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Research Article

Preliminary phytochemical screening and cytotoxic potentials from leaves of *Sanchezia speciosa* Hook. f

Md. Abu Shuaib Rafshanjani[1], Shumaia Parvin[2*], Md. Abdul Kader[2], Tahmida Sharmin[2]

[1]*Department of Pharmacy, North South University, Plot 15, Block B, Bashundhara R/A, Dhaka-1229, Bangladesh*
[2]*Department of Pharmacy, University of Rajshahi, Rajshahi 6205, Bangladesh*

***Correspondence Info:**
Shumaia Parvin
Assistant Professor,
Department of Pharmacy,
University of Rajshahi, Rajshahi-6205, Bangladesh
Telephone: +88-0721-711110 (off)
Fax: +88-0721-71114
E-mail: lassli.rubd@gmail.com

Abstract

Background: *Sanchezia speciosa* Hook.f is an important medicinal plant with several ethnomedicinal properties. In the present experiment the leaves of this plant were screened for the presence of major phytochemical groups. The phytochemicals are the wide variety of compounds produced by plants manipulated widely in the pharmacognostic drug development and in the treatment of the major ailments.
Methods: Phytochemical screenings were performed by various functional group tests. All the leaves extracts were subjected to brine shrimp lethality bioassay and standard anticancer drug vincristine sulphate was used as positive control.
Results: Phytochemical investigation of the leaves extracts showed the presence of alkaloids, glycosides, flavonoids, triterpenoids, carbohydrates, steroids, phenolic compounds, saponins and tannins. The ethyl acetate fractionates of the leaves extracts were positive for all the phytochemicals tested while n-hexane fractionates were negative for flavonoids and phenolic compounds. The n-hexane and ethyl acetate soluble fractionates showed significant cytotoxic activities with median lethal concentrations (LC_{50}) 19.95 µg/mL and 12.88µg/mL while LC_{50} for the vincristine sulphate was 10.96 µg/mL.
Conclusion: This study shows that the leaves of *Sanchezia speciosa* Hook. f may have potentials in control of carcinogenesis in the field of pharmacology.
Keywords: *Sanchezia speciosa* Hook.f, phytochemicals, *Artemia salina* Leach, brine shrimp lethality test.

1. Introduction

Traditional medicine system is based on different plants for many years around the world and still provides remedies in mankind [1]. Active pharmaceutical compounds can be isolated from these plants which have good medicinal properties[2]. According to World Health Organization (WHO, 1993), from 119 plant derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlate directly with their traditional uses as plant medicine [3].

In Bangladesh, most of the rural people rely on different forms of traditional medicine for their primary health care. Majority of the plants in Bangladesh did not undergone studies to investigate their bioactive compounds[4]. Therefore screening of Ayurvedic medicinal plant has potential in our country. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds[5]. For discovery and development of novel drugs, scientists are looking forward to the alternative sources and in last few decades, medicinal plants have been extensively studied for their bioactive principles to develop new lead molecules for pharmaceutical use.

Brine shrimp assay is very useful tool for the isolation of bioactive compounds from plant extracts[6]. The method is attractive because it is very simple, inexpensive and low toxin amounts are sufficient to perform the test in micro well scale. Brine shrimp *Artemia salina,* also known as sea

monkey, is a marine invertebrate about 1 mm in size[7]. It is used as "benchtop bioassay" for the discovery and purification of bioactive natural products and is an excellent choice for elementary toxicity investigations of consumer products. The shrimp lethality assay is based on the ability to kill laboratory-cultured *Artemia nauplii* (animal's eggs)[8].

Sanchezia speciosa Hook.f (Family: Acanthaceae) is a stout erect shrub commonly known as Fire Fingers in English. *Sanchezia* is named for Jose Sanchez, a nineteenth-century professor of Botany at Cadiz, Spain[9]. The plant is native to Ecuador and Peru. It is cultivated for both its attractive orange flowers and green leaves with yellow veins and is very popular as a hedge, screen or border plant. The species is commonly planted as an ornamental plant andfound in wet and shady areas and in many Pacific islands, Hawaii, Fiji and New Caledonia[10]. Traditionally the most important part used in this plant is the leaves and these are applied externally for wounds.

The present study aims to investigate the presence of various phytoconstituents and cytotoxic potentials of leaves extracts of commonly used plant *Sanchezia speciosa* Hook.f.

