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Research Article

CHROMATOGRAPHIC FINGER PRINT STUDIES ON SAPONINS OF *AERVA LANATA* (L.) JUSS. EX SCHULTES BY USING HPTLC

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ABSTRACT

The present study was designed to determine the saponins HPTLC profile of the medicinally important plant *Aerva lanata* (L.) Juss. Ex Schultes. which will help in crude drug identification and in standardization of the quality and purity for various pharmaceutical industries. HPTLC studies were carried out following Harborne and Wagner et al. method. The Chloroform-Glacial acetic acid-Methanol-Water (6.4: 3.2: 1.2: 0.8) was employed as mobile phase for saponins. Linear ascending development was carried out in 20cm x 10cm twin trough glass chamber (Camag, Mutenz, Switzerland) saturated with the mobile phase and the chromatoplate development for two times with the same mobile phase to get good resolution of phytochemical contents. The developed plate was sprayed with Anisaldehyde sulphuric acid reagent as spray reagent and dried at 100°C in hot air oven for 10 min. The methanolic extract of stem, leaves, root, flower and seeds of *A. lanata* showed the presence of 21 different types of saponins with 21 different Rf values with range 0.01 to 0.98. Maximum number (9) of saponins has been observed in roots followed by stem (8). Among the three different saponins of reproductive parts (flowers and seeds), two saponins with Rf values 0.45 and 0.82 are unique to reproductive parts only. The saponins with the Rf value 0.42 is present commonly in all the vegetative parts of the plant. The saponins with Rf values 0.46, 0.49, 0.63, 0.73, 0.80 and 0.92 showed their unique presence only in the stem. The saponins of root also showed their uniqueness by the expression 0.01, 0.11, 0.26, 0.54, 0.55 and 0.74 in the saponins profile. The saponins with the Rf value 0.97 is present commonly in the roots and flowers & seeds of the plant. The saponins with the Rf value 0.84 show their joint presence in stem and root of *A. lanata*. The developed HPTLC fingerprints will help the manufacturer to distinguish the adulterant and standardization of herbal formulations. Such chemo finger printing will act as biochemical markers for this medicinally important plant in the pharma industry and plant systematic studies.

Keywords: Saponins, HPTLC profile, Fingerprint, Phytochemistry, Secondary metabolites

INTRODUCTION

Natural products derived from food and medicinal plants are the potential sources of antioxidant molecules [1,2]. Herbal drugs have been in exercise by different civilizations in various parts of the world for centuries to treat a large number of diseases. Today, the plant based medicines are being used worldwide as medication and suggest a broad spectrum of activity since ancient times. But Indian herbal drugs have still low acceptability in the world market due to insufficient scientific validation. International agencies especially WHO emphasized on quality standards of complex herbal formulations through scientific validation of single raw drugs. The drug efficiency depends upon the several active principles and components present in it. Many natural (age, origin) & scientific (methodology of drug formulation) factors influence the proportion of various components in plant material.

