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Pharmacognostical Evaluation of *Leucas aspera* Link.

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Abstract – *Leucas aspera* Link. (Lamiaceae) is an important medicinal plant in indigenous systems of medicine in India and commonly known as 'Thumbai'. It has various ethnomedicinal values as various traditional communities find diverse medicinal properties. It is used as antipyretic, stimulant, expectorant and diaphoretic drug. The present communication deals with the detailed pharmacognostical evaluation of whole plant of *L. aspera* collected from five different geographical zones of the country-Uttar Pradesh, Orissa, Karnataka, West Bengal and Gujarat. The botanical characters and TLC fingerprint profile of all the samples were quite similar but some variations were observed in physicochemical parameters. However, some microscopical characters and TLC profile can be used as diagnostic characters for identification of *L. aspera*, for example amphistomachic leaves and two types of trichomes-abundant, non glandular, uniseriate, 1-3 celled and few glandular, 2-5 celled stalk with rounded tip. Presence of some components at R_fs-0.56, 0.65 and 0.76 under UV 366 and at R_fs-0.31, 0.43, 0.60, 0.76 and 0.82 under visible light after derivetization in TLC profile may also be used as diagnostic character.
Keywords : *Leucas aspera*, Thumbai, Pharmacognostical studies, antipyretic

Introduction

Leucas aspera Link. (Lamiaceae), commonly known as 'Thumbai' is an important drug in Indian classical systems of medicine and is used as antipyretic, stimulant, expectorant, aperient, diaphoretic, anti-rheumatic and as an insecticidal agent (Anonymous, 1962, Chopra *et al.*, 1956). Ethnobotanically it is also reported to be useful in malarial fever among the 'Kols' tribe of Banda district and in some tribes of south India (Anonymous, 1962; Saxena and Vyas, 1981). In north Bengal flowers are given with honey for cough and cold in children (Chopra et al., 1956). It is also used in the treatment of jaundice in south Kannada district of India (Shivaprasad and Chandrashekar, 2003). Besides, the juice of leaves is used as an external application for psoriasis, chronic skin eruptions and painful swelling. An alcoholic extract of leaves shows antibacterial activity against *Micrococcus pyogens* and *E. coli*. (Chopra *et al.*, 1956). Srinivas *et al*, (2000) reported the significant anti-inflammatory effect of *L. aspera* against carrageenia induced paw oedema and in cotton pellet induced granuloma in rats. Saundane *et al*, (2000) studied the therapeutic efficacy of different extracts of plant and found that, ethanol and distilled water extracts of *L. aspera* exhibited significant anti-inflammatory activity, whereas, significant analgesic effect was shown by petroleum ether and ethanol extract. Recently, it has been reported to have antioxidant activity too (Sadhu *et al.*, 2003). Two sterols oleonolic and ursolic acid and a triterpenoid lactone were reported as main constituents of this plant (Chopra *et al.*, 1956).

Although, Shome and Mehrotra (1990) reported comparative pharmacognostic and antagonistic studies of the flowers of two closely related plants i.e. *Leucas cephalotes* and *L aspera*, but till date no pharmacognostical standards were reported on the whole plant collected from different geographical zones of the country. Hence, the present study has been undertaken for evolving standard parameters for the identification and quality evaluation of *L. aspera*. The study includes morphological and anatomical characters of whole plant except flower; fluorescent powder analysis and evaluation of different physicochemical parameters along with the thin layer chromatography (TLC).

