Pharmacognostic evaluation of *Leucas cephalotes* spreng leaves

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**ABSTRACT**

**Background:** The reliability and integrity of traditional systems of medicine depend upon properly identified sample of drugs ready to use as therapeutic agents for safety reason of the consumers. **Objective:** Keeping this background in mind this study was carried out on the leaf of *Leucas cephalotes*, basically a folklore drug also used in Unani Medicine and Ayurveda, on pharmacognostical parameters for producing enough data for correct identification of the plant. **Materials and Methods:** Pharmacognostic parameters such as morphology, anatomy, macroometry, micrometry, and quantitative microscopy, observation of isolated elements, physicochemical evaluation, preliminary phytochemical evaluation and fluorescence analysis of leaf were carried out using standard methods described by Johnson, Trease and Evans, British pharmacopoeia, Jenkins et al. and Kokoshi et al. HPLC and Spectrophotometery of aqueous and methanol extracts of leaf were also made. **Results:** Detailed results have been shown in figures and tables. **Conclusion:** The study provided useful information which can effectively be used to ascertain the authenticity of the available sample of the drug. **Keywords:** anatomy, HPLC, micrometry, physicochemical studies, spectrophotometery.

**INTRODUCTION**

Medicinal plants and traditional systems of medicine have been indissolubly linked as medicinal plants are backbone of these systems. Since the credibility and reliability of these systems of medicine depend on appropriately identified crude drug samples, it becomes crucial to have authentic samples ready to use as therapeutic agents which are largely concerned with the safety of consumers because herbal drugs can be used safely only when their safety, efficacy and quality standards are up to the mark. Reproducibility of the effectiveness of herbal formulations is another concern for which availability of homogenous starting material is inevitable. Unfortunately, these values have not been given due consideration in traditional systems of medicine. In view of the continuously rising demand and interest of people in herbal drugs that have called for greater exactitude in appraisal of these drugs, it becomes critical to ascertain standard samples of crude single drugs for referential information.[1] The rapid expansion of various aspects of crude drugs has necessitated a systemic approach to study these drugs with methodical and appropriate methods of standardization.[2]

Sophisticated analytical instruments play significant role in the evaluation of crude drugs and formulations and are used for standardization. Though, it is obligatory to use instrumental techniques to obtain information required for solving analytical problems, significance of classical methods of analysis can’t be underestimated. Therefore, combination of some physical and chemical operations on the samples of crude drugs substantiated with modern analytical tools will be better for checking the genuineness of crude drug samples.
Leucas cephalotes (LC) commonly known as Gumma in India, belonging to the family Lamiaceae, primarily a folklore medicine also used in Unani medicine and Ayurveda, is an annual herb and an upland rainy season weed, usually found in roadsides, meadows, waste lands and cultivated grounds throughout the greater part of India. The entire plant as well as its different parts, in isolation, are used medicinally. Whole plant possesses stimulant, laxative, diaphoretic, antiseptic, anthelmintic, insecticidal, germicidal, emmenagogue, expectorant and antipyretic properties. Though, fruit and seed of this plant have been evaluated for some pharmacological actions and chemical constituents, but, very few reports on its pharmacognostic aspects are available. Therefore, pharmacognostic study of the leaf of this plant was taken up for generating data for referential information.

**MATERIALS AND METHODS**

**Material**

**Collection and authentication of the plant**

Fresh plant was collected from the forest of Satpura range of Burhanpur (MP), India, in the month of July. The plant was identified and authenticated by botanists of National Ayurveda Dietetic Research Institute, Bangalore vide authentication no. Drug Authentication/SMPU/NADRI/BNG/2009-10/896. Fresh material was used for morphological and anatomical studies, whereas the material was dried well in shade and powdered in electric grinder for other studies. The study was carried out in the Pharmacognosy laboratory and Central Instrumentation Facility Laboratory, National Institute of Unani Medicine (NIUM), Bangalore.

**Preparation of extract**

Coarse powder of air dried drug (100 g) was subjected to Soxhlet apparatus for 8 h for hot extraction with distilled water, methanol, acetone, di-ethyl ether, petroleum ether and chloroform, separately. The extracts were filtered and the filtrate was evaporated to dryness. The percentage yield of each solvent was calculated with reference to the air dried drug and expressed in gm%±SEM.

**Macroscopic studies**

Fresh leaf was examined by naked eye for morphology and organoleptic characters.

