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Phytochemical Screening and Antimicrobial Activity of *Sansevieria roxburghiana* Schult. and Schult. F.

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Abstract: *Sansevieria roxburghiana* is an Indian herb used for various ailments by traditional healers. In this study we have carried out phytochemical analysis and antimicrobial investigation of different solvent and aqueous extracts of the leaves and rhizome of *Sansevieria roxburghiana* against a panel of clinically significant bacterial and fungal strains. Phytochemical studies revealed the presence of carbohydrates, saponin, flavonoids, phenols, alkaloid, anthocyanin and β-cyanin, glycosides, proteins and phytosterols. Susceptibility testing by disc diffusion assay revealed significant antimicrobial activity of methanol and acetone extracts of leaves against Gram-positive bacteria such as *Micrococcus luteus*, *Bacillus cereus, Enterococcus* spp., *Staphylococcus aureus*, Gram-negative bacteria such as *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescence, Salmonella typhi*, *Salmonella paratyphi, Klebsiella pneumoniae, Shigella sonnei* and *Escherichia coli,* fungal strains *Cryptococcus* spp.and *Candida albican.* Ethyl acetate extracts of rhizomes also exhibited appreciable antimicrobial activity against most of the pathogens tested. The minimum inhibitory concentrations (MIC) of the various extracts by Agar Dilution method ranged from 1.0 to 8.0mg/ml. The leaf extracts exhibited better antimicrobial activity than rhizomes. The study findings provide supportive evidence for the use of *Sansevieria roxburghiana* in traditional medicines.

Key words: Antibacterial activity · *Sansevieria roxburghiana* · Medicinal plants · Agar Dilution · Alkaloids

INTRODUCTION

Sansevieria roxburghiana belongs to the family Dracanaceae, commonly referred to as bowstring hemp, piles root [1] and Jaang Mattai in Tamil (Vernacular). *Sansevierian*s are popular garden or indoor plants with long rhizomes and fibrous roots having ability to flourish under low light conditions and requiring minimal attention [2]. A number of species such as *Sansevieria cylindrica*, *Sansevieria ehrenbergii*, *Sansevieria guineensis*, *Sansevieria longiflora*, *Sansevieria roxburghiana*, *Sansevieria trifasciata* and *Sansevieria zeylanica* are grown as ornamental plants [3]. The medicinal uses of *Sansevieria* species include treatment for abdominal pains, ear ache, diarrhea and hemorrhoids [4, 5]. Traditionally, in treating earaches and hemorrhoids, the leaves are heated and the warm juice is squeezed onto the affected area. The leaf sap is applied directly to infected sores, cuts and grazes. It is also used to treat fungal and scabies infection [2, 6]. The medicinal properties of *Sansevieria* species is well documented [7-9]. Previous studies on *Sansevieria* species have documented anti-inflammatory activity [10, 11], analgesic property[12], antioxidant and antimicrobial activity [4, 13].

Even though there are various reports on the biological activity of the genus *Sansevieria* there is a paucity of data on antimicrobial properties of *S. roxburghiana*. The present study investigated the antimicrobial activity of leaves and rhizome of *S. roxburghiana* against various clinically significant bacteria and fungi.

MATERIALS AND METHODS

Collection of Plant Samples: Healthy, disease free leaves and rhizomes of *Sansevieria roxburghiana* were collected from the garden of Government Arts and Science College, Nandanam, Chennai, (India).

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The plants were authenticated at the Department of Botany. Washed and air dried fresh leaves and rhizomes were cut into small pieces and pulverized in a domestic blender and used for the preparation of aqueous and solvent extracts.

Test Microorganisms: The antimicrobial activity of *S. roxburghiana* was tested against clinical isolates of *Salmonella paratyphi, Shigella sonnie, Salmonella typhi*, *Bacillus cereus, Staphylococcus aureus*, *Micrococcus luteus, Enterococcus* spp*., Klebsiella pneumoniae, Proteus vulgaris* and *Cryptococcus neoformans, Candida albican* and standard strains of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853. The bacterial strains were maintained on Nutrient Agar (NA) and fungi on Sabouraud Dextrose Agar (SDA) at laboratory division, National Institute of Epidemiology, Chennai, India.