Experimental

Plant material was collected from Lucknow, Uttar Pradesh, India in the month of Nov.- Dec. and lodged in the department's herbarium vide voucher specimen number

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NBR-PH-222089. Samples of *L. aspera* were also procured from Orissa, Karnataka, West Bengal, and Gujarat. Microscopic studies were carried out on transverse sections (T.S.) by using saffranin as the staining agent (Johansen, 1940). Fluorescence powdered drug analysis was carried out according to the methods described by Chase and Pratt (1949) and Kokoski *et al.*, (1958). The Physico-chemical parameters *viz.*, successive Soxhlet extractives, total ash, acid insoluble ash, alcohol (Ethanol) and water soluble extractives were determined by Indian

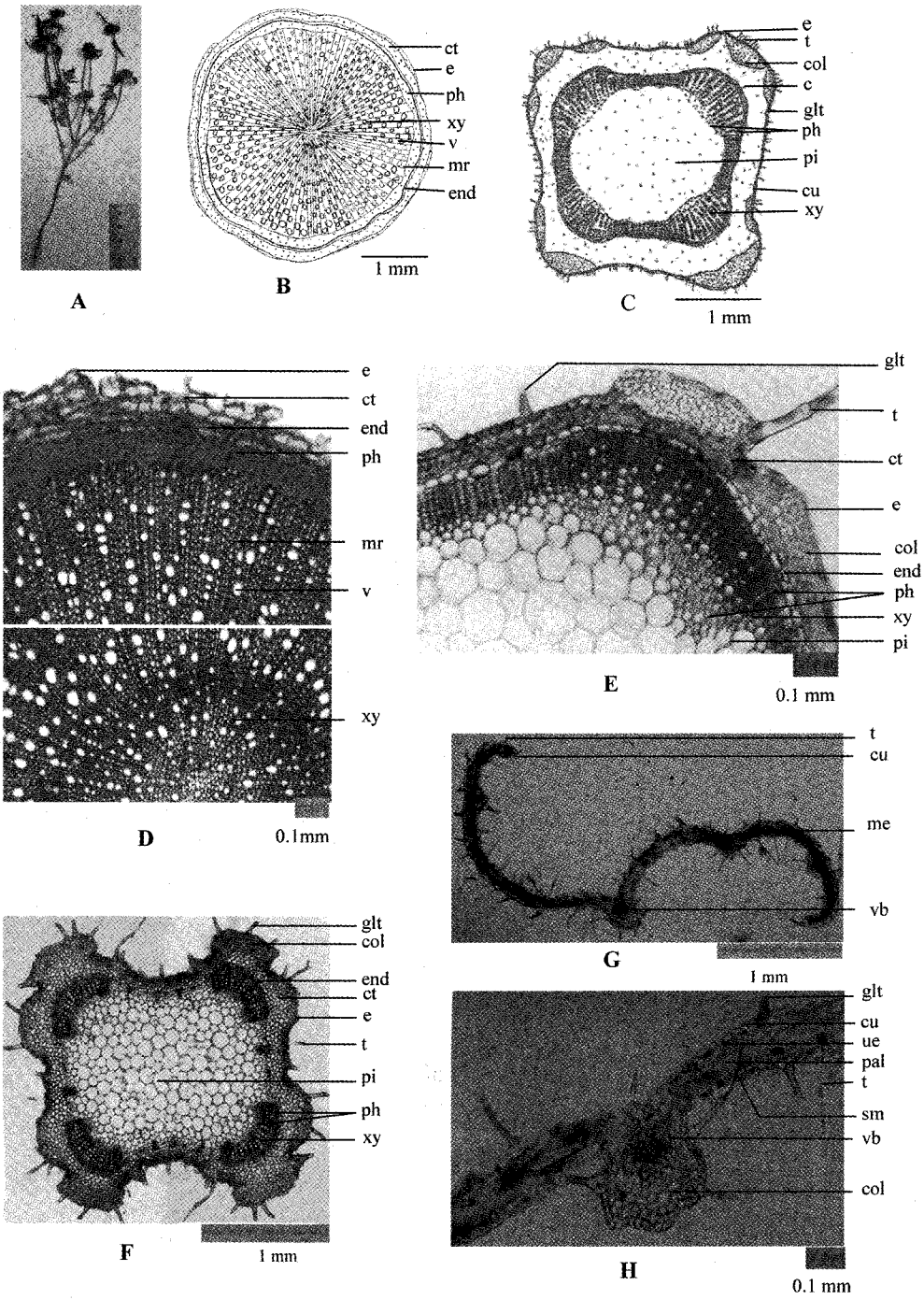

Fig. 1. Macro and Microscopy of *L. aspera.*

A. Crude drug, B. TS Root (Diagrammatic), C. TSStem (Diagrammatic), D. TS Root, E. TS Stem, F. TS Peduncle, G. TS Leaf (Diagrammatic), H. TS Leaf passing through midrib regionD. powder.

