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Research Article

GC-MS ANALYSIS AND ANTIBACTERIAL ACTIVITY OF CHROMATOGRAPHICALLY SEPARATED PURE FRACTIONS OF LEAVES OF *SANSEVIERIA ROXBURGHIANA*

DEEPA PHILIP[1], KALEENA P.K[2*], K. VALIVITTAN[1]

[1]Department of Biotechnology, St. Peter's University, Avadi, Chennai 600054, India, [2] Department of Zoology, Presidency College, Chennai 600005, India. *Email: stptrsbiotech@gmail.com

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ABSTRACT

The aim of the study was to investigate the antibacterial activity of chromatographically separated pure fractions of leaves of *Sansevieria roxburghiana* and to screen the phytochemical compounds by GC-MS method. Two fractions out of the three showed pronounced activity at 1mg/ml against gram positive and gram negative bacterias responsible for various infections. In the GC-MS analysis, 16 bioactive phytochemical compounds were identified in the methanolic extract of *S. roxburghiana.*

Keywords: TLC, Glycosides, *S. Roxburghiana,* Disc diffusion, 1, 2-Benzenedicarboxylic Acid.

INTRODUCTION

Plants are valuable source of new natural products. Despite the availability of different approaches for the discovery of therapeuticals, natural products still remain as one of the best reservoirs of new structural types. Farnsworth[1] claims that 119 characterized drugs are still obtained commercially from higher plants and that 74% were found from ethnobotanical information. When one considers that a single plant may contain up to thousands of constituents, the possibilities of making new discoveries become evident. The crucial factor for the ultimate success of an investigation into bioactive plant constituents is thus the selection of plant material[2]. Isolation of pure, pharmacologically active constituents from plants remains a long and tedious process. For this reason, it is necessary to have methods to estimate the presence of phytochemical compounds which should eliminate unnecessary separation procedures. Chromatography techniques are the most useful and popular tools used for this purpose. Chromatography is an analytical technique dealing with the separation of closely related compounds from a mixture[3].

Thin-layer chromatography (TLC) is the simplest and cheapest method of detecting plant constituents because the method is easy to run, reproducible and requires little equipment[4]. *Sansevieria roxburghiana* is a medicinal herb belongs to the family Dracanaceae, with lots of medicinal properties[5,6,7]. No phytochemical investigation has yet been carried out on *S. roxburghiana.* However, previous phytochemical studies on some other *Sansevieria* species revealed the presence of steroidal glycosides and saponins some of which were found to possess antimicrobial, cytotoxic, cytostatic activities[8,9,10]. Here an effort is done to evaluate the antibacterial activity of pure fractions separated from TLC of leaves of *S. roxburghiana* and to analyse the phytochemicals present through GC-MS.

MATERIALS AND METHODS

Plant material

Botanically identified and authenticated leaf materials of *Sansevieria roxburghiana* were procured from the premises of Government Arts and Science College, Nandanam, Chennai, (India). Washed and air dried fresh leaves were subjected to methanol and acetone extract preparation.

The experiment was carried out in the Laboratory division of National Institute of Epidemiology,Chennai, India and Sophisticated analytical instrumentation facility (SAIF), IIT, Chennai, India.

Solvent extracts

10g of pulverized leaf material was mixed with 100ml of solvent methanol and acetone and kept in rotary shaker at 100 rpm overnight and filtered with Whatman No.1 filter paper and concentrated to dryness at 40º C, lyophilized and stored at 4º C until further use.

Test Microorganisms

The antibacterial activity of TLC separated fractions of leaves of *S. roxburghiana* were tested against clinical isolates of gram positive *Staphylococcus aureus*, *Enterococcus* spp., and gram negative bacterias *Klebsiella pneumoniae, Proteus vulgaris, Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853. The bacterial strains were maintained on Nutrient Agar (NA) at laboratory division, National Institute of Epidemiology, Chennai, India.

Thin Layer Chromatography

The methanol and acetone Extracts of leaves of *S. roxburghiana* were subjected to Thin Layer Chromatography.

