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Phytochemical studies on the terpenoids of medicinally important plant *Aerva lanata* L. using HPTLC

Yamunadevi M[1], Wesely EG[2], Johnson M[3*]

[1]*Research & Development Department, Bharathiyar University, Coimbatore-641 046, Tamil Nadu, India*
[2]*Department of Botany, Arignar Anna Government Arts College, Namakkal-637 002, Tamil Nadu, India*
[3]*Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India-627 002*

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ABSTRACT

Objective: To elucidate the terpenoids profile of *Aerva lanata* (*A. lanata*) using high performance thin layer chromatography (HPTLC). **Methods:** Preliminary phytochemical screening was done and HPTLC studies were carried out. The *n*-hexane: ethyl acetate (7.2: 2.8) was employed as mobile phase for terpenoids. **Results:** The desired aim was achieved using *n*-hexane-ethyl acetate (7.2: 2.8) as the mobile phase. The methanolic extract of stem, leaves, root, flower and seeds of *A. lanata* showed the presence of 27 different types of terpenoids with 27 different R_f values in the range of 0.06 to 0.97. The developed HPTLC method for terpenoid profile is simple, precise and accurate and can be used for the identification and commercial application. **Conclusions:** HPTLC profile of terpenoids has been chosen here to reveal the diversity existing at biochemical level in *A. lanata*. Such finger printing is useful in differentiating the species from the adulterant and act as a biochemical marker for this medicinally important plant in the pharmaceutical industry and plant systematic studies.

1. Introduction

Aerva lanata (*A. lanata*) known as Polpala is a prostrate to decumbent, sometimes erect herb, found throughout tropical India as a common weed in fields and wasteland. Traditionally, *A. lanata* Linn. leaves are used as sap for eye complaints, an infusion is given to cure diarrhea and kidney stone, and 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. A variety of pharmacological activities of this ethnomedicinally important plant has been reported as follows: anthelmintic, demulcent, anti-inflammatory, diuretic, expectorant, hepatoprotective and nephroprotective activities[1]. Alcoholic extract of shoots of *A. lanata* has shown significant antidiabetic and antihyperglycaemic activities in rats[2]. Antimicrobial, cytotoxic, urolithiatic, hypoglycemic, antihyperlipidaemic, antiparasitic, antihelmentic activities have also been reported in *A. lanata* by various workers[3,4]. Although the preliminary phytochemical studies revealed the presence of various bioactive compounds other than alkaloids, there is no detail study on phytoprofiling of *A. lanata*. In the present study, effort has been made to elucidate the terpenoids profile of *A. lanata* using high performance thin layer chromatography (HPTLC).

*Corresponding author: Johnson M, Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India-627002.
Tel: + 91 97 86 92 43 34
Fax: + 91 462 2561 765
E-mail: ptcjohnson@gmail.com

2. Materials and methods

A. lanata was collected from natural habitats, Coimbatore district, Tamil Nadu, India, and authenticated by Dr. EG Wesely and the voucher specimens were deposited in the 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 following the method of Harborne[5]. HPTLC studies

were carried out following the method of Misra *et al*[6]. 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 was used. All the solvents used for HPTLC analysis were 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 precoated 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 thin-layer chromatography (TLC) twin through developing chamber after saturated with solvent vapor with respective mobile phase (terpenoids) and the plate was developed in the respective mobile phase up to 90 mm. The *n*-hexane: ethyl acetate (7.2: 2.8) was employed as mobile phase for terpenoids. 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 was developed twice 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 plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images under white light, UV light at 254 and 366 nm. The developed plate was sprayed with anisaldehyde sulphuric acid as spray reagent and dried at 100 ℃ in hot air oven for 3 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. Densitometric scanning was performed on Camag TLC scanner III and operated by CATS software (V 3.15, Camag).

3. Results

Preliminary phytochemical examination of methanolic extracts of *A. lanata* Linn. revealed the presence of steroids, terpenoids, flavoniods, alkaloids, glycosides, sugar, carbohydrate, proteins, ash content and aminoacids. 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 *n*-hexane-ethyl acetate (7.2: 2.8) as the mobile phase. The methanolic extract of stem, leaves, root, flower and seeds of *A. lanata* showed the presence of 27 different types of terpenoids with 27 different R_f values in the range of 0.06 to 0.97 (Table 1). In general more degree of terpenoid diversity was observed in vegetative parts when compared to the reproductive part. Maximum number (11) of terpenoid was observed in leaves followed by stem (9). Among the eleven

