WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 11, 2115-2128. Research Article ISSN 2277– 7105

# OPTIMIZATION OF EXTRACTION CONDITIONS AND FINGERPRINT DEVELOPMENT OF LEUCAS ASPERA LINN. BY HPTLC AND HPLC

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Article Received on
17 Sept 2015,

Revised on 09 Oct 2015,
Accepted on 31 Oct 2015,

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## ABSTRACT

Leucas aspera Linn. has been used in ayurvedic medicine for treatment of various ailments. An attempt has been made to optimize different extraction conditions for leucas aspera linn. Hence, it helps for the extraction of the phytochemical constituents and which is used for standardization of chromatographic techniques. Chromatographic fingerprint analysis of herbs and herbal drugs represents a comprehensive qualitative approach for the purpose of species authentication, evaluation of quality and ensuring the consistency and stability of herbs, herbal drugs and their related products. Therefore, aim of this study was to develop the high performance thin layer chromatography (HPTLC) and high performance liquid chromatography (HPLC) fingerprint profile of dried whole plant extract of leucas aspera linn. In the present research article HPTLC and HPLC fingerprint profile of raw material from whole plant of leucas aspera linn. in methanolic extract. HPTLC and HPLC method for the separation of active phytochemical constituents in leucas aspera linn. extracts has been developed. For HPTLC of these extracts was performed on silica gel $60F_{254}$ by semi-automatic applicator and using solvent system toluene: ethyl acetate: methanol (7:2:1, v/v/v) and for HPLC mobile phase A: 0.1% formic acid in water and B:acetonitrile were used. Hence, it was concluded that the established fingerprint provide theoretical and technical support for routine quality control, species identification, authentification and was appropriate for standardization of drug.

**KEYWORDS:** leucas aspera linn, Extraction, CAMAG HPTLC, Shimadzu HPLC, Fingerprint, authentification, standardization.

## INTRODUCTION

Natural products continue to form a significant proportion of drugs in current use and those of under investigation. It has been estimated that 56% of the lead compounds for medicines in the British National Formulary are natural products.[1] There are about 1250 Indian Medicinal Plants, which are used for formulating therapeutic preparation according to Ayurveda and other traditional system of medicine.[2] Phytochemical investigations carried out during the 1970s and 1980s have discovered a number of alkaloids and other pharmacologically active substances that are currently being studied and that can possible serve as models for new synthetic compounds.[3]

Leucas aspera linn. is a medicinal herb that belongs to the family Lamiaceae (Labiatae). It is popular as "Thumbai" throughout the Indian sub continent. Among several other colloquial names, "Dhronpushpi" is common in North India. L.aspera possesses immense medicinal properties- antipyretic.[4] arvicidal.[5] insecticidal.[6] anti-inflammatory.[7,9] antimicrobial.[10,17] and antioxidant.[18,20] Prajapati et al.[21] have reviewed various phytochemical constituents and pharmacological activities of L. aspera. Anatomical features of L. aspera were well explored; its morphology was quite interesting; crystals of varied shape and inclusions/exudates were noticed within and on the leaf and stem.[22] Current investigation is a sequel to it to elucidate the micro chemical (elemental) characteristics of various crystals, inclusions and exudates from various parts of L. aspera.

Leucas aspera Linn. has been used in ayurvedic medicine for treatment of various ailments. An attempt has been made to optimize different extraction conditions for Leucas aspera linn. Hence, it helps for the extraction of the phytochemical constituents and which is used for standardization of chromatographic techniques.[23,24,25,26]

Chromatographic fingerprint analysis of herbal drugs represents a comprehensive qualitative approach for the purpose of species authentication, evaluation of quality and ensuring the consistency and stability of herbal drugs and their related products.[27] In the recent year advancement in of chromatographic and spectral fingerprints plays an important role in the quality control of complex herbal medicines.[28] High Performance Liquid Chromatography (HPLC) is one mode of chromatography, one of the most used analytical techniques.[29] HPLC has over the past decade become the method of choice for the analysis of a wide variety of compounds.[30] as well as HPTLC is more efficient, faster method and the results are more reliable and reproducible. In combination with digital scanning profiling, HPTLC

also provides accurate and precise Rf values and quantitative analysis of sample by in situ scanning densitometry aided by formation of easily detected derivatives by post-chromatographic chemical reactions as required, as well as a record of the separation in the form of a chromatogram with fractions represented as peaks with defined parameters including absorbance (intensity), Rf, height and area.[31]

In the present research work, the plant materials selected were subjected to extraction using various solvents and from the extraction efficiency the best solvent was selected. In addition, the parameters chosen for the optimization of the experimental conditions, for getting the best extraction efficiency were i) Amount of the solvent ii) Time of extraction and Number of extractions.

