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COMPARATIVE ANALYSIS OF METHANOL EXTRACT (LEAF) OF THREE SPECIES OF SANSEVIERIA USING CHEMICAL AND BIOLOGICAL PARAMETERS

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ABSTRACT
Three different species of Sansevieria namely S.trifasciata, S.roxburghiana and S. cylindrica were compared using chemical and biological parameters. Morphological parameter were the length, width and foliage of leaf of the plants, S.roxburghiana leaves were longest and consisted of more foliage when compared to other species. 11 solvent systems were used in TLC fingerprinting and Chloroform solvent was unique as all the species showed bands at different RF values. All the species were inactive in cytotoxic activity using brine shrimp motility assay.

KEYWORDS: S. trifasciata, S. roxburghiana, S. cylindrica, brine shrimp assay, TLC, Methanol extract.

INTRODUCTION

Sansevieria genus belongs to family Asparagaceae, plants are cultivated for ornamental and medicinal purpose. A number of species of the family have been explored for medicinal purpose such as *Sansevieria liberica* has been reported to be of use in management of malarial fevers.[1] in folk medicine and in experiments also same has been supported. Another member of the family *Sansevieria trifasciata* is known for its wound healing capacities.[2] Leaf architecture of the genus is very significant and the members of the family present a vast variation in the leaf structure.[3] In the present study three species have been compared on the basis of leaf structure and their cytotoxic activity using brine shrimp assay. TLC base chemical fingerprinting has also provided an interesting pattern, same has been discussed.

MATERIALS AND METHODS

Plant collection and preparation of Solvent extract

Fresh leaves of three different species of Sansevieria i.e, S. trifasciata, S. roxburghiana and S. cylindrica were collected from the medicinal germplasm garden of Regional plant resource center (RPRC), Bhubaneswar. Leaves were washed in running tap water to remove dust and impurities. Morphological study of leaves was conducted, parameters studied were color, foliation and length of leaf. After that leaves were shade dried and made into powder using a mechanical grinder. Methanolic extract was prepared using cold percolation method.[4] After extraction the extract was concentrated by using Buchhi(R-200) Rotavapour under vacuum. Extract was stored in screw cap vials till further studies.

TLC analysis of methanolic extract of three species

TLC plates were prepared on 7cm glass slides using silica gel GF254 (Acme research Laboratory, Bombay). Slides were washed with detergent and dried. Clean and dried slides were wiped with ethyl acetate for removing surface adherents. 3 gms of silica was taken in 20 ml of distilled water and slurry was prepared by constantly stirring, and finally was poured over the slides and slides were left undisturbed till the drying of silica layer. Slides were activated at 100 degree Celsius before running the TLC. A total of 11 solvents were used for TLC analysis and these solvents were as follows

1) Ethyl acetate: Water: acetic acid(8:1:1)
2) Chloroform: hexane (3:2)
3) Ethyl acetate: benzene (9:1)
4) Benzene
5) Benzene: Chloroform (1:1)
6) Butanol: acetic acid: water (4:1:5)
7) Chloroform
8) Toluene
9) Acetonitrile
10) Chloroform: acetic acid (9:1)
11) Ethyl acetate: chloroform (1:9)

Spots were visualized either by naked eye, under UV at 365nm and 254nm or using Iodine vapour. Results were recorded and RF value of all the spots was calculated.

Retention factor= Distance moved by solute from origin/ Distance moved by solvent

Brine shrimp (Artemia salina) mortality assay

Cytotoxic activity study was carried out by brine shrimp lethality assay using standard protocols.[5] Brine shrimp (*Artemia salina*) eggs were hatched in artificial sea water, which was prepared using black salt 3.6 gm/ 200 ml distilled water. The eggs were incubated for 48 hours at temperature of about 28° C to get the desired growth of the larvae for biological evaluation. For each dose level 3 replicates were used. To each test tube of negative control, positive control and extracts, 20 numbers of brine shrimp and volume was made up to 10ml by adding salt water. Cytotoxic assay was carried out at three doses 200, 400 and 800µg/ml. Motility assessment of larvae was conducted at each hour up to four hours.

Motility readings were graded as below.
4+ = high motile
3+ = motile
2+ = sluggish
1+ = slow
Nil = no activity

RESULTS AND DISCUSSION

Comparative analysis of Sansevieria trifasciata(S1), *Sansevieria roxburghiana*(S2) and *Sansevieria cylindrica*(S3) was conducted using three parameters These were morphological studies on plant, brine shrimp assay and TLC fingerprinting using a number of solvents. Amongst the morphological parameters leaves were studied in details as per their length and width, their number in one plant (foliage). Results of the same are depicted in the Table 1. As can be seen from the table foliage of leaf is sufficient to distinguish between the three species. The three species also differed in the pattern over their leaves, S1 had vertical grooves all along the length, where as S2 was a light green as compared to others, S3 had beautiful striations on the leaf which were horizontal as opposed to the first one.