Pharmacopoeial methods (Anonymous, 1966). A known quantity of dried plant material was extracted in Soxhlet apparatus with hexane, chloroform, acetone, ethanol and water successively and percentage of each solid extractive was calculated by evaporating the solvents. The estimation of sugar, starch (Mont Gomery, 1957) and tannins (Anonymous, 1984) were also carried out. For TLC fingerprint profile 2 g of each the powdered material was refluxed separately with 25 ml methanol on a water bath for 30 minutes consecutively 3 times. The extract was filtered and concentrated under reduced pressure and taken as test solutions. The 15 µl of the each test solution were applied on precoated silica gel TLC plates (G60 F_{254} E. Merck) with the help of Camag Linomat IV Applicator. The plate was eluted in a solvent system-toluene: ethyl acetate: methanol (85: 15: 0.01) to a distance of 8.5 cm at room temperature (25°C). The plate was derivatized by spraying with anisaldehyde sulfuric acid reagent and then heated for 10 min at 110°C. the photographs were taken by Desaga video documentation unit under UV 366 before spraying and under visible light after derivatization.

Results

Macroscopy – Drug consists of whole plant of *Leucas aspera* Link. (Lamiaceae), a herbaceous, much branched, erect or diffuse annual, 30-60 cm high found more or less throughout India as a weed in cultivated field, waste lands and roadsides. Dried stem pieces are somewhat quadrangular in shape, longitudinally ribbed and furrowed, pubescent, wide central parenchymatous pith occupying the major region of the stem, surrounded by a discontinuous ring of xylem. Leaves subsessile, simple, linear, lanceolate measuring 3.0 to 6.8 cm in length and 0.7 to 1.0 cm wide, margin serrate, apex acute, surface somewhat pubescent, flowers in axillary whorls, bracteate, calyx 10, corolla white, bilipped about 1 cm in length, tube 5 cm long, pubescent above, anthers 4, epipetalous, didynamous. Colour is greenish, taste is slightly salty and pungent (Fig. 1 A).

Microscopy

Root: T. S. root circular in out line, the outermost layer is thick walled epidermis which become crushed at places, cortex parenchymatous 5 to 7 cells broad, consists of tangentially elongated, irregular cells, endodermis discernible, followed by 1 to 2 layered parenchymatous pericycle; phloem is well developed and consists of sieve tubes, companion cells and phloem parenchyma. Major portion of the root is occupied by secondary xylem which consists of vessels, fibres, tracheids and xylem parenchyma, uni to biseriate medullary rays are also distinct (Fig. 1 B &D).

Stem: T.S. stem somewhat quadrangular and ridged in outline, covered with thick papillose cuticle, epidermis, bears simple multicellular, 3 to 4 celled lignified trichomes measuring 2.3-16.1 mm and sessile glandular trichome measuring 0.92-1.38 mm in size with broad base and 3 to 4 celled head and stalked glandular trichome with single celled globular head, hypodermis, composed of 3 to 8 rows of collenchymatous tissue, underneath the ridges, cortex parenchymatous forms 3 to 5 rows, with somewhat thick walled cells, endodermis-distinct, pericycle is characterized by a row or two of big parenchymatous cells, intercepted at places by fibres. Vascular bundles are bicollateral, a large vascular bundle underneath the ridge and 3 to 4 small vascular bundles in between the ridges. After secondary growth these bundles united and formed a continuous ring. Xylem is well developed, radially arranged and consists of vessels, tracheids, fibres and xylem parenchyma. Phloem is narrow encircling the xylem ring, cambium not distinct, peri-medullary phloem surrounding the pith are well developed underneath the ridges, pith cells are thin walled and parenchymatous (Fig. 1 C & E).