Hence, the main objective of the present study is focused on HPTLC and HPLC fingerprint profile of raw material from whole plant of Leucas aspera linn. in methanolic extract. HPTLC and HPLC method for the separation of phytochemical constituents in leucas aspera Linn. extracts has been developed.

## MATERIALS AND METHODS

### (I) Sample Collection

Leucas aspera linn. whole plant material was collected from Alappuzha, Kerala respectively. The identity of the plant was authenticated by the Department of Botany, at the MS University of Vadodara. Plant material was cleaned and washed under tap water and dried at room temperature for two days, and then was kept in oven at 40°C for two days. The dried material was powdered and sieved through ASTM sieve (85/BS sieve) separately and was kept in separate airtight containers. Samples for HPTLC and HPLC fingerprinting were prepared by using optimized conditions for extraction.

### (II) Chemicals and reagents

HPLC grade methanol, toluene, chloroform, iso-propenol and ethanol obtained from Merck, Anisaldehyde sulphuric acid reagent, formic acid, water, and acetonitrile.

### (iii) Selection of extracting solvent.[23,24,25,26]

### 1) Optimization of solvent

In this experiment the amount of Leucas aspera L.whole plant powder taken was kept constant throughout the experiment. In different sets of volumetric flasks, accurately weighed

Leucas aspera L. whole plant powder were taken. In these different sets of volumetric flasks, equal amount of different solvents were added separately and kept for one hour. Then these extracts were filtered through Whatmann filter paper (No. 41) in previously weighed dry beakers separately and solvents were evaporated to dryness on a water bath. The dried residues were weighed and the percentage extraction of various solvents was calculated. From the percentage extraction values, the best solvent was optimized. The results are given in Table 1.00**.**

**2) Optimization of amount of solvent**

In this experiment, the amount of Leucas aspera L.whole plant powder taken was kept constant throughout the experiment. In different sets of volumetric flasks, accurately weighed Leucas aspera L**.**whole plant powder was taken. In these different sets of volumetric flasks, different amounts of methanol were added and kept for one hour. Then these extracts were filtered through Whatman filter paper no. 41 in pre-weighed dry beakers separately and solvent was evaporated to dryness on a water-bath. The dried residue was then weighed and the percentage extractions were calculated. From the percentage extraction values, the amount of solvent was optimized**.**

From the graph of volume of solvent (in $cm^3$) versus percentage extraction as shown in Figure 2.00, it was observed that the percentage extraction levels remain constant after certain volume of a solvent used for extraction.

The amount of methanol added and the corresponding percentage extraction is given in Table 1.01.

**3) Optimization of time**

For optimization of time of contact between powder and solvent, the optimized volume of solvent was added to the sample and the contents in the flasks were filtered after different time intervals. The above procedure was repeated and the percentage extractive values were calculated. From the percentage extractive values, the optimization time was determined.

For optimization of time, methanol was added to the samples kept in different flasks. The contents in the flasks were filtered after different time intervals and the percentage extractive values were calculated.

From the graph of time in minutes versus percentage extraction as shown in Figure 2.01**,** it was observed that the percentage extraction levels after certain time of extraction remains constant. The time in minutes and corresponding percentage extractive values are given in Table 1.02.

**4) Optimization of number of extractions**

For optimization of the number of extractions, the optimized amount of selected solvent was added to the sample in different sets of flasks and these flasks were kept aside for 90 minutes (optimized time) for both the plant materials. Then the contents of the flasks were filtered separately through Whatman filter paper no. 41 in pre-weighed dry beakers. The residues were again taken in a flask and extracted again using methanol for 90 minutes (optimized solvent and time). The above procedure was repeated.

The percentage extraction values were calculated. From these values number of extractions was optimized. From the graph of number of extractions versus percentage extraction as shown in Figure 2.03**,** it was observed that the percentage extraction levels after certain number of extractions remains constant. The number of extractions and the corresponding percentage extraction for methanol are given in Table 1.03**.**

**(IV) Sample preparation**

**Step 1:** Accurately weighed 250 mg of Leucas aspera linn. Whole plant powder then transferred to 20 ml test tube and 12.5 ml of methanol was added to it. The test tube was kept on test tube shaker for 90 minutes. The contents were filtered through Whatmann filter paper no. 41.

**Step 2:** The residue obtained in step 1 was subjected for further extraction using 12.5 ml of methanol for 90minutes and filtered through Whatmann filter paper no. 41.

