



Evaluation of bioactive compounds in *Sansevieria roxburghiana* leaves using UV-VIS, FTIR and HPLC techniques

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Abstract

Medicinal plants are assuming greater importance in the primary health care of individuals and communities in many developing countries. The bioactive components of *Sansevieria roxburghiana* leaves have been evaluated using HPLC, UV VIS and FTIR. The phytochemical screening of *Sansevieria roxburghiana* leaves showed that the presence of terpenoids and phenol were present in ethanol, aqueous, ether and hexane extracts. Among the four extracts, the ethanol extract have high concentrations of phenol, flavonoids, alkaloids and tannin were observed as compared with aqueous, ether and hexane extract. The UV- VIS profile showed the occurrence of peaks at 199-664 nm reveals the presents of flavonoids in the *Sansevieria roxburghiana*. The results of FTIR analysis confirmed the presence of phenol, alkanes, Alkenes, alcohol, aromatic, aliphatic amines and amine compound. The results of this study offer a platform of using *Sansevieria roxburghiana* leaf as herbal alternative for various diseases including diabetic, cardiovascular etc. The results of this study offer a platform of using *Sansevieria roxburghiana* leaves as herbal alternative for various diseases including cancer and diabetic.

Keywords: HPLC, UV-VIS, FTIR, *Sansevieria roxburghiana*, phytochemicals

Introduction

Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments. They are assuming greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase in demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as an alternative to allopathic medicines Ashis (2003) ^[1]. Phytochemicals simply means plant chemicals. "Phyto" is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. In addition, these compounds may be responsible for the beneficial effects of fruits and vegetables on an array of health related measures (Dahanukar *et al.* 2000) ^[2]. Chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs from medicinal plants Cox and Balick (1994) ^[3]. The valuable medicinal properties of different plants are due to presence of several constituents i.e. saponins, tannins, alkaloids, alkenyl phenols, glycol-alkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters Cox (1990) ^[4]. Among them some are act as synergistic and enhance the bioactivity of other compounds.

Within a decade, there were a number of dramatic advances in analytical techniques including HPLC, UV, FTIR, NMR and GC-MS that were powerful tools for separation, identification and structure determination of phytochemicals Roberts and Xia (1995) ^[5]. The aim of this study is to determine the bioactive compounds present in the

Sansevieria roxburghiana leaves extract with the aid of UV-VIS, FTIR and HPLC Techniques, which may provide an insight in its use of tradition medicine.

Materials and methods

Collection of plant materials

The *Sansevieria roxburghiana* leaves were collected from Ariyalur, Tamil Nadu, India from a herb. The plant were identified and authenticated by Dr. S. John Britto, The Director, the Rapinat Herbarium and center for molecular systematics, St. Joseph's college Trichy-Tamil Nadu, India. A Voucher specimen has been deposited at the Rapinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of extracts

The *Sansevieria roxburghiana* leaves were first washed well and dust was removed from the leaves. Then the leaves were dried at room temperature and coarsely powdered. The powder was extracted with ethanol, aqueous, petroleum ether and hexane for 24 hours. A semi solid extract was obtained after complete elimination of organic under reduced pressure. The extract was stored in desiccator until used.

Preliminary phytochemicals screening

Chemical tests were carried out on the alcoholic extract using standard procedures to identify the preliminary phytochemical screening following the methodology of Harborne (1973) ^[6] and Sofowara (1993) ^[7].

UV and FTIR Spectroscopic analysis

The extracts were examined under visible and UV light for proximate analysis. For UV and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using a high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 260-900 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400-4000 cm^{-1} and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

HPLC Analysis

Sample preparation: The sample was prepared according to the procedure. The extraction was carried out using 2 ml of fermented broth with 50 mL of 95% ethanol under 80 KHz, 45°C in ultrasonic extraction device for 30 min, repeated twice. The extract was collected and filtered; the filtrate was dried at 50°C under reduced pressure in a rotary evaporator. The dried crude extract was dissolved in the 100 ml mobile phases. After filtering through a filter paper and a 0.45 mm membrane filter (Millipore), the extract was injected into HPLC.

