Original Article

Secondary Metabolite Profiling Of Pharmacologically Active Compounds From Sansevieria Cylindrica Bojer Ex Hook. Using UV, FTIR And HPLC Analysis

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Abstract

Introduction: *Sansevieria cylindrica* Bojer ex Hook. plant is an indigenous plant of the African continent subtropical province that is planted for decorative purposes in Egypt and India. It belongs to the Asparagaceae family. The plant is effective against treatment for caries, small pox, snake bite, stomach cancer, inflammatory conditions, influenza, cough, ear pain, swellings, diarrhoea, boils and fever and also this plant possess antihelmintic, antirheumatic, diuretic, laxative, vermifuge, antibacterial activity, antitrypanosomal activity, antioxidant and antidiabetic activities, antimutagenic effect, cytotoxic activity.

Methods: The spectroscopic analysis i.e. UV, FTIR, and HPLC were used to analyse the bioactive components of *Sansevieria cylindrica* leaf extracts in ethanol and chloroform.

Result: Peaks in the UV profile indicate that flavonoids, alkaloids, and phenolic compounds are present in the plant extract. FTIR analysis confirmed the presence of phenol, alkanes, secondary alcohol, aromatic amine, carboxylic acid, nitro compound, and halogen compound. Finally, flavonones, cinnamic acid derivatives, steroidal saponin, and phenolic compounds were noticed in the two plant extracts by HPLC analysis.

Conclusion: The above three evaluations provide the basis for using *Sansevieria cylindrica* leaf as a herbal alternative for a variety of diseases.

Keywords: *Sansevieria cylindrica*, UV, FTIR & HPLC

INTRODUCTION

Man relied on the healing properties of medicinal plants prior to the introduction of chemical medicines. Plants are in high demand in international trade because they are very effective, inexpensive, have no side effects, and can be used as an alternative to allopathic medicines. Plants are believed to be a rich source of ingredients that can be used in the development of pharmaceutical, non-pharmacopoeial, or synthetic drugs. Quite apart from that, plants play an important role in the development of human cultures all over the world. Furthermore, some plants are regarded as important sources of nutrition, and as a result, they are recommended for their therapeutic properties.

Plants with medicinal properties are useful for curing human diseases and play an important role in healing due to the presence of phytochemical constituents. Flavonoid, alkaloid, phenol and tannins, carboxylic acids, terpenes and amino acids, and inorganic acids are some of the phytocontituents found in plants. These phytoconstituents give plants distinct features and characteristics. [1]As a necessary consequence, analysing these chemical constituents would assist in determining various biological activities of plants.

To determine and estimate the presence of such phytocontituents in medicinal plants, a variety of techniques can be used. The most useful and widely used tools for this purpose are spectroscopic techniques. UV-VIS spectroscopy is a simple, low-cost, and quick test for detecting phytocomponents. UV-visible spectroscopy employs light in the visible or adjacent ranges. The colour of the chemicals involved has a direct impact on absorption in visible ranges. In these parts of the electromagnetic spectrum, molecules undergo electronic transitions. [2] The Fourier Transform Infrared Spectrophotometer (FT-IR) was possibly the most powerful tool for identifying the different types of chemical

bonds/functional groups found in phytochemicals. The wavelength of light absorbed was the primary noticeable feature of the chemical bond, as shown by the annotated spectrum. The chemical bonds in a compound can be determined by interpreting its infrared absorption spectrum. Moreover, FTIR spectroscopy is a timesaving method for characterising and identifying functional groups. [3]

HPLC is a versatile, robust, and widely used chromatographic technique used in phytochemical and analytical chemistry to identify, quantify, and purify the individual components of a mixture. [4]Currently, this technique is gaining popularity among various analytical techniques as the primary choice for fingerprinting research for herbal plant quality control. [5]Several authors describe the use of high-performance liquid chromatography (HPLC) to characterise and quantify secondary metabolites in plant extracts, primarily phenol compounds, steroids, flavonoids, and alkaloids. [6, 7, 8, 9]
Sansevieria cylindrica Bojer ex Hook. is a perennial plant in the Asparagaceae family of plants. It is native to the African continent's subtropical regions and is cultivated for ornamental purposes in Egypt. [10] It is one of the most recommended plants for improving air quality and has the ability to capture 107 different types of toxins, including air pollution and cigarette smoke (nicotine). [11] Because the whole plant is useful for treating cuts, sprains, and broken bones, and the roots are useful for treating snakebites, it is used as a traditional remedy, particularly in remote rural areas where herbal forms of medicine are still commonly used as medicines.