**Step 3:** Extraction procedure was repeated again like step2. (The optimized condition for number of extractions was observed to be three in the case of leucas aspera Linn)

**Step 4:** The filtrates obtained in Step 1, 2 and 3 were pooled together, concentrated to half the volume and was used for the further analysis.

**(V) Instrumentation and chromatographic conditions**

**For Thin Layer Chromatography (Table no.1.05,1.06,1.07)**

A Camag HPTLC system (Muttenz, Switzerland) equipped with a sample applicator Linomat V, twin trough chamber, Camag TLC visualiser, HPTLC Scanner 4, winCATs software version (1.4.6) and Hamilton (Reno, Nevada, USA) Syringe (100µL).

**HPTLC instrument having following chromatographic conditions:**

Mobile phase: toluene: ethyl acetate: methanol (7:2:1, v/v/v)

Stationary Phase: Silica gel 60 $F_{254}$ HPTLC pre-coated plates

Detector: UV-Visible and fluorescence.

Wavelength: 254nm, 366nm, 580nm.

Instrument name: Camag HPTLC system

Application Volume: 10µL

Development Mode: CAMAG Twin Trough Chamber

Detecting reagent: Anisaldehyde sulphuric acid reagent

**For High performance liquid chromatography (Table no.1.08)**

A Shimadzu HPLC system, LC-2030 3D model (Low pressure quaternary gradient system) was used with inbuilt PDA detector. Shimadzu LAB SOLUTIONS software (ver. 5.8) was used to operate the HPLC.

**HPLC instrument having following chromatographic conditions:**

Mobile phase: A: 0.1% formic acid in water and B:acetonitrile

Column: Phenomenex C18 (250 x 4.6 mm, 5 µm) column

Detector: PDA Detector.

Wavelength: 254 nm

Instrument name: Shimadzu LC-2030 3D (Prominenence-i)

Injected volume: 20 µl.

Flow rate: 0.5ml/min.

## RESULTS AND DISCUSSION

The proposed optimization of extraction conditions for HPTLC and HPLC method can be used to develop a fingerprint for the identification of Leucas aspera linn. whole plant.(Table : 1.00,1.01,1.02,1.03,1.04 ) The Rf from HPTLC and Rt values from HPLC are tabulated in Table – 1.05,1.06 and1.07 for HPTLC and Table no:1.08 for HPLC can be considered as analytical or bio-marker for identification of plants.

**Table 1.00 Optimization of solvent for extraction leucas aspera L.**

| Sr. No. | Solvent | Weight of powder (g) | Amount of solvent ($cm^3$) | % Extract* |
|---|---|---|---|---|
| 1. | Petroleum ether | 1.0 | 10.0 | 1.64 |
| 2. | n- Hexane | 1.0 | 10.0 | 1.92 |
| 3. | Chloroform | 1.0 | 10.0 | 2.86 |

| | | | | |
|---|---|---|---|---|
| 4. | Ethyl acetate | 1.0 | 10.0 | 3.22 |
| 5. | Ethanol | 1.0 | 10.0 | 3.11 |
| 6. | Methanol | 1.0 | 10.0 | **7.72** |

*** Each observation is mean of three extractions.**

**Table 1.01 Optimization of amount of solvent for extraction leucas aspera L.**

| Sr. No. | Weight of powder (g) | Amount of solvent (cm3) | % Extract* |
|---|---|---|---|
| 1. | 1.0 | 10.0 | 3.27 |
| 2. | 1.0 | 25.0 | 5.94 |
| 3. | 1.0 | **50.0** | **7.50** |
| 4. | 1.0 | 75.0 | 8.29 |
| 5. | 1.0 | 100.0 | 8.38 |