HPLC conditions: Flavonoids were analysed using an RP-HPLC method Weerasak Samee and Suwanna Vorarat (2007) [8], Shimadzu Corp., Kyoto, consisting of a LC-10ATVp pump, SCL 10A system controller and a variable Shimadzu SPD-10ATVp UV VIS detector and a loop injector with a loop size of 20 μl . The peak area was calculated by a CLASSVP software. Reverse-phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250 \times 4.6 mm i.d., particle size 5 μm , Luna 5 μ C-18; phenomenex, Torrance, CA, USA) at 25°C. The gradient elution of solvent A [water-acetic acid (25:1 v/v)] and solvent B (methanol) had a significant effect on the resolution of

compounds. As a result, solvent gradients were formed, using dual pumping system, by varying the proportion of solvent A [water-acetic acid (25:1, v/v)] to solvent B (methanol). Solvent B was increased to 50% in 4 min and subsequently increased to 80% in 10 min at a flow rate of 1.0 mL/min. Detection wavelength was 280 nm.

Results and Discussion

The pharmacological activities of any plant sample are due to the presence of metabolites, secondary metabolites and secretory products in it. These usually consist of the phenolic compounds, alkaloids, tannins, saponins, carbohydrates, glycosides, flavonoids, steroids, etc. Most phenolic compounds such as flavonoids, glycosides, triperinoids, flavonols, carbohydrates and anthraquinones are found distributed throughout the plant kingdom Harborne (1973) [6]. Similarly, the polyphenolic compounds most commonly found in plant extracts are the phenolic acids, flavonoids and tannins (Naik *et al.* 2006) [9]. These compounds together with other phenolic structures of plant origin have been reported as scavengers of Reactive Oxygen Species (ROS) and are seen as promising therapeutic drugs for free radical mediated pathologies including diabetic, cardiovascular diseases Velavan (2011) [10]. Most flavonoidic compounds exhibit antipyretic, analgesic, anti-inflammatory, anti-arthritis, antioxidant and immunomodulatory properties (Balasundram *et al.* 2006; Gill *et al.* 2011) [11, 12]. These activities of flavonoidic compounds may be due to the presence of gallic acid, ellagic acid, quercetin, tannin acid, vanillin, resorcinol, catechin, etc.

In the present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *Sansevieria roxburghiana* investigated and summarized in Table-1. The phytochemical screening of *Sansevieria roxburghiana* leaves showed that the presence of terpenoids and phenol were present in ethanol, aqueous, ether and hexane extracts. Among the four extracts, the ethanol extract have high concentrations of phenol, flavonoids, alkaloids and tannin were observed as compared with aqueous, ether and hexane extract.

Table 1: Qualitative Analysis phytochemicals in different extracts of *Sansevieria roxburghiana* leaves

S.No	Secondary Metabolites	Ethanol extract	Aqueous extract	Pet. ether extract	Hexane extract
1	Tannin	++	++	-	-
2	Phlobatannins	-	-	-	-
3	Saponin	+	+	-	-
4	Flavonoids	++	++	+	-
5	Steroids	++	-	+	+
6	Terpenoids	++	++	++	++
7	Triterpenoids	++	++	-	-
8	Alkaloids	++	-	-	-
9	Anthroquinone	++	-	+	+
10	Polyphenol	++	+	+	+
11	Glycoside	++	-	+	+
12	Coumarins	-	+	-	-
13	Emodins	-	-	-	-
14	Anthocyanins	-	-	-	-

(+) Presence; (-) Absence; (++) Highest concentrations

Spectrophotometric analysis

The UV-VIS profile of plant extract was taken at the 100 to 800nm wavelength due to the sharpness of the peaks and proper baseline. The UV-visible spectra were performed to

identify the compounds containing σ - bonds, π -bonds, and lone pair of electrons, chromophores and aromatic rings. The profile showed the peaks at 199.6, 210.7, 398.8 and 664.5nm with the absorption 3.990, 3.999, 0.248 and

0.079 respectively (Fig-1 and Table 2). The flavonoids spectra typically consist of two absorption maxima in the ranges 230-290 nm (band I) and 300-350 nm (band II). The precise position and relative intensities of these maxima give valuable information on the nature of the flavonoids.

