Research Article

Quantitative Phytochemical Screening and HPTLC Fingerprinting of aerial parts of *Sarcostemma brevistigma*

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ABSTRACT

Preliminary quantitative phytochemical screening and HPTLC (High performance-Thin Layer Chromatography) finger print profile was carried out in *Sarcostemma brevistigma*. Quantitative analysis indicated that, different solvent extracts of *S. brevistigma* contained relatively higher levels of total flavonoids, total phenolics and tannins. HPTLC fingerprinting profile displayed the existence of 11 flavonoids**,** at the Rf in the range of 0.02 to 0.94 and 12 phenolic compounds were separated at the Rf in the range of 0.03 to 0.98. It was concluded that HPTLC fingerprint analysis of aerial parts of ethanolic extract of *S. brevistigma* can be used as a diagnostic tool for the correct identification of the plant and it is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations. Further, the separation and characterization of the bioactive compound from the plants is to be evaluated and reported in near future.

Keywords: Quantitative, HPTLC finger printing, flavonoids, phenols, screening.

INTRODUCTION

Natural product has always remained as a profile source for the discovery of new drug and used since the Vedic period.[1] Ayurveda has been a vibrant system of health care in India and has been plasticized, since 6000 years back, but growth as an industry, has commenced only a few years back. India's share in global export of medicine is around 10%. Therefore, there is a need to transform Ayurveda into a dynamic, scientifically validated, and proof based industry, which take its roots from the rich knowledge base of old tradition.[2,3]

A wide variety of active phytochemicals, including flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins, tannins, gallic acid, quercetin, phytosterols, alcohols, aldehydes have been identified from medicinal plants.[4] These phytochemicals are estimated by a variety of techniques such as spectroscopy and chromatography. Fingerprint analysis by HPTLC has developed into an effective and powerful tool for linking the chemical constituents profile of the plants with botanical identity and for estimation of chemical and biochemical markers.[5-7]HPTLC offers many advantages over other chromatographic techniques such as unsuppressed flexibility, choice of detection, user friendly, rapid and cost effective.[8] This method is widely used at industrial level for routine analysis of herbal medicines and it is a sophisticated and automated form of the thin-layer chromatography (TLC) with better and advanced separation efficiency and detection limits for qualitative and quantitative analytical tasks.[9,10] Attempts on applications of HPTLC for the purpose of phytochemical and biomedical analysis, herbal drug quantification, active ingredient quantification, fingerprinting of formulations and check for adulterants in the herbal formulations were successfully made by several researchers.[11-13]

Sarcostemma brevistigma (Somalatha) is an Asclepidiaceae species, distributed in temperate, subtropical and tropical regions. They are found across Africa, tropical Asia, and Australia and in parts of North America. In India it is distributed in most of the states, especially in Bihar, West Bengal, Karnataka, Tamil Nadu, Maharashtra and Kerala. This plant is widely used by tribal people of Pillur beat to treat various ailments including asthma, inflammation and skin infections and it is extensively used in traditional medicinal system of India. It has been reported to treat asthma, rheumatism, arthritis, joints pain, hypodermic diseases, snake bite, tumour, vesicant, whitlow, constipation, ascites and stomach distention.[14, 15]

In the present study the quantitative phytochemical screening and HPTLC finger printing of ethanol extracts of aerial parts of *S. brevistigma* has been done to identify the chemical constituents.

MATERIALS AND METHODS

Collection and identification of plant

Sarcostemma brevistigma was collected from Pillur Beat (Pillur slope RF and Nellithurai RF), Karamadai Range, Western Ghats, Tamil Nadu, and India. The authenticity of the plant was confirmed in Botanical Survey of India, Southern Circle, and Coimbatore by referring the deposited specimen. The voucher number of the specimen was BSI/SRC/5/23/2015/ Tech./2334.