The well developed quality standards can be achieved only through systematic evaluation of the plant material using modern analytical techniques including chromatographic ones. TLC and HPTLC are methods commonly applied for the identification, assay and the testing of purity, stability, dissolution or content uniformity of raw materials (herbal and animal extracts, fermentation mixtures, drugs and excipients) and formulated products (pharmaceuticals, cosmetics, nutrients) [3]. HPTLC is a valuable tool for the investigation of herbal products with respect to different aspects of their quality. HPTLC analysis is comparatively short and many samples can conveniently be compared side by side on the same plate. This is particularly important for screening and inspection / selection of raw materials and for process control during manufacturing. HPTLC has been widely used for the phytochemical evaluation of the herbal drugs, due to its simplicity and minimum sample clean up requirement. HPTLC results are not only reported as peak data but can also be presented and communicated as images. Finger print analysis by HPTLC has become an effective and powerful tool for linking the chemical constituents' profile of the plants with botanical identity and for the estimation of chemical and biochemical markers [4-10]. A lot of works have been done for the HPTLC profile of medicinal plants in pharmaceuticals industries [11-15]. Only a few reports are available for the HPTLC profile of *A. lanata* [16-19]. But there is no report on the saponin profile of *A. lanata*. Traditionally, leaves of *Aerva lanata* (L.) Juss. Ex Schultes. are used as sap for eye-complaints; an infusion is given to cure diarrhoea and kidney stone; and the root is used in snake bite treatment. A leaf decoction preparation is used as gargle for treating sore throat and is also used in various complex treatments against guinea-worm. In addition to the traditional uses, the plant is reported for a number of pharmacological activities viz., anthelmintic, demulcent [20], anti-inflammatory [21], diuretic [22], expectorant, hepatoprotective [23] and nephroprotective [24], anti-diabetic, anti-hyperglycaemic [25,26], anti-microbial, cytotoxic [27], urolithiatic [28], hypoglycemic, anti-hyperlipidemic [29], anti-parasitic and anti - helmentic activities [30]. In order to identify the bioactive compounds responsible for the above pharmacological activities, phytochemical studies have been carried out by several researchers with the report of different kinds of bioactive compounds particularly alkaloids such as: canthin-6-one and beta-carboline, aervine (10-hydroxycanthin- 6-one), methylaervine (10-methoxycanthin-6-one), aervoside (10-β-dglucopyranosyloxycanthin-6-one) and aervolanine (3-(6-methyoxy-β-carbolin-1-yl) propionic acid) from leaves of *A. lanata* [31]. Hence, the present study was designed to determine the saponins HPTLC profile of the medicinally important plant *A. lanata* which will help in crude drug identification and in standardization of the quality and purity for various pharmaceutical industries as well as in the search for development of high yielding plant varieties either by plant breeding or biotechnological studies.

MATERIALS AND METHODS

Aerva lanata (L.) Juss. Ex Schultes. was collected from natural habitats, Rasipuram, Nammakkal, Tamil Nadu, India, and authenticated by Dr. E.G. Wesely and the specimens voucher were deposited in the St. Xavier's College Herbarium for further reference. The fresh leaves were shade dried and powdered using the electric homogenizer. The powdered samples were extracted with 150 mL of petroleum ether, methanol and ethyl acetate for 8 - 12 h by using the

Soxhlet apparatus. Preliminary phytochemical screening was done by following the standard method described by Harborne [32], HPTLC studies were carried out following Wagner et al [3]. For the present study CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 with 12 bit CCD camera for photo documentation, controlled by WinCATS 4 software were used. All the solvents used for HPTLC analysis was obtained from MERCK. The samples (5µL) were spotted in the form of bands of width 5 mm with a Camag microlitre syringe on pre-coated silica gel glass plate 60F-254 (20 × 10 cm with 250 µm thickness (E. Merck, Darmstadt, Germany) using a Camag Linomat IV (Switzerland). The plates were pre-washed by methanol and activated at 60°C for 5 min prior to chromatography. The sample loaded plate was kept in TLC twin trough developing chamber (after saturated with solvent vapor) with respective mobile phase (alkaloids) and the plate was developed in the respective mobile phase up to 90 mm. The Chloroform-Glacial acetic acid-Methanol-Water (6.4: 3.2: 1.2: 0.8) was employed as mobile phase for saponins. Linear ascending development was carried out in 20cm x 10cm twin trough glass chamber (Camag, Mutenz, Switzerland) saturated with the mobile phase and the chromatoplate development for two times with the same mobile phase to get good resolution of phytochemical contents. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25 ± 2°C). The developed plate was dried by hot air to evaporate solvents from the plate. The developed plate was sprayed with Anisaldehyde sulphuric acid reagent as spray reagent and dried at 100°C in hot air oven for 10 min. The plate was photo-documented at UV 366 nm and daylight using Photo-documentation (CAMAG REPROSTAR 3) chamber. Finally, the plate was fixed in scanner stage and scanning was done at 366 nm. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images under White light, UV light at 254 and 366 nm. Densitometric scanning was performed on Camag TLC scanner III and operated by CATS software (V 3.15, Camag). Yellow-brown, blue-violet coloured zones at Day light mode were present in the tracks, it was observed from the chromatogram after derivatization, which confirmed the presence of Saponin in the given standard and may be in the samples.

