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HPTLC Analysis: Identification of Flavonoid from *Senna alata* (L.) Flowers

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ABSTRACT

The aim of the study is to develop the high performance thin layer chromatography (HPTLC) fingerprint profile of flavonoid of *Senna alata* (L.) HPTLC method for the separation of the active constituents in extracts has been developed using solvent system Toluene: Ethyl acetate: Formic acid (5:4:1). The preliminary phytochemical results of *S.alata* indicated that the presence of alkaloids, flavonoids, saponins, tannins and glycosides. In HPTLC analysis, it showed the presence of flavonoid quercetin in standard as well as the sample with Rf value 0.57. In future, these fingerprinting images will be helpful in the identification and quality control of the drug and ensure therapeutic efficacy.

INTRODUCTION

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. This knowledge is accessible from thousands of medical texts and manuscripts. The substances having medical value have been extensively used for treating various disease conditions. Herbs being easily available to human beings have been explored to the maximum for their medicinal properties. Products of primary metabolism such as amino acids, carbohydrates and proteins are vital for the maintenance of life processes while others like alkaloids, phenolics, steroids, terpenoids are products of secondary metabolism and have toxicological, pharmacological and ecological importance [1]. Many medicinal plants, traditionally used for thousands of years are present in a group of herbal preparations of the Indian traditional health care system (Ayurveda) and proposed for their interesting multilevel activities. Amongst the medicinal plants used in Ayurvedic preparations for their therapeutic action, some have been thoroughly investigated and some need to be explored [2].

Flavonoids comprise a large group of plant secondary metabolites characterized by a Diphenyl propane structure (C6-C3-C6). Numerous preclinical and some clinical studies suggest that flavonoids have potential for the prevention and treatment of several diseases. Some epidemiological studies support a protective role of diets rich in foods with flavonoids and a reduced risk of developing cancer and cardiovascular diseases [3-4]. Preclinical *in vitro* and *in vivo* investigations have shown plausible mechanisms by which flavonoids may confer cancer and cardiovascular protection [5]. In addition to their preventive potential, certain flavonoids may be useful in the treatment of several diseases. Some evidence supporting the therapeutic potential of flavonoids come from the study of plants used in traditional medicine to treat a wide range of diseases, which has shown that flavonoids are common bioactive constituents of these plants [6]. Flavonoids have been reported to possess many useful properties, including anti-inflammatory activity, oestrogenic activity, enzyme inhibition, antimicrobial activity, anti-cancer, anti-allergic activity, antioxidant activity, vascular activity and cytotoxic antitumour activity [7] .

Senna alata (L.) Roxb) belongs to the Fabaceae family (subfamily Caesalpinioideae) and commonly known as candle bush, with reference to the shape of its inflorescences, or ringworm tree because of a traditional Use [8]. It is commonly referred to as “Asuwon oyinbo” by the

Yoruba ethnic group in Southwestern Nigeria [9-10]. It is widely available in the tropics and has very important applications in folkloric medicine [11]. In the northern part of Nigeria, particularly in Adamawa and Taraba States, the root, stem and leaves are used by practitioners of herbal medicines to treat burns, skin and wound infections, diarrhea, gastrointestinal and upper respiratory tract infections [12]. In Ghana and Ivory Coast, decoctions of the leaves and roots are used to treat diarrhea, dysentery, and other gastrointestinal problems. The leaves are well known for their laxative property and due to the high content of chrysophanic acid. The leaf extract is also used for skin diseases. In addition, leaves are also used for various diseases of the liver [13-14]. The macerated juices of the young fresh leaves are used to treat eye infections and parasitic diseases [15]. The decoction of the stem bark and roots are used to treat urinary tract infections, bronchitis, and asthma [16]. The main objective of this study is aimed to analyze the HPTLC fingerprinting profile for flavonoids in the flower extract of *S.alata*.

MATERIALS AND METHODS

Collection of plant material

The flowers of *Senna alata* were collected from its natural habitat in and around Mannargudi, Thiruvarur district, Tamilnadu, India.

Preparation of plant extract

Fresh flowers were shade dried at room temperature for 10 days and powdered coarsely using electric blender. The powder (10gm) was taken and mixed with ethanol (150ml). The mixture was boiled until and it was reduced to one third. The extract was filtered with a muslin cloth. The filtrate was transferred into china dish and was allowed to evaporate using water bath. The obtained paste form of the extract was used for phytochemical studies.