The plant is effective in treating caries, small pox, snake bite, stomach cancer, inflammatory conditions, influenza, cough, ear pain, swellings, diarrhoea, boils, and fever, and it also has antihelmintic, antirheumatic, diuretic, laxative, vermifuge, antibacterial activity,[12] antitrypanosomal activity, [13] antioxidant and antidiabetic activities, [14, 15] antimutagenic effect, [16] cytotoxic activity.[17]

Therefore, the current study used UV-Vis, FTIR, and HPLC to profile the bioactive compounds in ethanol and chloroform extracts of *S.cylindrica* leaves.

MATERIALS AND METHODS

Collection of Plant Material and Preparation of Plant Extracts

Plants collected from Holy Cross College Campus (Autonomous), Nagercoil, India, were identified by a taxonomist from Rapinat Herbarium and Center for Molecular Systematics, St. Joseph's College, Trichy, Tamil Nadu. It was deposited at the Rapinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil Nadu, India. Plant materials were then washed separately and dried for a few days in the shade. The dried leaves were ground into coarse powder by grinding machines and stored at room temperature for future use. The sample could be extracted using two solvents, ethanol and chloroform, and then evaporated to dryness using a rotary evaporator. The resulting residue was then analysed.

UV-Visible spectroscopic analysis:

A UV-visible spectrophotometer (Perkin Elmer, USA Model: Lambda 950) with a slit width of 2nm and a 10-mm cell at room temperature was used for the analysis. For proximate analysis, the extract was examined under visible and UV light at wavelengths ranging from 200 to 800nm. The extract was centrifuged at 3000 rpm for 10 minutes before being filtered through Whatman No. 1 filter paper for UV-VIS spectrophotometer analysis. The sample is diluted to a ratio of 1:10 in the same solvent. [18]

Fourier transform infrared FT-IR spectroscopic analysis:

The Fourier Transform Infrared Spectrophotometer (FTIR) is one of the most effective tools for determining the types of chemical bonds (functional groups) present in compounds. As shown in the annotated spectrum, the wavelength of light absorbed is characteristic of the chemical bond. Chemical bonds in a molecule can be determined by interpreting the infrared absorption spectrum Five milligrams of the isolated material were mixed with 100 mg of KBr pellet before being loaded into FTIR spectroscopy (Shimadzu, IR Affinity 1, Japan) with a Scan range of 500 to 4000 cm-1.

HPLC analysis

A Shimadzu system was used for high performance liquid chromatography (Controller, CBM20Alite, pump; LC-10 AT VP, PDA detector, SPD-M20A; Shimadzu, Kyoto, Japan). Samples were diluted to 1ml and the sample injection volume was kept at 20µl. A reverse phase-HPLC system with a C18G column with a diameter of 250 mm 4.6 mm and a mobile phase of 70% acetonitrile and 30% water (0.2% acetic acid) was used for separation. Throughout the analysis, the flow rate was kept constant at 1ml/min, and the acquisition was carried out at 25 ° c for 20 minutes. [19]

RESULTS AND DISCUSSION:

Spectroscopic techniques have evolved into a valuable technique for secondary metabolite profiling, as well as qualitative and quantitative assessment of pharmaceutical and biological materials.

UV-VIS analysis was performed to identify phytoconstituents present in two extracts of S. cylindrica. UV-visible spectra were used to identify compounds with σ-bonds, π-bonds and lone pair of electrons, chromophores, and aromatic rings.

