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Evaluation of analgesic, cytotoxic and antioxidant activities of *Sansevieria roxburghiana* Schult. and Schult. f.

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ABSTRACT

Objective: To investigate the crude methanolic extract of whole plant part of *Sansevieria roxburghiana* Schult. and Schult. f. (Agavaceae) and the possibility of analgesic, cytotoxic and antioxidant activities. **Methods:** The analgesic activity was assessed by acetic acid induced writhing test in mice. The cytotoxic activity was evaluated by brine shrimp lethality bioassay while antioxidant effect was measured by 1, 1-diphenyl-2-picrylhydrazyl-hydrate (DPPH) free radical scavenging assay. **Results:** The ethyl acetate soluble fraction of the crude extract was found to have significant (P<0.001) analgesic activity at the oral dose of 100 mg/kg body weight. In brine shrimp lethality bioassay, the aqueous soluble fraction exhibited maximum toxicity towards the shrimp with LC_{50} value of 0.735 μg/mL compared to 0.544 μg/mL exhibited by standard vincristine. The crude methanolic extract along with its all partitionates revealed mild to moderate free radical scavenging activity. **Conclusions:** These primary findings suggest that the extract might possess some chemical constituents that are responsible for analgesic, cytotoxic and antioxidant activities.

1. Introduction

Sansevieria roxburghiana (*S. roxburghiana*) Schult. and Schult. f. is a herbaceous perennial plant with short fleshy stem, occurring in eastern coastal region of India, also in Sri Lanka, Indonesia and tropical Africa[1]. This plant has been traditionally used as a cardiotonic, expectorant, febrifuge, purgative, tonic in glandular enlargement and rheumatism[2-4]. The rhizome of the plant also showed antitumor and antidiabetic activity against the experimental animals[5,6]. The leaves of the plant have antibacterial activity against gram positive and gram negative bacterias responsible for various infections[7].

As a part of our ongoing investigations on local medicinal plants of Bangladesh[8,9], in this paper, we reported the analgesic, cytotoxic and antioxidant activity of the whole plant part of *S. roxburghiana*.

2. Materials and methods

2.1. Plant material

For this present investigation, *S. roxburghiana* was collected from Dhaka, July 2008 and was identified at Bangladesh National Herbarium, where a voucher specimen has been deposited for future reference (DACB-35975). The collected plant parts were dried for one week and ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

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2.2. Preparation of the extract

About 500 g of powdered material was taken in a clean, flat bottomed glass container and soaked in 1.5 L of 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. Then it was filtered through Whatman filter paper number 1. The extract was concentrated with a rotary evaporator at low temperature (40-45 ℃) and reduced pressure. The concentrated methanolic extract was partitioned by the modified method of Kupchan[10] and the resultant partitionates *i.e*, petroleum ether, carbon tetrachloride, chloroform, ethyl acetate and aqueous soluble fractions were used for the experiment.

2.3. Experimental animals

Young Swiss-albino mice of either sex aged 4-5 weeks, average weight 20-25 g were used for the experiment. The mice were purchased from the animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B). They were kept in standard environmental condition (at 24.0±0.0 ℃ temperature and 55%-65% relative humidity and 12 hour light/12 hour dark cycle) for one week for acclimation after their purchase and fed ICDDRB formulated rodent food and water ad libitum. The set of rules followed for animal experiment were approved by the institutional animal ethical committee[11].

2.4. Drugs and chemicals

Both the acetic acid and dimethyl sulfoxide (DMSO) were obtained from Merck, Germany. Tween-80 was obtained from BDH Chemicals, UK. Normal saline solution was purchased from Orion Infusion Ltd. Bangladesh. Diclofenac sodium (Voltalin) was obtained from Novartis Ltd., Bangladesh.

2.5. Analgesic activity by acetic acid induced writhing method

The analgesic activity of the samples was evaluated using acetic acid induced writhing method in mice[12]. In this method, acetic acid was administered intraperitoneally to the experimental animals to create pain sensation. As a positive control, any standard NSAID drug can be used. In the present study, diclofenac sodium was used to serve the purpose. About 100 mg/kg body weight of the plant extract was administered orally to the Swiss-albino mice after an overnight fast. Test samples and vehicle were administered orally 30 min prior to intraperitoneal administration of 0.7% v/v acetic acid solution (0.1 mL/10g) but diclofenac sodium was administered 15 min prior to acetic acid injection. Then the animals were placed on an observation table. Each mouse of all groups were observed individually for counting the number of writhing they made in 15 min commencing just 5 min after the intraperitoneal administration of acetic acid solution. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated group was compared to that of a control group while diclofenac sodium (10 mg/kg) was used as a reference substance (positive control).

2.6. Cytotoxic activity

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds[13]. Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. For cytotoxicity screening, DMSO (Dimethyl sulfoxide) solutions of the plant extractives were applied to *Artemia salina* in a one day *in vivo* assay. For the experiment, 4 mg of each of the extracts were dissolved in DMSO and solutions of varying concentrations (400, 200, 100, 50, 25, 12.5, 6.25, 3.123, 1.563, 0.781 μg/mL) were obtained by serial dilution technique. The solutions were then added to the pre-marked vials containing ten live *Brine shrimp* nauplii in 5 mL simulated sea water. After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent of lethality of the *Brine shrimp* nauplii was calculated for each concentration. The median lethal concentration (LC_{50}) of the test samples were obtained by plotting percentage of the shrimp killed against the logarithm of the sample concentration.