**Microscopic studies**

Transverse sections of leaf were cut according to the method described by Johnson. The sections were stained, mounted and observed under microscope. Photographs were taken by digital camera (Sony10.1MP). Micrometry of various cells was done with the help of a micrometer (stage micrometer and ocular micrometer) by the method described by Trease and Evans and expressed in micron (µm). Study of isolated elements was also carried out.

**Physico-chemical studies**

For estimation of ash values, extractive values, and pH, standard methods described in British pharmacopoeia were applied. Moisture content was determined by the method of Jenkin et al. Florescence analysis of powdered drug was carried out according to the method of Kokoshi et al.

**Preliminary phytochemical studies**

Preliminary phytochemical screening for detection of various phytochemical was done by the method of Bhattacharji and Das.

**High performance liquid chromatography (HPLC)**

HPLC of aqueous extract of leaf was run on an ultra fast liquid chromatography (UFLC) system (Shimadzu, Japan) with a LC-20AD pump and 20A auto-sampler, Phenomenex Luna C18 (2) column (250×4.6 mm id) 5 micron was maintained at 40°C. Mobile phase solvents were filtered through 0.45 µ membrane Millipore, PVDF under vacuum. The sample for analysis was filtered through the 0.22 µ membrane. The mobile phase A, solvent was double distilled water. The mobile phase B, solvent was HPLC analytical grade methanol. The flow rate was 0.5 ml/minutes using methanol: water (70:30) as mobile phase solvent, under a pressure of 100 f/sq.cm, run time of 10 minute and an injection volume of 20 µL at 240, 205, 254, and 238 nm. Analyst 1.4 software was used to control all the parameters.

**Spectrophotometry**

Spectrum scan curves of aqueous and methanol extracts of leaf were obtained by using UV-Vis Spectrophotometer 3000 (Labindia). After preheat time, spectrophotometer was assessed to spectrum scanning mode. The parameters were set, the photometric mode was assessed to Abs, scanning speed was set as middle, and the wave
length range was set to 190–660 nm. Base line correction was performed with the blank cell, and then samples of extracts of drug were scanned.

**RESULTS**

Leaf shortly petioled, oblong-elliptic, opposite, decussate, simple, petioles pubescent; lamina ovate, widest in the middle and tapering to pointed apex, bases obtuse, margin serrulate, crenate, tips acute, unicostate, reticulate, upper surface pubescent, lower surface puberulent, the veins more conspicuous and membranaceous, petioles 6–11 mm long, lamina 3.5–7.0 cm × 1.5–3 cm. and green in colour (Figures 1a & b). Transverse section of leaf was through midrib and lamina showed the single layered epidermis of elongated epithelial cells with wavy cell wall measured 46.12–62.26–80.71 µ in length and 34.59–36.89–46.12 µ in breadth. Numerous covering trichome uniseriate, multicellular, acute tipped, 1–4 celled present on upper and lower surface of the section measured 57.65–85.32–126.83 µ in length and 11.53 µ in breadth. The Diacytic (Caryophyllous or cross celled) stomata measured 23.06 µ in length and 11.53 µ in breadth. Mesophyll comprises of palisade and spongy parenchyma cells. Palisade cells arranged in single layer, compact with radially elongated cells below upper epidermis measured 34.59–43.81–46.1 µ in length and 8.12–10.84–11.53 µ in breadth. Spongy parenchyma multilayered, isodiametric, measured 34.59–50.73–69.18 µ in length and 34.59–46.12–80.71 µ in breadth. In the midrib portion the palisade cells absent and epithelial cells followed by the double layer of the collenchyma cells present at below upper epidermis measured 11.53 µ in length and 11.53 µ in breadth and thickness of layers of collenchymas above lower epidermis measured 57.65–76.09–103.75 µ. Transverse section of midrib vascular bundles slightly arc shaped with lignified Xylem and non lignified Phloem measured 172.95–244.53–299.78 µ in length and 115.3–138.36–172.95 µ in breadth. The size of xylem measured 8.12–13.15–23.06 µ in length and 8.12–10.84–11.53 µ in breadth. Phloem measured 8.12 µ (Figures 2a–2d). Results of micrometry and quantitative microscopy of leaf and micrometry of trichome are given in Tables 1, 2, 3, respectively. Isolated elements are given in Figures 3l–3h. Results of fluorescence analysis of powder are shown in Table 4.

