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Research Article

CHROMATOGRAPHIC STUDIES ON BIOACTIVE COMPOUNDS OF ETHANOLIC LEAF EXTRACT OF *AERVA LANATA* BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY TECHNIQUE

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ABSTRACT

Objective: This study was designed to determine the bioactive compounds such as alkaloids, glycosides, phenol, and tannins by high performance thin layer chromatography (HPTLC) which will help in crude drug identification and in the standardization of *Aerva lanata* in pharmacological industries.

Methods: HPTLC studies were conducted as Harborne described. The toluene-acetone-formic acid (4.5:4.5:1); ethyl acetate-ethanol-water (10:1.35:1); ethyl acetate-ethanol-water (8:2:1.2); toluene-ethyl acetate-formic acid-methanol (3:3:0.8:0.2) were employed as mobile phase for phenol, alkaloid, glycoside, and tannin profiles.

Result: The ethanolic extract of leaves of *A. lanata* illustrated the presence of 11 different types of phenol with 11 different Rf values with range 0.06-0.95, 10 different types of alkaloid with 10 different Rf values from 0.02 to 0.92, 12 different types of glycoside with 12 different Rf values from 0.02 to 0.96, 9 different types of Tannin with 9 different Rf values from 0.07 to 0.93.

Conclusion: This study supplements valuable information about known and unknown bioactive compounds with the bioactivity of *A. lanata*. Further, pharmacological studies on structure of the bioactive compounds can be formulated to treat diseases. Thus, the ethanolic extract of *A. lanata* plant can be utilized as a useful medicinal herb for alleviation of various illness and disorder.

Keywords: High performance thin layer chromatography, Alkaloids, Glycosides, Phenols, Tannins.

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INTRODUCTION

Herbal drugs have been in exercise in various parts of the world for centuries to treat a number of diseases. Today, the plant based medicines are being used worldwide as medications and suggest a broad spectrum of activity since ancient times. The drug efficiency depends on the several active principles and compounds present in it.

Like other plants, medicinal plants synthesize a vast range of organic compounds that are traditionally classified as primary and secondary metabolites. Primary metabolites are compounds that have essential roles associated with photosynthesis, respiration, growth, and development. These include phytosterols, acyl lipids, nucleotides, amino acids, and organic acids. Other phytochemicals, many of which accumulate in surprisingly high concentration in some species, are referred as secondary metabolites. It plays a key role in protecting plants from herbivorous and microbial infection, as attractants for pollinators and seed dispersing animals, as allelopathic agents, ultra violet (UV) protectants and signal molecules in the formation of nitrogen-fixing root nodules in legumes.

In recent years, the role of some secondary metabolites as protective dietary constituent has become an increasingly important area of human nutrition research. Medicinal plants antioxidant activity is mainly due to the presence of secondary metabolites. Based on their biosynthetic origins plant secondary metabolites can be divided into four major groups. Flavonoids, phenolics, terpenoids, and alkaloids are containing compounds.

The well-developed quality standards can be achieved only through systematic evaluation of the plant material using modern analytical techniques including thin layer chromatography (TLC) and high performance TLC (HPTLC). HPTLC is 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 [1]. To identify the bioactive compounds responsible for pharmacological activities, phytochemical studies have been conducted by several workers with the report of different kinds of bioactive compounds particularly saponins, steroids, terpenoids, etc. The main constraint in the use of traditional remedies is the lack of standardization of raw material, manufacturing process, and the final product. HPTLC is a valuable tool for identification because it can provide chromatographic fingerprints that can be visualized and stored as electronic images. However, to fulfill the lacuna, this study was intended to resolve the alkaloid, glycoside, tannin, and phenol profile present in the methanolic extract of leaves of *Aerva lanata*, which will be useful for the proper identification of commercial samples [2,3].