RESULTS

Diverse compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. The separation was achieved using Chloroform-Glacial acetic acid-Methanol-Water (6.4: 3.2: 1.2: 0.8) as the mobile phase (Fig.1.A-D). The methanolic extract of stem, leaves, root, flower and seeds of *A. lanata* showed the presence of 21 different types of saponins with 21 different Rf values with range 0.01 to 0.98 (Tables 1-5; Fig. 1 F- K; Fig. 2 A- E). In general, maximum number bands of saponins have been observed in vegetative parts when compared to the reproductive parts, it confirms the diversified saponin presence in the vegetative parts. Maximum number (9) of saponins has been observed in roots followed by stem (8). Among the three different saponins of reproductive parts (flowers and seeds), two saponins with Rf values 0.45 and 0.82 are unique to reproductive parts only. Five different types of saponins have been observed in the leaves of *A. lanata*. Among the five different saponins of leaves, only one (0.24) is showed similarity with other vegetative parts (root and stem). The saponin with Rf value 0.69, 0.85, 0.91 and 0.98 are not present in other aerial parts of the plant, they showed their unique presence only in the leaves of *A. lanata*. The saponins with the Rf value 0.42 is present commonly in all the vegetative parts of the plant. The saponins with Rf values 0.46, 0.49, 0.63, 0.73, 0.80 and 0.92 showed their unique presence only in the stem. The saponins of root also showed their uniqueness by the expression 0.01, 0.11, 0.26, 0.54, 0.55 and 0.74 in the saponins profile. The saponins with the Rf value 0.97 is present commonly in the roots, flowers and seeds of the plant. The saponins with the Rf value 0.84 show their joint presence in stem and root of *A. lanata* (Table -5).

Table 1: HPTLC – Saponin profile of the Methanolic extracts of Aerva lanata Root (Sample A)

Peak	Rf	Height	Area	Assigned substance
Standard	0.27	159.1	7662.0	Saponin
1	0.01	10.6	56.0	Unknown
2	0.11	11.2	300.2	Unknown
3	0.26	36.2	1399.1	Saponin 1
4	0.42	100.9	4059.6	Saponin 2
5	0.54	13.4	168.5	Unknown
6	0.55	17.0	313.7	Saponin 3
7	0.74	49.8	1537.7	Saponin 4
8	0.84	30.8	439.9	Saponin 5
9	0.97	23.6	140.2	Saponin 6

Table 2: HPTLC – Saponin profile of the Methanolic extracts of Aerva lanata Stem (Sample B)

Peak	Rf	Height	Area	Assigned substance
1	0.42	20.6	545.5	Saponin 1
2	0.46	31.3	458.6	Unknown
3	0.49	33.7	996.7	Unknown
4	0.63	23.5	280.4	Unknown
5	0.73	22.2	927.9	Unknown
6	0.80	28.1	154.2	Unknown
7	0.84	17.2	267.2	Saponin 2
8	0.92	26.5	613.7	Saponin 3

Table 3: HPTLC – Saponin profile of the Methanolic extracts of Aerva lanata Leaves (Sample C)

Peak	Rf	Height	Area	Assigned substance
1	0.42	75.7	3680.6	Saponin 1
2	0.69	14.7	350.7	Saponin 2
3	0.85	95.0	2167.6	Saponin 3
4	0.91	155.6	6440.0	Saponin 4
5	0.98	46.3	407.7	Saponin 5