Preliminary phytochemical screening

Phytochemical analysis of the extract was conducted as per the standard procedure [17]. By this procedure, the presence of several phytochemicals like alkaloids, flavonoids, tannins, saponins, esters, resins, sugars and glycosides were tested.

High performance thin layer chromatography (HPTLC) Analysis

Instrument	:	CAMAG Automatic TLC Sampler 4 (ATS4) with win CATS software.
Stationary phase	:	plates silica gel 60 F_{254} pre coated layer (20 cm X 10 cm)
No. of tracks	:	3 band length: 8 mm.
Mobile phase	:	Toluene: Ethyl acetate: Formic acid:(5:4:1)
Standard	:	Quercetin
Sample	:	Brown powder
Solubility	:	Methanol
Standard concentration		1µl/ml
Standard preparation	:	Weigh 5mg of standard Quercetin in 5ml of methanol
Standard injection volume	:	1, 2, 4,6,8,10,12,14,16,18µl
Sample concentration	:	5,10µl
Sample preparation	:	Weigh about 100mg of hydro-alcoholic extract and dissolved in 5ml of 7:3 Alcohol: Water
Sample application volumes (µl)	:	1, 2,4,8,12,16
Development mode	:	Ascending mode
Scanning wavelength	:	254 nm and 366nm
Measurement mode	:	absorbance
Evaluation	:	A band Rf value of 0.55 corresponding to Quercetin is visible in test solution tracks

Preparation of the plates

The plates used for HPTLC was silica gel 60 F_{254} (E.MERCK KGaA). 50µg/ml of the standard was applied in the form of bands using LINOMAT IV applicator. The volumes applied were 1, 2, 4, 6, 8, 10, 12, 14, 16 and 18µl. The sample concentration was 10mg/ml and the different volumes were 1, 2, 4, 8, 12 and 16µl. The mobile phase used was toluene: ethyl acetate: formic

acid: methanol (5.5:3:1:0.5). The chromatograph was developed for15 minutes, dried at room temperature and scanned at 254nm and 366nm.

Procedure

Applied 1µl of standard solution and 5, 10µl of test solution on a percolated silica gel 60F254 TLC Plate of uniform thickness 0.2mm using sample applicator. Developed the plate in the solvent system to a distance of 8cm. Scanned the plate densitometrically at 254nm using TLC Scanner. Observed the plate under UV light at 254nm & 366nm using CAMAG REPROSTAR 3.

RESULTS

The qualitative analysis of bioactive compounds for the ethanolic flower extract of *S.alata* have been analyzed in this study and there is wide range of phytochemical compounds present in the extracts shown in Table 1**.**

Table 1: Phytochemical screening of ethanolic extract of *Senna alata*

S.No	Phytoconstituents	Results
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	+
4	Quinones	-
5	Tannins	+
6	Steroids	-
7	Glycosides	+
8	Coumarins	-
9	Reducing Sugar	-

+ indicates presence whereas – indicates absence

High performance thin layer chromatography (HPTLC) is a valuable tool for reliable identification. The TLC procedure was optimized with a view to separate the compounds and to identify one of the phytochemical flavonoids in the extract. Initially, toluene: ethyl acetate: formic acid: methanol in varying ratios was tried along with several combinations of other

solvents. The developing system consists of toluene: ethyl acetate: formic acid: methanol (5.5:3:1:0.5 v/v/v/v) gave a sharp and well-defined band with Rf 0.60 for quercetin. The peak display of 1µl of standard (Figure 2) showed the presence of the bioactive compound flavonoid. The identity of the quercetin bands in sample chromatograms was confirmed by the chromatogram obtained from the sample with that obtained from the reference standard solution.

HPTLC analysis of ethanol extract of *Senna alata* flower was carried out along with the standard flavonoid quercetin and toluene: ethyl acetate: formic acid: methanol (5.5:3:1:0.5) as the mobile phase. The identity of the bands of quercetin in the methanol extract was confirmed by comparing the UV-Vis absorption spectra with those of standards using a CAMAG TLC scanner 3 (Figure 1). The standard quercetin has Rf value of 0.57 for quercetin (Table 2 and Figure 3). The 3-D spectrum of all tracks scanned at 254nm is shown (Figure 4).