METHODS

A. lanata was collected from natural habitats, Coimbatore, Tamil Nadu, India. It was authenticated by (CPCSEA/No: 158/1999/CPCSEA). The fresh leaves were shade dried and (5 g) of plant material was extracted with ethanol in Soxhlet apparatus for 3 hrs. Cooled, filtered the content and concentrated using vacuum flash evaporator. Dissolved the content with 1 ml ethanol and centrifuged at 3000 rpm for 5 minutes. This solution was used as test solution for HPTLC analysis.

HPTLC analysis

2 µl of test solution and 2 µl of standard solution were loaded as 5 mm band length in the 3 × 10 Silica gel 60F_{254} TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with solvent vapor) with respective mobile phase (phenols: toluene-acetone-formic acid at the ratio of 4.5:4.5:1; alkaloids: ethyl acetate-methanol-water at the ratio 10:1.35:1; glycoside: ethyl acetate-ethanol-water at

the ratio 8:2:1.2; tannin: toluene-ethyl acetate-formic acid-methanol at the ratio 3:3:0.8:0.2) and the plate was developed up to 90 mm. Linear ascending development was carried out in 20 cm × 10 cm twin trough glass chamber saturated with the mobile phase and the chromatoplate development for two times with the same mobile phase to get food resolution of phytochemical contents. 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 at visible light, UV 254 nm, and UV 366 nm. The developed plate was sprayed with respective spray reagent (phenol: folin–Ciocalteu reagent, tannin: 5% ferric chloride reagent, glycoside: Libermann-Burchard reagent, alkaloid: dragendorff's reagent followed by 10% ethanolic sulfuric acid reagent) and dried at 100°C in a Hot air oven. The plate was photo-documented in visible light and UV 366 nm mode using photo documentation (CAMAG REPROSTAR 3) chamber. After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 254 nm. The peak table, peak display, and peak densitogram were noted. The software used was win CATS 1.3.4 version.

RESULTS

HPTLC finger printing analysis of phenols

HPTLC fingerprint analysis of ethanol extract of *A. lanata* illustrated the presence of 11different types of phenol with 11 different Rf values with range 0.06-0.95 (Table 1). Ethanol extract of *A. lanata* is compared with standard quercetin along with peak densitogram (Figs. 1-3). Blue, Brown colored zone at visible mode was present in the track, it was observed from the chromatogram after derivatization, which confirmed the presence of phenol/phenol carboxylic acid in the given standard and may be in the sample.

HPTLC finger printing analysis of alkaloid

HPTLC fingerprint analysis of ethanol extract of *A. lanata* illustrated the presence of 10 different types of alkaloid with 10 different Rf values from 0.02 to 0.92 (Table 2). Ethanol extract of *A. lanata* is compared

Table 1: Phenolic profile of the ethanolic extract of the leaf of *A. lanata*

Track	Peak	Rf	Height	Area	Assigned substance
Sample A	1	0.06	38.7	766.7	Unknown
Sample A	2	0.15	15.5	600.4	Unknown
Sample A	3	0.22	54.8	1195.8	Unknown
Sample A	4	0.32	10.9	293.9	Unknown
Sample A	5	0.43	35	905.9	Unknown
Sample A	6	0.59	10.8	222.2	Unknown
Sample A	7	0.66	37.8	868.1	Unknown
Sample A	8	0.75	385.8	18826.5	Phenolic 1
Sample A	9	0.81	276	12465.9	Unknown
Sample A	10	0.88	196	8805	Unknown
Sample A	11	0.95	242.5	10706.6	Unknown
STD	1	0.77	280.1	7949.1	Quercetin

A. lanata: Aerva lanata

Table 2: Alkaloid profile of the ethanolic extract of the leaf of *A. lanata*

Track	Peak	Rf	Height	Area	Assigned substance
Sample A	1	0.02	132.1	2940.3	Unknown
Sample A	2	0.11	13.8	521.8	Unknown
Sample A	3	0.30	114.9	4493.6	Unknown
Sample A	4	0.34	154.1	8741.1	Unknown
Sample A	5	0.44	50.3	2189.7	Unknown
Sample A	6	0.57	336.2	16341.5	Alkaloid 1
Sample A	7	0.66	121.8	3936.6	Unknown
Sample A	8	0.75	287.9	18920.0	Unknown
Sample A	9	0.89	599.1	38489.9	Unknown
Sample A	10	0.92	526.2	19452.9	Alkaloid 2
STD	1	0.46	616.2	19452.9	Colchicine

