Phytol - A chemical constituent of *Leucas aspera* through HPTLC

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**Abstract:** The aim of this study was to evaluate phytochemical constituent of *Leucas aspera*. The plant extract is obtained by soxhlet extraction method by using ethanol as solvent. Phytochemical analysis was performed on plant extract to detect the presence of phytoconstituents and comparison is done by using HPTLC. Phytochemical screening revealed the presence of phenolics, alkaloids, tannins, terpenoid in *Leucas aspera*. HPTLC fingerprinting shows presence of caryophyllene in *Leucas aspera*. The HPTLC is also suitable for rapid and simple authentication.  

**Keywords:** HPTLC, extraction, *Leucas aspera*

I. INTRODUCTION

Phytochemical analysis of plants which were used in folklore has yielded a number of compounds with various pharmacological activities. Standardization of the plant material is need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physico-chemical characters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful (1). HPTLC is becoming a routine analytical technique because of its advantages of low operating cost, high sample throughput, simplicity and speed, the need for minimum sample clean up, reproducibility, accuracy, reliability and robustness (2). Since ancient times, the medicinal properties of plants have been investigated in the recent scientific developments throughout the world. Although the original hopes regarding their therapeutic usefulness were not immediately realized, recent researchers have demonstrated their involvement in the medicinal action of a number of plant drugs. It is very probable that a number of herbal remedies, the constituents of which are still unknown (3) *Leucas aspera* is reported to have antifungal, prostaglandin inhibitory, antioxidant, antimicrobial, antinociceptive and Cytotoxic activities (4). *Leucas aspera* is used in the traditional medicine of the Philippines to treat scorpion bites. It is also an antipyretic, it is a herb that has the ability to help reduce fever (5). In some forms of traditional medicine, the steam formed by crushing the Samoolam, also known as the plant's flowers, seeds, roots, berries, bark or leaves, can be inhaled to help treat nasal congestion, coughing, cold, headache and fever. In addition the juices of the flower can be extracted and used to help treat sinusitis, as well as headaches. The juice of the flowers can also be used to treat intestinal worms in children. Consequently, present study was focused on the qualitative estimation in the whole plant ethanolic extract of *Leucas aspera* for the identification of phytol by high performance thin layer chromatography.

II. MATERIALS AND METHODS

**A. Instrumentation**  
A Camag HPTLC system (Muttenz, Switzerland) equipped with a sample applicator Linomat V, twin trough plate development chamber, TLC Scanner3, win CATS software and Hamilton (Reno, Nevada, USA) Syringe (100μL).

**B. Material and reagents**  
HPLC grade ethanol, Benzene, Ethyl acetate were obtained from E. Merck, India.

**C. Plant material and extraction**  
*Leucas aspera* material was collected from Gangapur dam, Nasik, Maharashtra, India. 50g of air dried whole plant sample was powdered with the help of grinder and then extracted by soxhlet method for 72 hours using ethanol. The extract was then filtered and concentrated in Rota evaporate to obtain crude form of ethanol extract. The final solution for spotting was prepared by reconstituting the extract in ethanol to give a final concentration of 1 mg/ml. This solution was used further for HPTLC analysis as per the procedure mentioned below.

**D. Standard stock solutions and sample preparation**  
Stock solution of standards (1 mg/mL) was prepared by dilution in ethanol.

**E. Chromatographic conditions:**
a) **Instrumentation**
A Camag HPTLC system (Muttenz, Switzerland) equipped with a sample applicator Linomat V, twin trough plate development chamber, TLC Scanner3, win CATS software and Hamilton (Reno, Nevada, USA) Syringe (100µL).

b) **Adsorbent:**
TLC aluminum plate Precoated with silica gel GF254 (E. Merck)

**F. HPTLC analysis**
Chromatograph was performed on 10x10 cm aluminum packed TLC plate coated with 0.2 mm layer of silica gel 60F254 (E. Merck Ltd, Darmstadt, Germany) stored in a desiccator, application was done by Hamilton micro syringe (Switzerland), mounted on a Linomat V applicator. Spotting was done on the TLC plate, ascending development of the plate, migration distance 80 mm (distance to the lower edge was 10 mm) was performed with Benzene: Ethyl acetate (4:1) up to 80 mm as a mobile phase in a camag chamber previously saturated for 30 min. Various concentrations of the sample (2µl, 3µl L, 4 µl and 5 µl) were applied in four tracks. After development the plate was dried at 60°C in an oven for 5 minutes. Densitometric scanning was then performed with a Camag TLC Scanner 3 equipped with win CATS Software Version at λ max 246 nm. The chromatograms were recorded.

**III. RESULTS AND DISCUSSION**
Standardization and characterization of herbal drugs is a topic of continuous scientific interest in the herbal drug industry. With the advent of modern chromatographic systems there is an ever increasing intent to produce and develop easy, rapid, convenient and cost effective methods for standardization (6). For the standardization of ethanolic extract of whole plant, HPTLC is a sensitive and accurate tool that widely used for quality assessment of plant extract and its derived product formulation (7). The 3D spectra of plant extract and standard shown in Fig. 1.a indicate that all sample constituents were clearly separated without any tailing and diffuseness and showing all peaks at same Rf. The chromatogram of caryophyllene shown in Fig. 1.b with Rf in the Table 1.c showed that for ethanol extract of *Leucas aspera*, there are 5 spots at the following Rf 0.04, 0.07, 0.14, 0.21,0.84 as shown in Fig. 1.c.

[Graph and table images are not included in the text.]
1. b) HPTLC chromatogram of Standard Phytol

![HPTLC chromatogram image]

1. c) HPTLC chromatogram of Leucas aspera

**Table 1 – Rf value of phytol**

<table>
<thead>
<tr>
<th>Plate dimensions</th>
<th>10 x 10 cm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stationary phase</td>
<td>Silica gel G for TLC.</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Benzene: Ethyl acetate (4:1 v/v)</td>
</tr>
<tr>
<td>Marker compound</td>
<td>Phytol</td>
</tr>
<tr>
<td>Rf value</td>
<td>0.80</td>
</tr>
<tr>
<td>λ max</td>
<td>246</td>
</tr>
</tbody>
</table>

In the current work, quality of *Leucas aspera* was evaluated on the basis of phytoconstituent using a validated HPTLC method. From the above studies we can interpretate that the *Leucas aspera* extract contained considerable amount of phytol. In future this study will be helpful for the quantitative determination of phytoconstituents in *Leucas aspera*.

**IV. CONCLUSION**

Phytochemical analysis is very important laboratory process or scientific process. This process is identified essential components of any plant part such as bark, leaves, stem, root. No one knows exactly how many different medicinal plants are enormously important in both traditional and western medicine. Hence it is essential to analyze the phytochemical present in the plant through a potential technique. Based upon the HPTLC fingerprints, it can be concluded that this analytical technique is a convenient method to identify the presence of numerous constituents present in ethanolic extract of plant.

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**REFERENCES**