# Pharmacognostic Evaluation of Stem of Gumma (*Leucas cephalotes* Spreng)

[1]Mohammad Yusuf Ansari,
[2]Abdul Wadud,
[3]Uzma Jabeen
and
[4]Syed Mohd Faisal Iqbal

[1]Hakim Abdul Hameed
Unani Medical College,
3- Idgah Road,
Dewas-455001 (M.P.)

[2]National Institutes of Unani Medicine,
Kottigepalya, Magadi Main Road,
Bangalore-560091

[3]Department of Microbiology,
Bangalore University,
Bangalore-560091

[4]S.H.U. Tibbia College,
Ganpati Naka,
Burhanpur-450331 (M.P.)

## Abstract

The study has been carried out on the stem of Gumma (*Leucas cephalotes* Spreng, family Lamiaceae), primarily a folklore medicine also used in Unani medicine and Ayurveda, in order to generate sufficient pharmacognostical data for proper identification of the plant. Anatomy, observation of isolated elements, physicochemical evaluation, and micrometry and fluorescence analysis of the stem were performed by using the standard methods usually applied in pharmacognostical studies. HPLC of aqueous extract was run. Spectrum scan curves of aqueous and methanol extracts were also obtained. Detailed results are shown in figures and tables. The findings can serve as the source of information to ascertain the authenticity and standardization of the available sample of the plant.

**Keywords:** Gumma, *Leucas cephalotes* Spreng, Stem Anatomy, Standardization, HPLC, Spectrophotometery.

## Introduction

Herbal drugs can be used safely only when they are authentic, but unfortunately due attention has not been paid towards making these drugs up to the mark, hence, a number of crude drugs used in traditional systems of medicine need extensive screening for standardization. Botany and traditional systems of medicine have been indissolubly linked as plants have been indispensable for human life for basic needs, medicinal plants sustained human health for centuries making these plants the back bone of almost all traditional systems of medicine. Authentic samples of crude drugs to be used as therapeutic agents are concerned with the safety of consumers. Reproducibility of the effectiveness of herbal formulations is also a major health concern for which homogenous starting material is inevitable. Increasing interest of people in herbal drugs has called for scientific appraisal of these drugs (Patra et al., 2010). The fast expansion of various aspects of crude drugs has necessitated a systemic approach to study these drugs with a methodical approach by means of appropriate methods of standardization as per WHO guidelines (Shinde et al., 2007).

Sophisticated analytical instruments play a significant role in the evaluation of new products. The use of these instruments, in present days, is an enthralling part of chemical analysis. Though, it is necessary to use several instrumental techniques to obtain the information required solving analytical problems,

* Author for Correspondence

significance of classical methods of standardization can't be underestimated. Therefore, combination of classical physical and chemical operations on the samples of crude drugs substantiated with modern analytical tools will be better choice for checking the genuineness of crude drug samples.

*Leucas cephalotes* Spreng commonly known as *'Gumma'* in India, belonging to the family Lamiaceae (Khanam, 2005) is an annual herb and an upland rainy season weed (Rajan, 2004), usually found along roadsides, in meadows, waste lands and cultivated grounds throughout the greater part of India (Khare, 2007). This plant as a whole and its different parts are used in Indian Systems of Medicine (ISM) as stimulant and laxative (Pullaiah, 2000), diaphoretic (Kirtikar, 2003), antiseptic, (Anonymous, 2000), anthelminthic (Anonymous, 2003), insecticidal, (Dymock, 2005), germicidal (Chpora, 2002), fungicidal, (Anonymous, 1997), emmenagogue (Chatterjee, 2003), expectorant and antipyretic (Rastogi, 1999) drug. Though, the plant especially fruit and seed have been evaluated for some pharmacological actions (Bhukya, 2010; Sharma, 1978; Sailor, 2010; Singh, 1978) and chemical constituents (Miyaichi, 2006), but the plant has not been evaluated on pharmacognostical basis. The present study, therefore, was taken up to evaluate the stem of the plant on pharmacognostical parameters for the purpose of identification.

## Material and Methods

(i) Collection and authentication of the plant

Fresh plant was collected from the forest of Satpura range of Burhanpur (M P), India, in the month of July. The plant was authenticated by botanists vide authentication No. Drug Authentication / SMPU/ NADRI/ BNG/2009-10/ 896. Fresh material was used for anatomical studies whereas the material was dried well in shade and powdered in electric grinder for other studies.