The mean percentage values of total ash, acid insoluble ash, water soluble ash and water insoluble ash was found to be 6.24 ± 0.31; 15.19 ± 0.10, 6.35 ± 0.10, 5.78 ± 0.14. The percentage of moisture content with reference to air dried drug determined by Azeotropic volumetric method using Dean stark apparatus was found to be 6.8 ± 0.37. The extractive values determined in Petroleum Ether, Diethyl Ether, Chloroform, Acetone, Methanol, and Distilled Water where found to be 4.37 ± 0.15, 5.07 ± 0.30, 6.83 ± 0.12, 6.41 ± 0.09, 14.14 ± 0.17 and 38.79 ± 2.31, respectively. The preliminary phytochemical screening of leaf showed presence of phytosterols, fixed oil, carbohydrate, phenolic compound & tannin, protein amino acid, glycoside, cardiac glycosides and flavonoids.

HPLC analysis of aqueous extract (Aq Ext LC-L) at 254 nm showed three peaks (Figure 4). Spectrum scanning of aqueous extract (Aq Ext LC-L) gave nine peaks and three valleys whereas methanolic extract (Met Ext LC-L) gave 3 peaks and four valleys (Figures 5 a&b).

**DISCUSSION**

Quality of raw materials which plays central role in guaranteeing purity, safety, efficacy and stability of herbal preparations is often challenging, but it can be triumphed

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<tr>
<th>Table 1. Macrometry of leaf of Leucas cephalotes Spreng.</th>
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<td><strong>Leaf (l x b)</strong></td>
</tr>
<tr>
<td>Mean</td>
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<tr>
<td>SD</td>
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<tr>
<td>SEM</td>
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<tr>
<th>Table 2. Micrometry of trichomes of leaf of Leucas cephalotes Spreng.</th>
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<tr>
<td><strong>Large</strong></td>
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<tr>
<td><strong>Range</strong></td>
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<tr>
<td>Mean</td>
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<tr>
<td>SD</td>
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<tr>
<td>SEM</td>
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<th>Table 3. Quantitative microscopy of leaf of Leucas cephalotes Spreng.</th>
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<td><strong>Stomatal index</strong></td>
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<tr>
<td><strong>Range</strong></td>
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<tr>
<td><strong>92–115</strong></td>
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<tr>
<td><strong>4–6.5</strong></td>
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<tr>
<td><strong>1–15</strong></td>
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<td><strong>0.07</strong></td>
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over by making appropriate strategies for standardization of herbs and their preparations. The approach includes a range of classical and analytical methods such as macroscopic, microscopic, physico-chemical, phytochemical and analytical studies.

Microscopic characters of a plant material such as types and arrangements of different cells, typical shape of trichomes, stomata, vascular bundle and other cells, micro-metry and quantitative microscopy are not only helpful for identification but are also indispensable, specially for those parts of the plants which are available in pieces. Therefore, these methods are often used in association with other analytical methods.[23]

Physico-chemical standards such as ash values, extractive values, moisture content, pH, fluorescence analysis of powdered drug, and qualitative and quantitative analysis of chemical constituents are widely accepted parameters. Ash value is an important parameter for detection of adulteration in herbal drugs.[24] Another valuable parameter is the extractive value in different solvents, to check the quality of drug. A specific solvent extracts specific phytochemical in specific amount. The amount of extract in a particular substance plays an important role in establishing the index of the purity. Any adulteration or substitution may cause change in extractive values.[25] An excess of water in medicinal plant material encourages microbial growth and deterioration. Estimation of moisture content is important for the material which deteriorates quickly in the presence of water. Thus, estimation of moisture content may be a good parameter for checking the purity of the drug.[26] Herbal drugs are generally used in powder form which is more susceptible for adulteration. This problem can be solved by observing the powder of the drug under day light and U/V light after treating the powder with different chemicals because the fluorescence characters are diagnostic.

Phytochemical present in plants are mainly alkaloids, glycosides, glucosides, essential oil, tannins, resins, and flavonoids etc. Analysis of these constituents is a receptive parameter for standardization. These phytochemicals not only vary from species to species but also differ in different samples of the same drug; therefore it can be used as an approachable parameter in the quality control of drugs.[27]

Recently, it has been possible to use sophisticated analytical methods such as HPLC, HPTLC, UV/VIS Spectrophotometry for isolation and identification of chemical constituents present in the with high end results. HPLC is a fast, sensitive and most preferred chromatographic technique for routine assay of new drug as well as determination of adulterant of established drugs. UV/VIS absorption technique may be used for analysis of a variety of natural products. In the present study HPLC of aqueous extract and UV/VIS Spectrophotometry of aqueous and methanol extracts were carried out. These two studies done by us were of preliminary type, hence no major inference can be drawn and could not be interpreted with the reported phytochemicals, however, may be used as standard. Further study is needed in this regard. It was also not possible to compare our findings with any other data as no such study on has been done, hence our findings may also be considered as an addition to the existing reserve of knowledge.