TLC standardization

Thin-layer chromatography (TLC) of leaf extracts were performed on precoated 5cmx 20 cm TLC plates coated with 0.25 mm layers of silica gel 60. After drying, the extracts were applied to the TLC plate with a 1µL microfuge tubes. The plate was then developed in a glass chambers (Desaga, Germany). Two chromatography solvents were used, chloroform: methanol (19:1) and Ethyl acetate: acetone (9.5:0.5). After running for sufficient time, visualization of the bands was achieved by spraying the plate with iodine and exposing it to UV light[3, 11]. The Rf value was calculated.

Preparative TLC

Preparative TLC was used for analytical separations of larger quantities of materials. TLC plates were prepared using Silica gel in 1:3 ratio with DD water. Applicator was spread over the clean glass plate in the thickness of 0.1 mm, dried it and loaded the sample above the 2-3 cm bottom of the plate. Plate was run in solvent jar upto 3 cm below the top of the plate. After removing it was visualized in UV chamber for fluorescent compound, pancal-D for sterol and fatty acids and Iodine spraying for all the major bands observation. The major bands were scrapped out and dissolved in methanol, separated the supernatant and allowed it for drying as F_1, F_2 and F_3[11,12].

Antibacterial Activity

Antibacterial activity of TLC separated pure fractions of leaves of *S. roxburghiana* were conducted by a disc diffusion method using Kirby-Bauer technique[13]. Dried fractions F_1, F_2 and F_3 were dissolved in 5% DMSO so as to get a concentration of 1 mg/ml. 5µl of this were applied on the disc and allowed to diffuse for half an hour at 4ºC and incubated at 37ºC for 24hrs. 5% DMSO served as negative control (C).The plates were observed for the presence of inhibition of bacterial growth that was indicated by the clear zone around the disc.

GC-MS Analysis

Since the methanol and acetone fractions obtained from TLC showed almost similar Rf values, methanol fraction was subjected to GC-MS analysis. Active fractions were dissolved in HPLC grade methanol and subjected to GC and MS JEOL GC mate equipped with secondary electron multiplier. JEOL GCMATE II GC-MS (Agilent Technologies 6890N Network GC system for gas chromatography). The column (HP5) was fused silica 50 m x 0.25 mm I.D. Analysis conditions were 20 min. at 100°C, 3 min at 235°C for column temperature, 240°C for injector temperature, helium was the carrier gas and split ratio was 5:4. The sample (1 µl) was evaporated in a split less injector at 300°C. Run time was 22 min. The components were identified by gas chromatography coupled with mass spectrometry.

Identification of components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08 and Wiley08 library. The name, molecular weight and structure of the components of the test materials were ascertained.

Statistical analysis

Datas were expressed as mean±standard deviation. The data obtained were subjected to ANOVA test to determine whether there was significant difference between extract used and also between the lengths of incubation.

RESULTS AND DISCUSSION

TLC was used to separate the methanol and acetone extracts of leaves of *S. roxburghiana.* 3 prominent spots were shown for methanol and acetone extracts on TLC. The Rf values calculated are shown in Table 1. These values were compared with the GC-MS results (Table 3) of phytochemicals to elucidate the possible secondary metabolites present in these extracts and to understand possible activity of these phytochemicals on respective microorganisms.

Antibacterial activity of TLC separated pure fractions (F1, F2, F3) of leaf of *S. roxburghiana* at 1mg/ml (5µg/disc) against six pathogenic bacteria are shown in Table 2. It is clear from the data the antibacterial activity of the pure fractions were more pronounced with gram negative bacterias *Escherichia coli, Klebsiella pneumonia, Proteus vulgaris* except *Pseudomonas aeruginosa.* All the bacteria showed better zone of inhibition against F1 and F2 fractions except *P. vulgaris* that showed inhibition only against F1 fraction (Fig 1). None of the bacteria showed inhibition against F3 fraction.

In the GC-MS analysis, 16 bioactive phytochemical compounds were identified in the methanolic fractions of *S. roxburghiana.* The GC-MS retention time (RT) and percentage peak of the individual compounds are presented in Table 3. The identification of phytochemical compounds were based on the peak area, molecular weight and molecular formula (Fig 2). It is evident from this table that all fractions have a complex chemical composition.

