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Micro Chemical (Elemental) Analysis of *Leucas aspera* (Willd) Link Employing SEM-EDAX

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ABSTRACT
The plant, *Leucas aspera* (Willd) Link is well known for its varied medicinal uses. Present study deals with its micro chemical (elemental) characterization using Energy Dispersive X-ray Analysis (EDAX) detector fitted to Scanning Electron Microscope. The plant has very interesting morphology. Crystals of varied shape and inclusions/exudates were noticed within and on the leaf & stem surfaces. Various plant parts analysed were, stem surface, stem sections, stem inclusions, blebs on stem hairs, crystals of varied shape, root sections, abaxial and adaxial surfaces, flower, seed and seed caruncle. Lot of variation in elemental composition was observed in various plant parts. Major elements detected were Carbon, Oxygen, Calcium, Silica, and Aluminum. Other elements found were Iron, Sodium, potassium, Phosphorus and Chlorine.

Keywords: *Leucas aspera*, Micro chemical, Elemental Analysis, Plant crystals, SEM-EDAX.

INTRODUCTION

Leucas aspera (Willd) Link 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 [1], Larvicidal [2], insecticidal [3], anti-inflammatory [4-6], antimicrobial [7-14] and antioxidant. [15-17] Prajapati *et al.*, [18] 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. [19] 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*.

Trace elements have a greater role to play on human and animal health. [20] Several workers have correlated the trace element contents of the herbal drugs with therapeutic action. [21-25] Singh *et al.*, [26] have aptly described the trace elements as "inorganic switches". Much attention was given to the trace element content of the herbal drugs in Ayurveda and traditional Indian medicinal systems. [27]

Various methods used for elemental detection are- Atomic Absorption Spectroscopy [28-29], Energy Dispersive X-ray Florescence [30], Electro Thermal Atomic Spectroscopy [31], Energy Dispersive X-ray Analysis [32-35], Instrumental Neutron Activation Analysis [36] and Particle Induced X-ray Emission. [37] Of all the techniques, SEM-EDAX permits the visualization of various microstructures within the sample and it is a non destructive technique that permits multiple sampling in various regions/parts of the plant with a minimum quantity of the drug sample. It is highly useful in the characterization of crystals and inclusions. Hence an attempt was made to evaluate the elemental composition of various parts of *L. aspera* using SEM-EDAX.

MATERIALS & METHODS

Leucas aspera plants were collected in Moinabad area near Hyderabad, A.P., India. Small pieces (3mm) of stem and root, 5 mm^2 pieces of leaves and their hand cut transverse sections and various other parts were fixed in 4% glutaraldehyde in phosphate buffer (0.02M, 6.9 pH), washed with distilled water, dehydrated in an alcohol series, air dried and coated with gold in Hitachi HUS-5GB Vacuum Evaporator. All the chemicals used were of Analytical grade and procured from Loba Chemical. India. SEM-EDAX analysis was carried out using INCAx-sight Oxford detector fitted to Hitachi S-520 Scanning Electron Microscope at an

