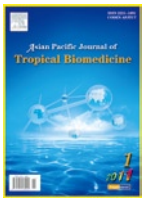
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb



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Chromatographic fingerprint analysis on flavonoids constituents of the medicinally important plant *Aerva lanata* L. by HPTLC technique

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ARTICLE INFO

Article history:
Received 16 July 2011
Received in revised form 7 August 2011
Accepted 28 August 2011
Available online 10 September 2011

Keywords:
Flavonoids
HPTLC profile
Fingerprint
Phytochemical screening
Aerva lanata
Chromatography

ABSTRACT

Objective: To identify the flavonoids HPTLC profile (bio-marker), at species level, for the identification and confirmation of crude drugs, HPTLC separation was initiated on different parts of *Aerva lanata* (*A. lanata*) L. from South India. **Methods:** Preliminary phytochemical screening was done by following the method of Harborne. HPTLC studies were carried out following Harborne and)Wagner *et al* method. The ethyl acetate-butanone-formic acid-water (5:3:1:1) was employed as mobile phase for flavonoids. **Results:** The methanolic extract of stem, leaves, root, flower and seeds of *A. lanata* showed the presence of 19 different types of flavonoids with 19 different Rf values with range 0.05 to 0.98. In general more degree of flavonoids diversity has been observed in vegetative parts when compared to the reproductive part. Maximum number of flavonoids has been observed in leaves followed by root and leaves. **Conclusions:** The results of the present study supplement the folkloric usage of the studied plants which possess several known and unknown bioactive compounds with bio-activity. By isolating and identifying these bioactive compounds new drugs can be formulated to treat various diseases. It can be concluded that constituents of *A. lanata* have effective components which can be utilized as useful herb for alleviation of various illness and disorder.

1. Introduction

Flavonoids comprise a large group of plant secondary metabolites characterized by a diphenylpropane structure (C6-C3-C6). They are widely distributed throughout the plant kingdom and are commonly found in fruits, vegetables and certain beverages. 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[1-3]. Preclinical *in vitro* and *in vivo* investigations have shown plausible mechanisms by which flavonoids may confer cancer and cardiovascular protection[4]. 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 comes 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[5]. Increasingly, flavonoids are becoming the subject of medical research. They 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 anti-tumour activity[6]. Flavonoids are widely distributed in plants fulfilling many functions. Flavonoids are the most important plant pigments for flower coloration producing yellow or red/blue pigmentation in petals designed to attract pollinator animals. The flavonoids quercetin is known for its ability to relieve hay fever, eczema, sinusitis and asthma. Epidemiological studies have illustrated that heart diseases are inversely related to flavonoid intake. Studies have shown that flavonoids prevent the oxidation of low-density lipoprotein thereby reducing the risk for the development of atherosclerosis. Consumers and food manufacturers have become interested

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in flavonoids for their possible medicinal properties, especially their putative role in prevention of cancers and cardiovascular diseases.

Aerva lanata (*A. lanata*) L. is an important medicinal plant, found throughout tropical India as a common weed in fields and wasteland[7]. Even now, wild collection of the species continues to be a source of raw drug in Ayurvedic preparations. Because of its popularity in folk medicine, *A. lanata* has become the subject of intense pharmacological and chemical studies for the last 30 years. Numerous studies have proven its versatile pharmacological activities: anthelmintic, demulcent, anti-inflammatory, diuretic, expectorant, hepatoprotective[8], nephroprotective[9], anti-diabetic activity, anti-hyperglycaemic activity in rats[10], anti-microbial, cytotoxic, urolithiatic, hypoglycemic, anti-hyperlipidemic[11], anti-parasitic and anti-helmentic activities[12]. In order to identify the bioactive compounds responsible for the above pharmacological activities phytochemical studies have been carried out by several workers 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*. The main limitation in the use of traditional remedies is the lack of standardization of raw material, manufacturing process and the final product. A biomarker on the other hand is a group of chemical compounds which are in addition to being unique for that plant material also correlates with biological efficacy. So the need arises to lay standards by which the right material could be selected and incorporated into the formulation. HPTLC is a valuable tool for reliable identification because it can provide chromatographic fingerprints that can be visualized and stored as electronic images[13-16]. A detailed exo-morphology, histo-morphology and physicochemical studies on the leaf and stem and micro-morphological of *A. lanata* have been carried out[17]. However, more work needs to be undertaken to fully characterize these compounds, to identify the active molecules with bioactive roles. To fulfill the requirement, the present study was intended to resolve the chemical profile and flavonoids constituents present in the stem, leaves, root and reproductive parts (Flower and Seed) of *A. lanata* L, which will be useful for the proper identification of commercial samples.