*** Each observation is mean of three extractions.**

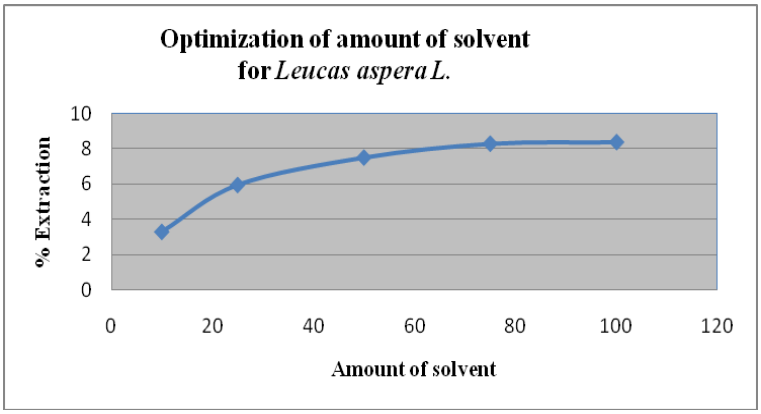


**Figure 2.00.Optimization of amount of solvent for extraction of Leucas aspera L.**

**Table 1.02 Optimization of time for extraction of leucas aspera L.**

| Sr. No. | Solvent | Weight of powder (g) | Time of extraction (min.) | % Extract* |
|---|---|---|---|---|
| 1 | Methanol | 1.0 | 30 | 7.04 |
| 2 | Methanol | 1.0 | 60 | 7.54 |
| **3** | Methanol | **1.0** | **90** | **7.93** |
| 4 | Methanol | 1.0 | 120 | 8.02 |
| 5 | Methanol | 1.0 | 150 | 8.08 |

*** Each observation is mean of three extractions.**

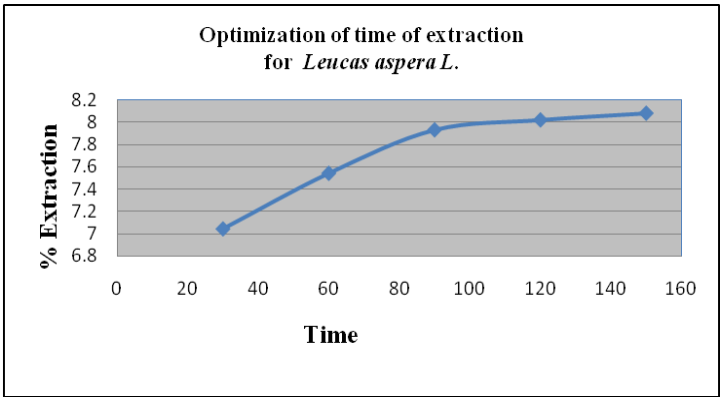


**Figure 2.01.Optimization of time for extraction of Leucas aspera L.**

**Table 1.03 Optimization of number of extractions Leucas aspera L.**

| Sr. No. | Weight of powder (g) | No. of Extractions (n) | % Extract* |
|---|---|---|---|
| 1 | 1.0 | 1 | 7.93 |
| 2 | 1.0 | 2 | 8.67 |
| **3** | **1.0** | **3** | **10.09** |
| 4 | 1.0 | 4 | 10.32 |
| 5 | 1.0 | 5 | 10.38 |

*** Each observation is mean of three extractions.**

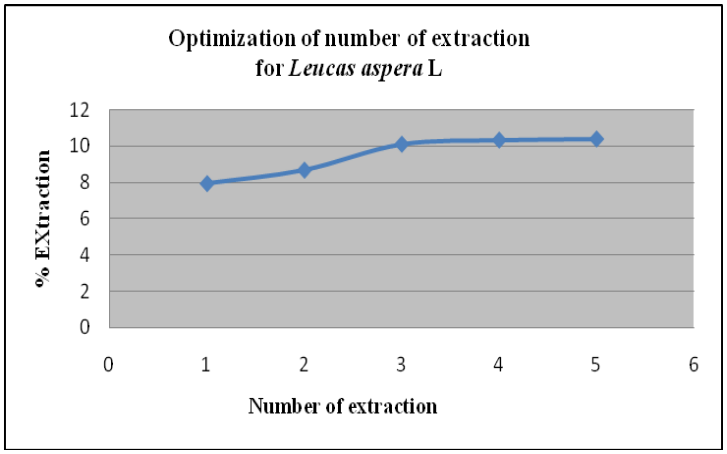


**Figure 2.03.Optimization of number of extractions of Leucas aspera L.**

The summary of the amount of solvent, time of extraction and number of extractions, which were optimized, are given in Table 1.04.

**Table 1.04 Optimization of extraction conditions leucas aspera L.**

| Sr. No. | Plant Species | Solvent | Amount of solvent ($cm^3$) | Time (min.) | No. of Extractions (n) | % Extract* |
|---|---|---|---|---|---|---|
| 1 | **Leucas asperaL.** | Methanol | 50.0 | 90 | 3 | 10.09 |

**Note: *All values are mean of three extractions.**

The maximum extractions of **Leucas aspera L.**whole plant were found in methanol, which was 10.09% . The optimum extraction conditions for selected herbs was shown in **Table1.04.** These optimized parameters can be used for bulk production of whole plant powder extracts of **Leucas aspera L.**whole plant.