The major phytoconstituents present in the GC-MS profile of TLC fraction of leaves of *S. roxburghiana* were 3,4-Dimethoxybenzoic anhydride (32.73%), 1,2-Benzenedicarboxylic Acid, BIS(2-Ethylhexyl) ester (17.30%), Palmitaldehyde, Diallyl Acetal (16.08), 1-Butyl 2-(8-Methylnonyl) Phthalate (15.78%), Delta.-Undecalactone (14.23%), n-Hexadecanoic acid (10.15%), 6,10,14-trimethyl-2-Pentadecanone (7.08%), Dodecanoic acid (6.58%) and 2,5-Dimethoxybenzhydrazide (6.35%).

The GC-MS spectrum showed presence of more long chain hydrocarbons. When the number of carbon atoms increase in the molecule, hydrophilicity is reduced and the lipophilicity is increased. Increased lipophilicity of a drug decreases its transport across intestinal epithelial cells[14,15]. Various aliphatic acids, aromatic aldehydes and ketones were also identified in the studied fractions. Compounds identified by GC-MS analysis possess various pharmaceutical applications. 3, 4-Dimethoxybenzoic acid; is used preferably as one of the principal anti-microbial preservatives used in foods and beverages. The destructive metabolic property of oxygen containing benzoic acid derivatives such as protocatechuic acid (3,4-dihydroxybenzoic acid) and veratric acid (3,4-dimethoxybenzoic acid) is used in the application for pharmaceuticals. The compound diethyl phthalate is used medicinally for the preparation of about 67 consumer formulations including bath preparations (oils, tablets, and salts), eye shadow, toilet waters, perfumes, other fragrance preparations, skin care preparations etc. so as a component in insecticide sprays, mosquito repellents and camphor substitute[16]. Dodecanoic acid, Tetradecanoic acid and n- Hexadecanoic acid have the property of antioxidant and antimicrobial activities[17]. It could be reasonably argued that the aromatic compounds comprising of aromatic carboxylic acids and esters are also responsible for the antimicrobial activity of *Sansevieria roxburghiana*.

In conclusion the study has revealed the presence of important separatable phytochemicals such as 3, 4-Dimethoxybenzoic Acid, Palmitaldehyde, 1, 2-Benzenedicarboxylic Acid, Delta.-Undecalactone etc. by GC-MS analysis in the leaves of *Sansevieria roxburghiana* showing potent antimicrobial activity. It has further confirmed by the presence of phytochemicals like diethyl phthalate that the plant extracts could be used for the treatment of various infections including skin transmitted infections. The results lend credence to the folkloric use of this plant in treating microbial infection and shows that *Sansevieria roxburghiana* could be exploited for new potent antimicrobial agents. Further study is required to see the specific medicinal property of these phytochemicals.

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Table 2: Antibacterial activity of TLC Separated pure fractions (F1, F2, F3) of leaf of *Sansevieria roxburghiana* by Disc Diffusion Method (Zone of Inhibition in mm at 5µg/d)[a,b]

S. No	Microorganism	F1	F2	F3
1.	*Escherichia coli*	11±0.57	11±0.62	NA
2.	*Pseudomonas aeruginosa*	6±0.39	8±0.40	NA
3.	*Enterococcus* spp.	10±0.47	9±0.59	NA
4.	*Klebsiella pneumonia*	10±0.64	12±0.38	NA
5.	*Staphylococcus aureus*	10±0.57	9±0.65	NA
6.	*Proteus vulgaris*	11±0.50	NA	NA

[a]Mean value ± SD, *n* = 3

[b]Statistical analysis data are expressed as means ± SD; NA No Activity.

Table 1: Retardation factors (Rf values) of acetone and methanol fractions of leaf of *S. roxburghiana* in different mobile phase