2. Materials and methods

A. lanata was collected from natural habitats, Coimbatore District, Tamil Nadu, India, and authenticated by Dr. EG Wesely and the specimens voucher were deposited in the St. Xavier's College Herbarium for further reference. The fresh materials were shade dried and powdered using the electric homogenizer. The powdered samples were extracted with 150 mL of solvent methanol for 8 - 12 h by using the soxhlet apparatus. Preliminary phytochemical screening was done by following the method of Harborne[18], HPTLC studies were carried out following Harborne[19] and Wagner *et al*[20]. For the present study CAMAG HPTLC system equipped with Linomat V applicator, Thin-Layer Chromatography (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 100 mg extract was dissolved in 5 mL of methanol and the solution was centrifuged at 3000 rpm for 5 min and used for HPTLC analysis as test solution. The samples (5 μL) 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 cm × 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 ℃ 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 (flavonoids) and the plate was developed in the respective mobile phase up to 90 mm. The ethyl acetate-butanone-formic acid-water (5:3:1:1) was employed as mobile phase for flavonoids. Linear ascending development was carried out in 20 cm×10 cm 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) ℃. The developed plate was dried by hot air to evaporate solvents from the plate. The developed plate was sprayed with 1% ethanolic aluminum chloride reagent as spray reagent and dried at 100 ℃ in hot air oven for 2 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).

3. Results

The results of the preliminary phytochemical studies confirms the presence of flavonoids, steroids, alkaloids, glycosides, terpenoids, sugars and amino acids in the methanolic extracts of *A. lanata* stem, leaves, root and reproductive parts. Different compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. The desired aim was achieved using ethyl acetate-butanone-formic acid-water

(5:3:1:1) as the mobile phase (Table 1 – 5); (Figure 1. A–E); (Figue 2. A – K). The methanolic extract of stem, leaves, root, flower and seeds of *A. lanata* showed the presence of 19 different types of flavonoids with 19 different Rf values with range 0.05 to 0.98 (Table 1 – 5). In general more degree of flavonoids diversity has been observed in vegetative parts when compared to the reproductive part. Maximum number of flavonoids has been observed in leaves followed by root and leaves [6,8]. Among the eight different flavonoids of stem, five flavonoids with Rf values 0.05, 0.15, 0.88, 0.94 and 0.98 are unique to stem only (Table 2). Six different types of flavonoids have been observed in leaves of *A. lanata*. Among six different flavonoids of leaves, 0.29 and 0.66 are unique to the leaves and they are not present in other vegetative and reproductive parts of the plant. The flavonoids with Rf values 0.12, 0.55 and 0.87 are showed their unique presence only in the root of *A. lanata*. Like that the flavonoids with Rf value 0.34, 0.63, 0.84 and 0.96 are present only in reproductive parts. The flavonoids with the Rf value 0.28 is present commonly in root and stem of the plant. The flavonoids with the Rf values 0.11 and 0.33 are showed their jointly presence in stem and leaves of *A. lanata*. The flavonoids with the Rf values 0.97 is expressed jointly in root and leaves of *A. lanata*. The flavonoids with the Rf values 0.14 is showed its occurrence commonly in reproductive parts and leaves of *A. lanata*.

Table 1
HPTLC–Flavonoids profile of the methanolic extracts of *A. lanata* L – Root.

Peak	Rf	Height	Area	Assigned substance
Standard	0.27	541.7	17490.8	Rutin standard
1	0.12	14.0	193.0	Unknown
2	0.14	26.6	479.4	Unknown
3	0.28	36.8	1046.1	Unknown
4	0.55	19.9	322.7	Unknown
5	0.87	158.6	5760.1	Flavonoid 1
6	0.97	29.9	513.5	Unknown

Table 2
HPTLC – Flavonoids profile of the methanolic extracts of A. *lanata* L – Stem.

Peak	Rf	Height	Area	Assigned substance
1	0.05	28.1	552.7	Flavonoid 1
2	0.11	213.7	4801.3	Flavonoid 2
3	0.15	316.0	7648.2	Flavonoid 3
4	0.28	65.7	1659.7	Unknown
5	0.33	168.0	6052.8	Flavonoid 4
6	0.88	15.3	461.3	Unknown
7	0.94	34.1	485.1	Unknown
8	0.98	31.8	304.8	Unknown

Table 3
HPTLC – Flavonoids profile of the methanolic extracts of *Aerva lanata* L – Leaf.