Occurrence of peaks at 207-380 nm reveals the presents of flavonoids in the *Sansevieria roxburghiana*. On comparison of the seeds and leaves spectra shows that the extracts has similar flavonoids and glycosides compounds reported Jasper *et al* (1958); Sofowara (1993) [13, 7].

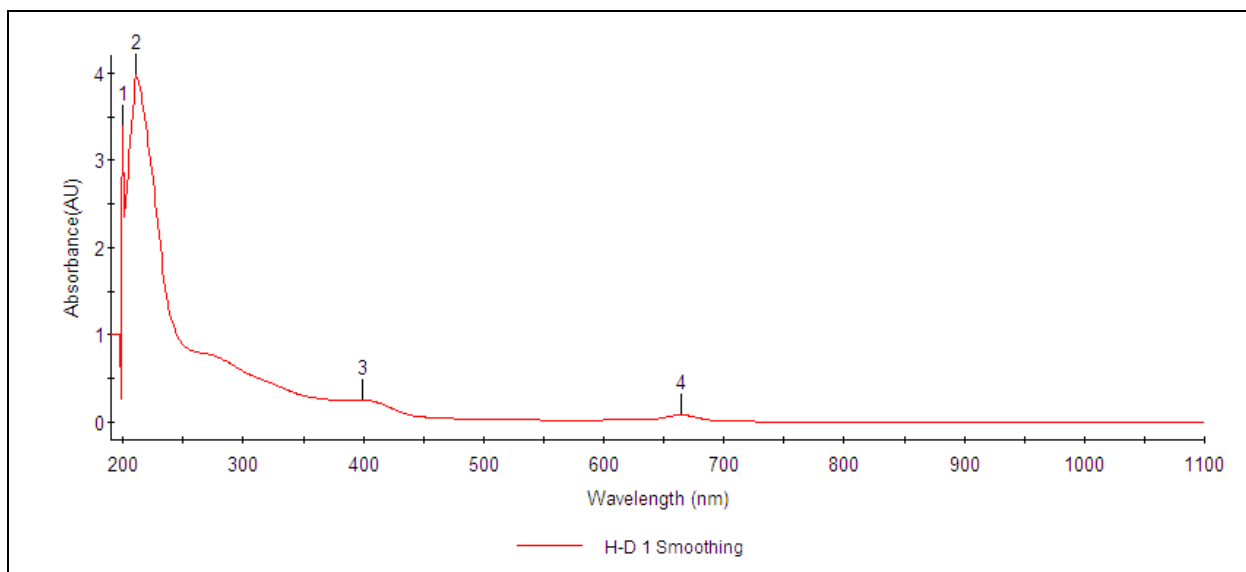


Fig 1: UV-Vis Spectral analysis of *Sansevieria roxburghiana* leaf extract

Table 2: UV-VIS Peak Values of Extract of *Sansevieria roxburghiana* leaf

S.No.	Wave length (nm)	Absorption Peak
1	199.6	3.990
2	210.7	3.999
3	398.8	0.248
4	664.5	0.079

Functional groups identification

The FTIR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of

the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of phenol, alkanes, Alkenes, alcohol, aromatic, Aliphatic amines and amine compound (Fig- 2, and Table- 3).