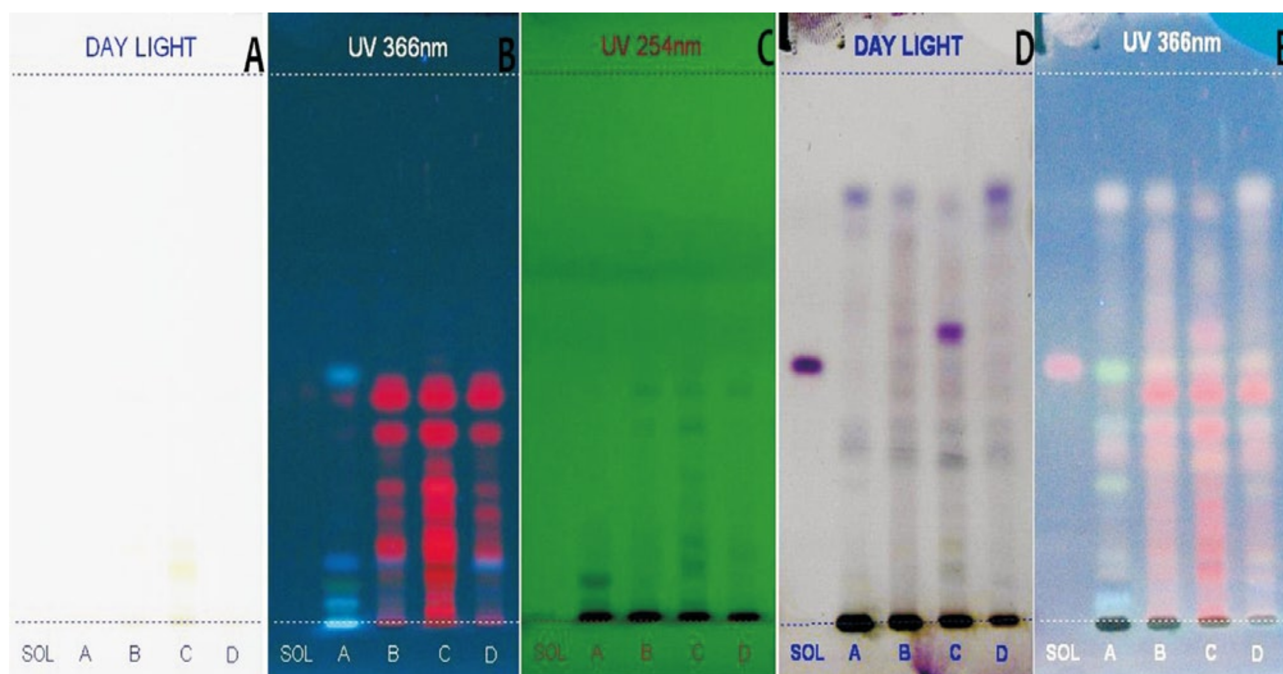

Figure 1. HPTLC studies on the terpenoids of medicinally important plant *A. lanata* L. (stem, leaves and roots).
A: HPTLC of the methanolic extract of *A. lanata* under daylight; B: HPTLC of the methanolic extract of *A. lanata* under UV 366 nm; C: HPTLC of the methanolic extract of *A. lanata* under UV 254 nm; D: HPTLC of the methanolic extract of *A. lanata* under daylight-after derivation; E: HPTLC of the methanolic extract of *A. lanata* under UV 366 nm after derivation.

Table 1
HPTLC terpenoid profile of the methanolic extracts of *A. lanata* L. (root, stem, leaf, flower and seeds).