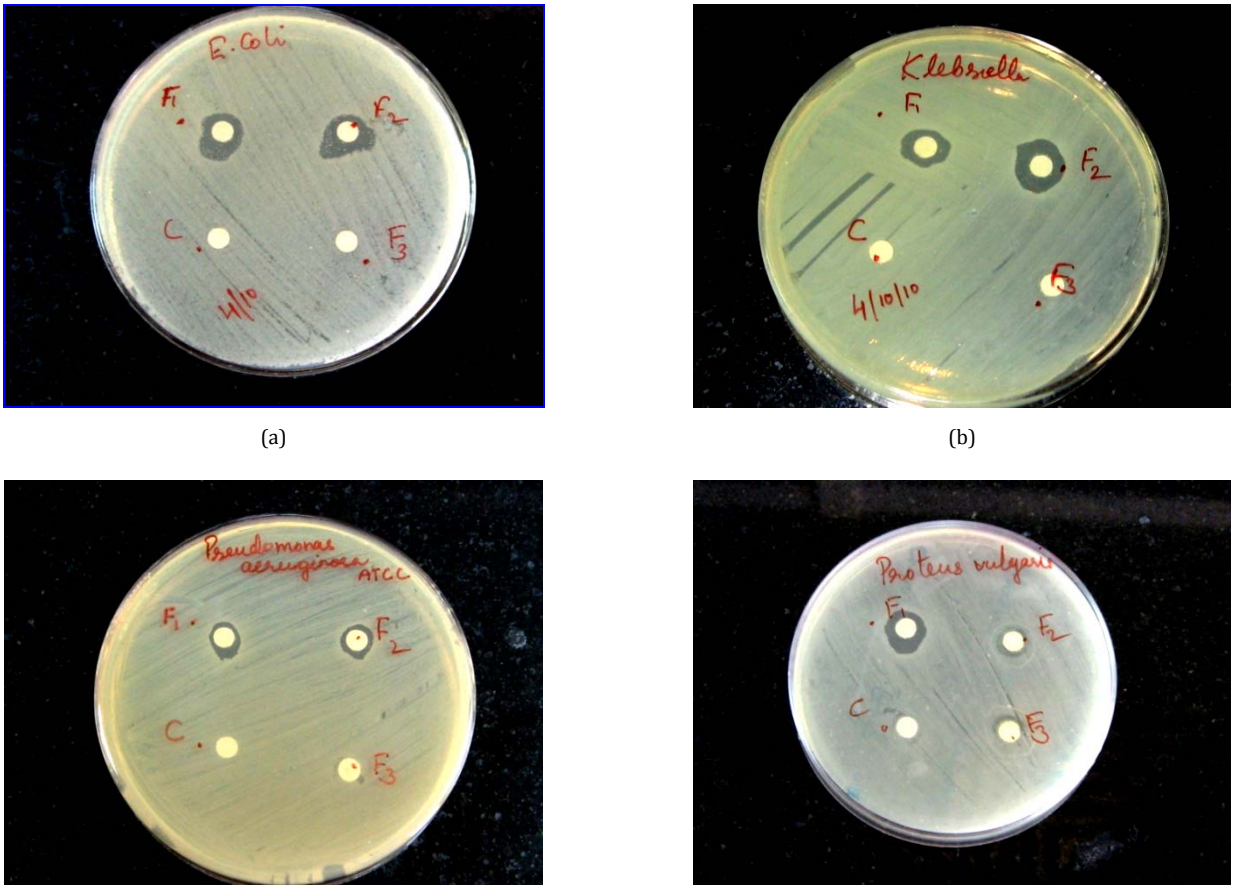
Spots	Ethyl acetate : acetone		chloroform: methanol	
	Acetone extract	Methanol extract	Acetone extract	Methanol extract
F_1.	0.7	0.8	0.8	N.D
F_2.	0.5	0.52	0.6	N.D
F_3.	N.D	0.4	0.3	0.3
F4.	N.D	N.D	N.D	0.1

N.D Not Detected.

Table 3: GC-MS analytical report of methanolic fractions of *S. roxburghiana.* leaves