Preparation of Extracts

Solvent Extracts: 10g of pulverized leaf/rhizome material was mixed with 100ml of solvent viz. methanol, acetone and ethyl acetate and kept in rotary shaker at 100 rpm overnight and filtered with Whatman No.1 filter paper and was concentrated to dryness at 40°C. Since the fresh plant material contain water, the extracts were further concentrated to dryness by means of a freeze-dryer to extract any excess water from the sample. It was stored at 4° C until further use.

Aqueous Extracts: Extracts were prepared using the modified method of Olivia Case [2]. 10 g of pulverized leaf/rhizome material was infused in 100 ml of hot (~95 °C) distilled water and left overnight under refrigeration (4 °C). After 24 h, the extracts were kept in rotary shaker at 100 rpm for 1h, filtered with Whatman No.1 filter paper and subsequently lyophilized at-47.5 °C. The frozen extract was then freeze-dried to a powder and stored at 4° C until further use.

Various concentrations of leaf and rhizome extracts were prepared in 5% Dimethyl Sulfoxide (DMSO) for determining antimicrobial activity.

Phytochemical Profiling: The phytochemical screening of the sample was carried out as described by [14, 15]. The samples were screened for the following components

Test for Carbohydrates

Molisch's Test: To 2ml of plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Formation of purple or reddish ring indicates the presence of carbohydrates.

Test for Tannins

Ferric Chloride Test: To 1ml of plant extract, 2ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for Saponins

Foam Test: To 1ml of plant extract, 5-10ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.

Test for Flavonoids

Sulphuric Acid Test: A fraction of the extract was treated with concentrated sulphuric acid and observed for the formation of orange colour.

Test for Alkaloids

Mayer's Test: To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

Test for Anthocyanin and Betacyanin

Sodium Hydroxide Test: To 2ml of plant extract, 1ml of 2N sodium hydroxide was added and heated for 5minutes at 100°C. Formation of bluish green color indicates the presence of anthocyanin and formation of yellow color indicates the presence of betacyanin.

Test for Glycosides

Sulphuric Acid Test: To 2ml of plant extract, 1ml of glacial acetic acid and 5%ferric chloride was added. Then few drops of concentrated sulphuric acid were added. Presence of greenish blue color indicates the presence of glycosides.

Test for Proteins and Aminoacid

Ninhydrin Test: To 2ml of plant extract, few drops of 0.2% Ninhydrin was added and heated for 5 minutes. Formation of blue colour indicates the presence of proteins.

Test for Steroids and Phytosterols

Sulphuric Acid Test: To 1ml of plant extract, equal volume of chloroform and few drops of concentrated sulphuric acid were added. Formation of brown ring indicates the presence of steroids and formation of bluish green color indicates the presence of phytosterols.

Test for Phenols

Ferric Chloride Test: To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green color indicates presence of phenols.

Testing for Antimicrobial Activity: Antibacterial activity of the leaf / rhizome extracts were determined using a modified Kirby-Bauer [16] disc diffusion method. All the bacterial test strains maintained on NA were freshly subcultured for 24-48hrs at 37 °C. Saline suspension of each test strain was prepared and turbidity matched to 0.5 McFarland standard to yield a bacterial suspension of 1.5×10^8 cfu/ml. Freshly prepared Mueller Hinton Agar MHA plates were seeded with the test inoculums to obtain a lawn culture. Sterile Whatmann No. 1 filter paper discs (~5mm diameter) impregnated with different concentrations of plant extracts (50, 100, 200 µg/disc) were placed on the inoculated MHA plates. 5% DMSO served as negative control, Norfloxacin (10µg) and Tetracycline (30µg) were used as standard antibiotics. Post incubation at 37°C for 24-48h, plates were read for zone of inhibition around the disc.

Anti-fungal susceptibility testing was carried out as described for antibacterial testing with SDA as the assay medium and Amphotericin B (100U/D) as standard antifungal agent.