Peak	Rf	Height	Area	Assigned substance
1	0.11	47.8	854.8	Unknown
2	0.14	237.4	5279.7	Flavonoid 1
3	0.29	13.6	289.0	Unknown
4	0.33	65.0	2336.9	Flavonoid 2
5	0.66	12.2	155.0	Unknown
6	0.97	63.1	999.1	Unknown

Table 4
HPTLC – Flavonoids profile of the methanolic extracts of *A. lanata* L – Flower and seeds.

Peak	Rf	Height	Area	Assigned substance
1	0.14	145.1	3745.6	Flavonoid 1
2	0.34	55.4	2980.2	Flavonoid 2
3	0.63	27.2	1184.4	Flavonoid 3
4	0.84	111.0	3615.4	Flavonoid 4
5	0.96	59.1	1146.6	Unknown

Table 5
Flavonoids profile of *A. lanata* L aerial and underground parts.

Rf	Root	Stem	Leaves	Flower
0.05	–	+	–	–
0.11	–	+	+	–
0.12	+	–	–	–
0.14	+	–	+	+
0.15	–	+	–	–
0.28	+	+	–	–
0.29	–	–	+	–
0.33	–	+	+	–
0.34	–	–	–	+
0.55	+	–	–	–
0.63	–	–	–	+
0.66	–	–	+	–
0.84	–	–	–	+
0.87	+	–	–	–
0.88	–	+	–	–
0.94	–	+	–	–
0.96	–	–	–	+
0.97	+	–	+	–
0.98	–	+	–	–

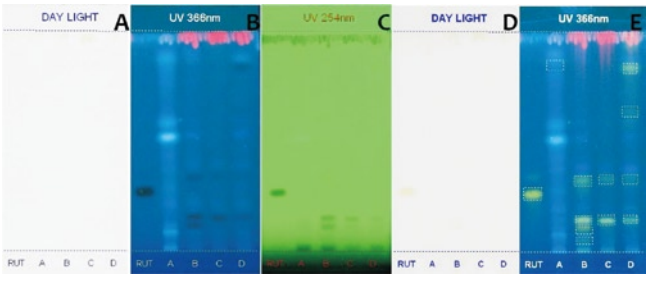

Figure 1. HPTLC studies on the flavonoids of the medicinally important plant *A. lanata* L. – vegetative and reproductive parts. A) HPTLC profile of the methanolic extract of *A. lanata* under daylight; B) HPTLC profile of the methanolic extract of *A. lanata* under UV 366; C) HPTLC profile of the methanolic extract of *A. lanata* under UV 254; D) HPTLC profile of the methanolic extract of *A. lanata* under day light – after derivation; E) HPTLC profile of the methanolic extract of *A. lanata* under UV 366 –after derivation.