(ii) Preparation of extract

A calculated amount of the coarse powder of air dried drug was subjected to Soxhlet apparatus for 8 h for hot extraction with distilled water, methanol, acetone, diethyl 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 g % $\pm$SEM for calculating extractive values and was used for further studies.

(iii) Microscopic studies

Transverse sections of the stem were cut according to the method described by Johnson (Johnson, 1940). The sections were stained, mounted and observed under microscope. Photographs were taken by digital camera (Sony10.1MP). Micrometry of various cells and study of isolated elements were carried out by the method reported (Trease and Evans, 2008).

(iv) Physicochemical studies

For estimation of ash and extractive values, standard methods described in British pharmacopoeia (Anonymous, 1980) were applied. Moisture content was determined by the reported method (Jenkin et al, 1957). Florescence analysis of powdered drug was carried out by reported the method (Kokoshi et al, 1958).

(v) Preliminary Phytochemical studies

Preliminary phytochemical screening for detection of various phytochemicals was carried out following the method (Bhattacharji and Das, 1969).

(vi) High performance Liquid Chromatography (HPLC)

HPLC of aqueous extract was run on an ultra fast liquid chromatography (UFLC) system (Shimadzu, Japan) with a LC-20AD pump and 20A auto-sampler, Phenomenex Luna $C_{18}$ (2) column (250 x 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 (7:3) as mobile phase solvent, under a pressure of 100 f/sq.cm, run time of 10 minute and an injection volume of 20μL. at 240, 205, 254, and 238 nm. Analyst 1.4 software was used to control all the parameters.

(vii) Spectrum scan

Spectrum scan curves of aqueous and methanol extracts 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 with the wave length range 190 – 660 nm. Base line correction was performed with the blank cell, and then samples of extracts of drug were scanned.

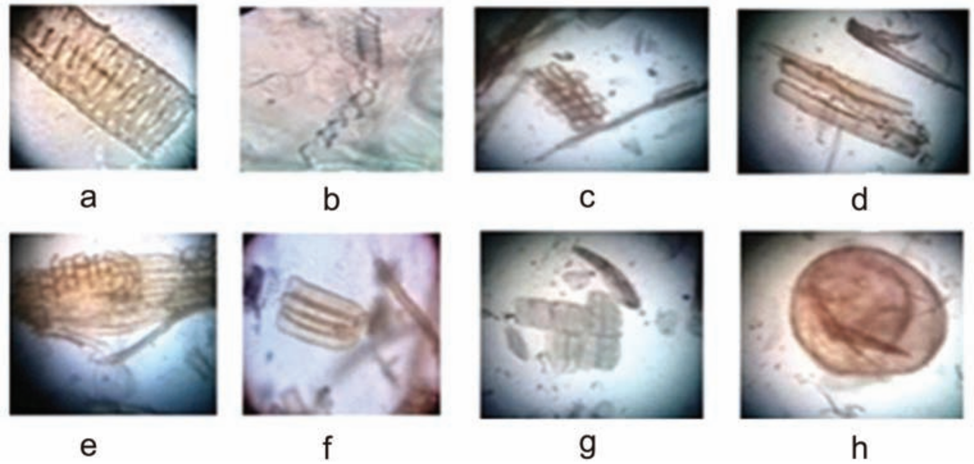
## Results

Detailed micrometry is shown in Table 1. Transverse section of stem was quadrangular in shape, covered with thick cuticle and numerous uniseriate, multicellular, 1-3 celled, of sharp tip covering trichomes. Occasional trichomes were sessile, glandular with broad base. The epidermis was composed of single layer of compactly arranged cells. The cortex underlying the epidermis was constituted a hypodermis layer, the outer most layer of the cortex composed of oval to circular collenchymatous cells. The pericycle made up of perenchymatous cells measured 11.53 – 18.44 – 23.06 μ in thickness. The vascular bundle was well developed, bicollateral with lignified xylem arranged radially, consisted of the vessel of protoxylem and metaxylem, phloem small. Pith portion was very clear and covered most of the part of the stem and made up of parenchymatous cells. Macerated stem showed the presence of reticulate vessel, tracheid, compound vessel, pith cells, fibers, annular vessel and trichomes (Fig.3a-3f). Isolated elements are shown in Fig.2.