Minimum Inhibitory Concentration (MIC): MIC determination for bacterial and fungal strains was done using agar dilution method [17]. Varying concentrations of each extract (0.125 to 16mg/ml) were prepared in 5% DMSO and filter sterilized (0.45µm). The extract was mixed with autoclaved and cooled MHA medium and dispensed into sterile petriplates. 5 µl of standardized inocula (matching 0.5 McFarland turbidity standards) of various test isolates was seeded on incubated MHA plates. Test strains on solvent free MHA and DMSO incorporated MHA served as growth and solvent controls respectively. MICs were read after 24-48h incubation at 37 °C.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of the leaf and rhizome extracts of *Sansevieria roxburghiana* (Table 1) revealed the presence of various chemical compounds such as alkaloids, saponins, flavonoids, phenols, glycosides, proteins, Anthocyanin, betacyanin, phytosterol, steroids and carbohydrates, some of which have been previously associated with antibacterial activity [14]. Since there are no reports available exclusively on *S. roxburghiana,* the phytochemical content of *S. roxburghiana* in the present study was comparable with the available literature on phytochemical content of related species such as *Sansevieria trifasciata* and *Sansevieria liberica* [12, 18, 19] respectively.

Antimicrobial activity of methanol, acetone, aqueous and ethyl acetate extracts of leaf and rhizome of *Sansevieria roxburghiana* were analysed against fourteen clinically significant organisms using disc diffusion method (Table 2). MIC values were determined for methanol, acetone and ethyl acetate leaf extracts (Table 4). All four extracts tested showed varying degree of antibacterial and antifungal activities at a concentration of 100 µg/disc which were compared with negative control 5% DMSO that showed no activity with any of the extracts (Fig. 1). The activity of methanol and acetone extracts of leaves (inhibition zone 9-18 mm) were found to be more pronounced than the ethyl acetate extract (inhibition zone 8-14 mm) against all the organisms tested.

Table 1: Phytochemical analysis of leaf and rhizome extracts of *Sansevieria roxburghiana*

		Acetone Extract		Ethylacetete Extract		Methanol Extract		Aqueous Extract	
S.No.	Secondary Metabolite	L	R	L	R	L	R	L	R
1	Carbohydrates	_	_	++	++	++	++	_	_
2	Saponin	_	_	++	+++	_	_	_	_
3	Tannins	_	_	_	_	_	_	_	_
4	Flavonoids	_	_	_	_	++	_	_	_
5	Alkaloids	+++	_	+	_	+++	_	_	_
6	Anthocyanide and Betacyanide	_	++	_	_	_	_	_	_
7	Glycosides	+	++	++	++	++	++	_	_
8	Protein	+++	+	_	_	++	++	_	_
9	Phytosterol and steroids	++	_	++	-	+++	_	_	_
10	Phenols	+	_	_	_	+	_	_	_

L-leaf extract R-rhizome extract, +++-Strongly positive, ++-positive, + Trace,-Not detected.

Table 2: Antimicrobial Activity of leaf extracts of *Sansevieria roxburghiana* by Disc Diffusion Method (Zone of Inhibition in mm at 100 µg/disc)

S.No	Microorganism	Acetone	Aqueous	Ethyl acetate	Methanol	Nx (10µg)	T(30µg)
1.	*Staphylococcus aureus*	12	0	9	10	23	22
2.	*Bacillus cereus*	11	0	8	12	23	23
3.	*Micrococcus luteus*	17	6	15	18	21	30
4.	*Enterococcus faecalis*	12	0	9	13	30	32
5.	*Escherichia coli*	11	0	12	13	35	33
6.	*Pseudomonas aeruginosa*	10	0	10	12	22	15
7.	*Pseudomonas fluorescence*	15	0	0	13	N.D.	21
8.	*Klebsiella pneumonia*	12	0	12	15	24	11
9.	*Salmonella typhi*	13	9	0	12	33	20
10.	*Salmonella paratyphi*	13	7	12	13	9	15
11.	*Proteus vulgaris*	13	0	15	14	R	20
12.	*Shigella sonnie*	10	0	0	13	18	N.D.
ApB (100U/D)							
13.	*Candida albicans*	12	0	11	14	14	-
14.	*Cryptococcuss neoformans*	11	0	8	10	17	-