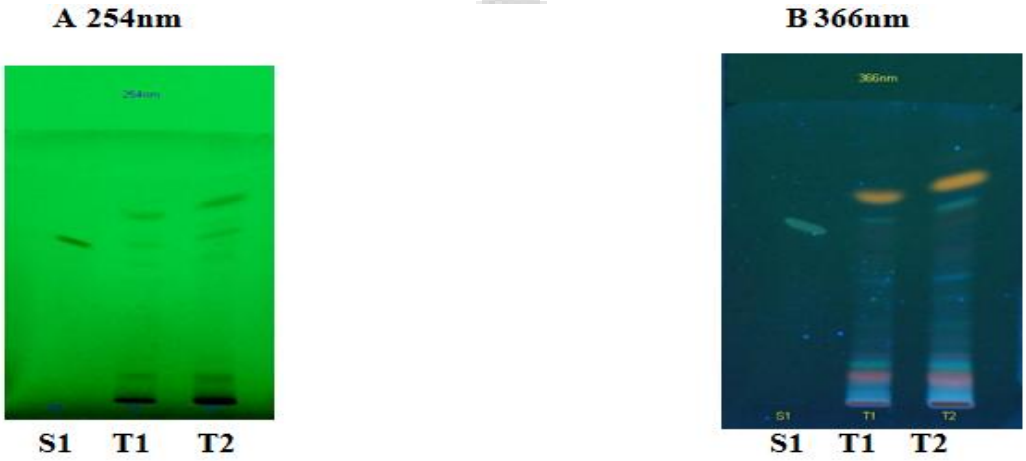

Figure 1: Chromatogram after derivatization, A) Under UV 254nm B) Under UV 366nm.

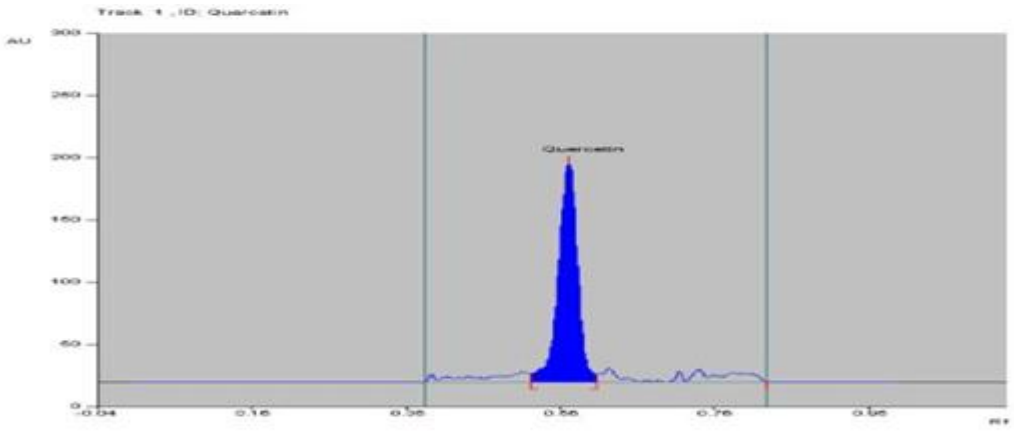

Figure : 2 Chromatogram of Standard Quercetin (1µl of standard)

Table 2: Shows peak table with Rf values, height and area of flavonoids and unknown compounds in methanol extract of *S.alata*

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.00	2.3	0.03	15.94	16.99	0.06	0.2	2408.0	14.22	Unknown
2	0.07	1.1	0.10	13.1	1.64	0.12	0.1	203.0	1.20	Unknown
3	0.22	4.6	0.26	16.2	2.01	0.27	4.7	295.7	1.75	Unknown
4	0.29	0.7	0.30	30.5	4.93	0.31	9.6	338.4	2.00	Unknown
5	0.32	9.2	0.32	24.3	3.03	0.34	8.3	193.0	1.14	Unknown
6	0.35	12.4	0.40	22.5	2.81	0.38	19.1	358.5	2.12	Unknown
7	0.38	19.1	0.47	34.0	4.24	0.41	23.9	551.4	3.26	Unknown
8	0.44	24.9	0.55	61.6	7.66	0.50	34.8	1697.5	11.21	Unknown
9	0.52	0.56	0.56	120.1	16.73	0.57	47.0	2534.5	14.07	Quercetin
10	0.57	0.58	0.58	55.3	6.90	0.61	26.1	1206.3	7.14	Unknown
11	0.62	0.66	0.66	195.1	24.34	0.71	38.9	6184.1	30.61	Unknown
12	0.71	0.72	0.72	40.5	5.05	0.74	22.6	714.0	4.22	Unknown
13	0.76	0.77	0.77	24.4	3.05	0.53	0.1	844.3	4.09	Unknown
14	0.92	0.95	0.95	12.8	1.60	0.97	0.1	201.8	1.19	Unknown