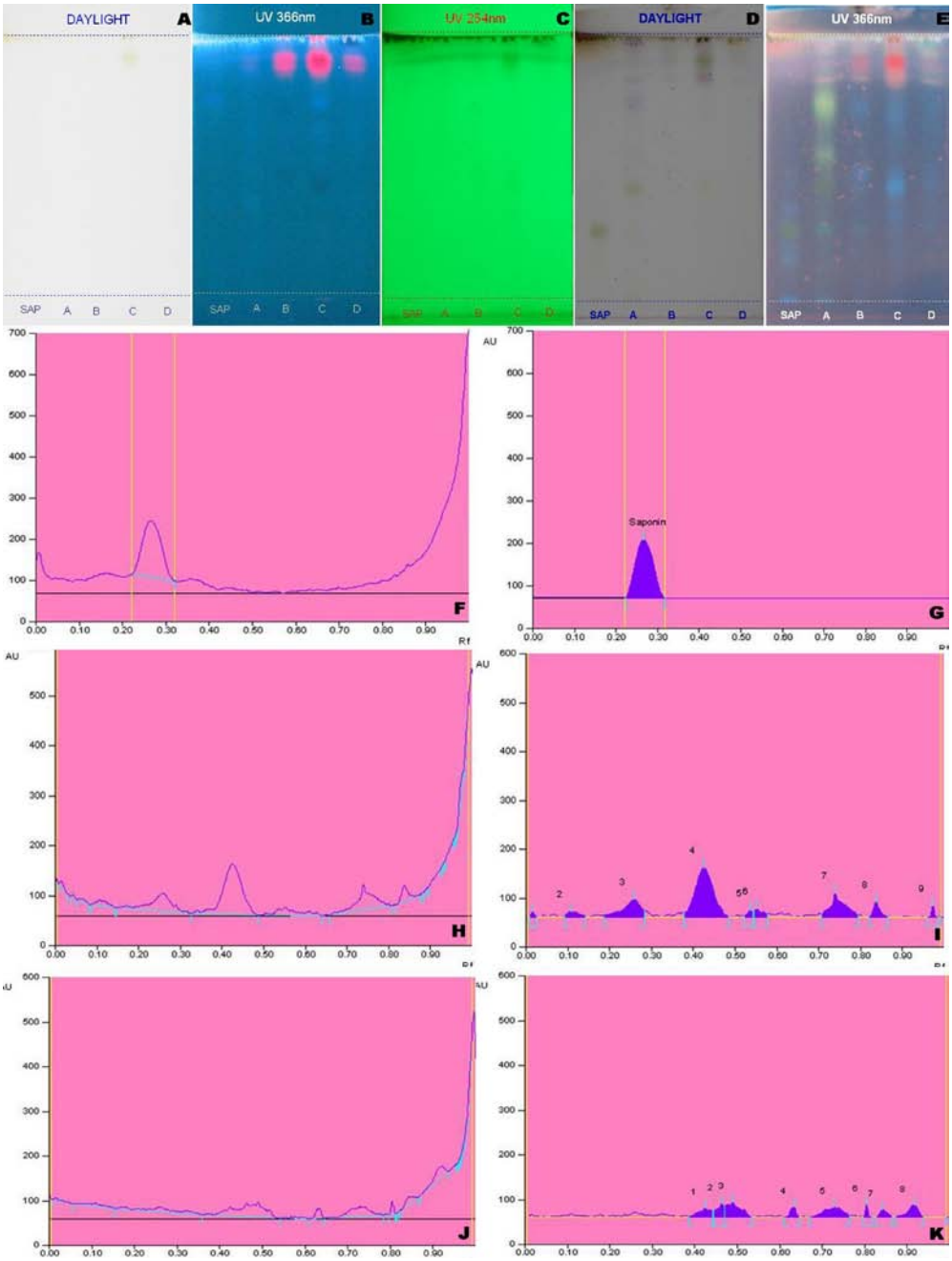

Fig. 1: HPTLC Studies on the Saponins of the medicinally important plant

***Aerva lanata* L. – Vegetative and Reproductive Parts**

A. HPTLC profile of the methanolic extract of *Aerva lanata* under Daylight

B. HPTLC profile of the methanolic extract of *Aerva lanata* under UV 366

C. HPTLC profile of the methanolic extract of *Aerva lanata* under UV 254

D. HPTLC profile of the methanolic extract of *Aerva lanata* under Day Light - After Derivation

E. HPTLC profile of the methanolic extract of *Aerva lanata* under UV 366 – After derivation

F. HPTLC Chromatogram of Standard Saponin [Scanned at 500nm]

G. HPTLC Chromatogram of standard Saponin Peak densitogram display [Scanned at 500nm]

H. HPTLC Chromatogram of *Aerva lanata* Root - Baseline display [Scanned at 500nm]

I. HPTLC Chromatogram of *Aerva lanata* Root - Peak densitogram display [Scanned at 500nm]