Parts of *A. lanata*	Peak	R_f	Height	Area	Assigned substance
Root	1	0.46	434.3	14285.0	Solanesol standard
	2	0.08	19.3	382.7	Unknown
	3	0.24	26.4	637.2	Unknown
	4	0.31	109.4	5761.9	Terpenoid 1
	5	0.45	45.9	2307.9	Unknown
	6	0.56	64.1	2434.3	Terpenoid 2
	7	0.57	63.2	2591.4	Unknown
	8	0.71	78.8	3014.4	Terpenoid 3
	9	0.77	185.7	7333.9	Terpenoid 4
Stem	1	0.30	103.2	4283.9	Terpenoid 1
	2	0.35	89.5	2755.0	Unknown
	3	0.41	96.9	3870.1	Terpenoid 2
	4	0.46	99.4	3342.9	Terpenoid 3
	5	0.52	125.8	5003.1	Terpenoid 4
	6	0.57	94.2	3966.2	Terpenoid 5
	7	0.69	106.9	5658.2	Terpenoid 6
	8	0.76	129.1	5524.1	Terpenoid 7
	9	0.88	10.1	196.1	Unknown
Leaf	1	0.09	56.4	1410.9	Terpenoid 1
	2	0.13	31.1	608.0	Terpenoid 2
	3	0.20	17.6	350.4	Terpenoid 3
	4	0.29	134.5	4687.9	Terpenoid 4
	5	0.34	88.0	2868.1	Terpenoid 5
	6	0.41	99.9	4362.9	Terpenoid 6
	7	0.45	91.5	2508.6	Terpenoid 7
	8	0.52	293.0	12801.0	Terpenoid 8
	9	0.62	72.8	3958.7	Terpenoid 9
	10	0.75	96.4	4171.5	Terpenoid 10
	11	0.97	11.1	114.1	Unknown
Flower and seeds	1	0.06	32.0	568.8	Terpenoid 1
	2	0.30	76.3	3189.8	Terpenoid 2
	3	0.35	63.0	1925.0	Terpenoid 3
	4	0.41	63.6	2150.5	Terpenoid 4
	5	0.46	66.5	2435.5	Terpenoid 5
	6	0.53	71.8	3008.1	Unknown
	7	0.64	66.6	3499.5	Terpenoid 6
	8	0.77	208.2	13641.4	Terpenoid 7

different terpenoids of leaves, eight terpenoids are unique to leaves only (Table 1). Eight different types of terpenoids were observed in root and reproductive parts (flowers-seeds). Among eight different terpenoids of underground part root, five are unique to the root only and they are not present in the aerial parts of the plant. Two different terpenoids with R_f values of 0.53, 0.64 are unique in the reproductive parts only. Like that the terpenoids with R_f values of 0.69 and 0.76 are present only in stem. The terpenoid with the R_f value of 0.41 is present commonly in all the aerial parts (stem, leaves, and reproductive parts) of the plant.

Table 2
Terpenoids profile of aerial and underground parts of *A. lanata* L.

R_f	Root	Stem	Leaves	Flower
0.06				+
0.08	+			
0.09			+	
0.13			+	
0.20			+	
0.24	+			
0.29			+	
0.30		+		+
0.31	+			
0.34			+	
0.35		+		+
0.41		+	+	+
0.45	+		+	
0.46		+		+
0.52		+	+	
0.53				+
0.56	+			
0.57	+	+		
0.62			+	
0.64				+
0.69		+		
0.71	+			
0.75			+	
0.76		+		
0.77	+			+
0.88		+		
0.97			+	

