Phytochemical Screening of *aerva lanata* using High Performance Thin Layer Chromatography (HPTLC) Method; A Review

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Abstract:

Aerva lanata species belonging to Amaranthaceae family is a perennial shrub. It is self- pollinating, bisexual plant having globose like structure has various phytochemical constituents. Aerva lanata has many pharmacological benefits like Anti-urolithiasis, Anti-microbial, Anti- hyperglycemic, hypolipidemic, Anti-ulcer. The present review shows the phytochemical screening using High Performance Thin Layer Chromatography (HPTLC) studies of *Aerva lanata*plant extract. HPTLC studies of methanolic extract of stems, leaves, root, flowers, and seeds of Aerva lanata plant extract constituted different colored phytochemical compounds with different Rf values. Phytochemical screening reflects the presence of terpenoids, steroids, glycosides. Thedeveloped HPTLC method for phytochemical profile studies is simple, precise and accurate and can be used for the identification and commercial application. The result obtained in the presentreview indicates that *Aerva lanata* plant has rich source of phytochemical compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases.

Keywords: *Aerva lanata*, HPTLC studies, Phytochemicals, Rf value.

INTRODUCTION:

Aerva lanata (Linn) Juss ex Schult is known in Indian medical literature (Chopra RN, *et al*,1956) as a common waysideweed, locally known as "bui." It is identified by its small bunches of woolly flowers that bloom on axillary branches. The plant is abundant on the plains in the warmer parts of India (Kirtikar KR, *et al*,1996) and is native in tropical Africa through Arabia and to the Philippines (Chattered A, *et al*, 1992) up to an altitude of 3000 m. *Aerva lanata* contains b-sitosteryl palmitate, a-amyrin, and b-sitosterol. The plant is also reportedto contain tannins, steroids, flavonoids, alkaloids, polysaccharides, and saponin (Chandra S, *et al*, 1990). The phytochemical profile of *A. lanata* has been studied extensively. In ethano medicine, A. *lanata* hasbroad uses. It is regarded as a valuable treatment for cough, sore throat, indigestion, wounds, headache, diabetes, and as a vermifuge for children. The herb is also used for diarrhea, such as with cholera and dysentery. It is also known to treat hemorrhage associated with pregnancy. Theflowers are used to treat gonorrhea and kidney stones. *Aerva lanata* has been documented for its pharmacologic properties including anti-asthmatic activity, urolithiasis effects, nephroprotective, antidiuretic, antidiabetic, antihyperglycemic, antimicrobial, cytotoxic, anti-HIV, immunomodulatory, anti-inflammatory, analgesic, anti-ulcer, and antioxidant activities (Zapesochnaya G, *et al*, 1992, Zadorozhnyi AM, *et al*,1986, Yuldashev AA, *et al*,2002, Wassel GM, *et al*,1987, Pervykh LN, *et al*,1993, Mallabaev A, *et al*, 1989).

HPTLC ANALYSIS TERPENOIDS IN AERVA LANATA:

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 metabolities. Mostof the thousands of terpenoids produced by plants have no discernible role in growth and development and are therefore often classified as secondary metabolities (Wink M, 2010).

METHOD:

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 (5µ L) were spotted in the form of bands of width 5 mm with a Camag microlitre syringe on precoated silicagel 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 prewashed by methanol and activated at 60ºC 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 asmobile 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 30min at room temperature [(25±2)ºC]. 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 wassprayed with anisaldehyde sulphuric acid as spray reagent and dried at 100ºC in hot air oven for3 min. The plate was photo-documented at UV 366 nm and daylight using photodocumentation (CAMAG REPROSTAR 3) chamber. Finally, the plate was fixed in scanner stage and scanningwas done at 366 nm. Densitometric scanning was performed on Camag TLC scanner III and operated by CATS software (V 3.15,Camag).

RESULT:

The HPTLC chromatogram developed using n-hexane: ethyl acetate solvent system showed thepresence of 27 peaks with maximum area under the curve indicating the possible quantity ofterpenoids in the methanolic extracts of root, stem, leaves, flowers and seeds of Aerva lanata (Table 1) (Yamunadevi M, *et al*,2011).