J. HPTLC Chromatogram of *Aerva lanata* Stem - Baseline display [Scanned at 500nm]

K. HPTLC Chromatogram of *Aerva lanata* Stem - Peak densitogram display [Scanned at 500nm]

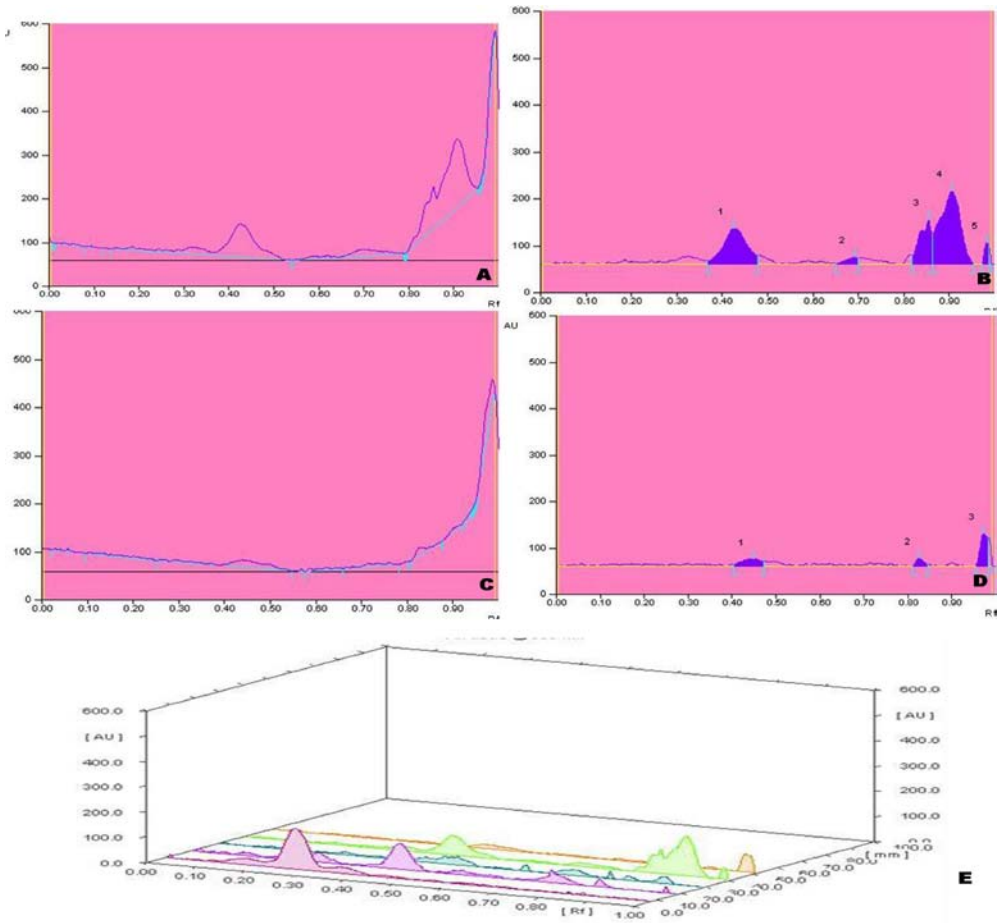

Fig. 2: HPTLC Studies on the Saponins of the medicinally important plant *Aerva lanata* L. – Leaves, Flowers and Seeds

A. HPTLC Chromatogram of *Aerva lanata* Leaves - Baseline display [Scanned at 500nm]

B. HPTLC Chromatogram of *Aerva lanata* Leaves - Peak densitogram display [Scanned at 500nm]

C. HPTLC Chromatogram of *Aerva lanata* Flowers and Seeds - Baseline display [Scanned at 500nm]

D. HPTLC Chromatogram of *Aerva lanata* Flowers and Seeds - Peak densitogram display [Scanned at 500nm]

E. 3D display of HPTLC Chromatogram of *Aerva lanata* – Root, Stem, Leaves, Flower and Seeds

Table 4: HPTLC – Saponin profile of the Methanolic extracts of Aerva lanata flowers and seeds (Sample D)