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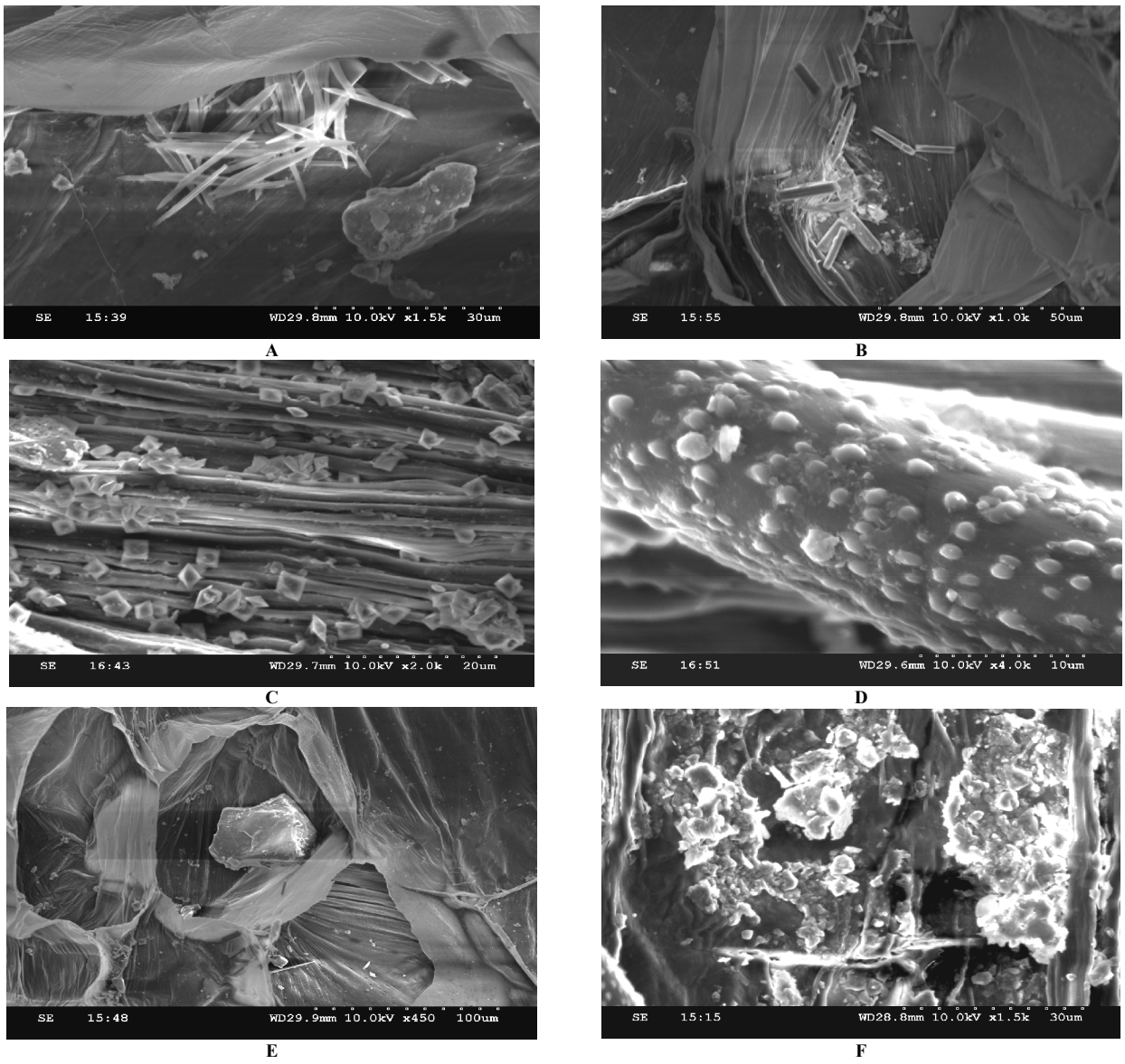

Fig. 1A-F: Scanning Electron Micrographs of various parts of *Leucas aspera*.
A. Cluster Of needle shaped crystals in stem (T.S); B. Rod shaped crystals in stem (T.S); C. Rhomboidal crystals on stem; D. Blebs on stem hair; E. Inclusion in Stem (TS); F. Exudates on stem

Table 1: Elemental composition (weight & Atomic weight* %) of stem

Element	Part studied		
	Stem Surface	Stem Section	Stem inclusion
Carbon	34.06 (44.15)	39.94(48.30)	51.54 (59.79)
Oxygen	50.37 (49.01)	54.39 (49.38)	44.07 (38.38)
Aluminum	1.20 (0.71)	ND	0.50 (0.26)
Phosphorus	ND	0.16 (0.08)	ND
Potassium	ND	0.31 (0.11)	ND
Calcium	10.98 (4.26)	3.92 (1.42)	2.60 (0.90)
Magnesium	ND	0.54 (0.26)	0.31 (0.18)
Silica	3.35 (1.86)	0.84 (0.44)	0.98 (0.49)

*Values in the parentheses; ND - Not Detected; $P < 0.001$ for all the values

acceleration voltage of 20KV. Full screen, window and spot modes were employed depending on the size of the plant part/component.

