Biological Activity Studies of the Extracts of *Sansevieria roxburghiana*

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Abstract

The cytotoxic potential of the different solvent extracts of *Sansevieria roxburghiana* were examined by using brine shrimp lethality bioassay. However, n-hexane, ethyl acetate, n-butanol and methanol extract exhibited quite potent activity in brine shrimp lethality bioassay with LC_{50} 4.89, 1.95, 10.69 and 12.51 µg/mL respectively. These results suggested that they might contains antitumar or cytotoxic agents. The ethyl acetate extract showed significant free radical scavenging activity with IC_{50}7.94 µg/mL and demonstrated excellent antibacterial activity.

Keywords: Bioassay, Cytotoxicity, Free radical scavengers, Antibacterial screening, Sansevieria roxburghiana.

Introduction

Bangladesh has a great treasure of medicinal plants. More than 500 plants have been reported to possess medicinal properties. A good number of medicinal plants are used for the treatment of sexual transmitted Infections such as gonorrhea, syphilis, herpes etc and other infectious diseases by traditional practitioners in Bangladesh. Some of them such as *Sansevieria roxburghiana*, *Sansevieria hycenthoides* etc. so far never been chemically investigated in details. Leaves of *Sansevieria roxburghiana* contain saponins[1], aconitic acid reducing sugars and inorganic salt[2]. Roots and Rhizomes contain an inert alkaloid, sansevirine, resin, and starch[3].These species under taken for investigation are to find the presence of ingredients which have cytotoxic, antimicrobial and antioxidant activities with a view to justifying their usage.

General bioassays that are capable of detecting board spectrum of bioactivity present in crude extracts are brine shrimp lethality bioassay (BSLT) and free radical scavenging activity test (FRST). Both techniques are easily mastered, low cost, and needs small amount of test material. BSLT is predictive cytotoxicity and pesticidal activity[4]. This test has been introduced in 1982[5] and employed for bioassay-guide fractionation of

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active cytotoxic and antitumor agents such as trilobacin from the bark of Asimina triloba[6] and cis-annonacin from Annona muricata[7]. FRST is also predictive of antioxidant activity and introduced in 1958[8]and employed for the detection of active free radical scavengers like vitamin C, vitamin E, flavonoids, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk[9]. There are a number of clinical

studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers[10]. *Sansevieria roxburghiana* (Common name–Murba ; Synonyms- Sansevieria,; Family–Agavaceae) is found in Bangladesh, India, Africa, Indonesia etc.[11] . In Bangladesh, *Sansevieria roxburghiana* are wildly distributed in Gazipur, Savar and Tangail. This plant is administered as a cooling medicine; given for the treatment of gonorrhea, purgative, tonic, expectorant and febrifugic etc[12].

Therefore, the present study was undertaken with an objective to evaluate the cytotoxic, antibacterial and antioxidant activities of the solvent extracts of *Sansevieriaroxburghiana.*

Materials and methods

Collection of plant material

Fresh leaves of *Sansevieriaroxburghiana* were collected from Gazipur in October, 2009 and identified by the taxonomist of Bangladesh National Herbarium, Dhaka, where a voucher specimen (No. 31563) has been deposited.

Preparation of the solvent extracts (Cold extraction)

Freshly collected leaves of *Sansevieriaroxburghiana* were dried in an oven at 38^0C and crushed in pieces. The crushed powder(390g) was extracted with methanol for 5 days. The extract was concentrated to gummy mass (45.9 g) using Buchi Rotary Evaporator. The methanol extract (13.8g) was then partitioned by separatory funnel by using n-hexane, then by ethyl acetate and finally by n-butanol. Then these extract were concentrated by using rotary vacuum evaporator to provide n-hexane (5.0 g), ethyl acetate (3.2 g), n-butanol (3.4 g) and water (5.8 g) extract.

General experimental procedure

The UV absorbance was performed with a PerkinElmer Shelton, CT06484USA, Lambda 25 UV/VIS spectrometer. Vacuum rotary evaporator (BUCHI, Rotavapor R-210 Switzerland) was used for evaporating solvents. All solvents were of analytical grade and obtained from commercial sources (Sigma-Aldrich, St. Louis, MO, USA).