Preparation of Plant Extract

The aerial parts were washed under running tap water, shade dried at room temperature, and powdered. The

powdered plant sample (50 g/250 ml) was extracted successively with ethanol, methanol, hexane and water using Soxhlet apparatus at 55-85°C for 8-10 hrs to extract the polar and non-polar compounds.[16] For each solvent extraction, the powdered pack material was air dried and then used. The solvents of the respective extracts were reduced under room temperature and stored at 4°C for further use. The dried plant extracts were then redissolved in dimethyl sulfoxide to get the solution of 10 mg/10 ml for each extract which was subjected to analysis of Quantitative phytochemicals.

Quantitative phytochemical studies

Determination of total phenolics and tannins

Ten microlitre aliquots of the extracts (10mg/2ml) were taken in the test tubes and made up to a volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteau phenol reagent and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min. and the absorbance was recorded at 725nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as tannic acid equivalents.

Using the same extract the tannins were estimated after treatment with polyvinyl polypyrrolidone (PVPP).[17] 100 mg of PVPP was weighed in to a 100 x 12 mm test tube and to this 1 ml distilled water and then 1 ml of the sample extracts were added. The content was vortexed and kept in the test tube at 4°C for 4 hrs. Then the sample was centrifuged (3000 rpm for 10 min at room temperature) and the supernatant was collected. This supernatant has only simple phenolics other than tannins (the tannins would have been precipitated along with the PVPP). The phenolic content of the supernatant was measured and expressed as the content of non-tannin phenolics on a dry matter basis. From the above results, the tannin contents of the sample were calculated as follows:

Tannin (%) = Total phenolics (%) - Non-tannin phenolics (%)

Determination of total flavonoids

0.5 ml aliquot of appropriately (10 mg/ 2 ml) diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5% $NaNO_2$ solution. After 6 minutes, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min and then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus water blank. The results were expressed as rutin equivalent.[18]

HPTLC analysis

The advancement of High Performance Thin Layer Chromatography (HPTLC) can provide an electronic image of chromatographic finger print and densitogram to detect the presence of a marker compound in the plant samples. Generally, this study is focused to confirm the secondary metabolites such as flavonoids and Phenols.

Sample preparation

100 mg of the aerial part of ethanolic extract of *S. brevistigma* was dissolved in 1ml of HPTLC grade methanol and centrifuged at 3000 rpm for 5 min. This solution was used as a test solution for HPTLC analysis.

Developing solvent system

Different solvent systems were used to develop HPTLC fingerprint profile for different secondary metabolite groups *viz*; flavonoids and phenols separately.[19-21]

Sample application

2 µl of sample and 3 µl of standard solution were loaded as 5mm band length separately on precoated silica gel $60F_{254}$ aluminum sheets (3 x 10 cm) using a Hamilton syringe with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

Development of chromatogram

After the application of spots, the chromatogram was developed in the twin trough glass chamber (20X10 cm) pre saturated with respective mobile phase.

Detection of spot

The air dried plates were kept in a photo documentation chamber (CAMAG REPROSTAR 3) and captured the images in visible light, UV 366 nm and UV 254 nm. The chromatogram was scanned by the densitometer at 405 nm after spraying with respective spray reagent and dried at 100°C in hot air oven. The peak number with its height, area and Rf values of fingerprint data were recorded by WIN CATS (1.3.4 version) software.

RESULTS

Table - 1 showed the total phenolics, flavonoids and tannin content of different solvent extracts (hexane, methanol, ethanol, aqueous) of the study plant. Total phenolics and tannin content were expressed as tannic acid equivalent (Fig.1). The total phenolic content was maximum in aqueous extract (6.04±0.04 mg/g) followed by hexane extract (4.26 ± 0.04mg/g) and methanols extract (4.06 ± 0.05mg/g). Minimum quantity was recorded in ethanol extract (3.09 ± 0.03mg/g). When compared to total phenolic content the tannin content was less in all the extracts. Maximum content of tannin was recorded in hexane extract (4.11 ± 0.03mg/g) followed by aqueous (3.95 ± 0.05mg/g) and methanol extract (3.78 ± 0.06 mg/g). The minimum content of tannin was noted in ethanol (2.98 ± 0.03mg/g) extract.

