ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Finger Printing of Alkaloids, Steroids and Flavonoids using HPTLC of *Leucas aspera* L. Whole Plant Methanolic Extract

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Abstract
In this study, *Leucas aspera* whole plant methanolic extract were examined for their phytochemical profile by high-performance thin layer chromatographic (HTPLC) method. Alkaloids, Steroids and Flavonoids profile were analysed. Our results revealed that at least two types of alkaloids were seen in this methanolic extracts. Six types of flavonoids and two types of steroids were found in the *Leucas aspera* whole plant methanolic extract. Alkaloid and flavonoid were found to have higher concentration when compared to steroids which were in lower concentration in the methanolic extract.

Key words: *Leucas aspera*, Alkaloid, Flavonoid, Steroid

1. INTRODUCTION

Herbs have been found to have a wide variety of uses like curing certain ailments, skin remedies and have found wide application in health industry. The reason for the medicinal effect of these plants has been found out to be the presence of certain bioactive compounds known as phytochemical compounds in them, which chemically, can be diverse ranging from alkaloids to saponins etc., these bioactive compounds exhibit an effect against a variety of microbial strains, and have been used depending upon the nature of the ailment. New technologies in fields like biotechnology have made it possible to identify, screen and isolate these compounds from their respective plants and make use of them for experiments and commercial purposes.

Leucas aspera is found in open, dry, sandy soil, being a weed on road sides and locally abundant and used as an indigenous system of medicine [1]. The plant is an erect or diffusely branched, annual herb and leaves are linear, blunt-tipped and the margins scalloped. *L. aspera* possesses different activities especially anti-inflammatory activity and is used against cobra venom poisoning and also as an analgesic [2]. Preliminary chemical examination of *L. aspera* revealed presence of triterpenoids in entire plant. Whole plant is reported to contain oleanolic acid, ursolic acid and 3-sitosterol. Glucosides, tannins, saponins, sterols, oleic, linoleic, palmitic, stearic, oleanolic and ursolic acids have been isolated from the leaves of this plant [3]. The plant is used as an insecticide and indicated in traditional medicine for coughs, colds, painful swellings, and chronic skin eruptions [4]. Traditionally the decoction of the whole plant is taken orally for analgesic–antipyretic, antirheumatic, antiinflammatory and antibacterial treatment and its paste is applied topically to inflamed areas [5]. Leaves are useful in treatment of chronic rheumatism, psoriasis, scabies, chronic skin eruptions [5]. In this study, the whole plant methanolic extract were evaluated for the chemical profile by HTPLC analysis.

2. RESULTS AND DISCUSSION

The whole plant methanolic extract was collected from *Leucas aspera L.* and the extract was analysed for phytochemical profiling using high-performance thin layer chromatography (HPTLC). Three different chemical profile were analysed viz. Alkaloid, Steroid and Flavonoid.

2.1. HPTLC Fingerprinting Profile for Alkaloids

HPTLC profile of methanol extract for alkaloid was recorded in Table 2. Bright orange coloured zone at visible light mode in reference track, it was observed from the chromatogram after derivatization and brown coloured zone after spraying the 10% ethanolic sulphuric acid reagent, which confirmed the presence of Alkaloids in the methanolic extract (Figure 1, 2). The Rf value of the extract was found to be 0.12, 0.18, 0.27, 0.53, 0.59, 0.90 of peak 1, 2, 3, 4, 5, 6 respectively. Among them peaks 4, 5 were found as alkaloids. The Rf value, peak height and area of the respective alkaloids were given in table 2. Nicotine was used as reference standard.

Previous studies indicated that the alkaloids present in juice of the leaves could be useful for treating psoriasis, chronic skin eruptions and chronic rheumatism. Alkaloids may also posses antibacterial activity against *Staphylococcus epidermidis* and *Klebsiella pneumoniae*, and *Escherichia coli* [6].

2.2. HPTLC Fingerprinting Profile for Steroids

HPTLC Steroid profile of methanol extract were given in Table 3. Blue-violet colored zones at Visible light mode were present in the track, it was observed from the chromatogram after derivatization, which confirmed the presence of Steroids in the methonalic extract of *Leucas aspera* (Figure 3, 4). The

Rf value of the extract was found to be 0.02, 0.53, 0.62, 0.82 of peak 1, 2, 3, 4 respectively. Among them peaks 1, 4 were found as steroids. Stigmasterol was used as reference compound.