**High performance thin layer chromatography**

**At 254 nm**

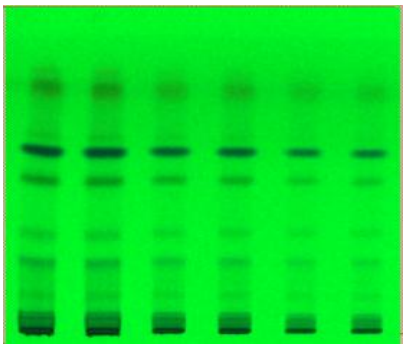

**Fig. 2.04 - HPTLC Chromatogram Image at 254 nm**

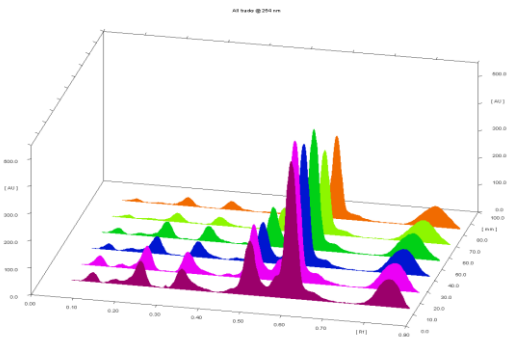

**Fig. 2.05 - 3D plot of all tracks at 254 nm**

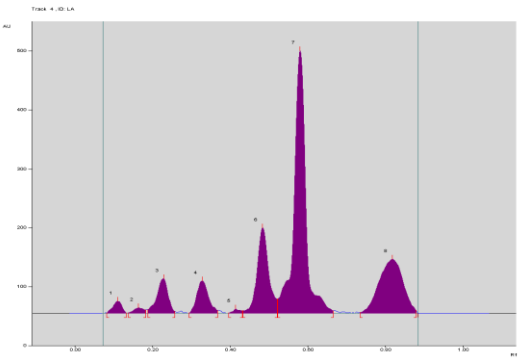

**Fig. 2.06 - Fingerprint at 254 nm**

Track 4, ID: LA

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.08 Rf | 0.7 AU | 0.11 Rf | 20.4 AU | 2.45 % | 0.13 Rf | 0.4 AU | 393.6 AU | 1.70 % |
| 2 | 0.14 Rf | 0.8 AU | 0.16 Rf | 9.3 AU | 1.12 % | 0.18 Rf | 5.8 AU | 193.9 AU | 0.84 % |
| 3 | 0.19 Rf | 6.6 AU | 0.23 Rf | 58.7 AU | 7.05 % | 0.26 Rf | 3.6 AU | 1415.6 AU | 6.13 % |
| 4 | 0.29 Rf | 0.8 AU | 0.33 Rf | 54.9 AU | 6.59 % | 0.37 Rf | 5.3 AU | 1362.8 AU | 5.90 % |
| 5 | 0.40 Rf | 0.5 AU | 0.41 Rf | 7.5 AU | 0.90 % | 0.43 Rf | 4.1 AU | 111.6 AU | 0.48 % |
| 6 | 0.43 Rf | 4.1 AU | 0.48 Rf | 145.0 AU | 17.41 % | 0.52 Rf | 24.1 AU | 3642.0 AU | 15.77 % |
| 7 | 0.52 Rf | 24.5 AU | 0.58 Rf | 445.3 AU | 53.46 % | 0.67 Rf | 4.5 AU | 11239.8 AU | 48.68 % |
| 8 | 0.74 Rf | 1.7 AU | 0.82 Rf | 91.7 AU | 11.01 % | 0.88 Rf | 5.0 AU | 4729.1 AU | 20.48 % |

**Table 1.05 – Peak list and Rf values at 254 nm**

**At UV (254nm)**

It is evident from Table -1.05 that in 10 µL methanolic extract of leucas aspera linn. whole plant, there are 8 spots at the Rf $_{max}$ 0.11, 0.16, 0.23. 0.33, 0.41, 0.48, 0.58 and 0.82 as shown in Fig. 3(2.06), indicating the occurrence of at least 8 different components in 10 µL methanolic extract. Out of 8 components, the components with Rf $_{max}$ values 0.48, 0.58 and 0.82 were found to be more predominant as the percentage area was more with 15.77%, 48.68% and 20.48% respectively. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 7.0 %.