Table 1: Morphological study of three Sansevieria species.

Species	Parameters	Plant A (in cm)	Plant B (in cm)	Plant C (in cm)	Plant D (in cm)	Average
Sansevieria trifasciata	Length	40	35	34.5	44	38.375 ± 3.89
	Width	1.2	0.8	0.9	1.2	1.025 ± 0.17
	Foliage	9	8	9	8	8.5 ± 0.5
Sansevieria roxburghiana	Length	152.5	137.2	126.7	120	134.1 ± 12.26
	Width	7.4	7.9	9.8	7.0	8.025 ± 1.07
	Foliage	3	2	3	3	2.75 ± 0.43
Sansevieria cylindrica	Length	45.5	47.6	52.7	58.5	51.075 ± 6.075
	Width	6.6	4.2	5.1	4.7	5.15 ± 0.89
	Foliage	5	5	4	5	4.75 ± 0.43

Brine shrimp lethality assay is a rapid test system for studying the cytotoxicity of plants. This has a correlation with anti tumor activities and is also used as a bioassay guided system to isolate cytotoxic principles from the plants.[6] All the three species showed no variation in the results as all were found to be inactive against this model of brine shrimp assay thus the plants are considered to be safe for ingestion by grazing animals.

A number of solvents were tried to differentiate between the three species of Sansievieria. As can be seen from Table 2 the solvents in which all the three species showed similar results were Ethyl acetate: chloroform, Chloroform: Acetic acid, BAW, benzene, chloroform : hexane and ethyl acetate: water: acetic acid, while the solvents in which two of the species showed similar results were Toluene, acetonitrile, Benzene : chloroform. The most important solvent systems which showed difference in all the three species was chloroform where when the samples were visualized using iodine vapours all the species gave spots with different RF values.

Thus this solvent is most suitable for the differentiation between the three species. Overall study presented interesting information that morphologically different species do have some inner similarities as well.

Table 2: Thin layered chromatographic analysis of Sansevieria species.

S.no.	Solvents	S_1	S_2	S_3
1	Ethyl Acetate: Water : Acetic Acid (8:1:1)	UV_{254} – Complete Purple streak UV_{365} – Complete Streak Iodine Visualization – Complete –Yellow Streak	UV_{254} – Complete Purple UV_{365} – Complete Streak Iodine Visualization – Complete –Yellow Streak	UV_{254} – Complete Purple UV_{365} – Complete Streak Iodine Visualization – Complete –Yellow Streak
2	Chloroform : Hexane (3:2)	U_{365} – Red Flouroscent Iodine Visualization – Rf = 0.12, 0.45	U_{365} – Red Flouroscent Iodine Visualization – Rf = 0.12, 0.45	U_{365} – Red Flouroscent Iodine Visualization – Rf = 0.12, 0.45
3	Ethyl acetate:	Iodine Visualization – Rf =	Iodine Visualization – Rf =	Iodine Visualization – Rf

	Benzene (9:11)	0.18, 0.3	0.18, 0.3	= 0.18, 0.3
4	Benzene	UV_{254} – Red Spot Iodine Visualization – Rf = 0.125, 0.232	UV_{254} – Red Spot Iodine Visualization – Rf = 0.125, 0.232	UV_{254} – Red Spot Iodine Visualization – Rf = 0.125, 0.232
5	Benzene: Chloroform (1:1)	Iodine Visualization – Rf = 0.12, 0.24, 0.40	Iodine Visualization – Rf = 0.40	Iodine Visualization – Rf = 0.12, 0.30, 0.40
6	Butanol: acetic acid: water BAW (4:1:5)	UV – Complete Streak Iodine – Complete Streak	UV – Complete Streak Iodine – Complete Streak	UV – Complete Streak Iodine – Complete Streak
7	Chloroform	Iodine Visualization – Rf = 0.2, 0.34, 0.51	Iodine Visualization – Rf = 0.14, 0.21, 0.35, 0.46	Iodine Visualization – Rf = 0.12, 0.33
8	Toulene	Rf = 0.125, 0.17	Rf = 0.125, 0.17	No Bands
9	Acetotonitrile	UV – Purple Streak UV – Bands Iodine Visualization – Streak up to Rf 50. Rf = 0.403	UV – Purple Streak UV – Bands Iodine Visualization – Streak up to Rf 50. Rf = 0.403	No Bands
10	Chloroform: Acetic Acid (9:1)	UV – Complete Streak Rf = 0.17, 0.26, 0.86	UV – Complete Streak Rf = 0.17, 0.26, 0.86	UV – Complete Streak Rf = 0.17, 0.26, 0.86
11	Ethyl Acetate: Chloroform (1:9)	Iodine Visualization – Rf = 0.12, 0.17, 0.48	Iodine Visualization – Rf = 0.12, 0.17, 0.48	Iodine Visualization – Rf = 0.12, 0.17, 0.48

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