The mean percentage values of total ash, acid insoluble ash, water soluble ash and water insoluble ash were found to be 8.14 + 0.10, 1.41 + 0.07, 3.80 + 0.07, and 4.61 + 0.07. The extractive values in petroleum ether, diethyl ether, chloroform, acetone, methanol, and distilled water were found to be 2.56 + 0.02, 3.60 + 0.12, 3.35 + 0.08, 3.55+ 0.06, 10.24 + 0.13, and 18.52 + 0.51. The percentage of moisture content was found to be 4 + 0.44, respectively (Table 1, 2). The result of fluorescence analysis of powder is shown in table 2. Preliminary phytochemical screening demonstrated the presence of glycoside, carbohydrate, phenol compounds, tannin, protein and amino acids.

HPLC analysis of the aqueous extract showed two peaks. Spectrum scanning of the aqueous extract exhibited five peaks and two valleys whereas that of methanol displayed three peaks and one valley (Fig.4, 5a, 5 b).

Fig. 1. *Leucas cephalotes* Spreng : A twig with stem, fruit and flower




(2a) Reticulate vessel (10 x 10), (2b) Annular vessel (10 x 45), (2c) Compound vessel (10 x 45), (2d)Vascular bundles (10 x10), (2e) Vascular bundles (10 x10), (2f) Compound vessel (10 x10), (2g) Parenchymatous cells (10x10), (2h) Pith cell (10x10).

Fig. 2. Study of isolated elements of stem of Leucas cephalotes Spreng

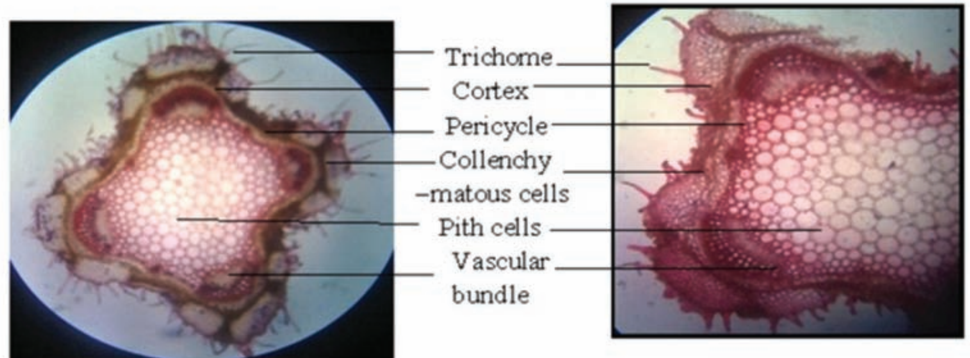



Fig. 3a. T.S of Stem (10 x 5)

Fig. 3b. T.S of Stem portion (10x5)

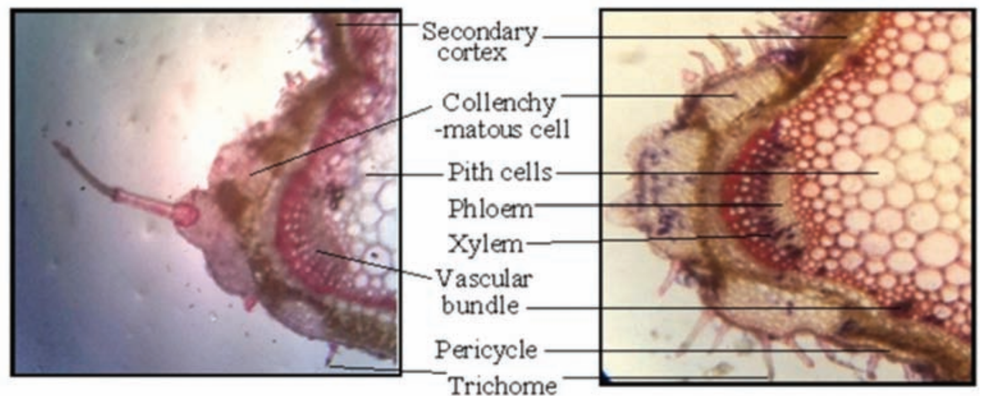



Fig. 3c. T.S. stem showing large Trichome (10 x 10)

Fig. 3d.T.S of Stem portion (10 x10)