The UV-Vis profile of the ethanol extract (Figure 1; Table 1) was chosen from 200 nm to 800 nm due to the wide range of distinct peaks and the appropriate baseline. The UV-Vis profile (Figure.1) revealed the presence of five absorption peaks at 228, 282, 307, 400, and 666 nm, with absorption spectra of 3.73, 3.07, 2.54, 1.207, and 0.47. The range for chloroform extract was chosen from 200 nm to 400 nm due to the broadness of distinct peaks and the proper baseline. The UV-Vis profile (Figure.2) revealed three absorption peaks at 210, 212, and 259 nm, with absorption spectra of 3.35, 3.33, and 1.47, respectively.

In the UV-VIS spectra of ethanol extract, the appearance of one or more peaks in the region from 200 to 800 nm indicates the presence of unsaturated groups and heteroatoms such as S, N, O. [20] The precise position of the peak between 230 and 285, as well as the relative intensities of these maxima, provide important information about the nature of the flavonoids. These absorption bands are typical of flavonoids and their derivatives. [21] Previous research has found that absorption bands at 234-676 nm are typical of alkaloids, flavonoids, and phenolic compounds. [22, 23] Carotenoids have a peak range of 400-450 nm. [23] The presence of peaks at 600-700 nm indicates the presence of chlorophyll in the extract. [24, 25] When it comes to chloroform extract, the presence of a peak in this range confirms the presence of flavonoids, alkaloids, and phenolic compounds in this extract. [22, 23]

The presence of flavonoids, alkaloids, and phenolic compounds in the plant extract is confirmed by UV-VIS spectroscopic analysis. In this case, the presence of these secondary metabolites in the extract is indicated by the presence of all peaks in this range.

Table 1. UV- Vis spectrum peak values of *S. cylindrica* ethanol extract

S.No.	Wavelength (nm)	Absorption peak
1	230	3.73
2	282	3.07
3	307	2.54
4	400	1.207
5	666	0.47

Figure 1: UV-Vis Spectral analysis of *S. cylindrica* ethanol extract

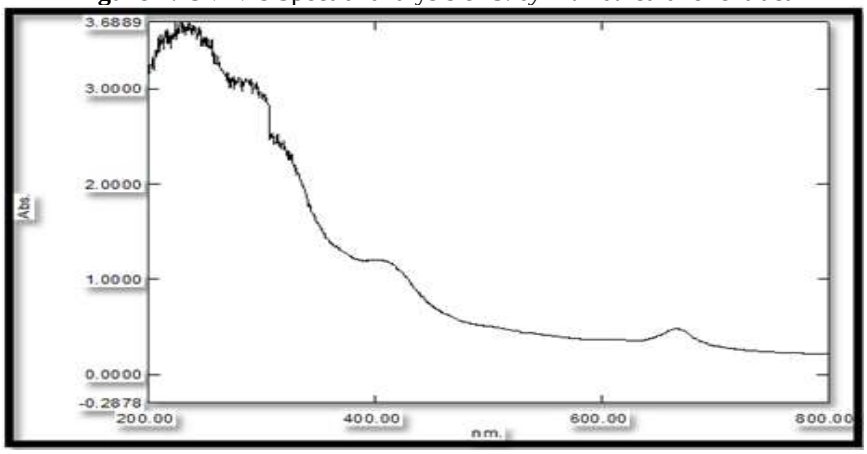
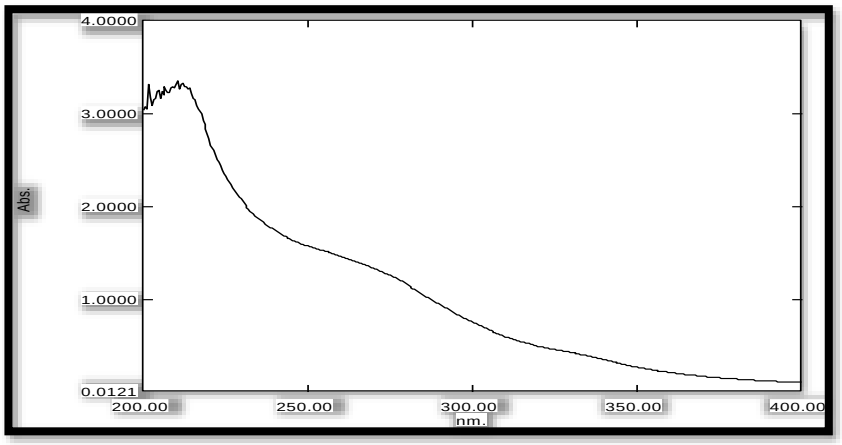