2.7. Antioxidant activity

The free radical scavenging activity (antioxidant capacity) of the plant extractives on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was estimated by the method established by Brand-Williams *et al*[14]. Here, 2.0 mL of a methanol solution of the sample (extractive/standard) at different concentrations (500 μg/mL to 0.977 μg/mL) were mixed with 3.0 mL of a DPPH methanol solution (20 μg/mL). After 30 min of reaction at room temperature in dark place the absorbance was measured at 517 nm against methanol as blank by a UV-Visible spectrophotometer. Inhibition of free radical DPPH in percent (I %) was calculated as follows:

$$(I\%) = (1-Asample/Ablank)\times 100$$

where, Ablank is the absorbance of the control reaction (containing all reagents except the test material) and Asample

is the absorbance of the sample. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted with inhibition percentage against extractive/standard concentration.

2.8. Statistical Analysis

Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the vehicle group. P values <0.05 were considered to be statistically significant compared with the control.

3. Results

Analgesic activity of the crude methanol extract and its all Kupchan fractions were tested by acetic acid induced writhing model in mice. The ethyl acetate, the chloroform and the petroleum-ether soluble fraction of crude methanolic extract of *S. roxburghiana* demonstrated significant analgesic activity with writhing inhibition of 62.5%, 60% and 56.66% respectively compared to 66.66% exhibited by standard diclofenac sodium (Table 1).

Table 1

Effect of the crude methanolic extract and different fractions of *S. roxburghiana* on acetic acid-induced writhing in mice.

Test samples	Group	Writhing count					Writhings* (mean±SEM)	% of writhing	% of inhibition
		M-1	M-2	M-3	M-4	M-5			
Control	A	23	24	24	25	24	24.00±0.62	100.00	0.00
Standard	B	8	9	8	6	9	8.00±0.84**	33.33	66.66
CM	G	12	14	12	12	17	13.40±1.52**	55.83	44.16
PESF	H	11	12	9	9	11	10.40±0.56*	43.33	56.66
CTSF	I	15	15	14	14	14	14.40±1.78	60.00	40.00
CFSF	J	7	9	10	11	11	9.60±0.50	40.00	60.00
EASF	K	8	8	8	11	10	9.00±0.23**	37.5	62.50

Here, CM: methanolic crude extract of *S. roxburghiana*; PESF: petroleum ether soluble fraction; CTSF: carbon tetrachloride soluble fraction; CFSF: chloroform soluble fraction, EASF: ethyl acetate soluble fraction of methanolic crude extract of *S. roxburghiana*. M-1= Mice 1, M-2 = Mice 2, M-3 = Mice 3, M-4 = Mice 4, M-5 = Mice 5.

Results are presented as mean±SEM, (n=5), *: P<0.05, **: P<0.001 Dunnett's t-test as compared to control.

Brine shrimp lethality bioassay indicates cytotoxicity of extracts. The crude extract along its Kupchan fractions showed significant toxicity against *Artemia salina* (Table 2). Among all the fractions, the aqueous soluble fraction exhibited maximum toxicity towards the shrimp with LC_{50} value of 0.735 μg/mL. In case of antioxidant screening, all the partitionates revealed mild to moderate free radical scavenging activity (Table 2).

Table 2

Cytotoxic and antioxidant activities of test samples of *S. roxburghiana*.

Sample	LC_{50} (μg/mL)	IC_{50} (μg/mL)
VS	0.544	–
BHT	–	71.02
CM	2.01±0.35	554.40±0.34
PESF	2.66±1.33	568.20±1.25
CTSF	3.20±0.98	185.70±0.22
EASF	1.14±0.36	124.50±1.51
AQSF	0.735±1.330	147.20±0.41

The values of LC_{50} and IC_{50} are expressed as mean±SD (n=3); VS: vincristine sulphate (Std.); BHT: butylated hydroxy toluene (Std.); –: not determined.

4. Discussion

Plants are employed as important source of medication in many traditional medications[15]. Analgesic activity of the methanol extracts along with its Kupchan fractions of *S. roxburghiana* Schult. and Schult. f. were tested by acetic acid induced writhing model in mice. Acetic acid induced writhing in mice attributed visceral pain finds much attention of screening analgesic drugs[16]. Pain sensation is elicited by triggering localized inflammatory response resulting the release of free arachidonic acid from tissue phospholipid[17].

The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability[18]. The acetic acid induced writhing method was found effective to evaluate peripherally active analgesics. The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition[19]. The ethyl acetate soluble fraction produced maximum writhing inhibition comparable to the standard drug diclofenac sodium. On the basis of this result it can be concluded that the methanol extract of *S. roxburghiana* possesses analgesic activity.

Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor, *etc* of the compound[13]. The aqueous soluble fraction of crude methanolic extract demonstrated significant activity against the *Brine shrimp* nauplii; LC_{50} was found at 0.735 μg/mL. However, further investigations using carcinoma cell line are necessary to isolate the active compound(s) responsible for the activity.

In case of antioxidant screening, all the partitionates revealed mild to moderate free radical scavenging activity. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule[20]. Most of the polar compounds such as phenolic and flavonoid substances are potent inhibitors of reactive oxygen species attack[21]. The biological properties, including cytotoxic and antioxidant properties, of flavonoids are considered in an evaluation of the medicinal and nutritional values of these compounds.

Conflict of interest statement

We declare that we have no conflict of interest.

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