No	RT	Name of the compound	Mol. F	MW	Area %
1.	8.65	Diethyl Phthalate	$C_{12}H_{14}O_4$	222	2.17
2.	9.13	6-Methyl-1-octanol	$C_9H_{20}O$	144	1.03
3.	10.31	2-propyldecan-1-ol	$C_{13}H_{28}O$	200	0.47
4.	11.75	2(4H)-Benzofuranone	$C_{11}H_{16}O_3$	196	3.10
5.	12.16	3,3-Dimethylhexanal	$C_8H_{16}O$	128	2.69
6.	12.44	Diisobutyl phthalate	$C_{16}H_{22}O_4$	278	3.40
7.	14.22	6,10,14-trimethyl-2-Pentadecanone	$C_{18}H_{36}O$	268	7.08
8.	14.38	2,5-Dimethoxybenzhydrazide	$C_9H_{12}N_2O_3$	196	6.35
9.	15.22	3,4-Dimethoxybenzoic anhydride	$C_{18}H_{18}O_7$	346	32.73
10	15.82	1,2-Benzenedicarboxylic Acid, Bis(2-Ethylhexyl) Ester	$C_{24}H_{38}O_4$	390	17.30
11.	17.11	Dodecanoic acid	$C_{12}H_{24}O_2$	200	6.58
12.	17.28	Palmitaldehyde, Diallyl Acetal	$C_{22}H_{42}O_2$	338	16.08
13.	18.61	1-Butyl 2-(8-Methylnonyl) Phthalate	$C_{22}H_{34}O_4$	362	15.78
14.	19.93	Delta.-Undecalactone	$C_{11}H_{20}O_2$	184	14.23
15.	21.3	Methyl Hexadecanoate	$C_{17}H_{34}O_2$	270	5.34
16.	21.58	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	10.15

The major compounds having 95% comparison with the compounds in the Wiley and NIST libraries



(a) (b)

(c) (d)

Fig. 1: Antibacterial activity of TLC separated pure fractions (F1, F2, F3) of leaf of *S. roxburghiana* against (a) *E.coli, (b) K. pneumonia, (c) P. aeruginosa, (d) P.vulgaris.*

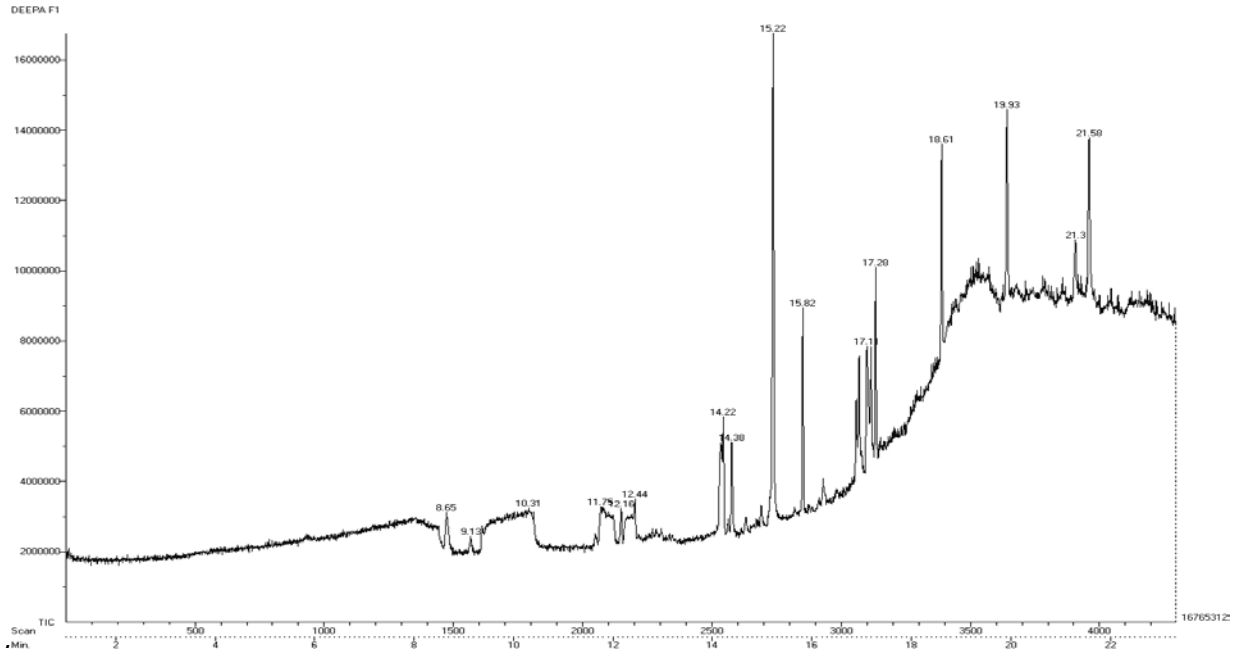


Fig. 2: GC -MS chromatogram of TLC fraction of leaf of *Sansevieria roxburghiana*

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