Peak	Rf	Height	Area	Assigned substance
1	0.45	15.3	641.5	Unknown
2	0.82	16.3	265.5	Saponin 1
3	0.97	70.3	1058.3	Saponin 2

Table 5: HPTLC – Saponin profile of the Methanolic extracts of *Aerva lanata*

Rf- Value	Root	Stem	Leaves	Flowers & Seed
0.01	+			
0.11	+			
0.26	+			
0.42	+	+	+	
0.45				+
0.46		+		
0.49		+		
0.54	+			
0.55	+			
0.63		+		
0.69			+	
0.73		+		
0.74	+			
0.80		+		
0.82				+
0.84	+	+		
0.85			+	
0.91			+	
0.92		+		
0.97	+			+
0.98			+	

DISCUSSION

Saponin is an important class of natural products that can be found primarily in roots, petals and foliage of many plants, as well as in some marine animals [34]. In the present study we observed the saponin presence in the stem, leaves, root, flower and seeds of *A. lanata.* Saponins occur constitutively in many plant species, in both wild plants and cultivated crops. Their structures are characterized by the presence of a steroid or triterpene group, referred to as the aglycone, linked to one or more sugar molecules. The presence of both polar (sugar) and non-polar (steroid or triterpene) groups provide saponins with strong surface-active properties which then are responsible for many of its adverse and beneficial biological effects [35].

In cultivated crops the triterpenoid saponins are generally predominant, while steroid saponins are common in plants used as herbs or for their health-promoting properties [36]. Triterpenoid saponins have been detected in many legumes such as soyabeans, beans, peas, lucerne, etc. and also in alliums, tea, spinach, sugar beet, quinoa, liquorice, sunflower, horse chestnut, and ginseng. Steroid saponins are found in oats, capsicum peppers, aubergine, tomato seed, alliums, asparagus, yam, fenugreek, yucca and ginseng. Yamuna et al., observed the steroid and triterpenoids presence in the stem, leaves, root, flower and seeds of *A. lanata.* Yucca schidigera is the most common commercial source of steroid saponins [37]. Saponins are generally known as non-volatile, surface active compounds that are widely distributed in nature, occurring primarily in the plant kingdom [38-40]. Saponins have a diverse range of properties, which include sweetness and bitterness [41-43], foaming and emulsifying properties [44], pharmacological and medicinal properties [45], strong haemolytic properties, as well as antimicrobial, insecticidal, and molluscicidal activities [46]. Saponins have found wide applications in beverages and confectionery, as well as in cosmetics [47] and pharmaceutical products [48]. Saponins have a potential as pharmaceutical synthons and have been used in hormone synthesis [49]. Most saponins possess a variety of bioactivities (e.g., cardiac, antifungal, hemolytic activities and abilities to affect metabolism and biosynthesis); they are among the major effective components in nutriceutical products [50]. The results of the present study and previous studies confirmed that the stem, leaves, root, flower and seeds of *A. lanata* possess the steroid, triterpenoids and saponins. It confirmed the pharmacological activities of *A. lanata* and suggest that the plant can be used to control insects, molluss etc. The biological and biochemical properties of saponins suggest that the *A. lanata* possess cardiac, antifungal, hemolytic activities and abilities to affect metabolism and biosynthesis. The HPTLC profile developed for the identification of *A. lanata* is simple, precise, specific, accurate, rapid and cost effective. This HPTLC profile may be used effectively for the identification plant and its derived products. The methanolic HPTLC chromatographic finger prints and could be used for the quality assessment of the plant.

CONCLUSION

The developed HPTLC fingerprints will help the manufacturer to distinguish the adulterant and standardization of herbal formulations. Such chemo finger printing will act as biochemical markers for this medicinally important plant in the pharma industry and plant systematic studies.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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