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Screening and Characterization of Antimicrobial Agents from *Sanseveria roxburghiana* and *Sanseveria trifasiata*

Danie Kingsley, Ritika Chauhan, Pamela Sinha and Jayanthi Abraham
Microbial Biotechnology Laboratory, School of Biosciences and Technology,
VIT University, 632014, Vellore, Tamil Nadu, India

Abstract: Present study was aimed to screen the effect of phytochemical compounds against various medically important pathogens from *Sansevieria roxburghiana* and *Sansevieria trifasiata* majorly known as Indian herb. *Sansevieria*s are the most popular ornamental plants with long rhizomes and fibrous roots possessing traditional healing properties. The acetone and methanol extraction was performed by soxhlet apparatus for the leaves of *S. roxburghiana* and *S. trifasiata* against bacterial strains. The obtained extracts were further analyzed for antimicrobial effect against Gram Positive as well as Gram negative bacteria and combined effect of extract and antibiotics was also studied. The phytochemical compounds were analyzed through thin layer chromatography and were screened for isolation of bioactive compound.

Key words: Antibacterial, antibiotics, synergistic effect, soxhlet apparatus

INTRODUCTION

Different approaches for the discovery of new therapeutics and natural products from plant sources remain the reservoir for the new structural types. The plant *Sansevieria* and number of species such as *Sansevieria cylindrica*, *Sansevieria ehrenbergii*, *Sansevieria guineensis*, *Sansevieria longiflora*, *Sansevieria roxburghiana*, *Sansevieria trifasciata*, *Sansevieria zeylanica* are mostly grown as ornamental plants. *Sansevieria* plant and their spp are well known to produce commercial fiber products which are used in making the bowstrings, fish nets, baskets, coarse fabrics (Joyner and Gangstad, 1951). The plant possesses medicinal properties and plays an important role in the discovery of novel drugs used as medicine. *S. roxburghiana* is an herbaceous plant with short fleshy stem and has been traditionally used as a cardiotonic, expectorant, febrifuge, purgative, tonic in glandular enlargement and rheumatism (Dhiman and Kumar, 2006; Pulliah, 2006; Khare, 2007). The two species of *Sansevieria* plant *S. roxburghiana* and *S. trifasiata* are widely distributed in ornamental coast, root and leaves of plants are pharmacologically used by the tribal as medicine. *S. roxburghiana* root is one of the plant having medicinal properties which is cultivated and exclusively used in China and India. The advantage of herbal medicine derived from plants is normally known free from side effects, toxicity and are economically important. The medicinal properties of *Sansevieria* species include treatment for abdominal pains, ear ache, diarrhea and haemorrhoids (Aliero *et al*., 2008; Van Wyk *et al*., 1997). In previous years, the leaves of *Sanseveria* were heated and the warm juice was squeezed onto the affected area to treat ear aches, haemorrhoids, infected sores, cuts and grazes. *S. roxburghiana* and *S. trifasiata* roots contain various chemical compounds such as amino acids, vitamins, carbohydrate, minerals, alkaloids, carotenoids, flavonoids phytates, saponins and tannins (Ikewuchi *et al*., 2010). The isolation of pure, pharmacologically active constituents from plants remains a long and tedious process.

In the present investigation *S. roxburghiana* and *S. trifasiata* plants were used to screen pharmacologically important phytochemical constituents against Gram positive and Gram negative bacterial strains.

MATERIALS AND METHODS

Sample collection: The healthy, disease free leaves of *S. roxburghiana* and *S. trifasiata* were collected from VIT University, Vellore, Tamil Nadu, India. The leaves were washed with the water and air dried fresh leaves were subjected to methanol and acetone extract preparation.

Corresponding Author: Jayanthi Abraham, Hexagon-109A, Laboratory of Microbial Biotechnology, School of Biosciences and Technology, VIT University, 632014, Vellore, Tamil Nadu, India Tel: +91-9843580709 Fax: 0416-2243092

Preparation of extracts: Finely ground powder of *S. roxburghiana* and *S. trifasiata* was extracted using acetone and methanol in a Soxhlet extractor not exceeding the boiling point of the solvent. The extracts were filtered through Whattman Filter paper No. 1. and then concentrated under reduced atmospheric pressure. The dry extracts were stored at -20°C. The extracts were further dissolved in 5% dimethyl sulphoxide (DMSO) for further experimental assays.