A. lanata: Aerva lanata

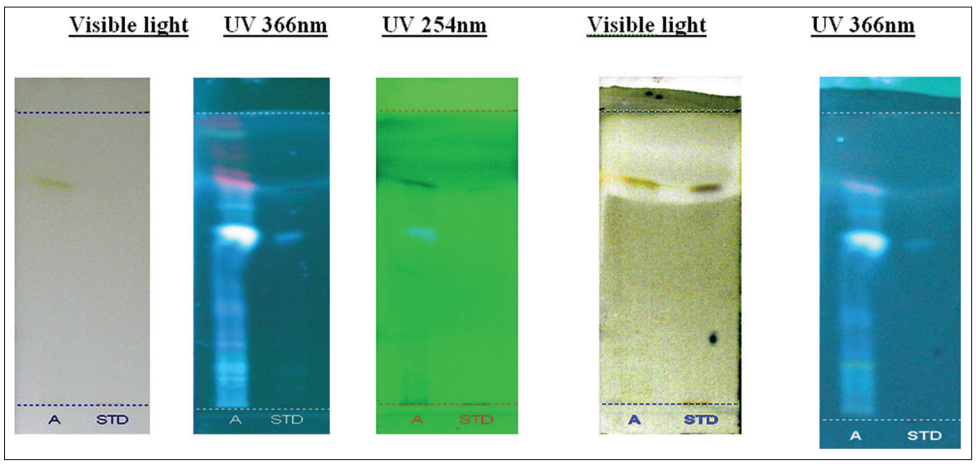

Fig. 1: High performance thin layer chromatography profile of the ethanolic extract of *Aerva lanata* and phenolic standard (quercetin)

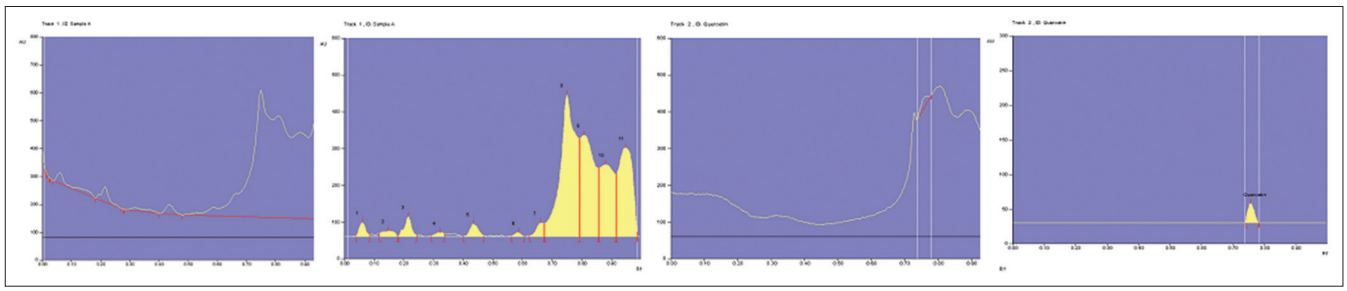

Fig. 2: High performance thin layer chromatography phenolic chromatogram of *Aerva lanata* leaf and phenolic standard (quercetin) - baseline display and peak densitogram display (scanned at 254 nm)

with Standard Colchicine along with peak densitogram (Figs. 4-6). Yellow, Brownish yellow colored zone at visible light mode were present in the tracks, it was observed from the chromatogram after derivatization, which confirmed the presence of alkaloid in the given standard and may be in the sample.