Table 1: 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			+	

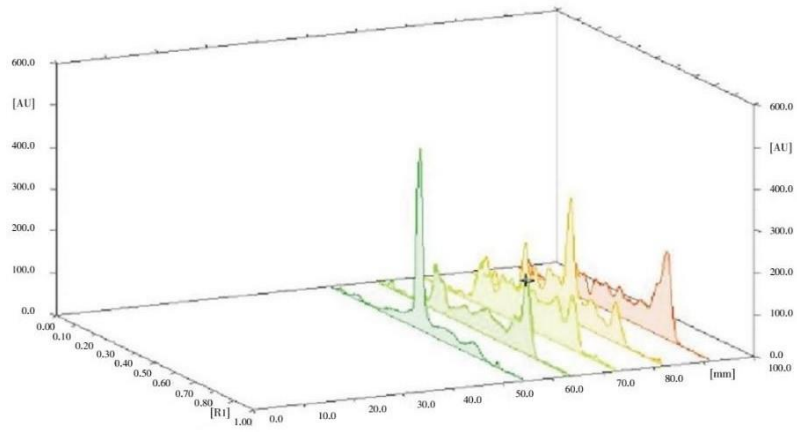

Fig:1 3d display of HPTLC Chromatograph of terpenoids in Aerva lanata- stem, root,leaves, flower and seeds

HPTLC ANALYSIS OF STEROIDS IN AERVA LANATA:

Steroids (naturally occurring or synthetic) such as methyl prednisolone, hydrocortisone,glucocortisteroids, corticosteroids, squalamine, oestrogens, androgens are also used for thetreatment of various diseases such as allergic reactions, arthritis some malignancies and diseasesresulting from hormone deficiencies or abnormal production. In addition, synthetic steroids (eg., mifepristone) that mimic the action of progesterone are widely used as oral contraceptive agents.
(Wagner H, *et al,*1996, Bhawani SA, *et al,* 2010)

METHOD:

A total of 100mg extract was dissolved in 5ml of methanol and the solution was centrifuged at 3000rpm for 5 min and used for HPTLC analysis as test solution. The samples (5µ L) were spotted in the form of bands of width 5 mm with a Camag microliter syringe on precoated silicagel 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 prewashed by methanol and activated at 60ºC 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 (steroids) and the plate was developed in the respective mobile phase up to 90 mm. The Chloroform-acetone (8:2) was employed as mobilephase for steroids. Linear ascending development was carried out in (20 cm× 10 cm) twin troughglass 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 30min at room temperature [(25±2)ºC]. 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 wassprayed with anisaldehyde sulphuric acid as spray reagent and dried at 100ºC in hot air oven for3 min. The plate was photo-documented at UV 366 nm and daylight using photodocumentation (CAMAG REPROSTAR 3) chamber. Finally, the plate was fixed in scanner stage and scanningwas done at 366 nm. Densitometric scanning was performed on Camag TLC scanner III and operated by CATS software (V 3.15, Camag).

RESULT:

The methanolic extract of stem, leaves, root, flower and seeds of A.lanata showed the presence of 30 different types of steroids with Rf values 0.04-0.97 (Table 2) (Yamunadevi M, et al,2011).

Table 2: Steroids profile of aerial and underground parts of *Aerva lanata*

Rf	Root	Stem	Leaves	Flower	No. of Bands
0.04				+	1
0.05	+		+		2
0.07				+	1
0.10			+		1
0.11	+				1
0.12		+			1
0.14			+		1
0.20	+		—		1
0.22		+		+	2
0.23			+		1
0.25	+				1
0.26				+	1
0.27		+			1
0.28			+		1
0.33	+	+			2
0.35			+		1
0.44	+				1
0.45		+	+	+	3
0.48	+				1
0.53	+	+			2
0.54				+	1
0.60	+			+	2
0.61		+	+		2
0.65		+			1
0.71	+				1
0.72				+	1
0.77				+	1
0.80			+		1
0.92			+		1
0.97			+		1

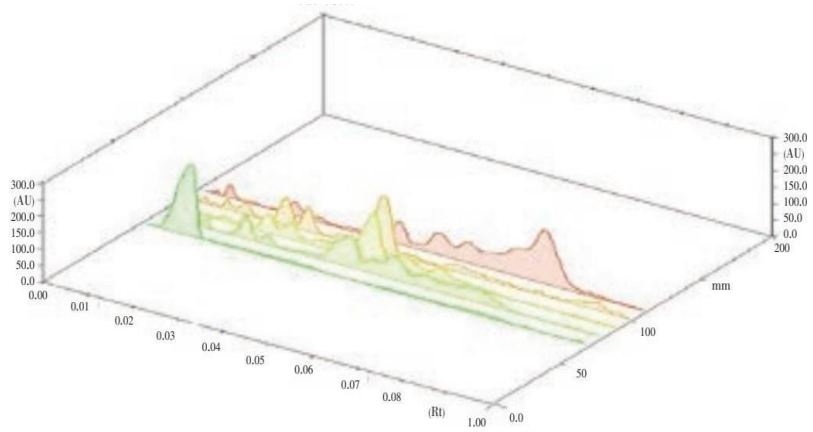

Fig:2 3d display of HPTLC Chromatogram of steroids in root, stem, leaves, flower and seeds of*Aerva lanata*