RESULT & DISCUSSION

Fig. 1A-F depicts crystals of various shapes, blebs on stem hair, inclusions in stem and exudates on stem. EDAX data from various parts and crystals are presented in Tables 1-4. Lot of variation in elemental composition was observed in various parts of *L. aspera*. Major elements detected and their ranges were as follows - Carbon: 34.06-61.92; Oxygen: 18.83-57.82; Calcium: 1.32-22.76; Silica: 0.61-4.04; Magnesium: 0.2-0.93 and Aluminum: 0.40-1.20. Calcium was predominant in almost all the plant parts; its ranges were quite higher than the usually reported values of many other plants. High Ca contents were also reported for *Gleditsia tricanthos* [38], *Beta vulgaris* and *Atriplex.* Ca values were low in root. Potassium & phosphorus were restricted to stem only. K levels were high in rhomboidal crystals. Iron, sodium and chlorine were found in needle shaped crystals.

Plant crystals are hitherto be believed as calcium carbonate or calcium oxalate. Current investigation proved it otherwise; similar observations were made by us earlier in *Lippia nodiflora.* [35] But, for EDAX Analysis it would not have been possible to observe the same with any other analytical tool. Blebs on stem hairs showed high iron content. Silica was present in almost all the parts studied except the caruncle. However, its content was high on leaf surface. This could be the reason for the roughness of the leaf surface and

the smoothness of the caruncle. Several workers have estimated various heavy metal elements in *Leucas aspera*, whole plant with respect to various geological locations and experimental conditions. [29, 36-37, 39-43] Their analyses were not holistic, but restricted to selected heavy metals and the plant *L. aspera* was good at accumulating few heavy metals. Dalvi *et al.*, [29] have stressed the need for making heavy metal analysis, an integral part of the standardization of herbal medicines and also reported higher concentrations of Iron in *L. aspera*.

Table 2: Elemental composition (weight & Atomic weight* %) of blebs on the hairs & crystals from stem of *Leucas aspera*

Element	Part studied			
	Bleb	Needle like Crystal	Diamond shaped Crystal	Cuboid Crystal
Carbon	52.82 (69.58)	34.77 (44.45)	54.89 (65.78)	47.21 (56.06)
Oxygen	18.83 (18.61)	52.33 (50.22)	33.00 (29.69)	45.82 (40.85)
Aluminum	ND	ND	0.40 (0.22)	ND
Phosphorus	ND	ND	ND	ND
Potassium	0.67 (0.27)	ND	ND	0.57 (0.21)
Calcium	22.76 (8.98)	10.06 (3.85)	10.96 (3.94)	2.360.84
Magnesium	0.20 (0.13)	ND	ND	ND
Silica	1.37 (0.77)	1.45 (0.79)	0.74 (0.38)	4.04 (2.05)
Iron	ND	0.44 (0.12)	ND	ND
Sulphur	3.35 (1.65)	ND	ND	ND
Sodium	ND	066 (0.42)	ND	ND
Chlorine	ND	0.32 (0.14)	ND	ND

*Values in the parentheses; ND - Not Detected; P < 0.001 for all the values

Table 3: Elemental composition (weight & Atomic weight* %) of various parts of *Leucas aspera*

Element	Part studied		
	Root section	Adaxial Leaf Surface	Abaxial Leaf Surface
Carbon	42.68 (50.64)	38.23 (46.81)	37.56 (46.69)
Oxygen	53.21 (47.40)	54.40 (50.01)	52.39 (48.89)
Aluminum	ND	0.40 (0.22)	0.59 (0.33)
Calcium	1.32 (0.47)	4.72 (1.73)	5.82 (2.17)
Magnesium	0.93 (0.54)	0.66 (0.40)	ND
Silica	1.86 (0.94)	1.58 (0.83)	3.63 (1.93)

*Values in the parentheses; ND - Not Detected; P < 0.001 for all the values

Table 4: Elemental composition (weight & Atomic weight* %) of various Parts of *Leucas aspera*

Element	Part studied		
	Flower	Seed	Seed Caruncle
Carbon	36.57 (46.66)	39.48 (47.74)	57.22 (64.28)
Oxygen	49.54 (47.46)	55.20 (50.11)	42.06 (35.47)
Calcium	10.38 (3.97)	4.19 (1.52)	0.72 (0.24)
Magnesium	ND	0.52 (0.31)	ND
Silica	3.50 (1.91)	0.61 (0.32)	ND