Leaf: T.S. passing through midrib shows deep groove on the upper side and broad ridges and furrows on lower side, lamina narrow shows 2 to 4 side veins and get curved at its extremities. The structure of side veins is similar to the midrib but they varying in size. Meristele consists of bicollateral vascular bundle; lower position of the meristele is occupied by the collenchymatous cells. Single layered thick walled upper epidermis interrupted by abundant glandular and few non glandular trichomes followed by single layered palisade cells. The mesophyll cells are parenchymatous. Both simple and glandular trichomes are present on both the surfaces measuring 0.69-3.00 mm, the former being plenty uniseriate 2 to 3 celled with broad base and pointed apex, the latter, stalked with 2 to 4 celled and unicellular head, the sessile glandular trichome with 2 to 6 celled head.

Epidermal cells are sinuous on upper surface but the cell margins not discernible on lower surface in surface view, stomata present on both the surfaces and are diacytic to anomocytic type. The size of stomata and stoma are 19.4-29.1 µm and less than 9.7 µm respectively in diameter. The palisade ratio, vein islet and vein termination numbers are 3-7, 16-39, 21-46 respectively; stomatal index are 9 to 18 on upper surface and 15 to 27 on lower surface and stomatal number are 10 to 55/mm^2 and 75 to 185/mm^2 on upper and lower surfaces respectively

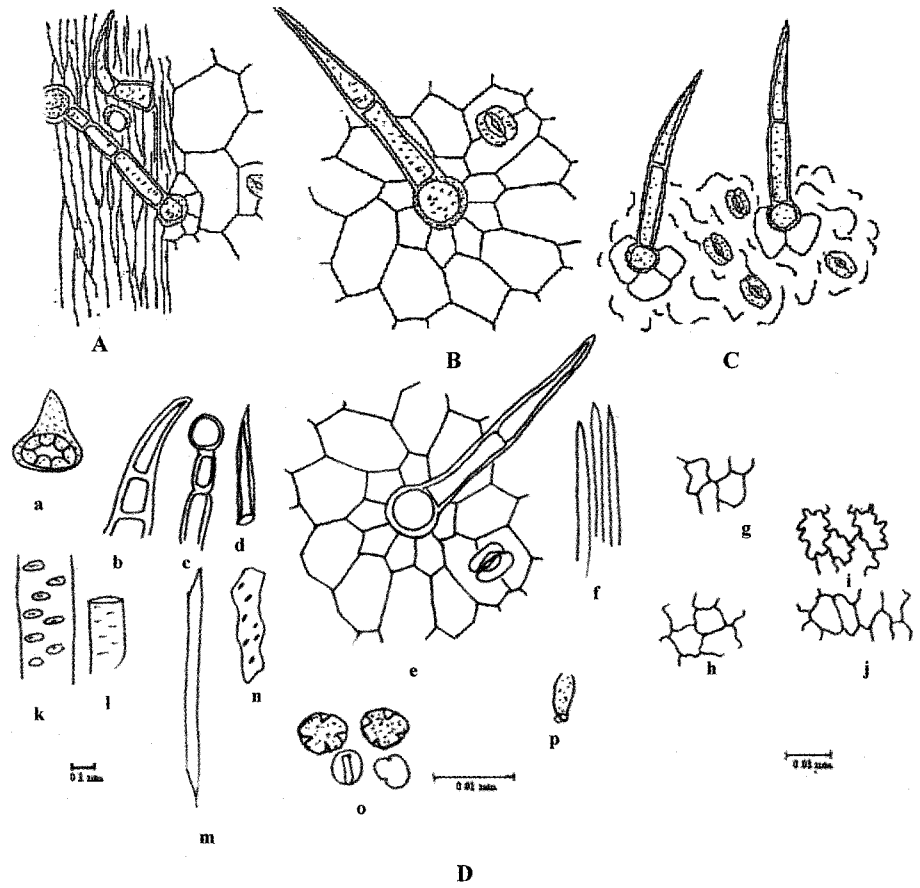

Fig. 2. Microscopy of Leaf surface and Powder.
A. Upper surface showing midrib region, B. Upper surface of Leaf C. Lower surface of Leaf.

