent was collected from Khulna, Bangladesh, during the month of October, 2011 on the day time. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (Accession No: DACB- 35987) and a voucher specimen was also deposited there.

Preparation of plant extract

The collected plant parts (roots) were separated from undesirable materials or plants or plant parts. They were air dried for three weeks. The plant parts were ground into a fine powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. A glass jar with plastic cover was taken and washed thoroughly. The jar was rinsed with ethanol and dried. Then 150gm of the dried powder was taken in the jar. After that 95% ethanol (750ml) was poured into the jar up to 1 inch height above the sample surface as it can sufficiently cover the sample surface. The plastic cover with aluminum foil was closed properly to resist the entrance of air into the jar. This process was performed for 8 days. The jar was shaken and stirring several times during the process to get better extraction. After the extraction process the extract was filtered by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper. The filtrate was collected in a beaker. The filtrate was condensed using a rotary Evaporator and after that it was kept under ceiling fan to evaporate the ethanol completely. It rendered a gummy concentrate (6 gm) of reddish brown color. The gummy concentrate was designated as crude extract of ethanol. The yield value was 3%.

Experimental animals

Swiss-albino mice (18-25 gm) of both sexes were used for the experiment. They were purchased from Jahangirnagar University, Bangladesh and were kept standard environmental condition (relative humidity 55-65%, room temperature 25.0 ± 2 °C, and 12 hrs light dark cycle) for one week for adaptation after their purchase and fed with standard food regularly. The study was conducted according to the guidelines of Institutional Animal Ethics Committee (Zimmermann 1983).

Chemicals and drugs

Glacial acetic acid was purchased from Sigma chemicals, USA. Diclofenac sodium and ascorbic acid were collected from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals were of analytical grade.

Phytochemical test

Preliminary phytochemical analysis of the *Clerodendrum viscosum* Vent plant extract was carried out by following standard procedure described by Ghani (Ghani 2003).

Analgesic activity test

Analgesic effect of the plant extract was investigated by acetic acid induced writhing method as followed by Ali *et al* (2012). Briefly, experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III, group- IV consisting of 5 mice in each group. Each group received a particular treatment i.e. control, positive control and the two doses of the extract. Each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly. Test samples, control and Diclofenac-Na were given orally by means of a feeding needle. A thirty minutes interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%, 10 ml/kg) was administered intraperitoneally to each of the animals of a group. After an interval of five minutes, which was given for absorption of acetic acid, number of squirms (writhing) was counted for 15 minutes.

In vitro Anti-oxidant Activity test

The anti-oxidant potential of the ethanolic extract was determined on the basis of their scavenging activity of the stable 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. The aliquots of the different concentrations (1-500 µg/ml) of the extract were added to 3 ml of a 0.004% w/v solution of DPPH. After 30 minutes, absorbance of each samples were determined by UV spectrophotometer at

517 nm. IC_{50} was determined from % inhibition vs concentration graph. The formula used for % inhibition ratio was $\% \text{ inhibition} = (1 - \text{Sample OD} / \text{Blank OD}) \times 100$.

Results

Phytochemical test

Phytochemical analysis of the ethanolic extract of the roots of *Clerodendrum viscosum* Vent indicated the presence of carbohydrates, glycosides, tannins, saponins, flavonoids and steroids types of compounds (Table 1).

Analgesic activity test

Ethanolic extracts of the plant extracts at the doses of 250 and 500 mg/kg body weight exhibited significant inhibition of writhing reflex 38.59% ($P < 0.01$) and 59.07% ($P < 0.01$) respectively while the standard drug diclofenac inhibition was found to be 77.78% ($P < 0.001$) at a dose of 25 mg/kg body weight (Table 2).

Anti-oxidant Activity

The plant extract displayed free radical scavenging activity in the DPPH assay ($IC_{50} = 32.5$ approx. $\mu\text{g/ml}$) which is comparable to that of ascorbic acid ($IC_{50} = 7$ approx. $\mu\text{g/ml}$), a well-known standard antioxidant (Fig 1).

Discussion

The phytochemical evaluation shows the presence of carbohydrates, glycosides, tannins, saponins, flavonoids and steroids in the ethanolic root extract of *Clerodendrum viscosum* Vent.