Table 2. UV- Vis spectrum peak values of *S. cylindrica* chloroform extract

S.No.	Wavelength (nm)	Absorption peak
1	210	3.53
2	212	3.33
3	259	1.47

Figure 2: UV-Vis Spectral analysis of *S. cylindrica* chloroform extract



FTIR analysis:

The use of FTIR technique allows, pointing out the implication of the different functional groups of guest and host molecules by analyzing the significant changes in the size and position of the absorbance bands. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio. The frequency range and functional group obtained from absorption spectra are presented in Table 3 & 4.

The strong peak at 598.86 cm^{-1} in both the extracts was attributed to halogen compound. [26] The band at 651.89 and 684.68 cm^{-1} was also attributed to halogen compound (C-Br). [27] The presence of C-O bond in 1081.03, 1269.07, 1113.81 and 1194.82 cm^{-1} is due to the appearance of strong absorption peak. [28] The appearance of peak at 3163.04 is due to O-H stretching. [29] The peaks which showed C–N/C–O/C–H stretching coincides with the findings of [30]. An occurrence of peak at 598.86 cm-1 in both the extracts represent the aromatic H out of plane bending. [31-33] The identified peaks at 1113.81 cm^{-1} and 1194.82cm^{-1} could be due to presence of C–O stretching due to an ester group and secondary alcohol. [34] No bond was found in the region of 2220 to 2260 cm-1 indicating that no cyanide group was present in the samples. This implies that the plant has no toxicity in both the extracts. [35] The presence of peak at 1399.26 cm^{-1} confirmed the presence of carboxylic acid group. [36]

The results of FTIR analysis confirmed the presence of phenol, alkanes, secondary alcohol, aromatic amine, carboxylic acid, nitro compound and halogen compound. All these compounds belong to secondary plant metabolites as per researcher explanations. [37,38] Fourier transform infrared transmission is very useful in plant characterization because it reveals the presence of inorganic and organic compounds in plants reveals the presence of functional groups serves as an indicator of different medicinal properties or biological activities of two leaf extracts of *S. cylindrica*.

Table 3. FTIR peak values of *S. cylindrica* ethanol extract

No	Peak	Intensity	Characteristic Absorptions (cm^{-1})	Possible Functional Group	Compound Class
1	598.86	92.01	600-500	-H bending (out of plane)	Aromtic ring
2	651.89	95.841	690-515	C-Br stretching	Halogen compound
3	753.15	96.565	858-733	C-H bending	Monosubstituted compound
4	1113.81	92.615	1124-1087	C-O stretching	Secondary alcohol
5	1152.39	96.901	1160-1120	C- N stretching	Aromatic amine
6	1194.82	96.93	1210-1163	C-O stretching	Ester
7	1399.26	71.657	1395-1440	C-O-H bending	Carboxylic acid
8	1509.19	97.914	1550-1500	N-O stretching	Nitro compound
9	2916.17	97.49	2855-2975	C- H stretching	Alkane
10	2987.53	96.85	2900-3100	C- H stretching	Alkene
11	3163.04	80.884	3200-2700	O-H stretching	Alcohol