Cytotoxicity bioassays

The cytotoxic activity was performed by brine shrimp lethality bioassay method[5]. The test samples for crude MeOH extracts as well as n-hexane (4.0 g), ethyl acetate (2.2 g), n-butanol (2.4 g) and water (3.8 g) extract were dissolved in DMSO and serial dilution were

made as 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781 and 0.3095 µg/mL. Vincristine sulphate (positive control) was dissolved in DMSO and serial dilution were made as 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625, 0.0781 µg/mL. Then each of these test solutions was added to test tubes containing 12 shrimps in simulated brine water (5 mL) and incubated at room temperature for 24 h. After 24 h, the median lethal concentration (LC_{50}) of the test samples was determined by a plot of percentage the shrimps against the logarithm of the sample concentrations (Finney method). Vincristine sulphate (LC_{50}= 0.52) was used as positive control in this assay to compare the cytotoxicity of the test samples. Results are presented in Table-1.

Antibacterial screening

The test samples were dissolved separately in specific volume of chloroform or methanol depending their solubility. The antibacterial screening was then carried out by the disc diffusion method[13,14]. The diluted samples were applied on to sterile blank discs (Oxoid, UK) at a concentration of 100 (g/disc for this test where Streptomycin 10 g/disc, Oxoid, UK) used as a standard. Results are presented in Table-2.

Free radical scavenging activity

The free radical scavenging activity was assayed spectrophotometrically by DPPH method [7]. The DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical has a deep violet color due to its unpaired electron and radical scavenging activity can be followed spectrophotometrically by a loss of absorbance at 525 nm. Sample stock solutions (1 mg/mL) were diluted to final concentrations of 100, 50, 10, 5 and 1 (g/mL in 70% ethanol or DMSO. DPPH ethanol solution (0.2 mM, 0.5 mL) was added to 1 mL of sample solutions of different concentrations, shaken well by vortex and allowed to react at room temperature. The absorbance values were measured after 10 min at 525 nm by UV/Vis spectrophotometer. The free radical scavenging activity of samples was calculated according to the formula:

DPPH radical scavenging activity (%)= [1-(Abs sample-Abs blank)/Abs control] × 100

where Abs sample is the absorbance of the experimental sample, Abs blank is the absorbance of the blank, Abs control is the absorbance of the control.

As a blank, 70% EtOH or DMSO solvent (0.5 mL) and sample solution (1.0 mL) were used. DPPH solution (0.5 mL, 0.2 mM) and 70% EtOH or DMSO solvent (1.0 ml) was used as a negative control. The ascorbic acid (vitamin C) was used as a positive control. Each treatment was replicated thrice. Results are presented in Figure-1.

Results and discussion

The cytotoxic activity of the different solvent extracts were determined by using brine shrimp lethality bioassay. The LC_{50} for vincristine sulphate (positive control), n-hexane, ethyl acetate and n-butanol extract obtained from Finney method were found to be 0.52, 4.89, 1.95, 10.69 and 12.51 µg/mL, respectively (Table 1). In comparison with the positive control (vincristine sulphate), it is mentioned that all the test samples were lethal

to brine shrimp nauplii. However, ethyl acetate extract (LC_{50} 1.95) demonstrated quite potent activity in brine shrimp lethality bioassay. These positive results suggested that they may contain antitumor or pesticidal active compounds.

The antibacterial activity of different solvent extracts were subjected to screening at 100 µg/disc of seven types of bacteria by using disc diffusion method. The moderate to good zone of inhibition exhibited by ethyl acetate (EE) and n-butanol (BE) extract against almost all tested pathogenic microorganisms having the zone of inhibition of 9±1 mm each(Table2).

The free radical scavenging activity of the solvent extracts (n-hexane, ethyl acetate and n-butanol) were assayed by using DPPH method. The IC_{50} for Vit-C (Ascorbic acid) and ethyl acetate extract were found to be 3.74 and 7.94 µg/mL respectively (Fig. 1). In comparison with the positive control (ascorbic acid), it showed significant antioxidant activity exhibited by the crude ethyl acetate extract. These findings suggest that the EtOAc extract may contain flavonoid/phenolic compounds which have the antitumor potentials.