Total flavonoid content of different solvent extracts (hexane, methanol, ethanol, aqueous) of the study plant powder was expressed as rutin equivalent. The ethanol extract showed maximum flavonoid content (11.25 ± 0.06mg/g) whereas, methanol (10.45± 0.02 mg/g) and aqueous (7.98 ± 0.01 mg/g) extracts exhibited moderate amount. The minimum content of flavonoid was noticed in hexane (5.45 ± 0.04 mg/g) extract.

Table 1: Estimation of total phenolics, tannin and total flavonoid content of different solvent extracts of *S. brevistigma*

S.No.	Extraction medium	Total phenolics (mg TAE/g extract)#	Tannin (mg TAE/g extract)#	Total flavonoid (mg RE/g extract)#
1.	Hexane	4.26±0.04	4.11±0.03	5.45 ± 0.04
2.	Methanol	4.06±0.05	3.78±0.06	10.45±0.02
3.	Ethanol	3.09±0.03	2.98±0.03	11.25±0.06
4.	Aqueous	6.04±0.04	3.95±0.05	7.98±0.01

Values are means of three independent analyses of the extract± Standard deviation (n=3). TAE = Tannic acid equivalent RE = Rutin equivalent

HPTLC profile of ethanolic extract was generated in solvent system in order to ascertain the total number of chemical moieties (Table 2).

HPTLC profile of the ethanolic extract of aerial part of *S. brevistigma* for flavonoids was recorded in Tables 2 and 3, Fig. 2, 3 and 4. The best solvent system evaluated was Chloroform- Methanol – Formic acid (8.5:1:0.5).

Yellow or yellowish blue coloured fluorescence zone at UV 366 nm and Black, blackish blue coloured fluorescence zone at UV 254 nm mode was observed from the chromatogram, which confirmed the presence of flavonoids in the sample and standard. 12 compounds were separated and among them 11 were flavonoids at the Rf in the range of 0.02 to 0.94.

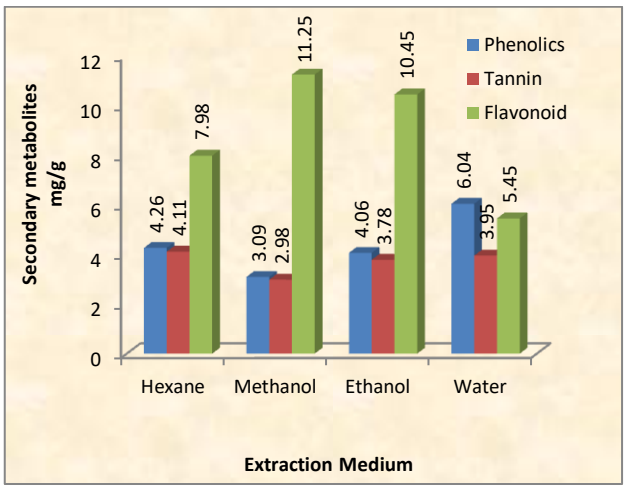

Figure 1: Estimation of total phenolics, tannin and total flavonoid content of different solvent extracts of *S. brevistigma* plant powder

The highest peak area was 29225.9AU and that of the lower was 1322.8AU observed at Rf of 0.10 and 0.94.

HPTLC profile of the ethanolic extract of aerial part of study plant for phenols was recorded in Tables 2 and 4, Fig. 5, 6 and 7. The best solvent system evaluated was Chloroform- Ethyl acetate – Formic acid (5:4:1). Blue, bluish brown coloured fluorescence zone at UV 366 nm and Blue, bluish black coloured fluorescence zone at UV 254 nm mode were observed from the chromatogram, which confirmed the presence of phenols in the sample and standard. 12 compounds were separated and all of them were phenols at the Rf in the range of 0.03 to 0.98. The highest peak area was 28084.3AU and that of the lowest was 872.5AU, observed at Rf of 0.03 and 0.89.