**At 366 nm**

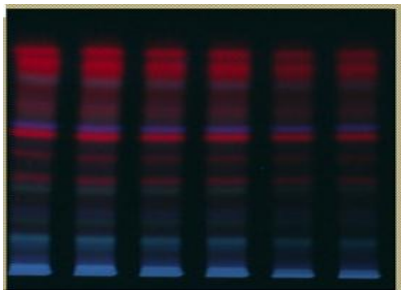

**Fig. 2.07- HPTLC Chromatogram Image at 366 nm**

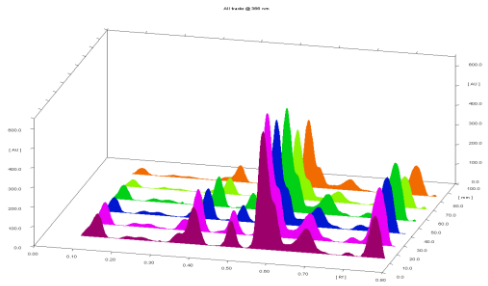

**Fig. 2.08 - 3D plot of all tracks at 366 nm**

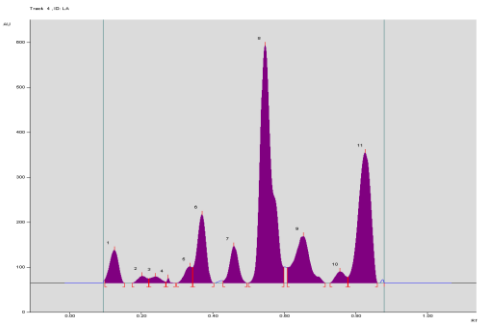

**Fig. 2.09- Fingerprint at 366 nm**

Track 4, ID: LA

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.10 Rf | 6.8 AU | 0.13 Rf | 73.1 AU | 5.50 % | 0.15 Rf | 0.1 AU | 1511.7 AU | 4.44 % |
| 2 | 0.17 Rf | 0.1 AU | 0.20 Rf | 15.3 AU | 1.15 % | 0.22 Rf | 9.9 AU | 321.8 AU | 0.94 % |
| 3 | 0.22 Rf | 10.0 AU | 0.24 Rf | 13.9 AU | 1.05 % | 0.27 Rf | 2.4 AU | 330.0 AU | 0.97 % |
| 4 | 0.27 Rf | 2.1 AU | 0.28 Rf | 10.0 AU | 0.76 % | 0.30 Rf | 0.1 AU | 52.7 AU | 0.15 % |
| 5 | 0.30 Rf | 0.1 AU | 0.34 Rf | 36.5 AU | 2.74 % | 0.34 Rf | 34.5 AU | 631.4 AU | 1.85 % |
| 6 | 0.34 Rf | 34.9 AU | 0.37 Rf | 152.1 AU | 11.44 % | 0.40 Rf | 0.2 AU | 3136.9 AU | 9.20 % |
| 7 | 0.43 Rf | 4.8 AU | 0.46 Rf | 82.0 AU | 6.17 % | 0.50 Rf | 0.1 AU | 1577.8 AU | 4.63 % |
| 8 | 0.50 Rf | 0.1 AU | 0.55 Rf | 527.8 AU | 39.71 % | 0.60 Rf | 35.2 AU | 14055.5 AU | 41.24 % |
| 9 | 0.61 Rf | 34.5 AU | 0.65 Rf | 103.8 AU | 7.81 % | 0.72 Rf | 0.2 AU | 3683.5 AU | 10.81 % |
| 10 | 0.73 Rf | 0.1 AU | 0.76 Rf | 25.0 AU | 1.88 % | 0.78 Rf | 13.0 AU | 551.6 AU | 1.62 % |
| 11 | 0.78 Rf | 13.3 AU | 0.83 Rf | 289.7 AU | 21.79 % | 0.86 Rf | 0.1 AU | 8231.2 AU | 24.15 % |

**Table 1.06– Peak list and Rf values at 366 nm**

**At fluorescence (366nm)**

It is revealed from Table -1.06 that in 10 µL methanolic extract of leucas aspera linn. whole plant, there are 11 spots at the Rf $_{max}$ 0.13, 0.20, 0.24. 0.28, 0.34, 0.37, 0.46, 0.55, 0.65, 0.76 and 0.83 as shown in Fig. 6(2.09), indicating the occurrence of at least 11 different components in 10 µL methanolic extract. Out of 11 components, the components with Rf $_{max}$ values 0.37, 0.55. 0.65and 0.83 were found to be more predominant as the percentage area was more with 9.20%, 41.24%, 10.81% and 44.15% respectively. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 8.5 %.