(Fig. 1 G & H and Fig. 2 A-C).

Peduncle: T.S. passing through peduncle, is similar to that of the stem except the more ridged margins and 5 to 15 rows of collenchymatous tissue, strongly developed underneath the ridges. Pith is also collen- chymatous (Fig. 1 F).

Powder – Greenish brown with pleasant odour and slightly salty and pungent taste, shows fragments of epidermal cells of leaf, stem, petal and sepals, patches of collenchyma, sessile and stalked glandular trichomes, single celled and multicelled non-glandular trichomes, stomata, vessels, tracheids, hairs of sepal, palisade cells, pollen grains, fragments of fibres and seeds (Fig. 2 D). Powder shows bright yellow colour with 50% KOH and bright green colour with 50% H_2SO_4, when observed under UV 254 nm.

Phytochemical studies

The percentage of total ash, acid insoluble ash, successive Soxhlet extractives, total sugars, starch, water and alcohol

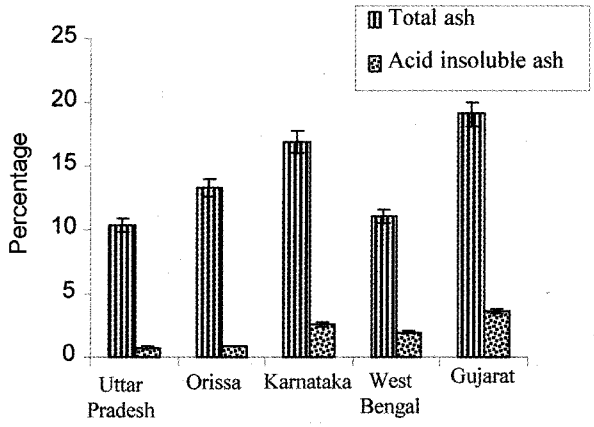

Fig. 3. Ash content in different samples of *L. aspera.*

(Ethanol) soluble extractives, tannins were estimated and the results are depicted in Figs. 3-6. Total ash and acid insoluble ash were maximum i.e. 19.08 and 3.54% respectively in Gujarat sample; the value is almost double as compared to Uttar Pradesh sample which has only 10.34 and 0.77% total ash and acid insoluble ash

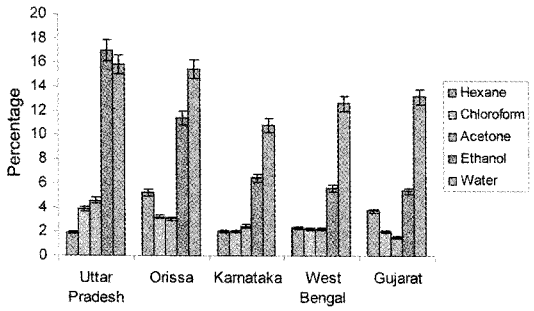

Fig. 4. Successive Soxhlet extractives in different samples of *L. aspera.*

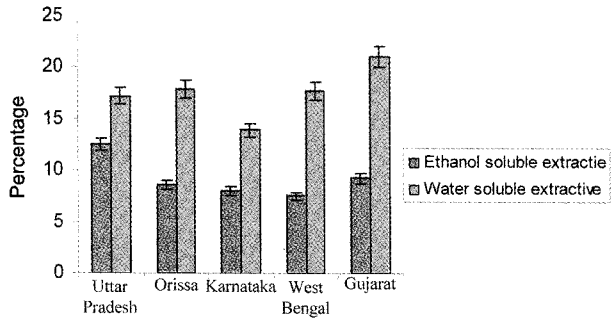

Fig. 5. Alcohol and water soluble extractives in different samples of *L. aspera.*