Table 2: Mobile phase, spraying reagent used and the colours observed for various secondary metabolites by using HPTLC for ethanolic extract of aerial parts of *S. brevistigma*

Name of the compounds	Mobile phase	Spraying reagent	Colour of the spot		
			Visible light	UV 366 nm	UV 254 nm
Flavonoids	Chloroform 85% - Methanol 10% - Formic acid 5% (8.5:1:0.5)	1% Ethanolic aluminium chloride reagent	Nil	Yellow, Yellowish blue	Black, blackish blue
Phenols	Chloroform 50% - Ethylacetate 40% - Formic acid 10% (5:4:1)	$FeCl_3$ reagent	Nil	Blue, bluish brown	Blue, bluish black

DISCUSSION

The secondary metabolites are compounds which are responsible for therapeutic efficacy of the drugs. Quantitative analysis of aqueous, hexane and methanol extracts of *S. brevistigma* indicated that the species contained relatively higher levels of total phenolics, flavonoids and tannins. The total flavonoid content was maximum in all the extracts. The phenolic content was maximum in aqueous extract and minimum in ethanol extract whereas, tannin content was maximum in hexane extract and minimum in ethanol extract.

Table 3: HPTLC profile of the ethanolic extract of aerial part of *S. brevistigma* for flavonoids.

Track	Peak	Rf	Height (mm)	Area (AU)	Assigned substance
Ethanolic extract of aerial part of *S. brevistigma*	1	0.02	712.8	24206.0	AutoGenerated1
	2	0.10	423.5	29225.9	AutoGenerated4
	3	0.23	231.0	4743.5	unknown *
	4	0.29	296.5	9678.9	AutoGenerated10
	5	0.35	297.4	8896.6	AutoGenerated9
	6	0.40	503.6	17396.9	AutoGenerated2
	7	0.44	311.7	13330.8	AutoGenerated8
	8	0.59	164.2	5429.0	AutoGenerated11
	9	0.67	291.3	10308.6	AutoGenerated7
	10	0.73	291.5	7856.3	AutoGenerated6
	11	0.75	289.4	9541.0	AutoGenerated5
	12	0.87	55.3	1488.6	AutoGenerated13

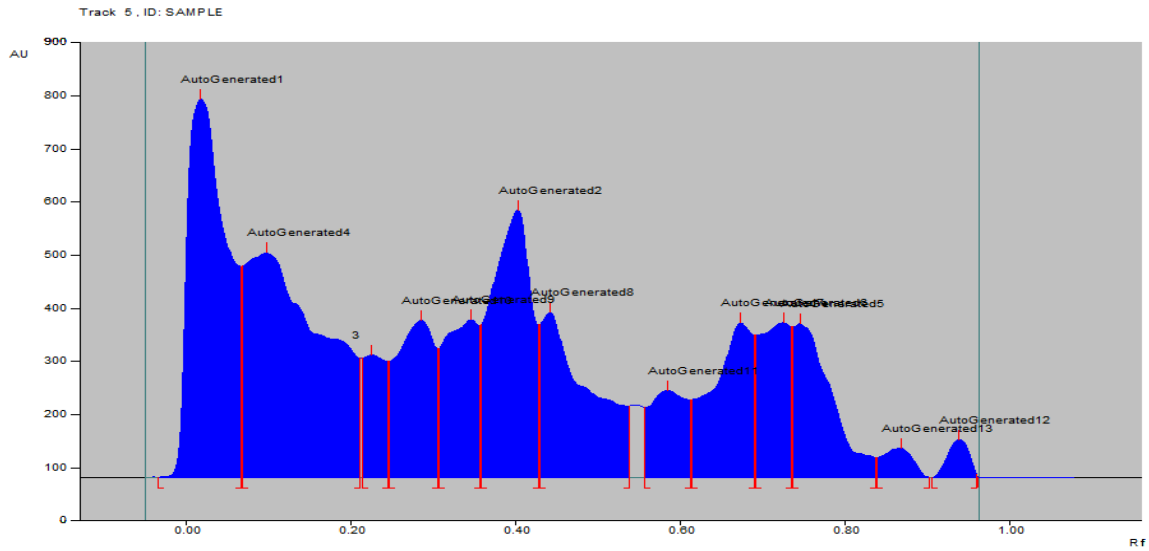

Figure 2: HPTLC densitogram for ethanolic extract of aerial part of *S. brevistigma* for flavonoids