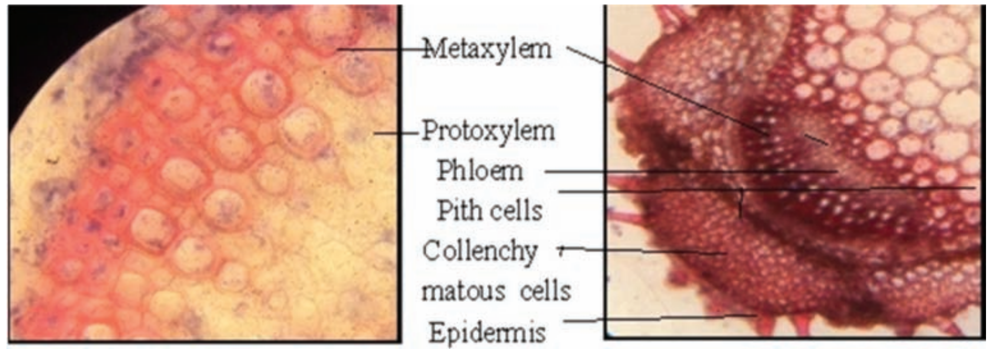



Fig. 3e. T.S. stem showing Vascular

Fig. 3f. T.S. of stem portion (10 x 10)

Fig.3. Microscopy of stem of *Leucas cephalotes* Spreng

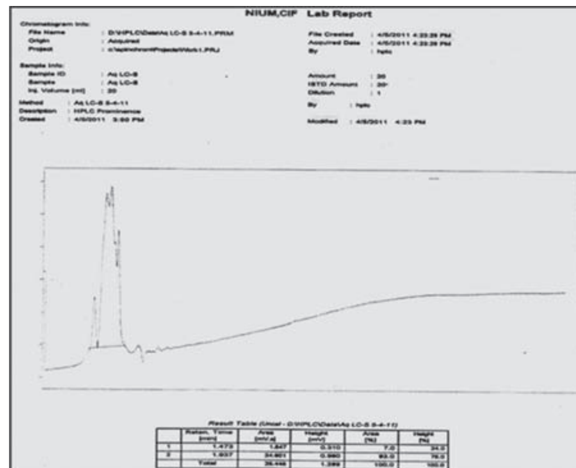
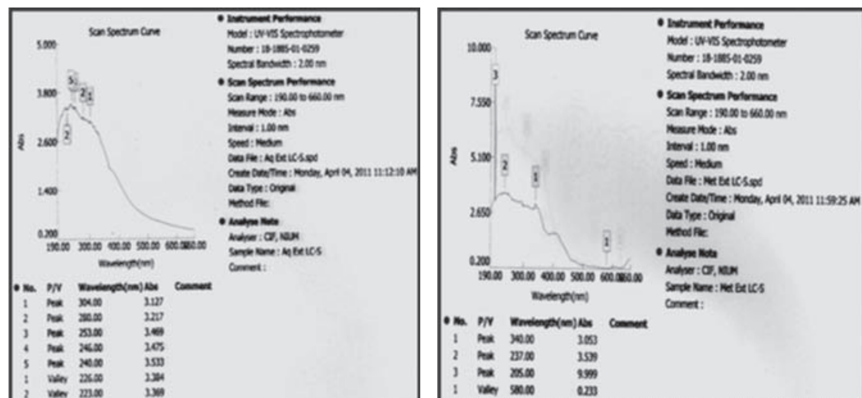



Fig. 4. HPLC of aqueous extract of stem of *Leucas cephalotes* Spreng




| No. | P/V | Wavelength(nm) | Abs | Comment |
|---|---|---|---|---|
| 1 | Peak | 304.00 | 3.127 | |
| 2 | Peak | 280.00 | 3.217 | |
| 3 | Peak | 253.00 | 3.469 | |
| 4 | Peak | 246.00 | 3.475 | |
| 5 | Peak | 240.00 | 3.533 | |
| 1 | Valley | 226.00 | 3.384 | |
| 2 | Valley | 223.00 | 3.369 | |

| No. | P/V | Wavelength(nm) | Abs | Comment |
|---|---|---|---|---|
| 1 | Peak | 340.00 | 3.053 | |
| 2 | Peak | 237.00 | 3.539 | |
| 3 | Peak | 205.00 | 9.999 | |
| 1 | Valley | 580.00 | 0.233 | |

Aqueous extract

Methanol extract

Fig. 5. UV/VIS Spectrophotometery of extract of stem of *Leucas cephalotes* Spreng