HPTLC fingerprint analysis of ethanol extract of *A. lanata* illustrated the presence of 12 different types of glycoside with 12 different Rf values from 0.02 to 0.96 (Table 3). Ethanol extract of *A. lanata* is compared with standard Swertiamarin along with peak densitogram (Figs. 7-9). Green colored fluorescent zone at UV 366 nm mode was present in the tracks, it was observed from the chromatogram after derivatization, which confirmed the presence of glycoside in the given standard and may be in the sample.

HPTLC finger printing analysis of tannin

HPTLC fingerprint analysis of ethanol extract of *A. lanata* illustrated the presence of 9 different types of Tannin with 9 different Rf values from 0.07 to 0.93 (Table 4). Ethanol extract of *A. lanata* is compared with standard gallic acid along with peak densitogram (Figs. 10-12). Green, brownish green and yellowish green colored zone at visible light mode were present in the tracks, it was observed from the chromatogram after derivatization, which confirmed the presence of tannin in the given standard and may be in the sample.

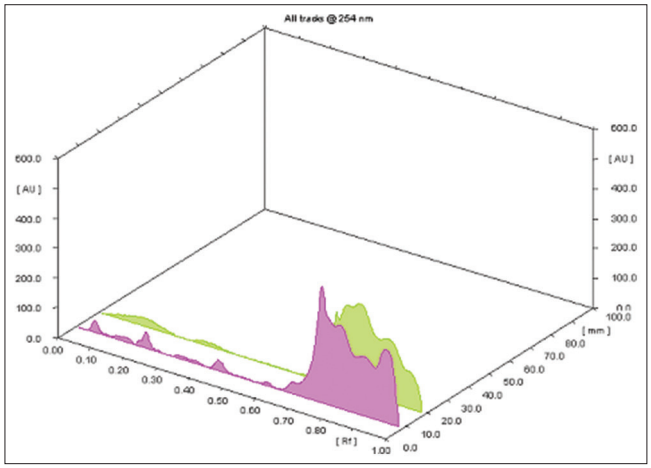

Fig. 3: 3D display of high performance thin layer chromatography phenolic chromatogram of *Aerva lanata*

Table 3: Glycoside profile of the ethanolic extract of the leaf of *A. lanata*

Track	Peak	Rf	Height	Area	Assigned substance
Sample A	1	0.02	190.4	2503.0	Unknown
Sample A	2	0.06	10.5	113.2	Unknown
Sample A	3	0.10	13.0	184.6	Unknown
Sample A	4	0.16	13.1	171.2	Unknown
Sample A	5	0.20	15.7	264.6	Unknown
Sample A	6	0.37	32.8	688.3	Unknown
Sample A	7	0.39	40.9	1051.5	Unknown
Sample A	8	0.56	16.1	227.1	Unknown
Sample A	9	0.62	43.5	1685.4	Glycoside 1
Sample A	10	0.70	12.2	279.4	Unknown
Sample A	11	0.92	264.6	14981.1	Unknown
Sample A	12	0.96	268.5	7975.3	Unknown
STD	1	0.73	150.3	2791.1	Swertiamarin