N.D Not Done, R Resistant,. Nx-Norfloxacin, T-Tetracycline, ApB-Amphotericin B. Values are the mean of three replicates

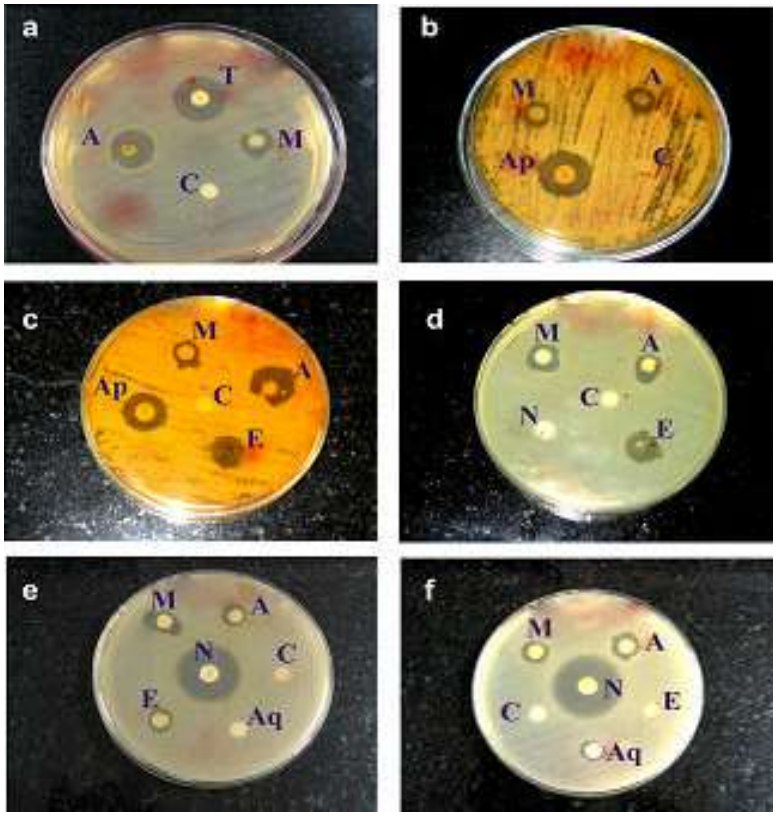

Fig. 1: Antimicrobial activity of leaf extracts of *Sansevieria roxburghiana* by disc diffusion method
a) *Pseudomonas fluorescence,* b) *Cryptococcus neoformans,* c) *Candida albican,* d) *Proteus vulgaris* e) *Pseudomonas aeruginosa,* f) *Salmonella typhi.*
M-Methanol Extract, A-Acetone Extract, Aq-Aqueous Extract, E- Ethyl acetate Extract, C-Control, N-Norfloxacin (10 µg), T-Tetracycline (30 µg), Ap-Amphotericin.

In comparison the aqueous extract showed less pronounced antimicrobial activity (Fig 1e, f). The poor activity of the aqueous extract against most bacterial strains investigated in this study is in agreement with previous reports [20, 21]. This could be due to the insolubility of the active compounds in water or the hot water could have caused denaturation of the active compounds. It is also observed from the results that

Table 3: Antimicrobial activity of rhizome extracts of *Sansevieria roxburghiana* by disc diffusion method (zone of inhibition in mm at 100 µg/disc)