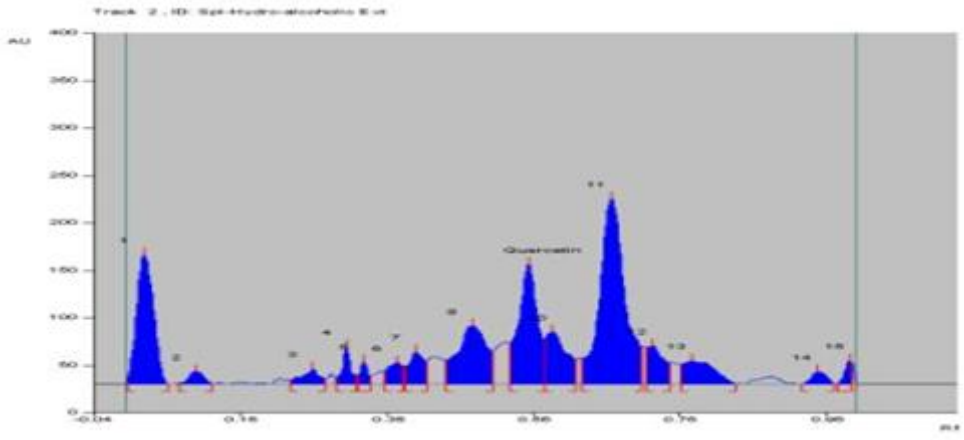

Figure : 3 Chromatogram of *S.alata* extract (5µl of standard)

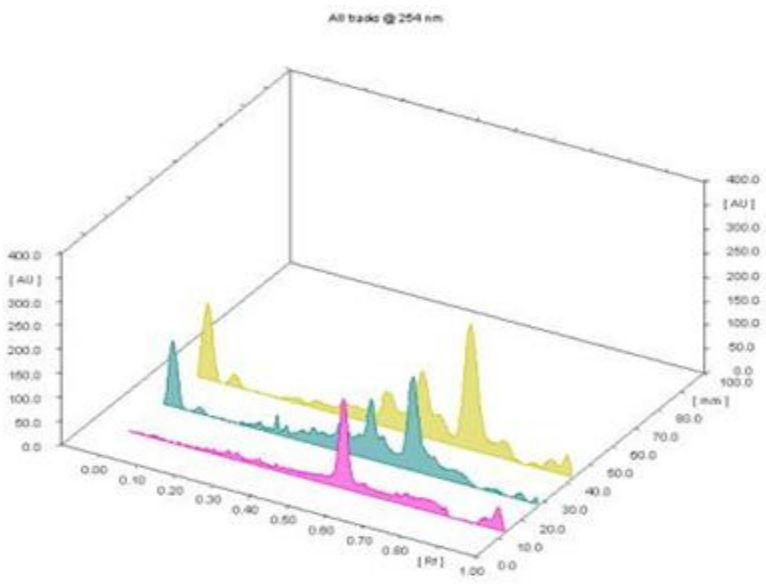

Figure: 4 3D display of HPTLC chromatogram of flower extracts of *S.alata*

DISCUSSION

In HPTLC profile, each and every metabolite has played specific role and function in harmony with other metabolites within the organization framework of the cells in the defense mechanism of the plants. Chromatographic fingerprinting of phytoconstituents can be used for the assessment of quality consistency and stability of herbal extracts or products by visible observation and comparison of the standardized fingerprint pattern [18]. According to WHO, it has emphasized the need to ensure the quality of medicinal plant products by using modern controlled techniques and applying suitable standards [19].

Flavonoids belong to a group of natural substances with variable phenolic structures and are found in fruit, vegetables, grains, bark, roots, stems, flowers, tea, and wine [20]. These natural products were known for their beneficial effects on health. Long before, flavonoids were isolated as the effective compounds. More than 4000 varieties of flavonoids have been identified, many of which are responsible for the attractive colors of flowers, fruit, and leaves [21]. Flavonoid intakes protect against coronary heart disease [22].

CONCLUSION

The HPTLC fingerprinting analysis showed that flavonoid compound quercetin is present within the ethanol extract of *S.alata*. Therefore, this study supports the use of flower extracts as

promising sources of potential antioxidants that may be effective as preventive agents against diseases. Future work would be interesting to know the chemical composition and better understand the mechanism of action of antioxidants present in the extract for development as drug for therapeutic application.

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