A. lanata: Aerva lanata

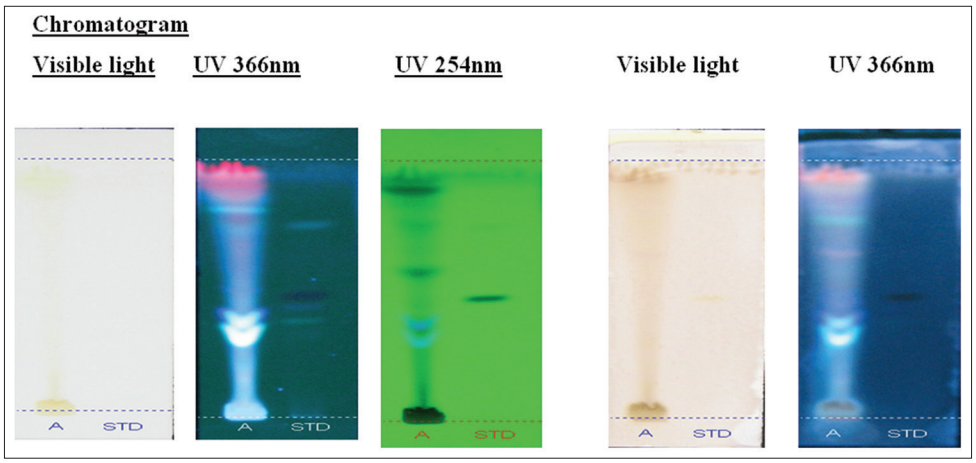

Fig. 4: High performance thin layer chromatography profile of the ethanolic extract of *Aerva lanata* and alkaloid standard (colchicine)

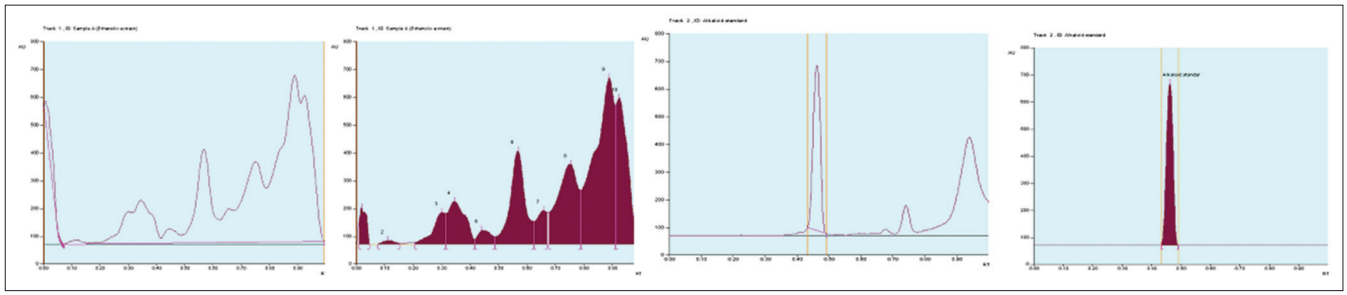

Fig. 5: High performance thin layer chromatography alkaloid chromatogram of *Aerva lanata* leaf and alkaloid standard (colchicine) - baseline display and peak densitogram display (scanned at 254 nm)

Table 4: Tannin profile of the ethanolic extract of the leaf of *A. lanata*

Track	Peak	Rf	Height	Area	Assigned substance
Sample A	1	0.07	23.4	431.4	Tannin 1
Sample A	2	0.15	44.1	956.0	Unknown
Sample A	3	0.26	82.6	5602.3	Unknown
Sample A	4	0.38	94.1	4464.5	Unknown
Sample A	5	0.64	400.1	36105.5	Tannin 2
Sample A	6	0.69	449.7	13767.3	Tannin 3
Sample A	7	0.73	574.2	22029.0	Tannin 4
Sample A	8	0.80	307.2	15856.7	Unknown
Sample A	9	0.93	157.2	8990.1	Unknown
STD	1	0.55	391.4	11243.1	Gallic acid

A. lanata: Aerva lanata

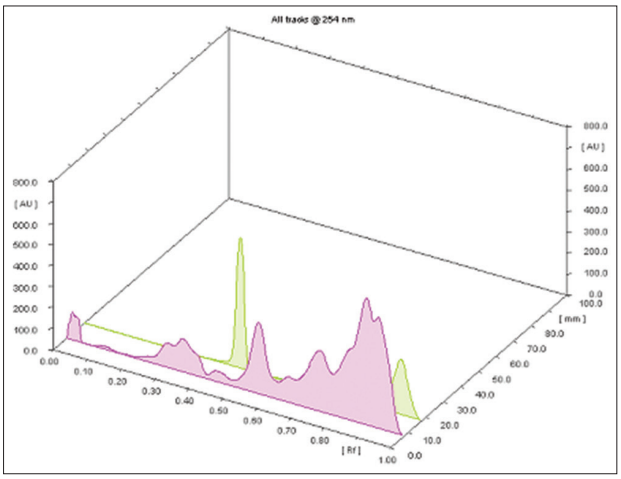

Fig. 6: 3D display of high performance thin layer chromatography alkaloid chromatogram of *Aerva lanata*

DISCUSSION

Authentication of medicinal plants in genetic and chemical level is a critical step in the use of these botanical materials for research and commercial preparations. For any living organisms, identity is very important to distinguish itself from other organisms within the populations and other populations. In plant taxonomy, during this molecular era, the morphological characters also play a key role in plant systematic study and are used as a tool for the classification of taxonomy [2].