Thin layer chromatography: TLC was used for analytical separations of phytochemical constituents present in the extracts. The different ratio of solvents was used chloroform: Methanol (19:1) and Ethyl acetate: Acetone (9.5:0.5). The samples were run and were visualized using iodine and exposing it to UV light. The R_f value was calculated by taking a average of both the plants leaves R_f Values. Then the major bands were scrapped out and dissolved in methanol, separated the supernatant and allowed it to drying as fragment (Karlsson, 1978; Wagner and Bladt, 1996).

Antimicrobial assay: The sensitivity of microorganisms to extracts of *S. roxburghiana* and *S. trifasiata* were tested by measuring the zone of inhibition of given concentration of the extract by the well-diffusion method. Clinical bacterial isolates were swabbed onto Muller-Hinton agar plates. Four wells were punctured onto the agar plate. The 50 mg mL^{-1} of dry extract with 100 µL of concentration were loaded into the wells. The petriplates were incubated for 24 h and the zone of inhibition was measured around the wells (Bauer *et al*., 1966).

Synergistic effect: The synergistic effect was determined with four bacterial antibiotics norflaxcin, tetracycline, erythromycin and chloramphenicol combined with methanol and acetone extract of *S. roxburghiana* and *S. trifasciata* against gram positive, gram negative bacteria.

The antibiotic discs were impregnated with obtained plant extracts and disc-diffusion method was used to measure the zone of inhibition on Muller Hinton agar. The inhibition rate of synergistic effect was determined in percentage (Fayaz *et al*., 2010):

$$\text{Synergistic effect (\%)} = \frac{\text{Zone of inhibition of antibiotics and plant extract}}{\text{Zone of inhibition of antibiotics only}} \times 100$$

RESULTS AND DISCUSSION

The methanol extract from the leaves *S. roxburghiana* and *S. trifasiata* showed good inhibition against all the pathogens. *S. roxburghiana* exhibited good inhibition effect against *Staphylococcus aureus* and *Pseudomonas aeruginosa* whereas *S. trifasiata* manifested good antimicrobial effect against *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* as shown in Table 1. The combined effect of antibiotics and plant extract has enhanced the antimicrobial effect of the extracts obtained against pathogenic microorganisms. The percentage inhibition of combined effect was calculated and it was observed that the leaves of *S. roxburghiana* possess antimicrobial effect (50%) against *Staphylococcus aureus* combined with norflaxcin whereas the leaf extract of *S. trifasiata* when combined with tetracycline it showed 36% of inhibition against *Staphylococcus aureus*. The synergistic effect of *S. roxburghiana* and *S. trifasiata* has been represented in Table 2 and 3, respectively. Both of the plant extracts were effective against Gram positive and Gram negative pathogenic microorganisms which is in good agreement with previous antibacterial studies conducted previously (Philip *et al*., 2011).

The 50 mg mL^{-1} of the methanol extracts manifested effective antimicrobial effect against pathogens. The components present in the plant extracts are responsible for the inhibition effects as all the antimicrobial and phytochemical agents are being carried by nature of solvent used. The leaves of *Sansevieria* can be directly used for cuts, grazes, infected sores, fungal infections and to study the were partially purified by preparative thin-layer chromatography and the major spot were observed on silica coated thin layer chromatographic plates with various solvent systems. The R_f values were calculated and recorded for the developed spots. Figure 1 shows the developed spots of leaves of *S. roxburghiana* with Chloroform: Methanol (60:40). The compounds separated through thin layer chromatography of *S. roxburghiana* and *S. trifasiata* with 50 mg mL^{-1} concentration exhibited good antimicrobial effect against pathogenic microorganisms.