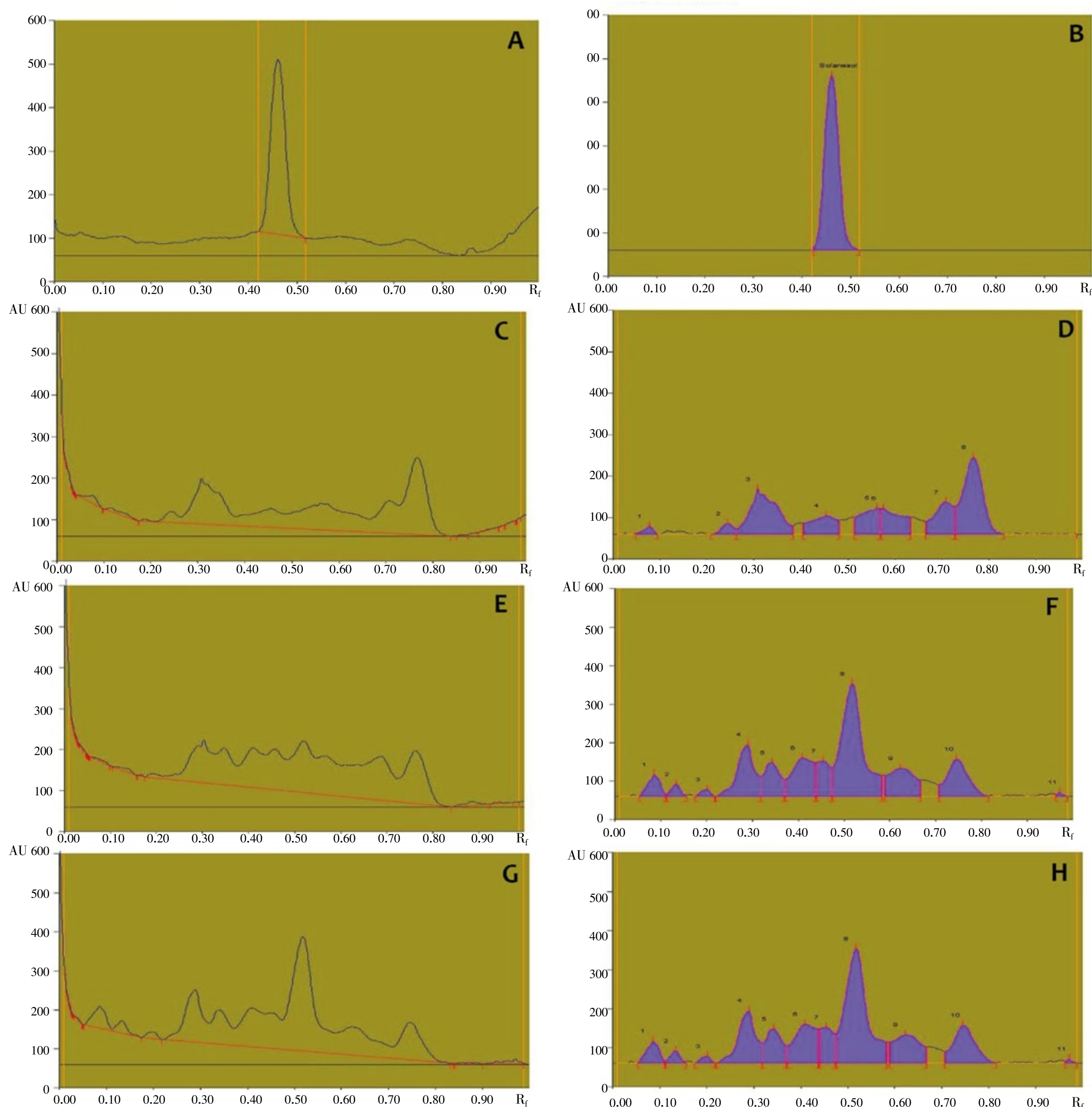

Figure 2. HPTLC chormatogram of medicinally important plant *A. lanata* L. (stem, leaves and roots).
A: HPTLC chromatogram of standard solanesol at 500 nm; B: HPTLC chromatogram of standard solanesol peak densitogram display at 500 nm; C: HPTLC chromatogram of *A. lanata* root-baseline display at 500 nm; D: HPTLC chromatogram of *A. lanata* root-peak densitogram display at 500 nm; E: HPTLC chromatogram of *A. lanata* stem-baseline display at 500 nm; F: HPTLC chromatogram of *A. lanata* stem-peak densitogram display at 500 nm; G: HPTLC chromatogram of *A. lanata* leaf-baseline display at 500 nm; H: HPTLC chromatogram of *A. lanata* leaf-peak densitogram display at 500 nm.

The well resolved HPTLC profiles of the methanolic extracts of *A. lanata* L. (stem, leaves, roots, flowers and seeds) were presented in Figure 1-3 and Table 1 to authenticate the presence of terpenoids in all the parts of *A. lanata*. HPTLC chromatogram of the standard solenosol and the terpenoids profile of *A. lanata* was depicted in Figure 2-3. This confirmed the presence of terpenoids in all the parts of *A. lanata*. The HPTLC chromatogram developed using n-hexane: ethyl acetate solvent system showed the presence of 27 peaks with maximum area under the curve indicating the possible quantity of terpenoids in the methanolic extracts of root, stem, leaves, flower and seeds of *A. lanata* (Table 2). It is generally realized that for monitoring quality, HPTLC fingerprinting is ideal which involves comparison between a standard and a sample. The use of markers ensures the concentration and ratio of components in the parts of the plant.