Phenolic acids are aromatic secondary plant metabolites, studied mainly for their properties against oxidative damage leading to various degenerative diseases, such as cardiovascular diseases, inflammation, and cancer [4,5]. Varied biological activities of phenolic acids were reported. It increases bile secretion, reduces blood cholesterol and lipid levels and antimicrobial activity against some strains of bacteria such as *Staphylococcus aureus* are some of biological activities of phenolic acids [6].

Tannins are a heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes with proteins, polysaccharides, alkaloids, nucleic acids, minerals, etc. Based on structural characteristics it is divided into four major groups gallotannins, ellagitannins, complex tannins, and condensed tannins. Tannins are widely used in the dyestuff industry as caustics for cationic dyes and also in the production of inks. Tannins in the form of proanthocyanidins have a beneficial effect on vascular health [7,8]. Tannins are used as astringents, stimulant, antiseptics, diuretics, wound healer, and antiulcer. They are useful as an anti-inflammatory agent and in the treatment of burns and other wounds based on their antihemorrhagic and antiseptic potentials [9-10].

Alkaloids are a natural product that contains heterocyclic nitrogen atoms. Alkaloids are naturally synthesis by large numbers of organisms, including animals, plants, bacteria, and fungi.

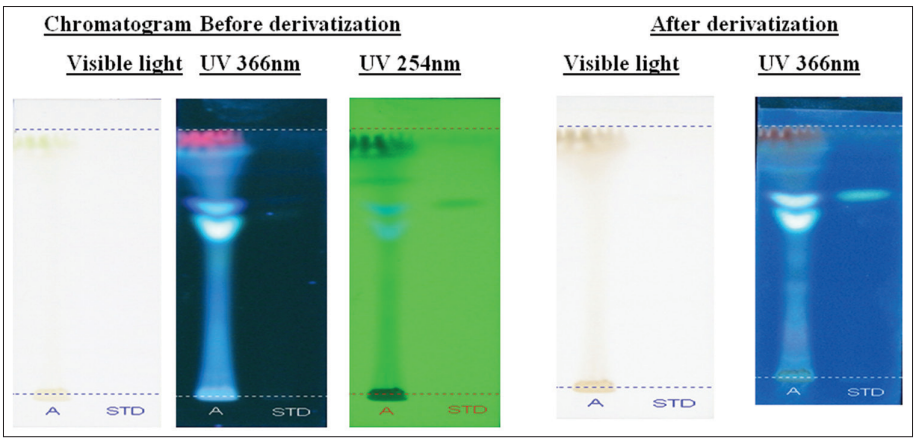

Fig. 7: High performance thin layer chromatography profile of the ethanolic extract of *Aerva lanata* and glycoside standard (Swertiamarin)