* Values in the parentheses; ND - Not Detected; P < 0.001 for all the values

In the present study, no harmful heavy metals were detected. It appears that the presence of the heavy metals depends on the locations from which plants are collected. Elemental composition of the plant varies from part to part. Many species were also found to exhibit phylogenic variation. [44-45] Patil and Gaikwad [45] have discussed the significance of various elements with respect to human physiology. *L. aspera* plants are often used as leafy vegetable; the rich calcium and iron contents of the plant might supplement the Ca and Fe to a great extent. Role of these elements in disease curing are well documented. However, the role of calcium in relation to the anti inflammatory activity for which the plant is widely known is to be explored further. Studies are underway in this regard.

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REFERENCES

1. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants, CSIR, New Delhi, 1956.
2. Rao DM, Sapthajyothi G, Reddy TRM, Prasad MVV, Subramanyam K. Larvicidal activity of essential oils from Indian medicinal plants against *Aedis aegypti* L. Indian J. Pharma. Res. 2009; 2(4): 762-764.
3. Chaudhury NA, Ghosh D. Insecticidal plants: Chemical examination of *Leucas aspera*. J. Indian Chem. Soc. 1969; 46(1): 95.
4. Goudgaon NM, Basavaraj NR, Vijayalaxmi. Anti-inflammatory activity of different fractions of *Leucas aspera* Spreng. Indian J. Pharmacol. 2003; 35: 397-398.
5. Kripa KG, Chamundeeswari DJ, Thanka Reddy CUM. Effect of hydro alcoholic extract of aerial parts of *Leucas aspera* (Willd) Link on inflammatory markers in Complete Freund's adjuvant Induced arthritic rats. Int. J. Green Pharma. 2010; 4(4): 281-287.
6. Saundane AR, Hidayatullah KM, Satyanarayan ND. Anti-inflammatory and analgesic activity of various extracts of *Leucas aspera* (Labiatae). Indian J. Pharma. Sci. 2000; 26: 144-146.
7. Rao BGVN, Rao P. Efficacy of some essential oils on pathogenic fungi. Flavan Industry 1972; 3(7): 368-370.
8. Thakur DK, Misra SK, Choudhuri PC. *In-vitro* trials of plant extracts and chemicals for their antifungal activity. Indian J. Animal Hlth. 1987; 26(1): 31-35.
9. Damayanti M, Susheela K, Sharma GJ. Effect of plant extracts and systemic fungicides on the pine apple fruit rotting fungus, *Ceratocystis paradoxa*. Cytobios 1996; 83: 155-165.
10. Begum M, Lokesh S, Raghavendrra VB. Role of leaf extracts of some medicinal plants in the Management of seed borne fungal diseases of Okra (*Abelmaschus esculentus* (L) Moench) Phytopath. and Plt. Protection 2009; 42(10): 950-955.
11. Satish S, Raghavendra MP, Raveesha KA. Antifungal potentiality of some plant extracts against Fusarium sp. Archives of Phytopath. and plt. Protection. 2009; 42(7): 618-625.
12. Rao BGVN. Antimicrobial action of some essential oils IV. Effect of organic compounds. Riechstoffe, Aronen Koerperflegemittel 1971; 21(1): 10-16.
13. Mangathayaru K, Laksmikant J, Shyamasundra N, Swapna R, Fathima GX, Vasantha J. Antimicrobial activity of *Leucas aspera* flowers. Fitoterapia 2005; 76(7-8):752-754.
14. Sapthajyothi G, Mahesh KYG, Muralidhara Rao Ramajaneyulu R. GC-MS analysis and inhibitory efficacy of *Leucas aspera* L. leaf volatile oil against selected microbes. Nigerian J. Nat. Prod. and med. 2007, 11: 79-81.
15. Sumathi A, Puspha A. Evaluation of enzymatic antioccidants in some Indian medicinal Plants. J. of Ecobiol. 2007; 21(2): 107-111.
16. Haripyaree A, Gureeshwar K, Damayanthi, M. Evaluation of antioxidant properties of some medicinal plants by sulfur free radical reactivity with curcumin asreference. Electronic J. Environ. Agri. and food chem. 2010; 9(2): 337-344.
17. Meghashri S, Vijayakumar H, Gopal S. Antioccidant property of a novel flavonoid from leaves of *Leucas aspera*. Food Chem. 2010; 122(1): 115-110.
18. Prajapati MS, Patel MS, Modi K, Shah MB. *Leucas aspera*: a review. Pharmacognosy Rev. 2010; 4: 85-87.
19. Yashvanth S, Rani SS, Rao AS, Madhavendra SS. Anatomical exploration of *Leucas aspera* (Willd) Link a medicinal herb and its pharmacognostic relevance. J. Pharm. Res. 2011; 4(12): 4777-4779.
20. Under Wood EJ. Trace metals in human and animal health. J. Human Nutrition 1981; 53: 37-48.

