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EVALUATION OF ANALGESIC, ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF ETHANOL EXTRACT OF CLERODENDRUM VISCOSUM VENT.

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ABSTRACT

Clerodendrum viscosum Vent. is a very common plant in Bangladesh which is locally familiar as "Bhat" or "Ghetu". Here, the ethanol extract of whole plant part of *C. viscosum* and its various solvent (petroleum-ether, chloroform and ethyl acetate) fractions were subjected for the appraisal of analgesic, antioxidant and cytotoxic activities. Analgesic activity was tested by acetic acid-induced writhing model in Swiss albino mice. All the plant samples at the oral doses of 100- and 200 mg/kg body weight were found to exhibit significant ($p<0.05$) pain reducing activity in test animals. Highest inhibition of writhing was 62.38% by the ethyl acetate soluble fraction at dose of 200 mg/kg body weight while the standard drug diclofenac sodium (50 mg/kg) produces 76.14% reduction of abdominal writhing. DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical quenching assay was employed to determine the antioxidant potential of the plant samples while cytotoxic activity was checked by brine shrimp lethality bioassay. In DPPH radical scavenging assay, the plant samples showed prominent antioxidant activity. Among all, the ethyl acetate fraction showed maximum antioxidant potential with IC_{50} value of 28.02±0.53 µg/ml. In cytotoxic assay, the petroleum-ether fraction demonstrated strong cytotoxicity with LC_{50} value of 1.42±1.12 µg/ml. In summary, *C. viscosum* extracts possess significant analgesic, antioxidant and cytotoxic activities which rationalize its traditional use in folk medicine.

INTRODUCTION

The use of natural products is rising in the world especially in developing countries such as Bangladesh, India, China, Arabic countries and Iran. The chemical diversity of plants has made them one of the attractive sources for the isolation of bioactive natural compounds [1]. *Clerodendrum viscosum* Vent. (Family: Verbenaceae) is a small tree which grows up to 4 feet high. The plant grows abundantly in waste places and graveyards in all

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areas of the country. The plant is rich in saponin, flavonoids, alkaloids [2]. Roots have antifungal flavonoids, cabruvin and quercetin, whereas seed oil contain fatty acids such as palmitic, oleic and linoleic acids. Leaf juice is used as laxative, anthelmintic and emetic. It is also beneficial for tumors, skin diseases and snake bite. Previous phytochemical investigation of this plant led to the isolation of sterols, sugars, flavonoids, saponins and (22*E*, 24*S*)-stigmasta-5,22,25-trien-3β-ol [3]. There are a small number of published reports regarding the biological activities of the crude extract of *C. viscosum* [3,4]. Therefore, as part of our continuing explore with medicinal plants [5-7], we evaluated the analgesic, antioxidant and cytotoxic activities of Kupchan fractions of ethanolic extract of *Clerodendrum viscosum.*

MATERIALS AND METHODS

Collection of plant materials and authentication

Whole plant parts of *Clerodendrum viscosum* were collected from Savar, Bangladesh. A voucher specimen (no. 37528) has been deposited in the Department of Botany, University of Dhaka for future reference.

Extraction and isolation

The powdered plant sample (900 g) was soaked in 2.5 L of ethanol for 7 days and then filtered with Whatman No. 1 filter paper. The collected filtrate was concentrated with a rotary evaporator (Heidolph, Germany) at low pressure and at 40-45ºC temperature to obtain a gummy concentrate that was designated as the crude ethanol extract of whole plant of *Clerodendrum viscosum*. The crude extract thus obtained was fractionated by modified Kupchan method [8] to yield petroleum-ether (1 g), carbon tetrachloride (1.1 g), chloroform (0.85 g) and aqueous (1.65 g) soluble materials.

Reagents and chemicals

Analytical grade reagents and chemicals were used in all the experiments. Diclofenac sodium was obtained as a gift sample from Square Pharmaceuticals Ltd. Bangladesh.

Test animals [9]

For analgesic activity test, Swiss albino mice of either sex (weighing between 25-30 g and aged 6-7 weeks) were purchased from the Animal Resource Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (icddr,b). They were housed in normal polypropylene cages and kept under controlled room temperature (24 ± 2ºC; relative humidity 60-70 %) in a 12 h light/dark cycle and fed icddr,b formulated rodent food and water (*ad libitum*). The animals were acclimatized to laboratory condition for one week prior to experiments and details of animal care should be provided. The protocols for conducting the experiments on the animals were permitted by the institutional ethical committee.