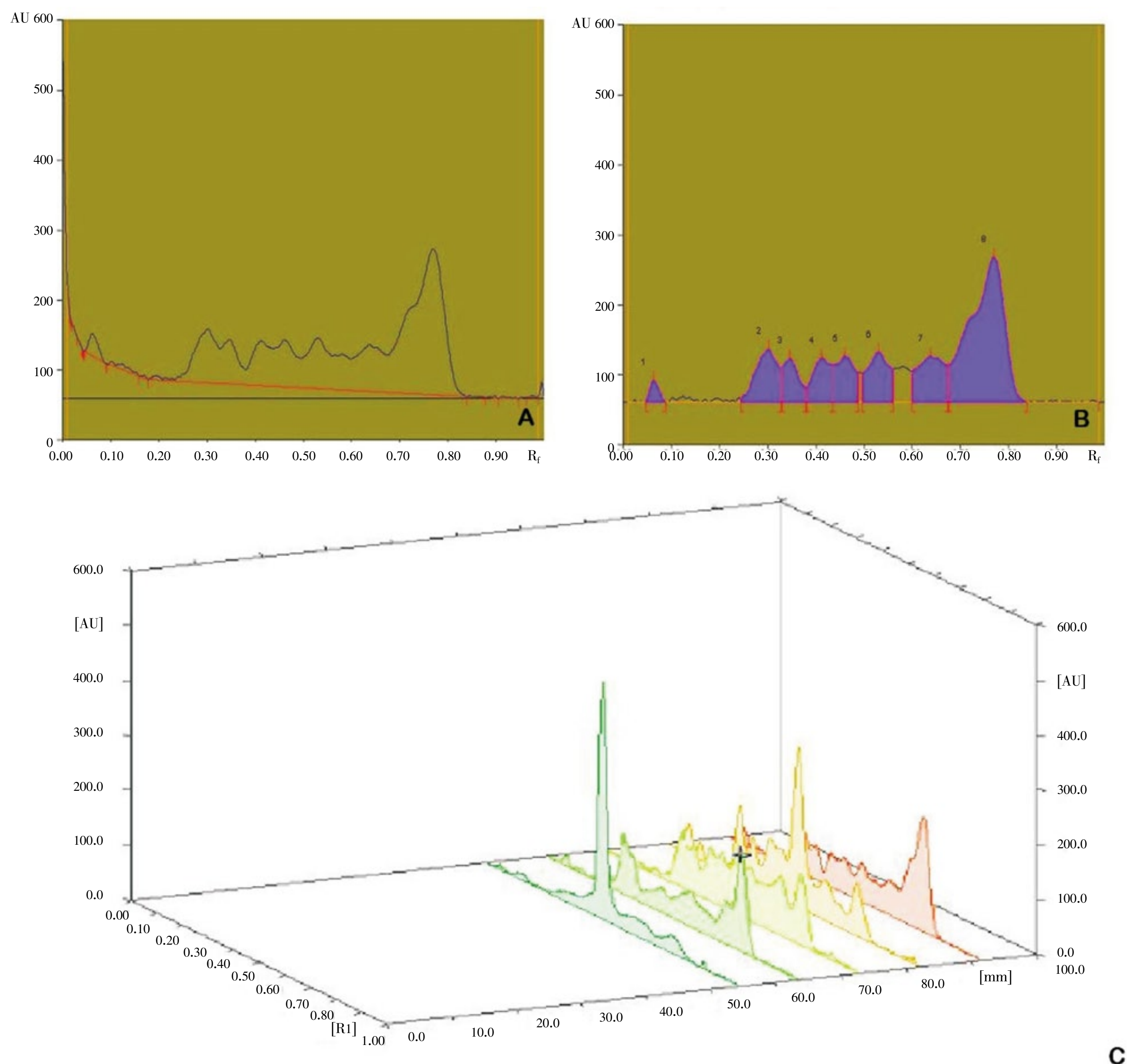

Figure 3. HPTLC chormatogram of medicinally important plant *Aerva lanata* L. (flowers and seed).
A: HPTLC chromatogram of *A. lanata* flowers and seeds-baseline display at 500 nm; B: HPTLC chromatogram of *A. lanata* flowers and seeds-peak densitogram display at 500 nm; C: 3D display of HPTLC chromatogram of *A. lanata*-root, stem, leaves, flower and seeds.

4. Discussion

Plants can produce many different types of secondary metabolites, which have been subsequently utilized by humans for their valuable characters in a diverse array of applications[7]. Secondary metabolites include compounds produced in response to stress, such as the case when acting as a deterrent against herbivores[8]. Currently, there is an increased interest in natural substances with valuable medicinal properties, such as terpenoids (hydrocarbon composition) and multiple C_5H_8. Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic, and other pharmaceutical functions. Terpenoids contribute to the scent of eucalyptus, the flavors of cinnamon, cloves, and ginger, and the color of yellow flowers. Well-known terpenoids include citral, menthol, camphor, salvinorin A in the plant *Salvia divinorum*, and the cannabinoids found in *Cannabis*. The steroids and sterols in animals are biologically produced from terpenoid precursors. Sometimes terpenoids are added to proteins, *e.g.*, to enhance their attachment to the cell membrane; this is known as isoprenylation[9]. These compounds and their derivatives also belong to other drugs such as validol, bromkamfora, menovasin, turpentine, *etc*. Turpentine is widely used as external drugs, and it is the main raw material for other products on the basis of terpenoids. The basis of turpentine is α-and β-pinene[10]. Basic research about terpenoids by various methods, including chromatography, was carried out in the early 60's late 70's of the last century[10]. A comprehensive review about terpenoids, their sources, structures, uses can be found[11]. All organisms naturally produce some terpenoids as part of primary metabolism, but many produce terpenoids *via* secondary metabolism. Terpenoids (also called "isoprenoids") constitute one of the largest families of natural products accounting for more than 40 000 individual compounds of both primary and secondary metabolisms. Most of them are of plant origin, and hundreds of new structures are reported every year[12-14]. Isopentyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) are the common five-carbon precursors of all