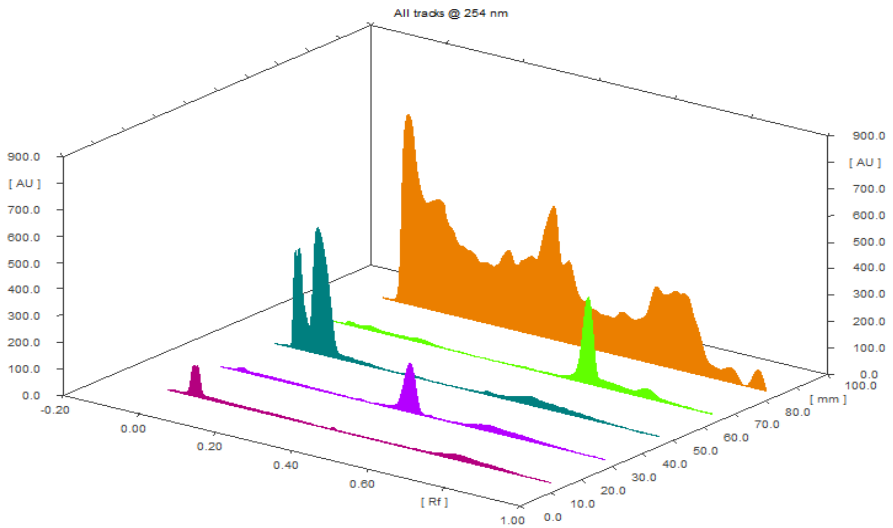

Figure 3: 3D diagram of HPTLC densitogram for ethanolic extract of aerial part of *S. brevistigma* for flavonoids

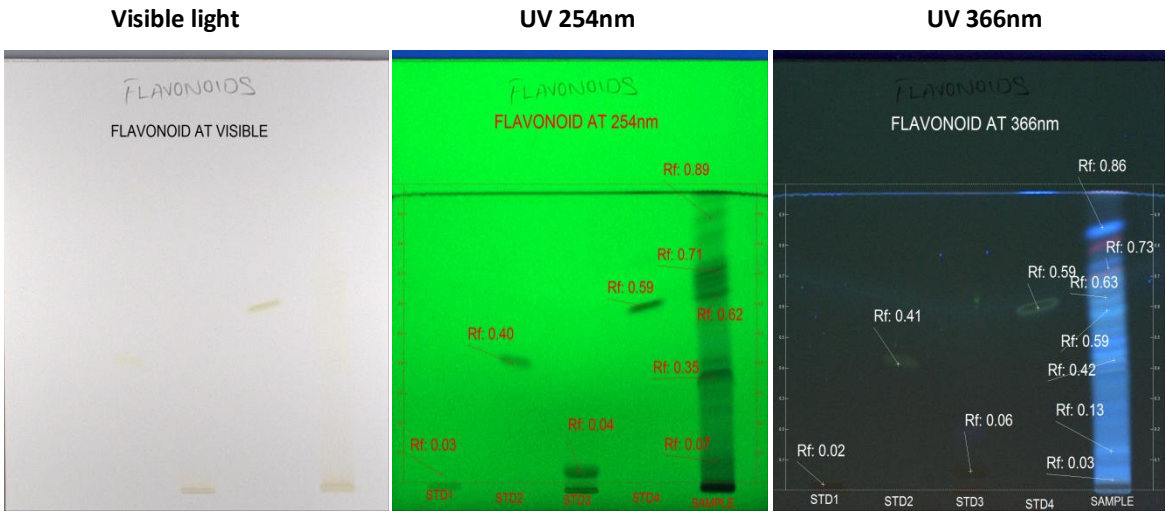

Figure 4: HPTLC fingerprinting profile for various secondary metabolites present in aerial parts of *S. brevistigma* for flavonoids.

