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Article

Biological activity studies of *Sensevieria hyacinthoides* extracts

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Abstract: Biological activity was studied of the different solvent extracts such as n-hexane, ethyl acetate, n-butanol, and methanol extracts of the rhizomes of *Sensevieria hyacinthoides.* The cytotoxic potential were examined by using brine shrimp lethality bioassay. Ethyl acetate extract exhibited quite potent activity in brine shrimp lethality bioassay with LC_{50} 1.95 µg/mL, respectively. These result suggested that it might contain antitumor or cytotoxic agent. The ethyl acetate extract showed significant free radical scavenging activity with IC_{50} 10.51 µg/mL and demonstrated excellent antibacterial activity. 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 3.91 and 10.51 µg/mL, respectively.

Keywords: antibacterial screening; bioassay; cytotoxicity; free radical scavengers; *Sensevieria hyacinthoides*

1. Introduction

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. *S. hycenthoides* commonly called as mother-in-law's tongue, devil's tongue, jinn's tongue, bow string hemp, snake plant and snake tongue and some of them such as *S. hycenthoides, S. roxburghiana* etc. so far never been chemically investigated in details. These species under took the investigation to find the presence of ingredients which have cytotoxic, antimicrobial and antioxidant activities 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 (Ghisalberti *et al.*, 1993). This test has been introduced in 1982 (Meyer *et al.*, 1983) and employed for bioassay-guide fractionation of active cytotoxic and antitumor agents such as trilobacin from the bark of *Asimina triloba* (Zhao *et al.*, 1992) and cis-annonacin from *Annona muricata* (Rieser *et al*., 1996). In Bangladesh and South Africa, *S. hyacinthoides* is grown in home gardens as an ornamental, medicinal, and spiritual plant (Ghani, 2003; Zobolo and Mkabela, 2006; Sultana *et al*., 2011; Maroyi and Mosina, 2014; Semenya and Potgieter, 2014; Mosina *et al*., 2015). The leaves and roots of *S. hyacinthoides* are marketed as herbal medicines in the Eastern Cape and KwaZulu-Natal provinces in South Africa (Cunningham, 1993; Dold and Cocks, 2002). In addition, FRST is also predictive of antioxidant activity and introduced in 1958 (Blois, 1958) 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 (Bendich and Langseth, 2001). 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 (Miller *et al.*, 2000). *S. hycenthoides* (Common name–Murba; Synonyms- S.; Family–Agavaceae) is found in Bangladesh, India, Africa, Indonesia etc. (Schaffner, 1930). In Bangladesh, *S. hycenthoides* 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 (Waricky and Hoehne, 1951). *S. hyacinthoides* is a succulent, robust, evergreen, stemless, and perennial herb which can grow up to 60 cm in height (Mwachala and Mbugua, 2007). *S. hyacinthoides* has fleshy creeping rhizomes that are sturdy, fibrous, and bright orange in color. The leaves are erect, rigid, loosely clustered, fibrous, flat, and arising from a horizontal underground rhizome. The leaves are lanceolate or narrowly elliptic in shape, the apex acute or obtuse, the blade leathery and dull green but mottled transversely with numerous more or less obscure pale green bands and the margins with a fine reddish line. The inflorescence is a many-flowered raceme, with stalkless flowers that are white, cream-colored or greenish-white to pale mauve in color. The flowers form small berry-like fruits, which are green at first, gradually becoming yellow when they ripen (Maoyi, 2019).

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

2. Materials and Methods

2.1. Collection of plant material

Fresh rhizomes of *S. hycenthoides* 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.

2.2. Preparation of the solvent extracts (cold extraction)

Freshly collected rhizomes of *S. hycenthoides* 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, USA. The methanol extract (13.8 g) was then partitioned by separatory funnel by using n-hexane, followed by ethyl acetate and n-butanol. These extracts were then 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).

2.3. General experimental procedure

The UV absorbance was performed with a PerkinElmer Shelton, CT 06484 USA, 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).

2.4. Cytotoxicity bioassays

The cytotoxic activity was performed by brine shrimp lethality bioassay method (Meyer *et. al.*, 1983). 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; Finney, 1952). 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.

2.5. 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 (Barry, 1980; Bauer, 1996). 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.

2.6. Free radical scavenging activity

The free radical scavenging activity was assayed spectrophotometrically by DPPH method (Rieser *et al*., 1996). 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).

3. 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.

It needs to add that Tkachenko et al. (2017a) evaluated the antioxidant activities of leaf extracts of *S. hyacinthoides* by assessing their in vitro effects against protein damage in equine erythrocytes using the OMP assay. The extracts reduced the concentration of ketonic derivatives of OMP when compared to untreated erythrocytes by 13.4% (Tkachenko *et al*., 2017a). Similarly, Tkachenko *et al*. (2017b) evaluated the antioxidant activities of leaf extracts of *S. hyacinthoides* by assessing the level of 2-thiobarbituric acid reactive substances (TBARS) as biomarkers of lipid peroxidation in equine erythrocyte suspension induced by treatment of the leaf extracts. The leaf extracts resulted in a significant increase of 29.7% of TBARS concentration in erythrocytes. These results suggest that *S. hyacinthoides* has a promising antioxidant and prooxidant potential.