2. Materials and methods

2.1. Plant material collection

Fresh, mature leaves of *Sanchezia speciosa* Hook.f were collected during the month of December 2012 from Natore city of Bangladesh and identified by Dr. AHM Mahbubur Rahman, Associate Professor, Department of Botany, University of Rajshahi, Bangladesh.

2.2. Plant materials extraction and fractionation

The fresh leaves were washed, sun dried and ground. The ground leaves(300 gm) were extracted with ethanol (2 litres) at room temperature for 6 days through occasional shaking and stirring[11], [12]. The extract was then concentrated by using a rotary evaporator in vacuum at 40ºC-50ºC. A portion of concentrated ethanol extracts of leaves were fractionated by the modified Kupchan partitioning method [13] in to n-hexane and ethyl acetate.

2.3. Phytochemical screening

The individual n-hexane and ethyl acetate soluble fractionates of ethanolic leaves extracts of *Sanchezia speciosa* Hook. f were subjected to phytochemical studies for the identification and presence of different phytoconstituents (Table 1). Test solution was prepared at 10% (w/v) concentration in distilled water unless otherwise mentioned in individual test. This investigation was carried out by using the standard protocols [14]-[16].

2.3.1. Tests for alkaloids

Dragendorff's test: Alkaloids were given reddish brown precipitate with Dragendorff's reagent (Potassium bismuth iodide solution).

Mayer's test: Alkaloids were given cream colour precipitate with Mayer's reagent (Potassium mercuric iodide solution).

Wagner's test: Alkaloids were given reddish brown precipitate with Wagner's reagent (solution of Iodine in Potassium Iodide).

2.3.2. Test for glycosides

Keller Killiani test: To plant extract, glacial acetic acid containing a trace amount of ferric chloride was added. Then it was transferred to a test tube where concentrated sulphuric acid was added carefully along the side of the test tube. Blue colour in the acetic acid layer indicated the presence of glycosides.

2.3.3. Tests for flavonoids

Alkaline reagent test: To the test solution few drops of sodium hydroxide solution was added, formation of an intense yellow colour which turned to colourless on addition of few drops of dilute acetic acid indicated the presence of flavonoids.

Ferric chloride test: To the test solution few drops of ferric chloride solution was added, intense green colour indicated the presence of flavonoids.

2.3.4. Tests for steroids and triterpenoids

Salkowski's test: Leaves extracts were treated in chloroform with few drops of concentrated sulphuric acid, shaken well and allowed to stand for some times, appearance of red colour in the lower layer indicated the presence of steroids and formation of yellow colour at the lower layer indicated the presence of triterpenoids.

Libermann-Burchard's test: Extract was treated with few drops of acetic anhydride, boiled and cooled, concentrated sulphuric acid was added along the side of test tube, brown ring at the junction of the two layers and the upper layer turned green, showed the presence of steroids and formation of deep red colour indicated the presence of triterpenoids.

2.3.5. Tests for phenolic compounds

Lead acetate test: To the test solution, few drops of 10% lead acetate solution was added, white precipitate indicated the presence of phenolic compounds.

Ferric chloride test: To the test solution, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

2.3.6. Tests for Carbohydrates

Fehling's test: Equal volume of Fehling's A (Copper sulphate in distilled water) and Fehling's B

(Potassium tartarate and Sodium hydroxide in distilled water) reagents were mixed and few drops of sample was added and boiled, a brick red precipitate of cuprous oxide indicated the presence of reducing sugars.

Molisch's test: 1 mL of test solution was treated with few drops of alcoholic α-napthol. Then 0.2 mL of concentrated sulphuric acid was added slowly along the side of the test tube, purple to violet colour ring appeared at the junction, indicated the presence of carbohydrates.

2.3.7. Test for saponins

Frothing test: A pinch of dried powdered leaves was added to 2-3 mL of distilled water. The mixture was shaken vigorously. Formation of foam indicated the presence of saponins.

2.3.8. Tests for tannins

Ferric chloride test: To the test solution few drops of ferric chloride test reagent were added. An intense green, purple, blue or black colour developed was taken as an evidence for the presence of tannins.

Lead acetate test: To the test solution few drops of 10%lead acetate were added. Formation of precipitation indicated the presence of tannins.