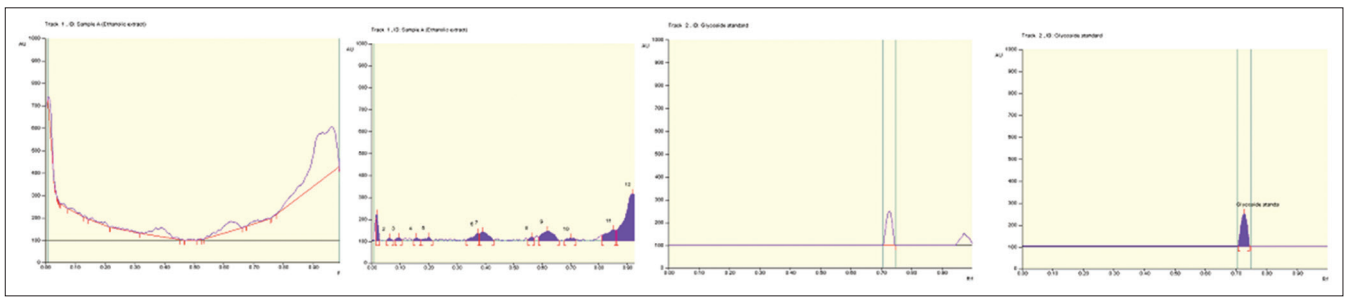

Fig. 8: High performance thin layer chromatography glycoside chromatogram of *Aerva lanata* leaf and glycoside standard (Swertiamarin) - Baseline display and peak densitogram display (scanned at 254 nm)

Alkaloids in nature are significant for the protecting and survival activity of plant because they ensure their survival against microorganisms, insects, and herbivores [11,12]. Alkaloids have many pharmacological activities including antihypertensive effects, antiarrhythmic effect, antimalarial activity, and anticancer actions. Some alkaloids have stimulant property as caffeine and nicotine, morphine are used as the analgesic and quinine as the antimalarial drug [13-15].

The result of this study showed and confirmed the presence of 11 different types of phenol with 11 different Rf values with range 0.06-0.95, 10 different types of alkaloid with 10 different Rf values from 0.02 to 0.92, 12 different types of glycoside with 12 different Rf values from 0.02 to 0.96, 9 different types of tannin with 9 different Rf values from 0.07 to 0.93. The developed HPTLC fingerprint will help the manufacturer for the quality control and standardization of herbal formulations.

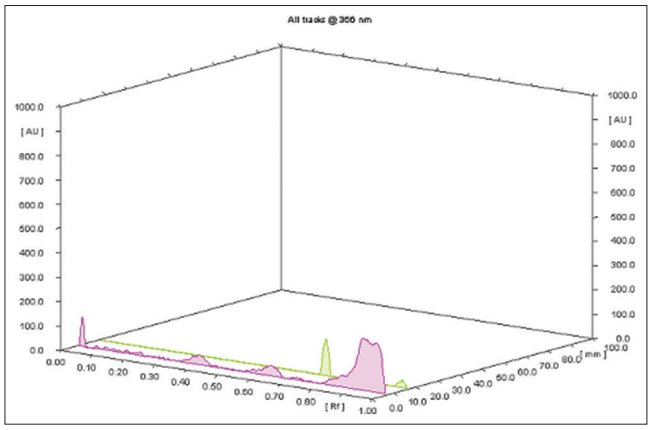

Fig. 9: 3D display of high performance thin layer chromatography glycoside chromatogram of *Aerva lanata*

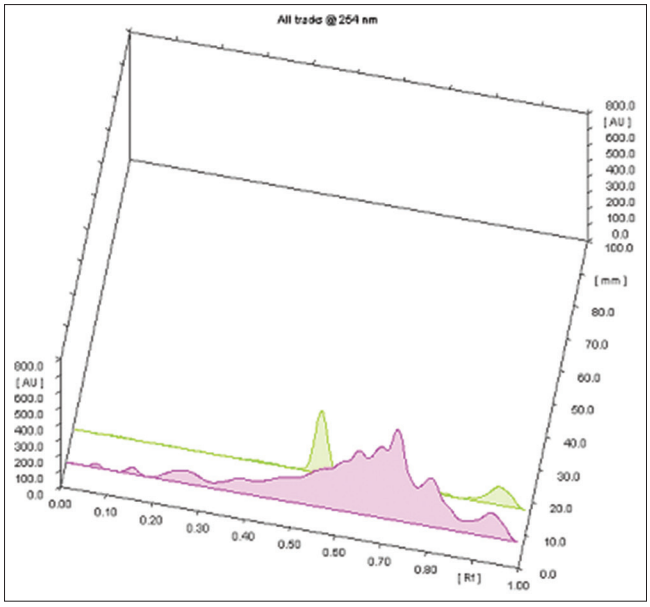

Fig. 12: 3D display of high performance thin layer chromatography Tannin chromatogram of *Aerva lanata*