The identified steroid compound can be purified and further study can done on Anti-inflammatory activity, as the recent study by Srinivasn et al [7] indicated that steroid compounds can be used against anti-inflammation.

Table 1. Details of standard, Mobile phase, Spray reagent for HPTLC Analysis of Alkaloid, Steroid and Flavonoid

Profile	Standard	Mobile Phase	Spray reagent
Alkaloid	Nicotine (NCT)	n-Butanol-Acetic acid-water (4 : 4 : 1)	Dragendroff's reagent followed with 10% ethanolic sulfuric acid solution reagent and dried at 100°C 3min
Steroid	Stigmasterol (SGL)	Toluene-acetone (9 : 1)	Anisaldehyde sulphuric acid reagent and dried at 110°C 3min
Flavonoid	Rutin (RUT)	Ethyl acetate-butanone-formic acid-water (5:3:1:1)	1% ethanolic aluminium chloride reagent and dried at 100°C 3min

Table 2. Peak table with RF valus, height and area of Alkaloids and unknown compounds

Track	Peak	Rf	Height	Area	Assigned substance
NCT	1	0.09	133.6	2636.7	Nicotine standard
LA	1	0.12	25.4	670.0	Unknown
LA	2	0.18	21.0	552.9	Unknown
LA	3	0.27	32.6	1071.1	Unknown
LA	4	0.53	411.6	38711.0	Alkaloid 1
LA	5	0.59	390.1	24288.0	Alkaloid 2
LA	6	0.90	19.3	440.4	Unknown

Table 3. Peak table with RF valus, height and area of Steroids and unknown compounds

Track	Peak	Rf	Height	Area	Assigned substance
SGL	1	0.39	300.1	7344.9	Stigmasterol standard
LA	1	0.02	31.6	193.5	Steroid 1
LA	2	0.53	13.4	175.0	Unknown
LA	3	0.62	15.8	408.8	Unknown
LA	4	0.82	53.2	2856.0	Steroid 2

2.3. HPTLC Fingerprinting Profile for Flavonoids

In flavonoid profile, Blue or yellow coloured fluorescent zones at UV 366nm mode were present in the track, it was observed from the chromatogram after derivatization, which confirmed the presence of Flavonoids in the whole plant extract (Figure 5, 6). The Rf value of the extract was found to be 0.03, 0.07, 0.16, 0.23, 0.28, 0.35, 0.47, 0.53, 0.78, 0.84, 0.94 of peaks 1 to 11. Among them peaks 4, 5, 6, 7, 8, 11 were found as flavonoids. The details values were presented in table 4. Rutin was used as standard compound.

Among the phytochemicals alkaloids were predominant followed by flavonoid and steroid in the whole plant methanolic extract. At least two types of alkaloids, six types of Flavonoinds and two types of steroids were observed. Comparatively lower concentration of steroid was found in the methanolic extract.

From the HPTLC finger printing 4 unknown compounds were present in Alkaloid profile and 2 unknown compounds were found in steroid profile and 5 unknown compounds were found in flavonoid profile. Further studies to identify these unknown compounds will elucidate their potential value in therapeutics.

Traditionally *Leucas aspera L* was used as an antipyretic and insecticide. Flowers are valued as stimulant, expectorant, aperient, diaphoretic, insecticide and emmenagogue. Leaves are considered useful in chronic rheumatism, psoriasis and other chronic skin eruptions. Crushed leaves are applied locally in snake bites [8]. Further studies on separation and purification of individual compounds will lead to identification of new phytochemical.

EXPERIMENTAL

2.4. Collection of plant materials

Whole plant of *Leucas aspera* were collected from Vellore and washed thoroughly 2-3 times with running tap water and once with distilled water, and then air dried and finally dried in hot air oven at 60°C for 48 hours.

2.5. Preparation of Methanol extract

Dried plant materials of *Leucas aspera* were powdered by using blender. 100 g of powdered whole plant were exhaustively extracted with methanol in the ratio of 1:5 (w/v) for 24 h using soxhlet apparatus. The extract was completely evaporated to dryness using rotary evaporator.