Table 4: HPTLC profile of the ethanolic extract of aerial part of *S. brevistigma* for phenols.

Track	Peak	Rf	Height (mm)	Area (AU)	Assigned substance
Ethanolic extract of aerial part of *S. brevistigma*	1	0.03	775.3	28084.3	AutoGenerated1
	2	0.10	562.7	10961.3	AutoGenerated4
	3	0.13	627.4	26118.4	AutoGenerated3
	4	0.25	251.4	13363.7	AutoGenerated8
	5	0.46	175.7	5443.4	AutoGenerated13
	6	0.53	225.2	10341.2	AutoGenerated9
	7	0.59	384.7	9578.6	AutoGenerated7
	8	0.66	216.5	6983.0	AutoGenerated10
	9	0.70	195.2	3591.9	AutoGenerated11
	10	0.72	194.4	4025.7	AutoGenerated12
	11	0.79	308.3	13787.7	AutoGenerated5
	12	0.89	51.6	872.5	AutoGenerated6

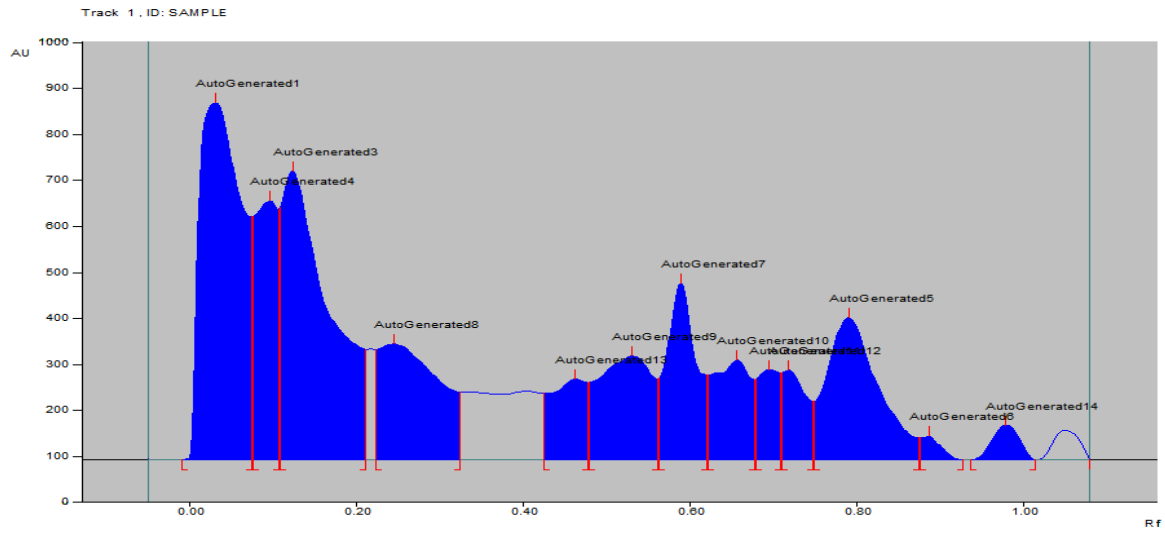

Figure 5: HPTLC densitogram for ethanolic extract of aerial part of *S. brevistigma* for phenols