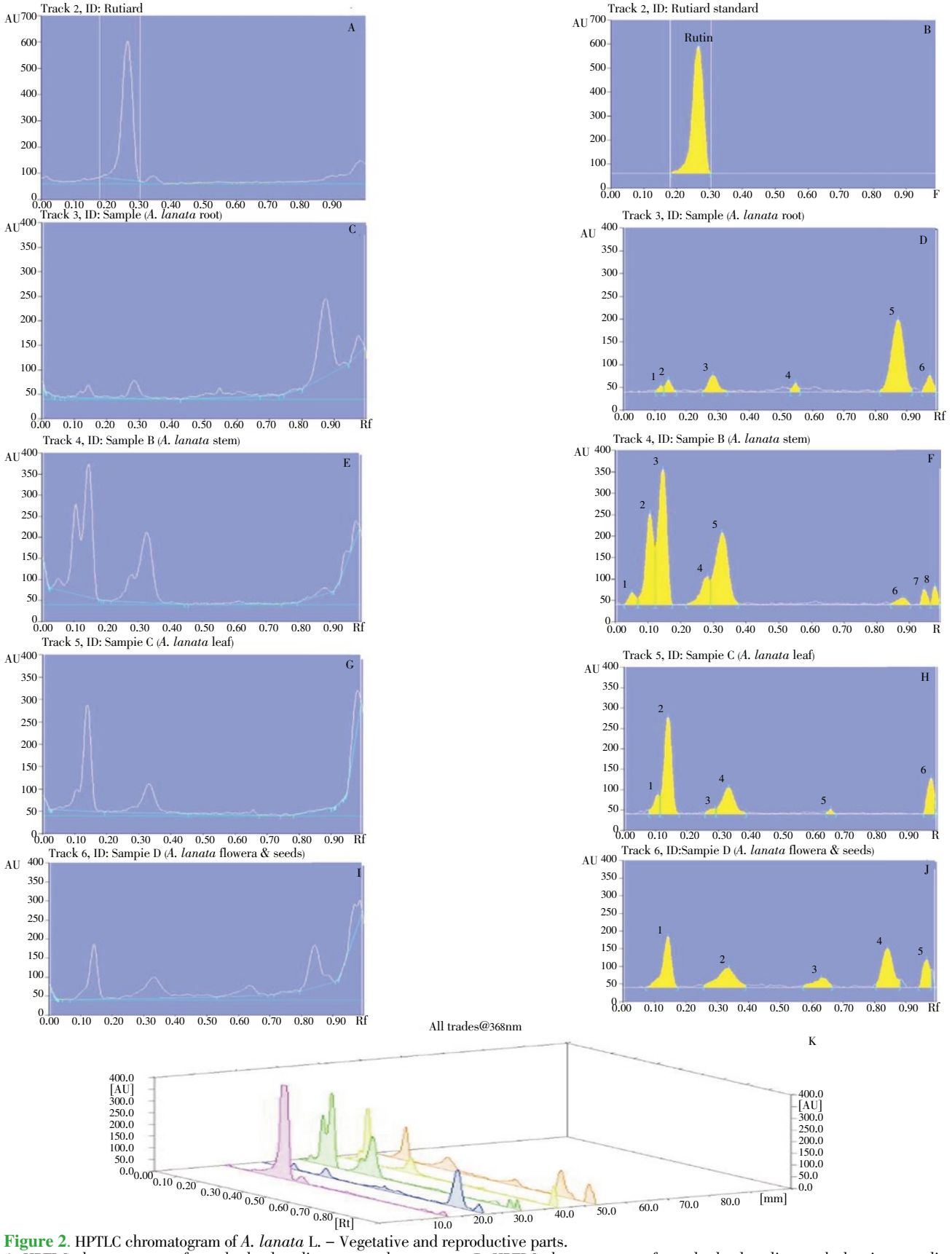

Figure 2. HPTLC chromatogram of *A. lanata* L. – Vegetative and reproductive parts.
A) HPTLC chromatogram of standard solasodine (scanned at 500 nm); B) HPTLC chromatogram of standard solasodine peak densitogram display (scanned at 500 nm); C) HPTLC chromatogram of *A. lanata* root – baseline display (scanned at 500 nm); D. HPTLC chromatogram of *A. lanata* root – peak densitogram display (scanned at 500 nm); E) HPTLC chromatogram of *A. lanata* stem – baseline display (scanned at 500 nm); F) HPTLC chromatogram of *A. lanata* stem – peak densitogram display (scanned at 500 nm); G) HPTLC chromatogram of *A. lanata* leaf – baseline display (scanned at 500 nm); H) HPTLC chromatogram of *A. lanata* leaf – peak densitogram display (scanned at 500 nm); I) HPTLC chromatogram of *A. lanata* flowers and seeds – baseline display (scanned at 500 nm); J) HPTLC chromatogram of *A. lanata* flowers and seeds – peak densitogram display (scanned at 500 nm); K) 3D display of HPTLC chromatogram of *A. lanata* – root, stem, leaves, flower and seeds.

4. Discussion

Flavonoids are ubiquitous in photosynthesising cells and therefore occur widely in the plant kingdom[10]. They are found in fruit, vegetables, nuts, seeds, stems and owers and represent a common constituent of the human diet. The results of the present study also confirm the flavonoids presence in the methanolic extract of stem, leaves, root, flower and seeds of *A. lanata* and supplemented the previous observations. In traditional medicines, medicinal plants have contributed hugely to the traditional and western medicines through providing ingredients for drugs or having played central roles in the drug discovery. The evaluation of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in herbal pharmacopoeia, pharmacognostical parameters and standards must be established. Chromatographic finger printing of phyto constituents 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[21]. In the present study we established the HPTLC profile for the vegetative and reproductive parts of *A. lanata* to identify and to differentiate the *A. lanata* from the other crude drugs and adulterants. HPLTC is useful alternative under circumstances where the other slower and more costly chromatographic methods are not appropriate. It was suitable methods to standardize raw herbs, active constituent enriched extracts and their formulations. The HPTLC method developed for the identification of *A. lanata* is simple, precise, specific, accurate, rapid and cost effective. This HPTLC procedure may be used effectively for the in plant and its derived products. Developed HPTLC chromatogram of methanol extracts of vegetative and reproductive parts of *A. lanata* may be treated chromatographic finger prints and could be used efficiently for identification, and quality assessment of the plant.

The developed HPTLC fingerprints will help the manufacturer for quality control and standardization of herbal formulations. Such finger printing is useful in differentiating the species from the adulterant and 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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