S.No	Microorganism	Acetone	Aqueous	Ethyl acetate	Methanol	Nx (10µg)	T (30µg)
1.	*Staphylococcus aureus*	0	0	12	0	23	22
2.	*Bacillus cereus*	0	0	11	8	23	23
3.	*Micrococcus luteus*	12	9	14	14	21	30
4.	*Enterococcus* spp	0	0	11	11	30	32
5.	*Escherichia coli*	0	0	11	0	35	33
6.	*Pseudomonas aeruginosa*	6	0	9	7	22	15
7.	*Pseudomonas fluorescence*	0	0	0	0	N.D.	21
8.	*Klebsiella pneumonia*	0	0	12	0	24	11
9.	*Salmonella typhi*	0	0	15	0	33	20
10.	*Salmonella paratyphi*	0	0	10	0	9	15
11.	*Proteus vulgaris*	11	0	11	0	R	20
12.	*Shigella sonnie*	0	0	0	0	18	N.D.
ApB (100U/D)							
13.	*Candida albicans*	12	0	14	12	14	-
14.	*Cryptococcuss neoformans*	0	0	8	0	17	-

N.D Not Done, R Resistant,. Nx-Norfloxacin, T-Tetracycline, ApB-Amphotericin B.

Values are the mean of three replicates

Table 4: Minimum Inhibitory Concentration (MIC) of leaf extract of *S. roxburghiana*

S.No.	Strains	Methanol (mg/ml)	Acetone (mg/ml)	Ethyl acetate (mg/ml)
1.	*Staphylococcus aureus*	8.0	8.0	>16.0
2.	*Escherichia coli*	8.0	8.0	16.0
3.	*Pseudomonas aeruginosa*	2.0	4.0	>16.0
4.	*Micrococcus luteus*	1.0	1.0	2.0
5.	*Bacillus cereus*	4.0	2.0	4.0
6.	*Shigella sonnei.*	4.0	>16.0	>16.0
7.	*Enterococcus* spp.	4.0	2.0	8.0
8.	*Salmonella typhi*	2.0	4.0	4.0
9.	*Proteus vulgaris*	1.0	1.0	>16.0
10.	*Klebsiella pneumoniae*	4.0	4.0	>16.0
11.	*Salmonella paratyphi*	4.0	4.0	>16.0
12.	*Candida albicans*	>16.0	>16.0	>16.0
13.	*Cryptococcuss* spp	>16.0	>16.0	>16.0

Values are the mean of three replicates

the methanol and acetone extract of leaf had wide antibacterial activity (Table 2) against both gram positive and gram negative bacteria as well as fungal strains (Table 4). The activity of the extracts against the Gram negative bacteria is noteworthy as these bacteria are known to exhibit high degree of resistance to conventional antibiotics [4].

Growth of *P.aeruginosa* and *S.typhi* was inhibited at a MIC value of 2mg/ml followed by *B.cereus, Enterococcus* spp*., K. pneumonia, S. paratyphi* and *Shigella sonnie*. *S. aureus* and *E. coli* showed highest MIC value of 8mg/ml. Antifungal activities against *Cryptococcus neoformans* and *Candida albican* was shown in disc diffusion testing (9-14mm) but MIC values were found to be >16mg/ml.

Acetone, ethyl acetate and methanol extracts of rhizomes also showed appreciable antimicrobial activity against some of the strains tested (Table 3). Ethyl acetate extracts of rhizome showed better antimicrobial activity compared to other solvent extracts. The results of this study reflect the potent antimicrobial phytochemicals present in solventextracts of the leaves and rhizomes of plant [22, 23]. The ability of the extracts to inhibit the growth of several bacterial and fungal species is an indication of the broad spectrum antimicrobial potential of *S. roxburghiana* which makes the plant a candidate for bio-prospecting for antimicrobial drugs. The antimicrobial activities of plant extracts could be attributed to the presence of different bioactive compounds [20]. The wider zones of inhibition of leaf extracts of *S. roxburghiana*

(Table 2) exhibit a better antimicrobial activity compared to rhizomes at the tested concentration.

The few variations in results between the disc diffusion and MIC results can be due to the different susceptibility of the bacterium to the plant extract, the rate of growth of bacteria, solvents used to extract the plant compounds and the rate of plant extract diffusion [24].

To the best of our knowledge this is the first time the antimicrobial activity of both leaves and rhizomes of *Sansevieria roxburghiana* against various pathogens are reported. Finding of this present study constitute supportive evidence to validate folkloric use of this plant as a remedy for various infections. Further investigations are required to isolate the active constituents responsible for the observed antimicrobial activity.

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