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 (Table 2).

In the study conducted by Poonam Sethi (2013), the ethanolic extract of rhizome of *Sansevieria roxburghiana* plant displayed remarkable antibacterial activity against the four pathogenic bacteria, *Salmonella typhi*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Maximum activity was seen in the case of *Pseudomonas fluorescens* where the zone inhibition diameter was 32 mm (300 µg/ml). The MIC study revealed that the value for the *Salmonella typhi* and *Escherichia coli* as 80 and 60 µg/ml for *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* (Poonam Sethi, 2013). Hanumanth Kumar and Pramoda Kumari (2015) reported about potential bioactive secondary metabolites and revealed the possible antimicrobial activities of leaf extracts of *Sansevieria roxburghiana*. Antimicrobial screening revealed significant antimicrobial activity against *Proteus vulgaris*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli* (Hanumanth Kumar and Pramoda Kumari, 2015). Furthermore, qualitative analysis, conducted by these authors, confirmed the presence of various primary and secondary plant metabolites such as alkaloids, terpenoids, flavonoids, saponins, steroids, phenols, tannins, and quinine in selected parts of *Sansevieria roxburghiana* (Tkachenko *et al.*, 2017c).

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 3.91 and 10.51 µg/mL respectively (Figure 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. hycenthoides* on brine shrimp nauplii.

Conc. (C) (µg /ml)	Log C	% Mortality				LC_{50} (µg/mL)				Vincristine sulphate			
		Sh. He	Sh. EA	Sh Bu	Sh. Me	Sh. He	Sh. EA	Sh. Bu	Sh. Me	Conc. (C) (µg /ml)	Log C	% Mortality	LC_{50} (µg/mL)
100	2.602	100	100	100	100					20	1.3	100	
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	4.89	1.95	10.69	12.51	0.1563	-0.806	20	0.52
0.3905	-0.408	0	8	0	0					0.078	-1.107	10	

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

Table 2. Antibacterial test results of the solvent extract of *S. hycenthoides.*

Material tested	MIC (µg/disc), diameter in mm						
	B. cereus	***B. megaterium***	***B. subtilis***	***S. aureus***	***E. coli***	***S. sonnei***	***S. dysenteriae***
MSh 100µg/disc	12	8	8	8	12	8	7
HSh 100µg/disc	NA	7	12	12	6	5	NA
ESh 100µg/disc	11	7	13	12	11	NA	8
BSh 100µg/disc	12	NA	7	8	NA	7	NA
Streptomycin 10µg/disc	22	23	17	18	28	18	27

MSh= MeOH extract, HSh= Hexane extract, ESh= EA extract, BSh= BuOH extract,
MIC = Minimum Inhibitory Concentration

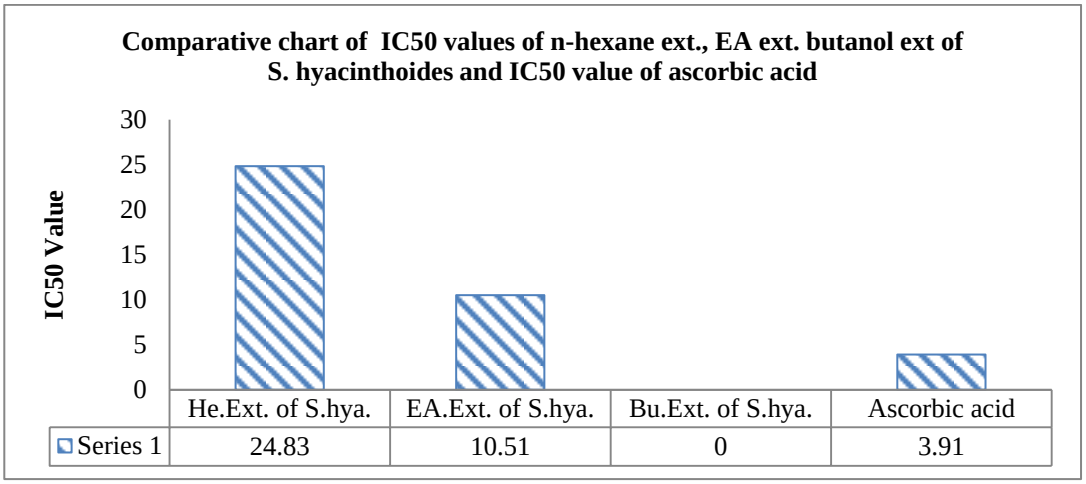

Figure 1. Comparative study of different crude extract of *Sensevieria hyacinthoides* and ascorbic acid.

4. Conclusions

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 *S. hyacinthoides* by local people. In the present study, it can be mentioned that only the ethyl acetate extract of *S. hyacinthoides* demonstrated excellent cytotoxic, antibacterial and free radical scavenging activity among the extracts. So, the above findings are recommended for the further investigation of the ethyl acetate part to evaluate active phytoconstituents.

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Conflict of interest
None to declare.

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