2.6. HPTLC Analysis for Alkaloid, Steroid and Flavonoid

A densitometric HPTLC analysis was performed for the development of characteristic finger printing of alkaloid, steroid and flavonoid profile. The *Leucas aspera* whole plant methanolic extract was dissolved with HPLC grade methanol 100 mg/0.5ml. The solution was centrifuged at 3000 rpm for 5 min and used for HPTLC analysis. 1 µl of sample and 5µl respective standards were loaded as 6mm band length in the 3 x 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 saturation with solvent vapor) with respective mobile phase up to 80 mm. The developed plate was dried using hot air to evaporate solvents from the plate and sprayed with respective spray reagent (Table 1). The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light and UV366nm. Before derivatization, the plate was fixed in scanner stage and scanning was done at 254nm. The Peak table, Peak display and Peak densitogram was identified.

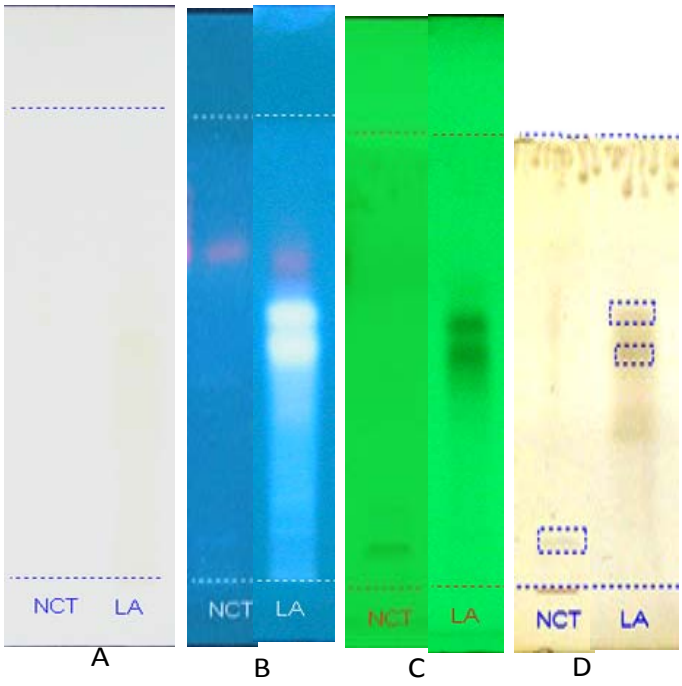

Figure 1. HPTLC finger printing profile for Alkaloid. Chromatograms of control and methonolic extract in HPTLC analysis. A. Before derivatization under day light, B. Under 366 nm, C. Under UV 254 nm, D. After derivatizatioin under day light, NCT – Nicotine, LA – Leucas aspera methonalic extract

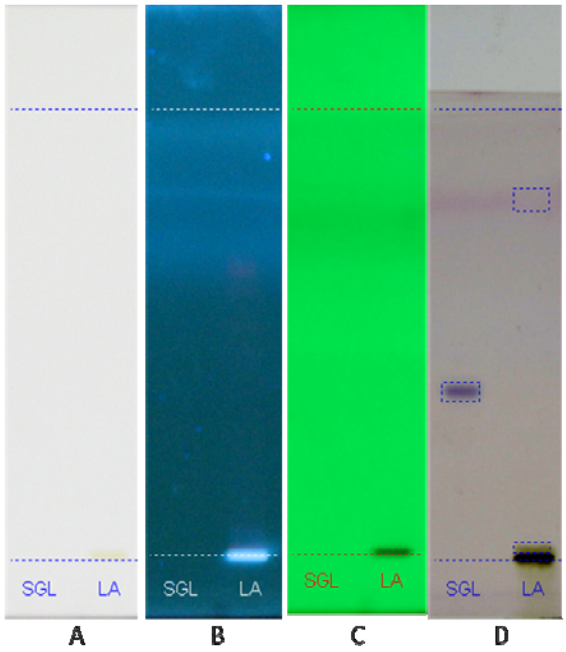

Figure 3. HPTLC finger printing profile for Steroid. Chromatograms of control and methonolic extract in HPTLC analysis. A. Before derivatization under day light, B. Under 366 nm, C. Under UV 254 nm, D. After derivatizatioin under day light, SGL – Stigmasterol, LA – Leucas aspera methonalic extract