Table 1: *Cytotoxic effects of the solvent extracts of S. roxburghiana on brine shrimp nauplii*

Conc.(C) (g/ml)	Log C	% Mortality				LC_{50}(g/mL)				Vincristine sulphate			
		Sr. He	Sr. EA	Sr. Bu	Sr. Me	Sr. He	Sr. EA	Sr. Bu	Sr. Me	Conc.(C) (g/ml)	Log C	% Mortality	LC_{50} (g/mL)
100	2.602	100	100	100	100	4.89	1.95	10.69	12.51	20	1.3	100	0.52
50	2.301	100	100	100	90					10	1	100	
25	2	90	100	85	67					5	0.698	90	
12.5	1.699	67	90	33	50					2.5	0.397	80	
6.25	1.398	58	80	25	30					1.25	0.096	70	
3.125	1.097	33	58	17	18					0.625	-0.204	60	
1.563	0.796	25	50	8	0					0.3125	-0.488	40	
0.781	-0.107	8	33	0	0					0.1563	-0.806	20	
0.3905	-0.408	0	8	0	0					0.078	-1.107	10	

Sr. He = n- Hexane extract of *S. roxburghiana,* Sr.EA = Ethyl acetate extract of *S. roxburghiana,* Sr. Bu = n-Butanol extract of *S. roxburghiana*, Sr. Me = Methanol extract of *S. roxburghiana*

Table 2: *Antibacterial test results of the solvent extract of Sansevieria roxburghiana (Leaves)*

Material tested	MIC (µg/disc), diameter in mm						
	B.cereus	*B.megaterium*	*B.subtilis*	*S.aureus*	*E.coli*	*S.sonnei*	*S.dysenteriae*
MSr 100µg/disc	12	8	8	10	13	13	7
HSr 100µg/disc	7	8	11	12	7	6	9
ESr 100µg/disc	8	7	13	9	12	NA	8
BSr 100µg/disc	11	NA	7	12	11	10	11
Streptomycin 10µg/disc	23	23	21	23	28	18	27

MSr= MeOH extract, HSr= Hexane extract, ESr= EA extract, BSr= BuOH extract, MIC = Minimum Inhibitory Concentration

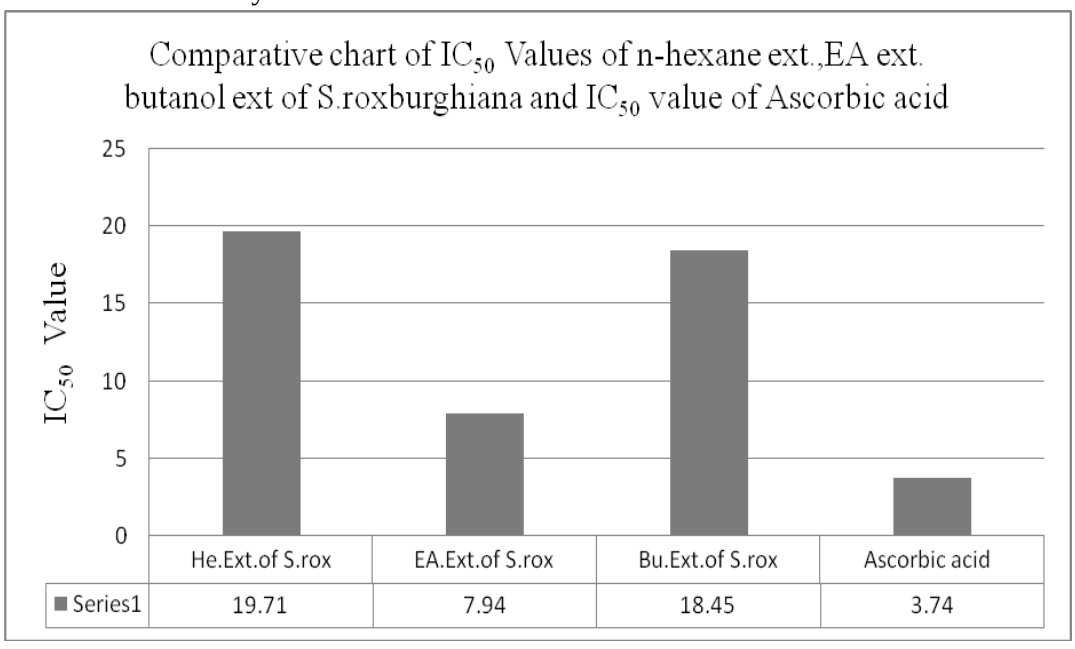

Figure 1 : *Comparative study of different crude extract of Sensevieriahyacinthoides and ascorbic acid.*

Conclusion

It is concluded that the antioxidant, antibacterial and cytotoxicity screening of the different solvent extracts were found to be consistent with the folk uses of *Sansevieria roxburghiana* by local people. In the present study, it can be mentioned that only the ethyl acetate extract of *Sansevieriaroxburghiana* demonstrated excellent cytotoxic, antibacterial and free radical scavenging activity among the extracts. So, the above findings recommended the further investigation of the ethyl acetate part to evaluate active phytoconstituents.

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