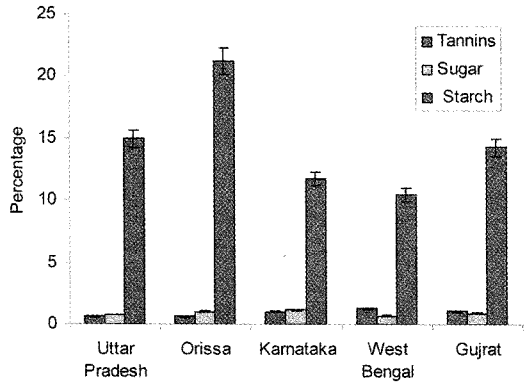

Fig. 6. Tannins, sugar and starch content in different samples of *L. aspera.*

Table 1. Characteristic marker components obtained from TLC

UV-366		Visible after spraying	
R_f Values	Colour of components	R_f Values	Colour of components
–	–	0.31	Grey
–	–	0.43	Grey
0.56	Red	–	–
0.60	Red	0.60	Grey
0.65	Blue	–	–
0.70	Red	–	–
0.76	Red	0.76	Green
–	–	0.82	Pink

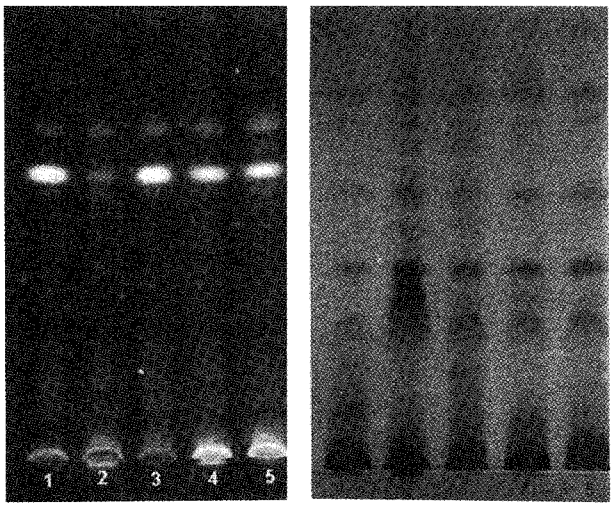

Fig. 7. TLC Finger-print profile of methanolic extract of *L. aspera.*
A. Under UV 366 nm. B. Under visible light after spraying with detecting reagent and heating at 110°C for 10 minutes.

respectively. Whereas, alcohol and water soluble extractives in successive Soxhlet extraction were maximum in Uttar Pradesh sample i.e. 17.02 and 15.87% respectively as compared to other samples.

The characteristic finger print profile of thin layer chromatograms can be used as marker for the quality evaluation of a particular sample. The characteristic marker components obtained from TLC are shown in Fig. 7 and Table 1. All the samples viz. Uttar Pradesh, Orissa, Karnataka, West Bengal and Gujarat showed very close resemblance in having almost more or less the same spots at the same R_fs. However, spot at R_f 0.70 is only visible in Orissa and Gujarat samples under UV 366 nm. After derivatization under visible light spot at R_f 0.31 is more prominent in Orissa sample as compared to other samples.

List of Abbreviations

col, collenchyma; ct, cortex; cu, cuticle; e, epidermis; end, endodermis; glt, glandular trichome; me, mesophyll; mr, medullary ray; pal, palisade cells; ph, phloem; pi, pith; sm, spongy mesophyll; t, trichome; ue, upper epidermis; v, vessels; vb, vascular bundle; xy, xylem; **Powder**: a-d, glandular and simple trichomes; e, epidermal cells leaf with stomata; f, hairs of calyx; g-h, epidermal cells of sepals; i-j, epidermal cells of petals; k-l, vessels; m, fibre; n, tracheid; o, pollen grain; p, fragment of seed; **TLC**: 1. Uttar pradesh, 2. Orissa, 3. Karnataka, 4. West Bengal, 5. Gujarat.

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