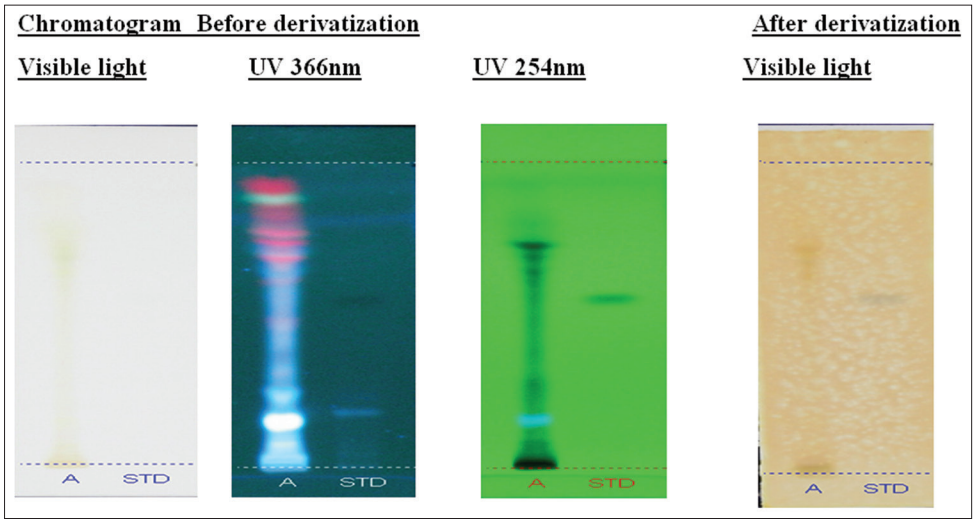

Fig. 10: High performance thin layer chromatography profile of the ethanolic extract of *Aerva lanata* and tannin standard (gallic acid)

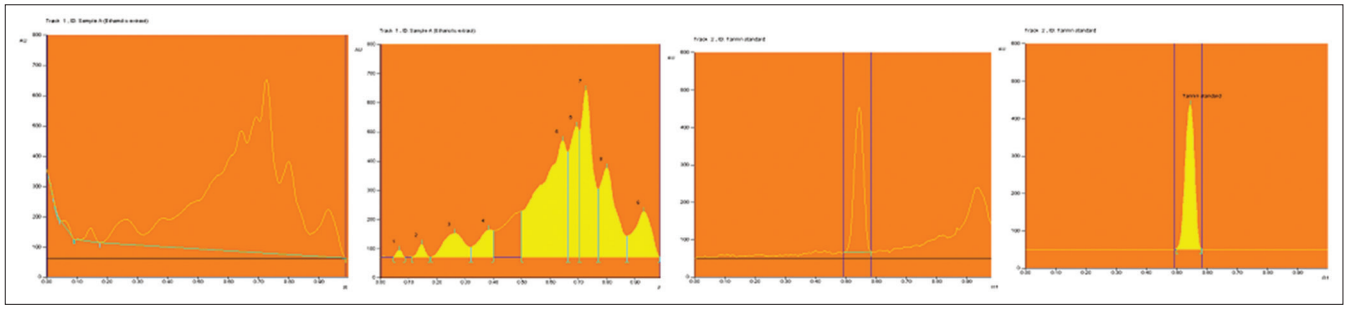

Fig. 11: High performance thin layer chromatography tannin chromatogram of *Aerva lanata* leaf and tannin standard (tannic acid) - baseline display and peak densitogram display (scanned at 254 nm)

CONCLUSION

The result of this study provided a valuable phytomarker for the identification and characterization of *A. lanata*. Further, pharmacological studies are going to isolate, identify, characterize, and elucidate the structure of the bioactive compound.

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