Our results show that the ethanolic plant extracts have potent analgesic activity in the acetic acid induced writhing method. Acetic acid causes pain and localized inflammation by the action of prostaglandins production [mainly, prostacyclines (PGI_2) and prostaglandin-E ($PG-E$)] which have been reported to stimulate the $A\delta$ -fibres that cause a sensation of sharp well localized pain (Ranolds *et al* 1982, Rang and Dale 1993). It has also been reported that acetic acid induces the increased level of PGE_2 and $PGF_{2\alpha}$ in the peritoneal fluid which is responsible for pain production (Bose *et al.*, 2010; Derardt *et al.*, 1980; Zakaria *et al.*, 2008; Zulfiker *et al.*, 2010; Bhalke *et al.*, 2009; Sulaiman *et al.*, 2008). There are various peripherally acting analgesic drugs such as ibuprofen, aspirin, diclofenac sodium and indomethacin that have been reported to inhibit acid induced writhing by inhibition of prostaglandin synthesis (Ishfaq *et al.*, 2004). Therefore it can be concluded that any agent that reduces the number of writhing will demonstrate analgesic effect by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Ferdous *et al.*, 2008). The result of the plant extract in acetic acid-induced writhing method suggests that the reduction of pain might be occurred due to the presence of analgesic properties in the extract via inhibition of prostaglandin synthesis.

In quantitative DPPH free radical scavenging antioxidant assay, our findings revealed that the ethanolic plant extracts possess significant anti-oxidant properties. The IC_{50} (inhibitory conc. 50%) of the plant extract showed about 32.5 $\mu\text{g/ml}$ whereas standard antioxidant compound showed about 7 $\mu\text{g/ml}$. It is now well established that free radicals (e.g. super oxide, hydroxyl radical, and nitric oxide) and other reactive species (e.g. hydrogen peroxide, single oxygen, hypochlorous acid) contribute to the pathology of many disorders including atherogenesis, neurodegeneration, chronic inflammation, and function of the immune system. Recent studies suggest that the inflammatory tissue damage is due to the liberation of reactive oxygen species from phagocytes invading the inflammation site (Conner and Grisham 1996). Consequently, the substances which relieve pain or inflammation may act by neutralizing (anti-oxidant) the oxidative group present in the inflammatory site. In our previous investigation the ethanolic plant extract reduces marked degree of pain in acetic acid induced pain in mice. From the previous data it can be assumed that the analgesic activity of the extract was may be due to reduction in production of arachidonic acid from phospholipids or may inhibit the enzyme system which may responsible for the production of prostaglandins. From the previous data on analgesic activity of the extract favor the presence of the antioxidant group present in *Clerodendrum viscosum* Vent. Further investigations are necessary to find out the active constituent responsible for this effect.

Conclusion

Our observation indicates that the ethanolic extracts of *Clerodendrum viscosum* Vent possess potent analgesic and anti-oxidant activities. Therefore, *Clerodendrum viscosum* Vent may be a potential source of compounds with analgesic and anti-oxidant activities and may contribute to national/ global economy if active compounds are isolated. Further investigation is required to find out the chemical constituents responsible for these effects.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Authors are grateful to the authority of the Khulna University for giving the opportunity to conduct such experiment and providing necessary chemical, instrument and utility support. Authors also like to express their cordial thanks to the experts of Bangladesh National Herbarium who helped for the identification of the plant.

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Table 1 Phytochemicals in the ethanolic root extract of *Clerodendrum viscosum* Vent.

Phytochemicals	Carbohydrate	Tannin	Flavonoid	Saponin	Gum	Steroid	Alkaloid
Inference	+	+	+	+	-	+	-

+ = presence; - =absence

Table 2 Effects of ethanolic root extracts of *Clerodendrum viscosum* Vent on acetic acid induced writhing in mice.

Treatment	Number of writhing	Inhibition rate (%)
Blank control (AA 10 mL/kg, i.p. + vehicle 10 mL/kg, p.o.)	34.2 ± 3.22	00.00
Positive control (AA 10 mL/kg, i.p. + diclofenac sodium 25 mg/kg, p.o.)	07.6 ± 2.92**	77.78
Test 1 (AA 10 mL/kg, i.p. + plant extract 250 mg/kg, p.o.)	21.0 ± 2.16*	38.59
Test 2 (AA 10 mL/kg, i.p. + plant extract 500 mg/kg, p.o.)	14.0 ± 3.86*	59.07

Results are presented as mean ± SEM, (n=5); *P<0.01, **P<0.001, significant compared to blank control; AA: acetic acid; i.p.: intraperitoneally; p.o.: per oral.

Fig1. Effects of ethanolic root extracts of *Clerodendrum viscosum* Vent on DPPH Scavenging activity