**At visible light**

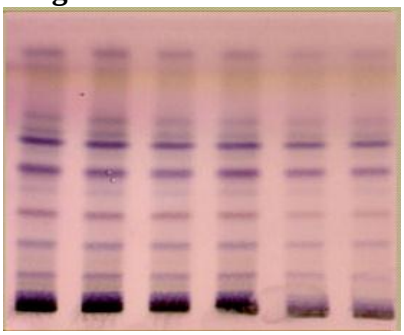

**Fig. 2.10 - HPTLC Chromatogram Image at 580 nm**

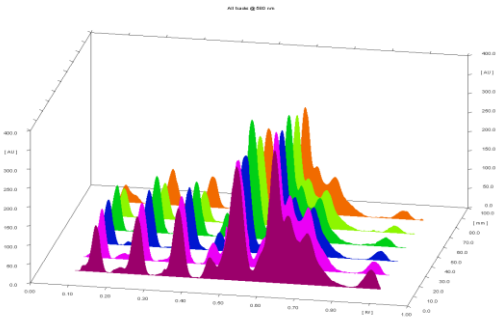

**Fig. 2.11- 3D plot of all tracks at 580 nm**

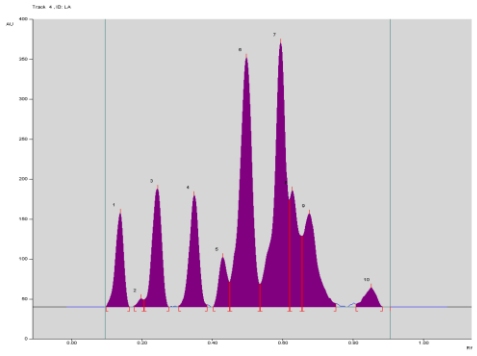

**Fig. 2.12- Fingerprint at 580 nm**

Track 4, ID: LA

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.10 Rf | 0.0 AU | 0.14 Rf | 117.5 AU | 8.35 % | 0.17 Rf | 0.2 AU | 2281.6 AU | 6.35 % |
| 2 | 0.18 Rf | 1.4 AU | 0.20 Rf | 10.6 AU | 0.76 % | 0.21 Rf | 9.0 AU | 142.8 AU | 0.40 % |
| 3 | 0.21 Rf | 9.3 AU | 0.24 Rf | 147.5 AU | 10.49 % | 0.28 Rf | 0.0 AU | 3205.7 AU | 8.92 % |
| 4 | 0.31 Rf | 1.5 AU | 0.35 Rf | 139.7 AU | 9.94 % | 0.39 Rf | 2.5 AU | 3108.1 AU | 8.64 % |
| 5 | 0.40 Rf | 0.4 AU | 0.43 Rf | 62.3 AU | 4.43 % | 0.45 Rf | 31.2 AU | 1257.2 AU | 3.50 % |
| 6 | 0.45 Rf | 31.8 AU | 0.50 Rf | 312.0 AU | 22.19 % | 0.54 Rf | 28.7 AU | 9237.7 AU | 25.69 % |
| 7 | 0.54 Rf | 29.0 AU | 0.60 Rf | 330.2 AU | 23.48 % | 0.62 Rf | 33.5 AU | 9255.5 AU | 25.74 % |
| 8 | 0.62 Rf | 134.7 AU | 0.63 Rf | 145.7 AU | 10.36 % | 0.66 Rf | 38.8 AU | 3038.6 AU | 8.45 % |
| 9 | 0.66 Rf | 88.8 AU | 0.68 Rf | 116.5 AU | 8.28 % | 0.75 Rf | 4.4 AU | 3758.5 AU | 10.45 % |
| 10 | 0.81 Rf | 3.5 AU | 0.85 Rf | 24.2 AU | 1.72 % | 0.88 Rf | 0.3 AU | 668.8 AU | 1.86 % |

**Table 1.07– Peak list and Rf values at 580 nm**

**At visible (540nm)**

It is revealed from Table -1.07 that in 20 µL methanolic extract of leucas aspera linn. whole plant, there are 10 spots at the Rf $_{max}$ 0.14, 0.20, 0.24. 0.35, , 0.43, 0.50, 0.60, 0.63, 0.68 and 0.85 as shown in Fig. 9(2.12), indicating the occurrence of at least 10 different components in 10 µL methanolic extract. Out of 10 components, the components with Rf $_{max}$ values 0.50, 0.60 and 0.68were found to be more predominant as the percentage area was more with 25.69%, 25.74%, and 10.45% respectively. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 9.0%.