Figure 4: FTIR Spectral analysis of *S. cylindrica* ethanol extract

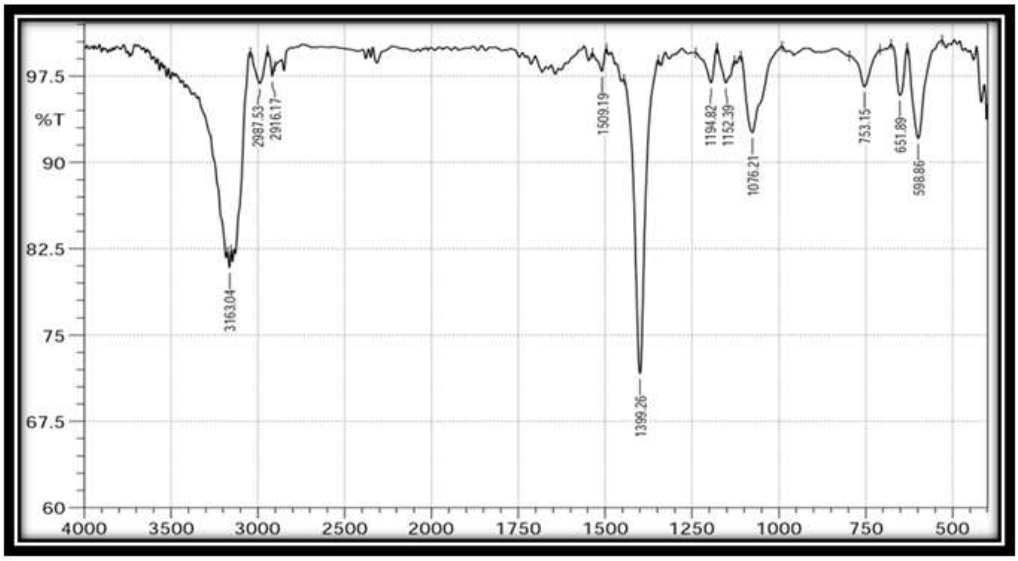
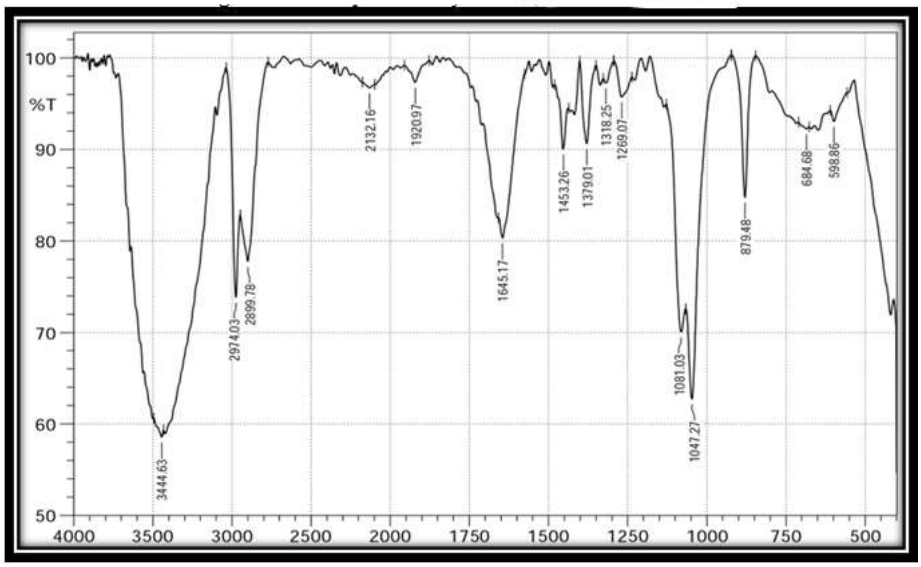