terpenoids. Terpenoids are defined as secondary metabolites with molecular structures containing carbon backbones made up of isoprene (2-methylbuta- 1, 3-diene) units. More than 36 000 terpenoids compounds have been identified, making terpenoids the largest class of plant metabolites. Most of the thousands of terpenoids produced by plants have no discernible role in growth and development and are, therefore, often classified as 'secondary' metabolites. Although comparatively few of these substances have been investigated in depth, they are thought to serve primarily in ecological roles, providing defense against and acting as attractants for animals that disperse pollen or seeds or as inhibitors of germination and growth of neighbouring plants[15]. After the discovery of the mevalonate (MVA) pathway in yeast and animals, it was assumed that IPP was synthesized from acetyl-CoA *via* MVA and then isomerized to DMAPP in all eukaryotes and some gram-positive prokaryotes[13,14]. Some exceptions have been described showing that interactions between the two biosynthetic pathways may exist[16]. Before 1993, the MVA pathway was the only known source of terpenoids. After isotope-labeling studies by Rohmer *et al*[17], it has been shown that there is an alternate pathway to terpenoids that do not originate from acetyl-CoA. The complete pathway has been finally elucidated in 2002[18]. This alternative MVA-independent pathway has been named the methylerythritol phosphate (MEP) pathway, which has been identified in both bacteria and plants[13,14]. Plants have an enormous capacity to synthesize huge amounts of diverse terpenoids, particularly *via* the combination of the terpenoid biosynthetic route and other secondary metabolic pathways. Plants use both pathways although they are compartmentalized: MVA to the cytoplasm and possibly to mitochondria to provide sterols, the side chain of ubiquinone, and sesquiterpenes (C_{15}), and MEP to plastids providing plastidial terpenoids, for example, isoprene (C_5), monoterpenes (C_{10}), diterpenes (C_{20}), including gibberellins and the phytyl tail of tocopherols and chlorophylls), and carotenoids (C_{40})[14]. Moreover, there is evidence that a certain degree of crosstalk between the MVA and MEP pathways can occur, implying that these pathways are not completely autonomous[19]. In addition to universal physiological, metabolic, and structural functions, many specific terpenoids function in various situations, including communication and defense. Members of the isoprenoid group also include industrially useful polymers (*e.g.*, rubber and chicle) and agrochemicals (*e.g.*, pyrethrins and azadirachtin). It is known that several herbal plants improve medical conditions. Such plants contain many bioactive phytochemicals. In particular, terpenoids are contained in many herbal plants, and several terpenoids have been shown to be available for pharmaceutical applications, for example, artemisinin and taxol as malaria and cancer medicines, respectively. Terpenoids are a large and diverse class of naturally occurring organic chemicals found in all classes of living organisms. Plant terpenoids are used extensively for their aromatic qualities and play a role in traditional herbal remedies. They are currently under investigation by numerous groups for antitumor, antibiotic, anticancer, antineoplastic, antibacterial, anti-inflammatory and other therapeutic properties[11]. Considering the wide therapeutic applications and importance of *A. lanata* an HPTLC method was developed to ensure the identity and quality of commercial samples. This shall help to obtain monograph of the future medicinally active plant. The method was validated by determining linearity, peak purity, and limit of detection and repeatability of terpenoids from aerial part extract of *A. lanata*. The developed HPTLC method for terpenoid profile is simple, precise and accurate and can be used for the identification and commercial application. For developing analytical method pure active chemical constituents should be isolated in further study and identification on the basis of reference standard shall be made. This profile helps in setting in house standards of the medicinal plants used extensively by herbal manufactures.

Conflict of interest statement

We declare that we have no conflict of interest.

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