**High performance liquid chromatography**

**At UV (254nm)**

It is revealed from Table -1.08 that in 20 µL methanolic extract of leucas aspera linn. whole plant, there are 15 peaks at the Rt 3.680, 3.783, 4.740. 5.064 , 5.234, 5.580, 6.176, 7.287, 8.030,8.723,8.523,9.679,11.063,11.259 and 12.626 as shown in Fig. 10(2.13), indicating the occurrence of at least 15 different components in 10 µL methanolic extract. Out of 15 components, the components with Rt values 3.680, 5.234,7.287, 8.030,8.723,8.523 and 12.626were found to be more predominant as the percentage area was more with 6.56%, 8.78%, 17.62%,5.63%,5.84%,6.12%and 27.78% respectively. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 5.50.

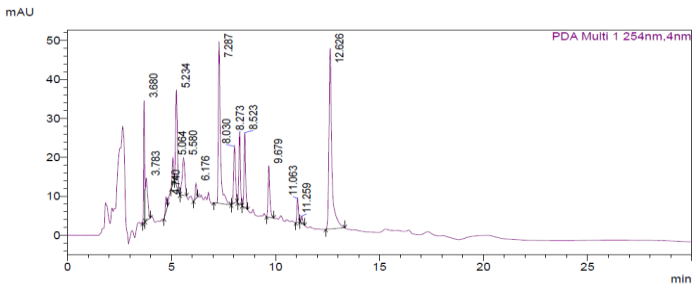


**Fig. 2.13– HPLC, Fingerprint( Chromatogram) at 254 nm**

**Table 1.08 HPLC Peak list and Rt $_{values}$ at 254 nm**

<Peak Table>
PDA Ch1 254nm

| Peak# | Ret. Time | Name | Area | Area% | Resolution(USP) | Tailing Factor | Height |
|---|---|---|---|---|---|---|---|
| 1 | 3.680 | RT:3.680 | 107072 | 6.568 | -- | -- | 31249 |
| 2 | 3.783 | RT:3.783 | 79732 | 4.891 | 0.495 | -- | 10959 |
| 3 | 4.740 | RT:4.740 | 18137 | 1.113 | 4.185 | 0.933 | 3650 |
| 4 | 5.064 | RT:5.064 | 32111 | 1.970 | 2.287 | 0.941 | 6784 |
| 5 | 5.234 | RT:5.234 | 143183 | 8.783 | 1.075 | 1.201 | 24315 |
| 6 | 5.580 | RT:5.580 | 86390 | 5.300 | 1.593 | 0.930 | 9631 |
| 7 | 6.176 | RT:6.176 | 18278 | 1.121 | 2.969 | 1.047 | 3881 |
| 8 | 7.287 | RT:7.287 | 287362 | 17.628 | 6.881 | 2.423 | 41558 |
| 9 | 8.030 | RT:8.030 | 91825 | 5.633 | 4.108 | 0.952 | 14483 |
| 10 | 8.273 | RT:8.273 | 95214 | 5.841 | 1.420 | 1.082 | 18245 |
| 11 | 8.523 | RT:8.523 | 99803 | 6.122 | 1.580 | 1.097 | 19270 |
| 12 | 9.679 | RT:9.679 | 78307 | 4.804 | 7.029 | 1.364 | 13099 |
| 13 | 11.063 | RT:11.063 | 29854 | 1.831 | 8.520 | 1.071 | 6048 |
| 14 | 11.259 | RT:11.259 | 9954 | 0.611 | 1.223 | 1.298 | 1784 |
| 15 | 12.626 | RT:12.626 | 452922 | 27.784 | 6.794 | 1.853 | 46389 |
| Total | | | 1630145 | 100.000 | | | 251344 |

## CONCLUSION

The availability of analytical methods for the analysis of ground plant material is paramount in ensuring quality in dietary supplement and herbal products. It is possible a manufacturer will purchase in bulk, ground plant material, but will only have the word of the supplier for sample authentication. As a powder, visual authentication of the plant and detection of adulterations are not possible. This fingerprint method, manufacturers as well as consumer groups can be used for QC of presence of leucas aspera linn. species in formulation or identification from raw materials.

These optimized extraction parameters can be used for bulk production of whole plant powder extracts of Leucas aspera L.whole plant. The HPTLC fingerprinting method had the advantages of simplicity, rapidity and visuality, whereas, the HPLC fingerprinting method had the advantages of specificity, powerful separation ability and the ability to derive detailed chemical information. Both methods improved the reliability of identification of traditional medicines.

**ACKNOWLEDGEMENTS:** Special thanks to Anchrom Enterprises Pvt.Ltd, India and Shimadzu Analytical (India) Pvt. Ltd. for providing their facility to complete the work.

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