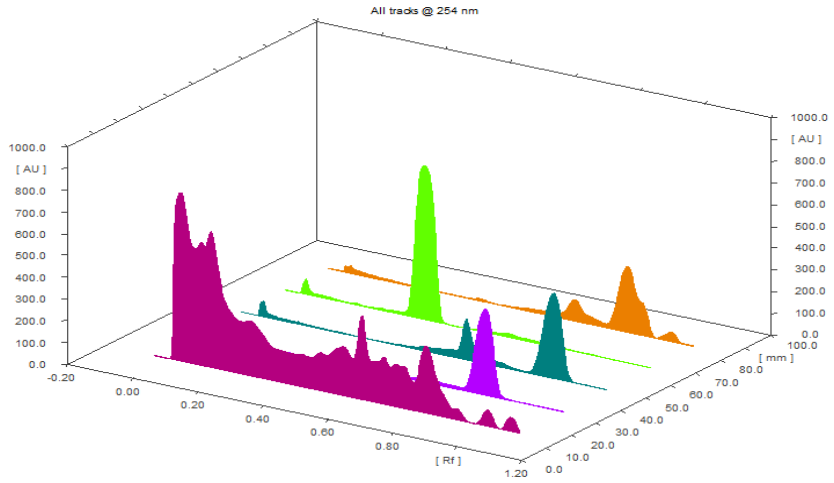

Figure 6: 3D diagram of HPTLC densitogram for ethanolic extract of aerial part of *S. brevistigma* for phenols

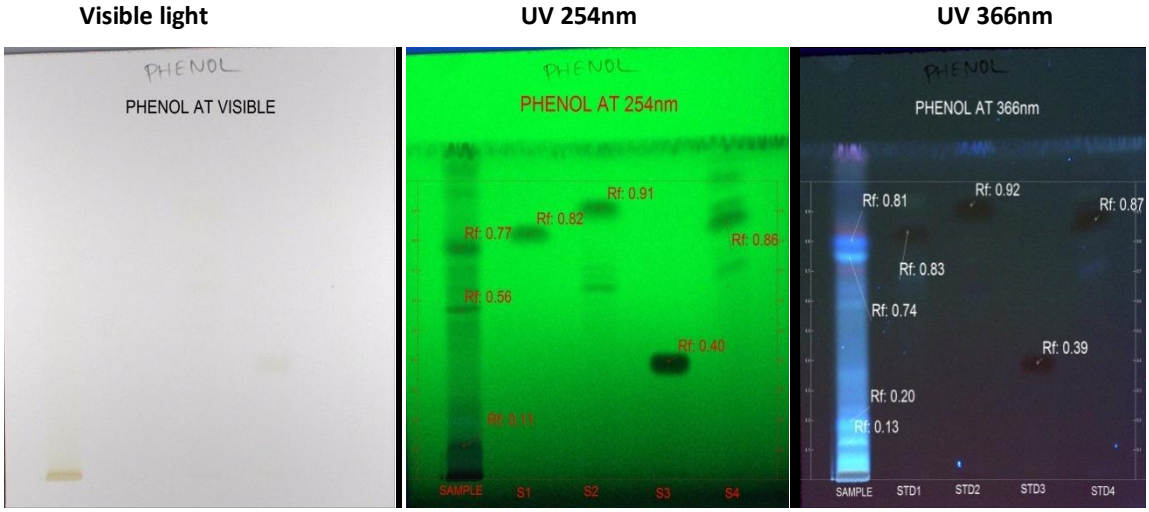

Figure 7: HPTLC fingerprinting profile for various secondary metabolites present in aerial parts of *S. brevistigma* for phenols.

The results showed that the plant extracts contain significant quantity of phenols and flavonoid compounds. Previously similar study was reported by Ahmad *et al*.[22] Therefore, the current preliminary quantitative phytochemicals screening might be proved valuable in the detection and further quantitative analysis of these therapeutically important compounds. The phenolic and flavonoid compounds are important antioxidants which act as antimicrobial, antiallergic, anti-inflammatory and anticancer agent. Phenolic compounds are most widely distributed phytochemicals which are derivatives of pentose phosphate, shikimate, and phenylpropanoid pathways in plants. These secondary metabolites play a vital role in reproduction and growth. These compounds also provide protection against harmful pathogenic microbes and predators.[22,23] Therefore, quantitative analysis of such vital compounds is extremely significant to determine the quality of drugs.