Test for analgesic activity [10]

Acetic acid induced writhing method was conducted to investigate the analgesic activity of the plant samples in mice. The test samples (100- and 200 mg/kg body weight), standard diclofenac sodium and the negative control were administered to the mice by oral route. 1% acetic acid was injected intraperitoneally to induce pain in mice. Any plant extract or compound with pain reducing property will have the capacity to lessen writhing sensation in mice relating to control group. The number of writhes was recorded for ten min after intraperitoneal injection of acetic acid. The percent inhibition of writhing was determined as follows:

$$\%\ \text{inhibition of writhing} = \frac{N_{Control} - N_{Test}}{N_{Control}} \times 100\%$$

Where, N = Mean number of abdominal writhing for each group.

Test for antioxidant activity [11]

Antioxidant capacity of the plant extractives was determined by DPPH radical scavenging assay. Here, the extract solution of each concentrations were added with 3.0 ml of ethanol solution of DPPH (20 µg/ml). Absorbance was measured at 517 nm after 30 min. The percentage of inhibition (I%) of the DPPH radical was estimated against blank:

$$(I\ \%) = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100\%$$

Where, A = Absorbance for each group at 517 nm. The DPPH radical scavenging activity curve was constructed for each test sample/standard and IC_{50} values (50% inhibition) were calculated.

Test for cytotoxic activity [12]

Cytotoxicity test was conducted by brine shrimp lethality bioassay. In the present study, vincristine sulfate was used as positive control. Dimethyl sulfoxide (DMSO) solutions of the plant samples were applied against *Artemia salina* in a 24 h *in vivo* assay. For the experiment, plant samples (4.0 mg) were dissolved in DMSO and by serial dilution technique, solutions

of varying concentrations (400 to 0.781 µg/ml) were obtained using DMSO.

Statistical analysis

Results were expressed as the mean ± SEM. Statistical analysis for *in vivo* study was carried out using one-way ANOVA followed by Dunnett's multiple comparisons. The results obtained were compared with the vehicle control group; $p \leq 0.05$ was considered as statistically significant.

RESULTS

In acetic acid-induced writhing model, all the test samples showed a dose-dependent pain-relieving activity in mice (Table 1). The ethyl acetate fractions and the chloroform fractions at a dose of 200 mg/kg body weight each, demonstrated the maximum 62.38% and 55.04% writhing inhibition, respectively, which were comparable to the standard diclofenac sodium (76.14% at a dose of 50 mg/kg body weight).

Table 1. Analgesic activity of *C. viscosum* by acetic acid-induced writhing test in mice.

Test sample	Dose (mg/kg)	Writhing count					Writhing (Mean ± SEM)	% writhing	% Inhibition of writhing
		M-1	M-2	M-3	M-4	M-5			
Control	0.1ml/10 g	23	21	22	20	23	21.8±0.58	100	-
Standard	50	5	4	6	6	5	5.2±0.37	23.8	76.14
CECV	100	13	14	12	13	14	13.2±0.37	60.55	39.45
	200	12	10	10	11	11	10.8±0.37	49.54	50.45
PEF	100	14	13	14	13	14	13.6±0.24	62.38	37.61
	200	11	10	11	9	11	10.4±0.39	47.70	52.29
CF	100	15	16	14	13	14	14.4±0.51	66.05	33.95
	200	10	10	11	9	9	9.8±0.37	44.95	55.04
EAF	100	10	11	10	12	11	10.8±0.37	49.54	50.45
	200	9	9	8	7	8	8.2±0.0.37	37.61	62.38

Here, Standard = Diclofenac sodium, CECV = Crude ethanol extract of *C. viscosum*; PEF = Petroleum-ether fraction; CF = Chloroform soluble fraction; EAF= Ethyl acetate soluble fraction of the crude ethanol extract of *C. viscosum*. M-1= Mice 1, M-2 = Mice 2, M-3 = Mice 3, M-4 = Mice 4, M-5 = Mice 5 and Number of animals in each group = 5. Results are presented as mean ± SEM, (n=5), $p<0.05$, Dunnett's *t*-test as compared to control.

In DPPH assay, the ethyl acetate soluble fractions of *C. viscosum* showed excellent antioxidant activity with a IC_{50} value of 28.02±0.53 µg/ml, compared to the standard butylated hydroxy toluene (IC_{50}= 16.32±1.17 µg/ml), while the carbon tetrachloride and petroleum-ether soluble fraction exhibited moderate activity with IC_{50} values of 38.73±0.72 and 86.37±0.78 µg/ml, respectively (Table 2). In the brine shrimp lethality bioassay, all the plant samples showed strong toxicity against *Artemia salina* (Table 2).