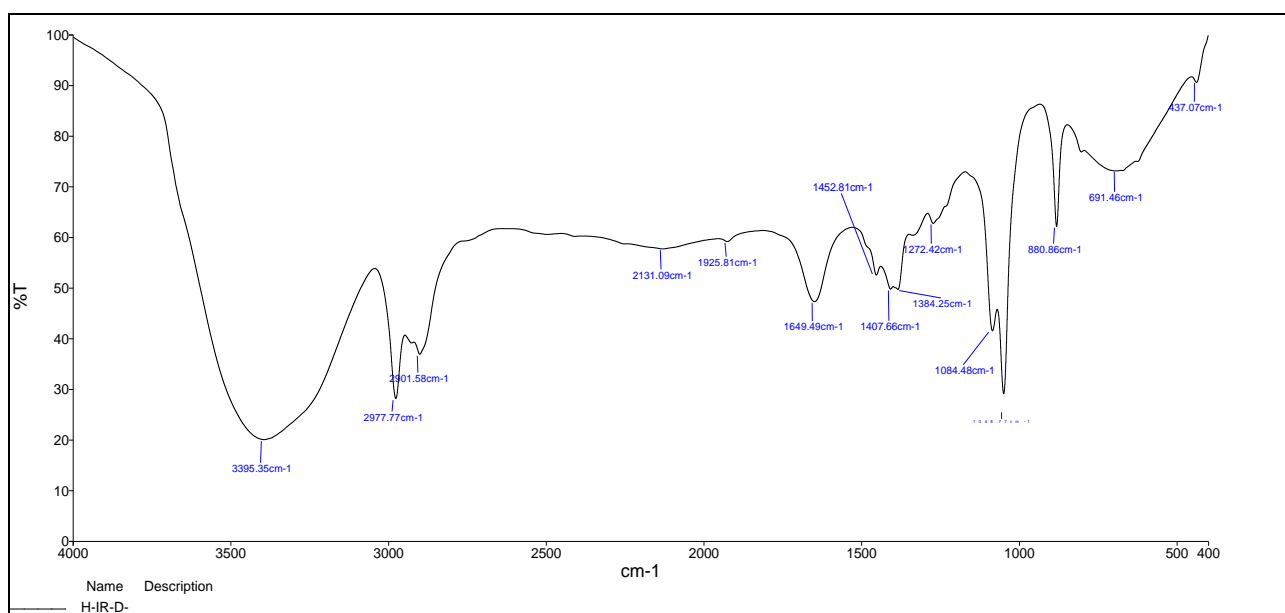


Fig 2: FTIR analysis of *Sansevieria roxburghiana* leaf extract

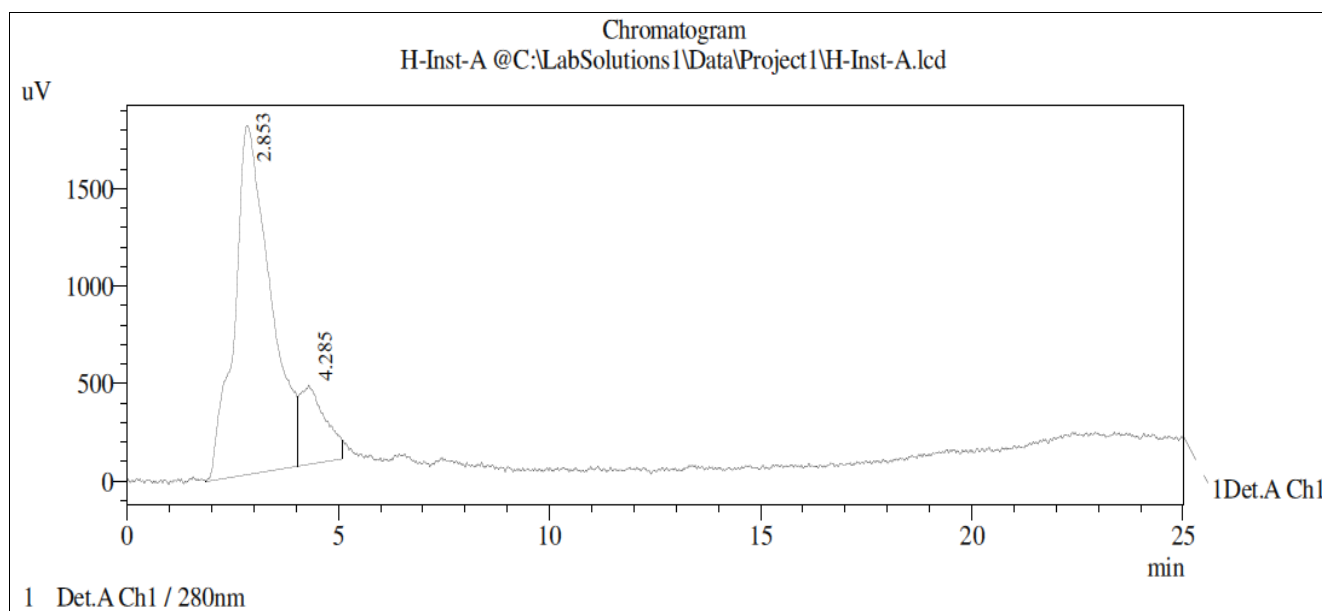
Table 3: FTIR Peak Values of Extract of *Sansevieria roxburghiana* leaf extract

S. No	Peak	Bond	Functional group
	3395.35	N-H stretch	1°, 2° amines, amides
	2977.77	C-H stretch	Alkanes
	2901.58	C-H stretch	Alkanes
	2131.09	-C≡C- stretch	Alkynes
	1649.49	N-H bend	1° amines
	1452.81	C-H bend	Alkanes
	1407.66	C-C stretch (in-ring)	Aromatics
	1272.42	C-N stretch	Aromatic amines
	1084.48	C-N stretch	Aliphatic amines
	1048.77	C-N stretch	Aliphatic amines
	880.86	C-H "oop"	Aromatics
	691.46	-C≡C-H: C-H bend	Alkynes

Determination of phytochemicals using HPLC

HPLC profiles of *Sansevieria roxburghiana* were analysed and two phenolic compounds namely Gallic acid (2.853min) and Ellagic acid (4.285 min), having different elution times could be obtained (Figure 3 and Table 4) when each compound was analyzed individually using the mobile

gradient phase consisting of methanol and 1% acetic acid in water during 30 minutes run time. Earlier review of (Gupta Mradu *et al* 2012; Michael Vagiri *et al* 2012; Nadia Alam *et al* 2011; Paranthaman *et al* 2012) [14 -17] supported the findings of these compounds.

**Fig 3:** HPLC analysis of *Sansevieria roxburghiana* leaf extract**Table 4:** HPLC analysis of *Sansevieria roxburghiana* leaf extract

Peak	Retention Time	Area %	Height %	Name of the compound**
1.	2.853	85.974	85.974	Gallic Acid
2.	4.285	14.026	14.026	Ellagic acid

** Compound identified by literature

Conclusion

The phytochemical screening shows that among the three extracts, the ethanol extract has higher concentrations of phenol, flavonoids, terpenoids, alkaloids and tannin were observed as compared with aqueous ether and hexane extract. The UV- VIS profile showed the occurrence of peaks at 199-664 nm reveals the presence of flavonoids in the *Sansevieria roxburghiana* leaves. The results of FTIR analysis confirmed the presence of phenol, alkanes, Alkenes, alcohol, aromatic, aliphatic amines and amine compound. HPLC profiles of *Sansevieria roxburghiana* leaves shows the presence of two phenolic compounds namely Gallic acid and Ellagic acid. The results of this

study offer a platform of using *Sansevieria roxburghiana* leaves as herbal alternative for various diseases including diabetic, cardiovascular etc.

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