Table 4. FTIR peak values of *S. cylindrica* chloroform extract

No	Peak	Intensity	Characteristic Absorptions (cm^{-1})	Possible Functional Group	Compound Class
1	598.86	93.081	500-680	-H bending (out of plane)	Aromtic ring
2	684.68	92.267	690-515	C-Br stretching	Halogen compound
3	879.48	84.754	895-885	C=C bending	Alkane
4	1113.81	62.732	1124-1087	C-N stretching	Aliphatic Amine
5	1081.03	70.06	1150-1085	C-O stretching	Aliphatic Ether
6	1269.07	95.744	1275-1200	C-O stretching	Alkyl Aryl Ether
7	1318.25	97.27	1215-1325	C=O stretching	Alkyl ketone
8	1379.07	90.656	1390-1310	O- H bending	Phenol
9	1453.26	90.043	1465-1450	C-H bending	Methyl group
10	1645.17	80.324	1620-1680	C=C stretching	Cyclic Alkene
11	1920.97	97.337	2000-1900	C=C=C stretching	Allene
12	2132.16	96.747	2322-2138	N=C=S stretching	Isothiocyanate
13	2899.78	77.745	2800-3200	C-H stretching	Alkane
14	2974.03	73.855	2855-2975	C-H stretching	Alkene
15	3444.63	58.551	3500 -3200	N-H stretching	Aliphatic primary amine

Figure 4: FTIR Spectral analysis of *S. cylindrica* chloroform extract



HPLC

Compounds in the extract were estimated using HPLC analysis. In the previous literatures, *S. cylindrica* extract was found to contain several compounds in varying amounts. HPLC analysis of the plant revealed the presence of several compounds such as steroidal saponins, which are considered the most distinctive metabolites of the genus *Sansevieria*. Other

compounds found in varying amounts in other members of the Asparagaceae family include phenolic acids, flavonones, cinnamic acid derivatives, and flavonoid derivatives. Family Aspargaceae belongs, was also reported as a source of stilbenes.[39]

There are seven peaks with different retention times in an ethanolic extract of *S.cylindrica* species (Table. 5 & Fig. 5). The peak with retention time 2.127min indicated the presence of a flavone compound,[40] 3.33min indicated the presence of cinnamic acid derivatives, [41] 6.4 min indicated the presence of a flavonone compound,[42] 2.337min indicated the presence of spirostane steroidal saponin, [43] 8.527min showed flavonone,[44] 12.780min showed furostane steroidal saponin.[45] Six peaks with different retention times are present in the chloroformic extract of *S.cylindrica* species (Table. 6 & Fig. 6). The presence of spirostanesteroidal saponin was demonstrated by retention times 2.943 and 7.613, respectively, [46, 47] 5.403min by the presence of the phenolic compound gallic acid, [48]and 9.490min by the presence of another phenolic compound ellagic acid. [49]

HPLC has gained popularity in fingerprinting study as well as in characterization and quantification of secondary metabolites. The chromatograms can be used to identify the unknown compounds by comparison with the chromatograms of known compounds reported in literature.[50] HPLC analysis of the plant extracts revealed the presence of flavonones, cinnamic acid derivatives, steroidal saponin, phenolic compounds in the two plant extracts.

Table 5: HPLC analysis of *Sansevieria cylindrica* ethanol extract

S.No.	Retention time [min]	Area [%]	Height [%]
1	2.127	16.7	40.7
2	2.337	68.4	47.9
3	3.333	10.8	6.7
4	6.400	1.1	1.6
5	8.527	0.6	0.8
6	10.260	0.6	0.5
7	12.780	1.9	1.8