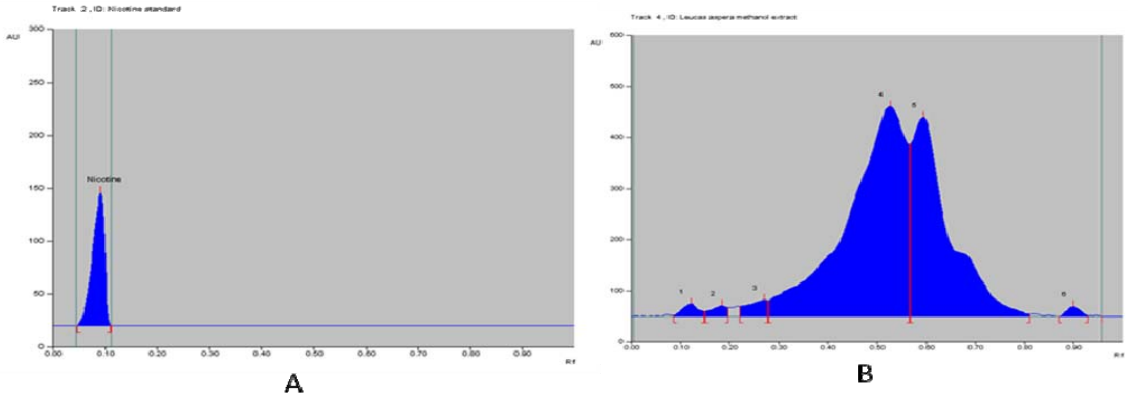

Figure 2. Peak densitogram display of control (A) and methonolic extract (B) in HPTLC analysis for Alkaloid profile

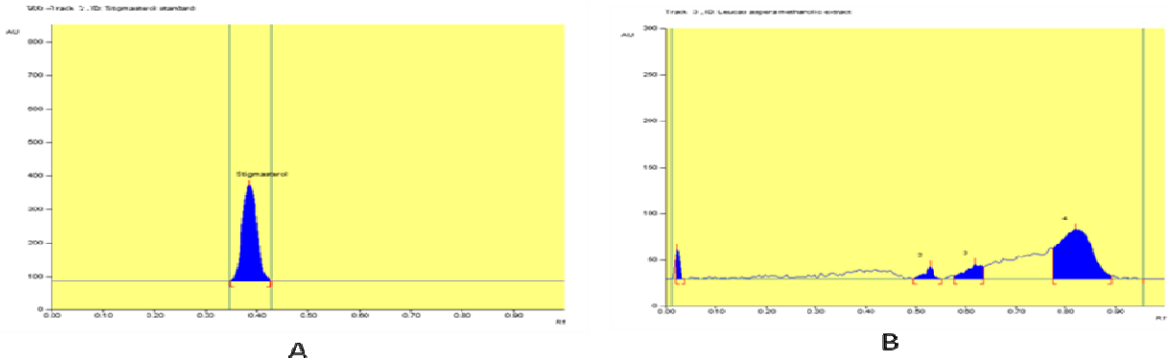

Figure 4. Peak densitogram display of control (A) and methonolic extract (B) in HPTLC analysis for Steroid profile