HPTLC is useful as a phytochemical marker[24,25] and more effective in the identification of plants through secondary metabolites.[26] It is considered as a rational method for more powerful and effective quality control of herbal drugs[27] and checking for the adulterants.[28] The qualitative analysis of ethanolic extract of aerial parts of *S. brevistigma* through HPTLC confirmed the presence of secondary metabolites flavonoids and phenols. This study exhibited the presence of 11 flavonoids and 12 phenlos with different Rf values and peak areas. The mobile phases for HPTLC used in study to separate the bioactive compounds were high polar solvent viz., Chloroform, methanol and formic acid. Many earlier reports were also suggesting this mobile phase of high polarity solvents for effective separation of bioactive compounds in many plant species.[11, 29, 30]

The well resolved HPTLC profiles showed the occurrence of the above said metabolites of medicinal importance, which support the traditional therapeutic uses of this species. The results indicated that the aerial part contain an appreciable amount of flavonoids and phenolics. Medically, the presence of these phenols and flavonoids explains the use of *S. brevistigma* in ethnomedicine [31,32] for the management of various ailments

This study is evidence for the presence of different types of flavonoids which were identified with different Rf levels. Putative therapeutic effects of many traditional medicines might be ascribed to the presence of flavonoids.[33] The presence of anti-carcinogenic [34-36] and antioxidative[37] properties of plants were because of the presence of flavonoids. Flavonoids were plant pigments, inhibits many bacterial strains, inhibit important viral enzymes, such as reverse transcriptase and protease and destroy some pathogenic protozones. Flavonoids were currently used as drugs or to prevent various diseases and in particular, efficient in preventing various types of cancer.[38] It has the ability to inhibit specific enzymes to stimulate some hormones and act as neurotransmitters.[39] Most flavonoids function in the human body as antioxidants[40] anticancer, antidiabetic, antiaging and prevention of cardiovascular diseases[41] and control inflammation.[42] Common examples for flavonoids are rutin, quercetin, catechin, kemperol, etc. The antioxidant properties of phenolic acids and flavonoids were due to their redox properties, ability to chelate metals and quenching of singlet oxygen.[32]

The preliminary HPTLC analysis of methanol extract of *S. brevistigma* showed the presence of various 12 phenolic compounds. The phenolics were one of the largest and most ubiquitous groups of plant metabolites.[43] A number of studies have focused on the biological activities of phenolic compounds which were antioxidants and free radical scavengers.[44] The antioxidant activity of phenolic compounds was mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, heavy metal chelators and hydroxyl radical quenchers.[32,45] They are strong antioxidants and might prevent oxidative damage to biomolecules such as DNA, lipids and proteins which play a role in chronic diseases such as cancer and cardiovascular diseases.[46] Previously similar study was reported by Varghese *et al*.[47] in *Citrullus lanatus*. The above stated reports justify the medicinal usage of *S. brevistigma*.

The present study is the first to report the HPTLC fingerprint of ethanolic extract of *S. brevistigma* aerial parts. From the HPTLC studies, it has been found that ethanol extract contains flavonoids and phenols. This densitometric HPTLC fingerprint profile may be used as marker for quality evaluation and standardization of the drug. Thus, HPTLC fingerprint profile along with their Rf values were recorded, which would serve as a reference standard for the scientist engaged in research on the medicinal properties of plant.[48]

CONCLUSION

People are demanding natural drugs for safety, due to various adverse effects of synthetic drugs. Therefore in recent years scientist are search in for alternative medicine to synthetic drugs. Some chronic diseases require long term therapy in that case synthetic drugs may produce side effects. Through various literature surveys we found that phenols and flavonoids are having curative property, hence phenols and flavonoids have been identified in this plant.. The developed fingerprint analysis of aerial parts of ethanolic extract of *S. brevistigma* will help to isolate and to identify new phenols and flavonoid, which will offer way to discover lead molecule for the development of drug.

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