2.4. Cytotoxicity screening

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds by Meyer method [17]. Here simple zoological organism (*Artemia salina* Leach) was used as a convenient monitor for the cytotoxicity screening. The eggs (Carolina, Biological Supply Company, Burlington, NC, USA) of the brine shrimp were collected and hatched in artificial seawater (3.8% NaCl solution) for 48 hours to mature shrimp called nauplii. Dissolution of n-hexane and ethyl acetate soluble fractions of leaves extracts were performed in artificial sea water using DMSO. Each 5 ml solution of different concentrations (5, 10, 20, 40, 80 µg/mL) of the two separate solvent fractionates were taken in different vials where brine shrimp nauplii were placed and observed for mortality for 24 hours. Artificial sea water medium containing DMSO was considered as negative control while standard vincristine sulphate was used as positive control. The resulting concentration-mortality data were transformed to Microsoft Excel statistical analysis for the determination of LC_{50} values of the plant extracts[18]-[21].

3. Results

In phytochemical investigation ethyl acetate fractionates from crude ethanol extracts of *Sanchezia speciosa* Hook.f leaves showed the presence of active biomolecules; alkaloids, glycosides, flavonoids, steroids, triterpenoids, phenolic compounds, carbohydrates, saponins and tannins as major phytochemical groups while the n-hexane soluble fractionates revealed the negative results for flavonoids and phenolic compounds (Table 1).

The results of brine shrimp lethality bioassay on n-hexane and ethyl acetate soluble fractionates of crude ethanol extracts from *Sanchezia speciosa* Hook.f leaves (% mortality at different concentrations and LC_{50} values) are depicted in Figure 1 and Table 2. The percentage of mortality increased with an increase in concentration. LC_{50} values of n-hexane and ethyl acetate fractionates were found to be 19.95 µg/mL and 12.88 µg/mL in compared with positive control vincristine sulphate having significant LC_{50} values of 10.96 µg/mL. Thus the ethyl acetate fractionates were more toxic to brine shrimp than the corresponding n-hexane fractions.

Table 1: Phytochemical screening results of the n-hexane and ethyl acetate fractionates of ethanolic leaves extract of *Sanchezia speciosa* Hook.f

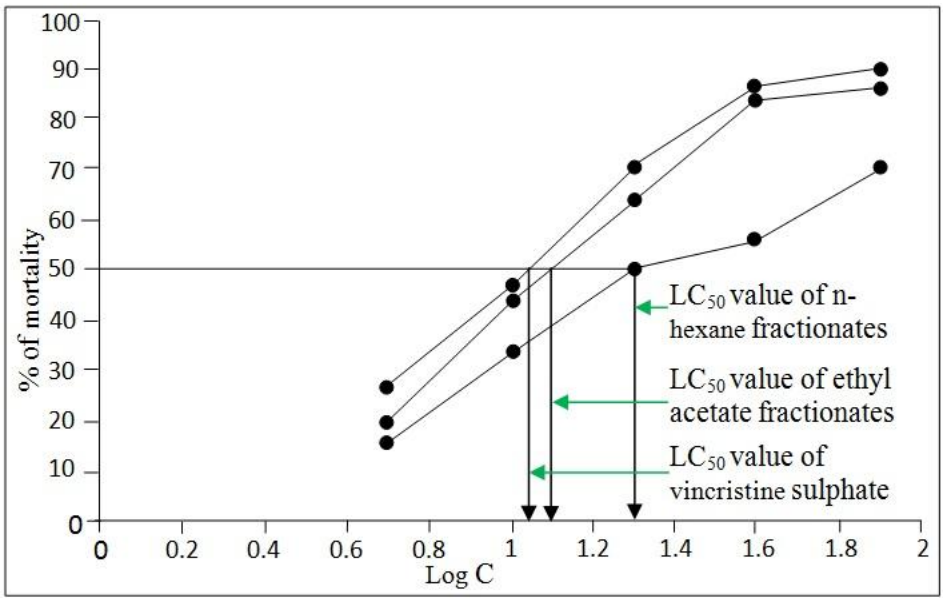
Chemical Groups	Tests	n-hexane fractionates	Ethyl acetate fractionates
Alkaloids	Dragendorff's test	+	+
	Mayer's test	+	+
	Wagner's test	+	+
Glycosides	Keller-Kiliani test	+	+
Flavonoids	Alkaline reagent test	-	+
	Ferric chloride test	-	+
Steroids	Salkowski's test	+	+
	Liebermann Burchard's test	+	+
Triterpenoids	Salkowski's test	+	+
	Liebermann Burchard's test	+	+
Phenolic compounds	Lead acetate test	-	+
	Ferric chloride test	-	+
Carbohydrates	Fehling's test	+	+
	Molish's Test	+	+
Saponins	Frothing test	+	+
Tannins	Ferric chloride test	+	+
	Lead acetate test	+	+

'(+)' indicates present and '(-)' indicates absent

Table 2: Results of brine shrimp lethality bioassay on n-hexane and ethyl acetate soluble fractionates of crude ethanol extracts from *Sanchezia speciosa* Hook.f leaves and for standard vincristine sulphate.