Table 1: Antimicrobial activity of methanol extracts from *S.roxburghiana* and *S. trifasiata* by disc- diffusion method

	Diameter zone of inhibition (mm)	
Test organisms	*S. roxburghiana*	*S. trifasiata*
Escherichia coli	11	12
Pseudomonas aeruginosa	15	12
Staphylococcus aureus	17	15
Bacillus cereus	5	NA

NA: No activity

Table 2: Diameter zone of inhibition of antibiotics combined with *S. roxburghiana* methanol extract against pathogens

		Diameter zone of inhibition (mm)		
Clinical pathogens	Antibiotic used	Antibiotic disc (a)	Methanol extract (b)	Fold increase percentage [(b-a)/a]*100 (%)
Escherichia coli	Norflaxcin	14	16	14.28
	Tetracycline	15	17	13.3
	Erythromycin	5	6	20
	Chloramphenicol	5	6	20
Pseudomonas aeruginosa	Norflaxcin	20	22	10
	Tetracycline	15	18	20
	Erythromycin	7	7	0
	Chloramphenicol	R	NA	NA
Staphylococcus aureus	Norflaxcin	20	30	50
	Tetracycline	15	17	13
	Erythromycin	6	8	33
	Chloramphenicol	10	12	16
Bacillus cereus	Norflaxcin	8	10	25
	Tetracycline	8	11	37.5
	Erythromycin	9	10	22.2
	Chloramphenicol	R	NA	0
Klebsiella pneumoniae	Norflaxcin	25	28	12
	Tetracycline	27	29	7.40
	Erythromycin	10	12	20
	Chloramphenicol	26	28	7.69

a: Antibiotic disc, b: Antibiotic disc impregnated with plant extract

Table 3: Diameter zone of inhibition of antibiotics combined with *S. trifasiata* methanol extract against pathogens

		Diameter zone of inhibition (mm)		
Clinical pathogens	Antibiotic used	Antibiotic disc (a)	Methanol extract (b)	Fold increase percentage [(b-a)/a]*100 (%)
Escherichia coli	Norflaxcin	11	13	18.1
	Tetracycline	12	14	16.6
	Erythromycin	7	9	28.5
	Chloramphenicol	5	6	20
Pseudomonas aeruginosa	Norflaxcin	22	23	4.5
	Tetracycline	10	13	30
	Erythromycin	7	7	0
	Chloramphenicol	NA	NA	NA
Staphylococcus aureus	Norflaxcin	21	25	19
	Tetracycline	11	15	36
	Erythromycin	6	8	33
	Chloramphenicol	7	9	28
Bacillus cereus	Norflaxcin	12	14	16.6
	Tetracycline	6	8	33.3
	Erythromycin	5	6	20
	Chloramphenicol	NA	NA	0
Klebsiella pneumoniae	Norflaxcin	25	27	8
	Tetracycline	21	23	9
	Erythromycin	20	26	30
	Chloramphenicol	27	30	11

a: Antibiotic disc, b: Antibiotic disc impregnated with plant extract

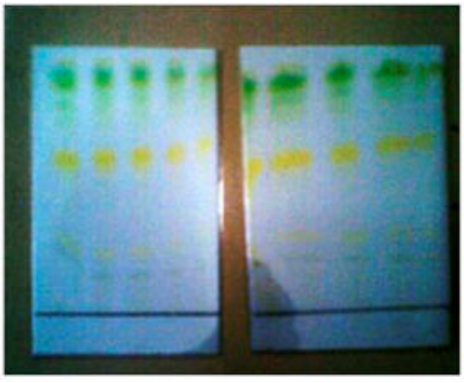

Fig. 1: Thin layer chromatography of methanol extract obtained from the leaves of *S. roxburghiana*

CONCLUSION

The present study on *Sansevieria roxburghiana* and *Sansevieria trifasciata* has revealed the presence of important compounds which were separated by thin layer chromatography. The extract obtained by leaves showed potent antimicrobial activity. It can be assume that the presence of the plant extracts could be used for the treatment of various infections because of its effective zone of inhibition. The result lends credence to the folkloric use of these plants in treating microbial infection and shows that *Sansevieria roxburghiana* and *Sansevieria trifasciata* could be exploited for new potent antimicrobial agents.

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