Table 4. Fluorescence analysis of powder of leaf of Leucas cephalotes Spreng.

<table>
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<tr>
<th>S. No.</th>
<th>Tests</th>
<th>Day light</th>
<th>U/V light</th>
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<tbody>
<tr>
<td>1.</td>
<td>Powder as such</td>
<td>Dark olive</td>
<td>Light green</td>
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<tr>
<td>2.</td>
<td>Powder+1NHCL</td>
<td>Dark yellowish brown</td>
<td>Light yellow</td>
</tr>
<tr>
<td>3.</td>
<td>Powder+1NaOH</td>
<td>Dark saddle brown</td>
<td>Lawn green</td>
</tr>
<tr>
<td>4.</td>
<td>Powder+1NaOH+Methanol</td>
<td>Dark olive</td>
<td>Green</td>
</tr>
<tr>
<td>5.</td>
<td>Powder+50% KOH</td>
<td>Saddle brown</td>
<td>Lime</td>
</tr>
<tr>
<td>6.</td>
<td>Powder+50% H2SO4</td>
<td>Saddle brown</td>
<td>Lawn green</td>
</tr>
<tr>
<td>7.</td>
<td>Powder+Conc.H2SO4</td>
<td>Dark red</td>
<td>Dark slate grey</td>
</tr>
<tr>
<td>8.</td>
<td>Powder+50% HNO3</td>
<td>Saddle brown</td>
<td>Lime green</td>
</tr>
<tr>
<td>9.</td>
<td>Powder+Conc.HNO3</td>
<td>Dark red</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>10.</td>
<td>Powder+ Acetic Acid</td>
<td>Olive green</td>
<td>Spring green</td>
</tr>
<tr>
<td>11.</td>
<td>Powder+ Iodine solution</td>
<td>Olive green</td>
<td>Spring green</td>
</tr>
<tr>
<td>12.</td>
<td>Powder+ Distilled water</td>
<td>Olive</td>
<td>Lawn green</td>
</tr>
<tr>
<td>13.</td>
<td>Powder+ Chloroform</td>
<td>Olive</td>
<td>Medium spring green</td>
</tr>
<tr>
<td>14.</td>
<td>Powder+ Acetone</td>
<td>Olive</td>
<td>Lime green</td>
</tr>
<tr>
<td>15.</td>
<td>Powder+ Picric Acid</td>
<td>Olive</td>
<td>Lime</td>
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</table>
CONCLUSION

In the light of the present study it can be concluded that the findings can serve as the source of information to ascertain the authenticity and standardization of the available sample of the drug.

CONFLICTS OF INTEREST

None

ACKNOWLEDGMENT

The authors are thankful to the authorities of National Institute of Unani Medicine, Bangalore for providing financial assistance and facilities for experimentation.

REFERENCES

8. National Institute of Science Communication and Information Sources (CSIR), Useful Plants of India, New Delhi; 2000.
Fig. 1. *Leucas cephalotes* Spreng

(1a) A twig with stem, fruit and flower  (1b) Leaf

Figure 2: Transverse sections of leaf of *Leucas cephalotes* Spreng

(2a) T.S. (10 X 10)  
(2b) T.S. (Pallisade cells)(10 x 45)  
(2c) Stomata of leaf (10 x 45)  
(2d) Trichome of leaf (10 x 10)

Figure 3: Isolated elements of leaf of *Leucas cephalotes* Spreng

(3a) A, Trichome, B, epithelial cells (10 x 10)  
(3b) A, Scalariform vessel, B, Parenchymatous cells (10 x 45)  
(3c) A, Vessel, B, Stomata (Diacytic), C, Epithelial cells (10 x 45)  
(3d) A, Compound vessels (10 x 45), (3e,f) A, Trichome (Base), B, Elongated epithelial cells (10 x 10)  
(3g) A, Trichome (Base), B, Elongated epithelial cells (10 x 10), (3h) Stomatal cell (10 x 45)
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**Figure 4:** HPLC of aqueous extract of leaf of *Leucas cephalotes* Spreng

**Figure 5:** Spectrophotometry of extracts of leaf of *Leucas cephalotes* Spreng

(5a) Aqueous extract  
(5b) Methanol extract

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