**Table 1:** Measurements of cells (Micrometry) of stem of *Leucas cephalotes* Spreng

| S. No. | Cells | Measurement (µm) |
|---|---|---|
| | | Length X Breadth (Range) |
| 1. | Trichome | 80.71 – 392.02 – 1360.54 x 11.53 – 18.44 – 23.06 |
| 2. | Outer cortex | 69.18 – 115.3 – 138.36 x 11.53 – 13.83 – 23.06 |
| 3. | Inner cortex | 23.06 – 32.28 – 46.12 x 11.53 – 16.14 – 23.06 |
| 4. | xylem | 8.12 – 15.46 – 23.06 x 8.12 – 14.77 – 23.06 |
| 5. | Phloem | 8.12 – 8.80 – 11.53 x 8.12 – 8.80 – 11.53 |
| 6. | Pith | 34.59 – 85.32 –126.83 x 34.59 –87.67–126.83 |

**Table 2:** Physicochemical values of stem of *Leucas cephalotes* Spreng

| S. No. | Particulars | Values | | | | | |
|---|---|---|---|---|---|---|---|
| 1. | Extractive values | Petroleum ether | Diethyl ether | Chloroform | Acetone | Methanol | Distilled water |
| | | 2.56 + 0.02 | 3.60 + 0.12 | 3.35 + 0.08 | 3.55+ 0.06 | 3. 10.24 + 0.13 | 18.52 + 0.51 |
| 2. | Ash values | Total ash | Acid insoluble ash | Water soluble ash | Water insoluble ash | x | x |
| | | 8.14 + 0.10 | 1.41 + 0.07 | 3.80 + 0.07 | 4.61 + 0.07 | x | x |
| 3. | Moisture content | 4 + 0.44 | x | x | x | x | x |

**Table 3:** Fluorescence analysis of powder of stem of *Leucas cephalotes* Spreng

| S. No. | Tests | Observations | |
|---|---|---|---|
| | | Day light | U/V light |
| 1. | Powder | Dark golden red | Golden red |
| 2. | Powder + 1NHCl | Yellowish green | Light yellow |
| 3. | Powder +1NNaOH + Methanol | Orange red | Yellowish green |
| 4. | Powder + 50%KOH | Dark golden red | Yellowish green |
| 5. | Powder + 50%H2SO4 | Dark golden red | Lime |
| 6. | Powder + Conc.H2SO4 | Dark golden red | Spring green |
| 7. | Powder + 50%HNO3 | Scarlet Dark | Slate grey |
| 8. | Powder + Conc.HNO3 | Light orange | Yellowish green |
| 9. | Powder + Acetic acid | Brilliant orange | Yellowish green |
| 10. | Powder + Iodine solution | Golden red | Spring green |
| 11. | Powder + Distilled water | Dark olive green | Yellowish green |
| 12. | Powder + Chloroform | Golden red | Medium spring green |
| 13. | Powder + Acetone | Dark golden red | Lime |
| 14. | Powder + Picric acid | Golden red | Yellowish green |

## Discussion

Quality of raw materials plays vital role in guaranteeing purity, safety, efficacy and stability of herbal preparations which is often challenging but can be triumphed over by making appropriate strategies for standardization. The approach includes a range of classical and analytical methods such as macroscopic, microscopic, physic chemical, phyto chemical 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, micrometry and quantitative microscopy are not only helpful for identification but are also indispensible, specially for those parts of the plants which are available in pieces (Lux, 2005).

Physicochemical standards such as ash values, extractive values, moisture content, 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 (Anonymous, 1992). Another valuable parameter is the extractive value taken in different solvents. A specific solvent extract is specific phytochemical in specific amount. Any adulteration or substitution may cause change in extractive value. The amount of extract in a particular substance plays an important role in establishing the index of the purity (Lux, 2005). 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 (Wallis, 2005). 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.

Phytochemicals present in plants are mainly alkaloids, glycosides, essential oil, tannins, resins, and flavonoids. 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 (Do, 2005).

Recently, it has been possible to use sophisticated analytical methods such as HPLC, HPTLC, and UV/VIS spectrophotometery for isolation and identification of phytochemicals with high end results. HPLC is a fast, sensitive and most

preferred chromatographic technique for routine assay of new drug as well as for determination of adulterant of established drugs. In the present study HPLC of aqueous extract and UV/VIS spectrophotometery of aqueous and methanol extracts were carried out. These two studies performed were of preliminary type, hence could not be interpreted with the reported phytochmicals, however, in combination with other methods may be considered as method development. It was also not possible to compare our findings with any other data as no such study has been reported earlier, hence our findings may be considered as an addition to the existing reserve of knowledge.

## 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.

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