Test samples	Conc. µg/ml	Log of conc.	No. of nauplii taken	No. of nauplii dead			Average No. of nauplii dead	Percent (%) of mortality	LC_{50} µg/ml
				Vial 1	Vial 2	Vial 3			
n-hexane fractionates	5	0.69	10	1	2	2	1.66	16.6	19.95
	10	1.0	10	3	3	4	3.33	33.3	
	20	1.3	10	6	4	5	5.00	50.0	
	40	1.6	10	5	6	6	5.66	56.6	
	80	1.9	10	7	8	6	7.00	70.0	
Ethyl acetate fractionates	5	0.69	10	1	3	2	2.00	20.0	12.88
	10	1.0	10	4	4	5	4.33	43.3	
	20	1.3	10	7	6	6	6.33	63.3	
	40	1.6	10	9	8	8	8.33	83.3	
	80	1.9	10	9	8	9	8.66	86.6	
Vincristine sulphate	5	0.69	10	2	3	3	2.66	26.6	10.96
	10	1.0	10	5	5	4	4.66	46.6	
	20	1.3	10	8	7	6	7.00	70.0	
	40	1.6	10	9	9	8	8.66	86.6	
	80	1.9	10	9	9	9	9.00	90.0	
Control	20 DMSO	00	10	0	0	0	0	0	---

Figure 1: Determination of LC_{50} values for n-hexane and ethyl acetate fractionates from crude ethanol extracts of *Sanchezia speciosa* Hook. f leaves and standard vincristine sulphate from linear correlation between logarithms of concentrations versus percentage of mortalities.



4. Discussion

Phytochemical screening is the first step in herbal medicine research to identify bioactive and novel lead compounds. Plant material consists of many different kinds of natural products with nature of different polarities leading to a different mode of solubility[22]. These secondary metabolites are secreted by the plants for their defense which are being used by peoples for various purposes and also are the key candidates in the medicinal value of plants [16][23].

The variation in results of brine shrimp lethality may be due to the difference in the amount and types of cytotoxic substances (e.g flavonoids, triterpenoids or tannins) present in the crude extracts of this plant leaves [24]. Moreover, McLaughlin *et al.*, 1993 also reported that alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity. Thus this significant lethality of the crude leaves extracts to *Artemia salina* Leach was attributed to the presence of potent cytotoxic compounds which needs further investigation.

Most of the pharmacologically active compounds are obtained from the natural sources. Because of the traditional use, most of them are

safely utilized nowadays. But when we move towards new discoveries from the medicinal plants, toxicity profile of the respective plant must also be checked in order to enhance safety[25]. Pharmacological evaluation of plants provides a good source for the development of novel and safe medicinal plants. The brine shrimp lethality bioassay has been established as a safe, practical and economic method for the determination of the toxicity of synthetic compounds[26] as well as higher plant products[27]. The brine shrimp nauplii has been used for a number of bioassay systems in which natural product extracts, fractions or pure isolates are tested at various concentrations [17]. In the present study, the two derived fractions of plant extracts showed LC_{50} values in safety range following the statement of Peteros and Uy [24] with LC_{50} values> 100 µg/ml.

Literature survey reveals that methanolic extract of *Sanchezia speciosa* Hook.f leaves had antioxidant and anticancer effects [28]. Rafshanjani *et al*., 2014 also reported *in vitro* antibacterial, antifungal and insecticidal activities of ethanolic extract and its fractionates from this plant [29]. To the best of our knowledge, it was our first attempt for phytochemical screening and determination of cytotoxic potentials using brine shrimp lethality test from leaves of *Sanchezia speciosa* Hook.f.

5. Conclusion

The n-hexane and ethyl acetate fractionates of *Sanchezia speciosa* Hook.f leaf can be regarded as a promising candidate for a plant derived antitumor agent in the field of pharmacology. Moreover, phytochemical screening and brine shrimp cytotoxicity assay results may be used for other researchers as a guide on which plant extracts/fractions will get priorities for further fractionation and isolation of these bioactive compounds.

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