Fig.5. HPLC analysis of *Sansevieria cylindrica* ethanol extract

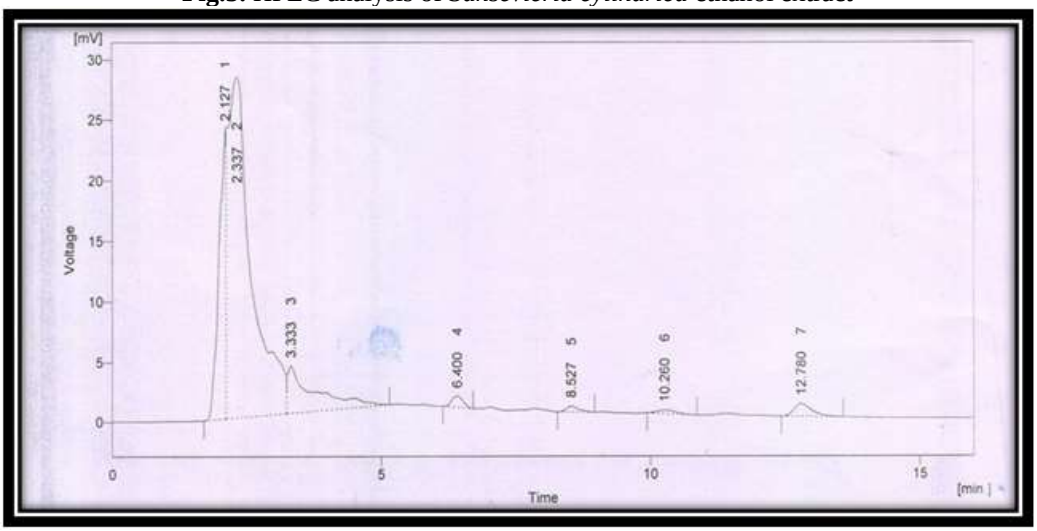
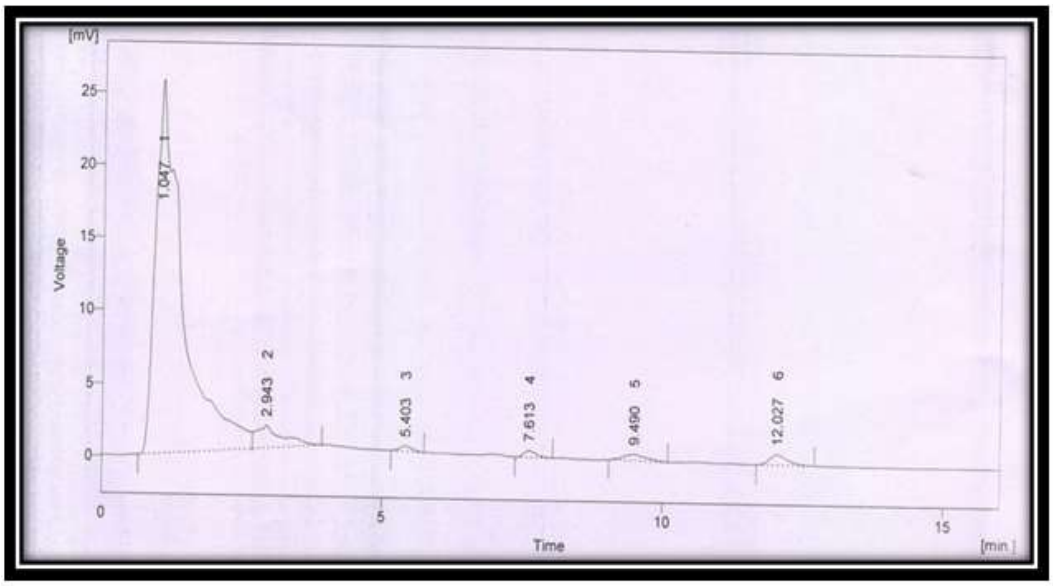


Table 6: HPLC analysis of *Sansevieria cylindrica* chloroform extract

S.No.	Retention time [min]	Area [%]	Height [%]
1	1.047	90.8	88.1
2	2.943	5.1	5.2
3	5.403	0.6	1.2
4	7.613	0.7	1.6
5	9.490	1.2	1.5
6	12.027	1.6	2.4

Fig.6. HPLC analysis of *Sansevieria cylindrica* chloroform extract



CONCLUSION:

The present study concludes that the presence of natural chemical compounds was detected in the selected solvent system. This study provided preliminary data for determining the chemical composition of *Sansevieria cylindrica* using UV, FTIR, and HPLC spectral techniques. UV spectrophotometric analysis revealed the presence of secondary metabolites in both the extract. The FTIR spectral analysis reveals the presence of distinct functional groups. The presence of flavonones, cinnamic acid derivatives, steroidal saponin, and phenolic compounds was revealed by HPLC analysis of plant extracts. The bioactive compounds found in leaf extracts have anticarcinogenic, anti-diabetic, antimicrobial, antioxidant, and anti-inflammatory properties.

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