Among all, the petroleum-ether fraction, the chloroform fraction and the ethanol extract exhibited maximum toxicity towards the shrimp with LC_{50} values of 1.42±1.12, 1.94±0.57 and 2.26±0.96 µg/ml, respectively.

Table 2. Antioxidant and cytotoxic activity of *C. viscosum*.

Test sample	IC50 (µg/ml)	LC50 (µg/ml)
BHT	16.32±1.17	--
VS	--	0.544±1.05
CECV	117.26±0.36	2.26±0.96
PEF	86.37±0.78	1.42±1.12
CF	106.21±1.23	1.94±0.57
CTF	38.73±0.72	15.28±0.62
EAF	28.02±0.53	4.18±0.84

Here, BHT = Butylated hydroxy toluene; VS = Vincristine sulfate. CECV = Crude ethanol extract of *C. viscosum*; PEF = Petroleum-ether fraction; CF = Chloroform fraction; CTF = Carbon tetrachloride fraction; EAF= Ethyl acetate fraction of the crude ethanol extract of *C. viscosum*. Results are presented as mean ± SEM, (n=3), $p<0.05$, Dunnett's *t*-test as compared to control.

DISCUSSION

In this study, we have tested the ethanol extract of *C. viscosum* whole plant and its different solvent fractions for analgesic, antioxidant and cytotoxicity activity. Analgesic action was evaluated by acetic acid-induced writhing model in mice. Intravenous injection of acetic acid triggers the release of prostaglandin in the body fluid which is responsible for pain sensation in experimental animals [13]. Non-steroidal anti-inflammatory agents act by hindering this sensory stimulation in response to inflammatory mediators such as prostaglandin [14]. Additionally, the level of analgesia can also be estimated by the percent reduction in the number of abdominal writhing [15]. Therefore, any compound that can reduce the level of writhing reflex will render analgesic effect preferably by a peripheral mechanism of pain inhibition [14].

In our experiment, the crude extract of *C. viscosum* whole plant and it's all Kupchan fractions (at doses of 100- and 200 mg/kg body weight) produced a dose-dependent and statistically significant ($p<0.05$) writhing reflex in acetic acid-induced mice. A similar pharmacological activity was found in experimental animals, where the ethanol extract of *C. viscosum* root at the doses of 250- and 500 mg/kg demonstrated about 38.59 % and 59.07% writhing inhibition, respectively [16].

The significant pain reduction by the plant extracts observed in our study might be due to the presence of bioactive principles in the extracts which can show analgesic action via the inhibition of prostaglandin biosynthesis. Previously it was confirmed that this plant extract contains bioactive phytochemicals including flavonoids, saponins, tannins etc. [16].

Free radicals such as reactive oxygen species (ROS), causes cell and tissue damage leading to pathological situations like inflammation, cancer, heart diseases and arthritis. Antioxidants with free radical scavenging activities have great importance in the prevention and treatment of diseases in which oxidants or free radicals are implicated. The uses of synthetic antioxidants have been limited because of their carcinogenicity. Alternatively, natural antioxidants are more desirable and safer [17]. In our study, all the plant samples showed significant antioxidant capacity that was established by DPPH free radical quenching capacity. Phytochemicals such as flavonoids, saponins, tannins, alkaloids present in these plant extract may scavenge these free radicals and thereby can protect the biological system from free radical induced oxidative stress [18].

Brine shrimp lethality bioassay was employed to determine the toxicity of plant extracts. Because it is a rapid, inexpensive and simple bioassay for testing plant extracts. In our study, the crude extract along with its Kupchan fractions showed significant toxicity against *Artemia salina*. The presence of different secondary metabolites like alkaloid, glycoside, steroids, tannin, and flavonoid in the extracts may play a key role to show the cytotoxic effect [19]. However, further studies using cancer cell line are necessary to isolate the active compound(s) responsible for the activity.

CONCLUSION

Clerodendrum viscosum Vent. exhibited significant analgesic, antioxidant and cytotoxic activities. The present study 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.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Sujan Chandra Das has conducted the laboratory experiments. Nazmul Qais has designed and guided Sujan Chandra Das for the animal study. Md. Ruhul Kuddus contributed in guiding the in vitro study and in preparing the manuscript. Choudhury M. Hasan supervised the overall study, and finalized the manuscript.

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