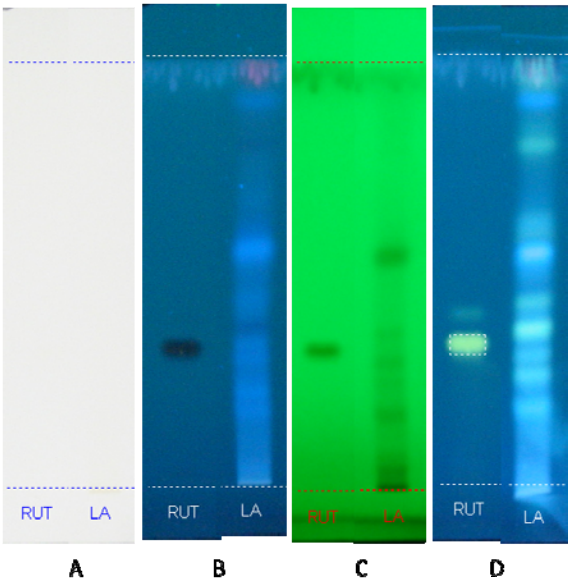

Figure 5. HPTLC finger printing profile for Steroid. Chromatograms of control and methonolic extract in HPTLC analysis. A. Before derivatization under day light, B. Under 366 nm, C. Under UV 254 nm, D. After derivatizatioin under day light, RUT – Rutinl, LA – Leucas aspera methonalic extract

Table 4. Peak table with RF valus, height and area of Flavonoids and unknown compounds

Track	Peak	Rf	Height	Area	Assigned substance
RUT	1	0.32	377.4	10448.2	Rutin standard
LA	1	0.03	21.5	327.4	Unknown
LA	2	0.07	22.8	243.4	Unknown
LA	3	0.16	146.5	6034.3	Unknown
LA	4	0.23	75.3	3037.4	Flavonoid 1
LA	5	0.28	121.9	4143.3	Flavonoid 2
LA	6	0.35	89.2	3384.6	Flavonoid 3
LA	7	0.47	62.4	1921.7	Flavonoid 4
LA	8	0.53	298.4	16699.7	Flavonoid 5
LA	9	0.78	58.1	1914.1	Unknown
LA	10	0.84	76.3	3646.2	Unknown
LA	11	0.94	194.8	12283.6	Flavonoid 6

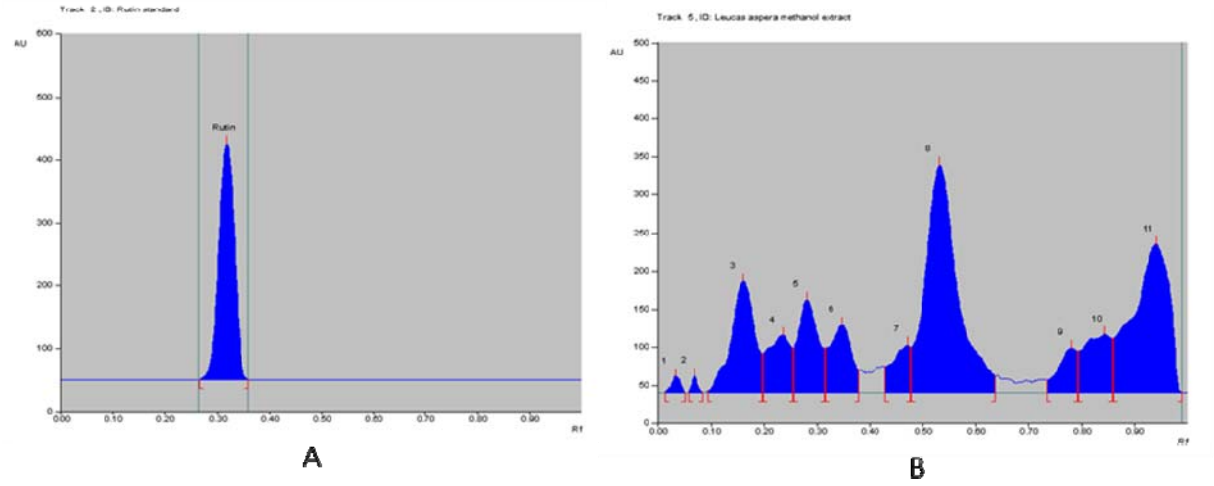

Figure 6. Peak densitogram display of control (A) and methonolic extract (B) in HPTLC analysis for Flavonoid profile

3. CONCLUSION

In recent years due to the adverse effects of allopathic medicine, people have started to think of alternative medicine. Particularly for diseases requiring chronic and long term medication, herbal and phytocompound based treatments are less toxic and effective. *Leucas aspera* collected from Vellore region seems to have promising phytochemical properties which will offer possible lead molecule for drug development.

ACKNOWLEDGEMENT

The authors thank VIT University for providing the necessary facility to carry out this work.

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