HPTLC ANALYSIS OF GLYCOSIDES OF AERVA LANATA:

Glycosides comprise a very wide range of compounds that are of common and ubiquitous occurrence in almost all plants. A considerable number of glycosides are of great medicinal value,all of which are of natural origin. These pharmaceutically valuable glycosides contribute to almost every therapeutic class, cardiac drugs, laxatives, counter irritants, analgesics, renaldisinfectants, anti-rheumatics, anti-inflammatory, anti-tuberculosis, expectorant and antispasmodic action (Pau 2010).

METHOD:

The 100 mg extract was dissolved in 5ml of methanol and the solution was centrifuged at 3000 r·min−1 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-coatedsilica 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 activatedat 60 °C for 5 min prior to chromatography. The sample loaded plate was kept in TLC twin troughdeveloping chamber (after being saturated with solvent vapor) with respective mobile phase (glycosides) and the plate was developed in the respective mobile phase up to 90 mm. The ethylacetate-ethanol-water (8 : 2 : 1.2) was employed as mobile phase for glycosides. Linear ascendingdevelopment was carried out in 20 cm × 10cm twin trough glass chamber (Camag, Mutenz, Switzerland) saturated with the mobile phase and the chromatoplate was developed for two timeswith the same mobile phase to afford good resolution of phytochemical contents. The optimizedchamber 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. Thedeveloped plate was sprayed with chloramine T reagent as spray reagent and dried at 100 °C inhot air oven for 3 min. The plate was photo- documented at UV 366 nm and daylight using CAMAG REPROSTAR 3 chamber. Finally, the plate was fixed in scanner stage, being scannedat 366 nm. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) andthe images were captured under white light, UV light at 254 and 366 nm. Densitometric scanningwas performed on Camag TLC scanner III and operated by CATS software (V 3.15, Camag).

RESULT:

The methanolic extract of stem, leaves, root, flower and seeds of *A. lanata* showed the presenceof 23 different types of glycosides with 23 different *R*f values with range 0.02 to 0.86

(Table 3) (Yamunadevi M, *et al*,2011).

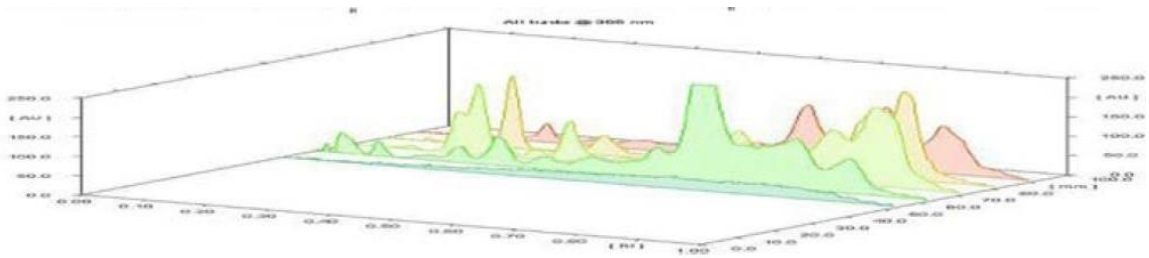

Fig 3. 3d display of HPTLC Chromatograph of glycosides in *Aerva lanata*- root, stem, leaves, flower and seeds

Table 3: Glycosides profile of *Aerva lanata L. aerial* and underground parts

R_f	Root	Stem	Leaves	Flower	No. of Bands
0.02	+				1
0.04	+				1
0.07				+	1
0.10	+				1
0.17		+	+		2
0.19	+				1
0.21		+	+	+	3
0.23	+				1
0.26		+			1
0.29	+				1
0.30		+		+	2
0.36		+	+		2
0.37	+			+	2
0.45	+				1
0.54				+	1
0.55		+	+		2
0.56	+		+		2
0.63	+	+	+	+	4
0.76				+	1
0.78	+	+	+		3
0.84			+		1
0.85		+		+	2
0.86	+				1

CONCLUSION:

Considering the wide therapeutic applications and importance of *Aerva lanata* a HPTLC methodwas developed to ensure the identity and quality of commercial samples. This shall help to obtainmonograph of the future medicinally active plant. The developed HPTLC method for phytochemical screening is simple, precise and accurate and can be used for the identification and commercial application. The Phytochemicals screened in *Aerva lanata* (Terpenoids, steroids,glycosides) play an important role in treatment of various diseases and can be used as traditionalherbal remedies.

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