21. Januja KM. Trace metal elements in *Allium cepa* (onion) and their therapeutic Importance. Hamdard Medicus 1990; XXXIII (2): 87-90.
22. Sailey A, Sahu R, Gupta B, Sandhi SM. Analysis of mineral elements of Medicinal plants used for the treatment of asthma, syphilis, diarrhea, skin diseases and rheumatism. Hamdard Medicus 1994; XXXVII: 18-22.
23. Sahito SR, Memon MA, Kazi TG, Kazi, GH, Jakhrani MA, Haque QU, Shar GQ. Evaluation of mineral contents in medicinal plant *Azadirechta indica* (Neem). J. Chemical Soci. of Pak. 2003; 25(2): 139-143.
24. Pirzada AJ, Iqbal P, Shaik W, Kazi TG, Ghani KU. Studies on the elemental Composition and antifungal activity of medicinal plant *Lippia nodiflora* L. against skin fungi. J. Pak. Assoc. Dermat. 2005; 15: 113-118.
25. Singh AK, Singh RP, Singh NP. Analysis of Macro and micro nutrients in some Indian Medicinal herbs grown in Jaunpur (UP) soil. Natural Sci. 2011; 3: 551-555.
26. Singh AK, Singh RP, Singh NP. Analysis of nutritional elements in Indian Medicinal herbs used to cure general weakness. Natural Sci. 2012; 4: 211-215.
27. Soderberg T, Halimans G. Treatment of leprosy with adhesive zinc tapes. Leprosy Rev. 1982; 56: 271-273.
28. Herber RFM, Stoeppler M. (Eds.) Trace element analysis in biological Samples. Elsevier NY.; 1994
29. Dalvi KM, Vaidya V V, Kekare MB, Chapanekar P, Shaw W. Determination of heavy metals from *Leucas aspera* using atomic absorption Spectroscopic technique. Nature, Environ. and Poll. Tech. 2007; 6(3): 525-527.
30. Joseph D, Lal M, Bajpai HN, Mathur PK. Levels of trace elements of a few Indian Species by energy dispersive x-ray fluorescence. J. Food. Sci.Tech. 1999; 36: 264-265.
31. Scancar J, Milacic R, Falnoga I, Cemazar M, Bukovec P. Use of nitric acid In sample pretreatment for determination of trace elements in various biological Samples by ETAAS. J. Pharmacol. & Biomed. Anal. 2000; 22: 993-1002.
32. Yashvanth S, Rani SS, Rao AS, Madhavendra SS. Microscopic and micro chemical evaluation (elemental Analysis) of the medicinal herb, *Lippia nodiflora* (Linn.) Rich (*Phyla nodiflora* Linn. Green). Asian Pac. J. Trop. Biomed. 2012; 2(S1): S124-S129.
33. Rechardt L, Lehtinen S, Wartiovaara J, Scanning electron microscopy and x-ray Microprobe analysis in detection of acetyl cholinesterase in cultured embryonal cancer cells. Histochem. and Cytochem. 1984; 329(11): 1154-1158.
34. Rai LC, Jensen TE, Rachlin JW. Morphometric and x-ray energy dispersive Approach to monitoring pH altered cadmium toxicity in *Anabaena flos-aquae*. Arch. Environ. Contam. Toxicol. 1990; 19: 479-487.
35. Pradhan AN, Agrahari AK, Meher A, Mishra MN, Elemental analysis by energy Dispersive x-ray spectroscopy (EDX) of *Capparis zeylanica* Linn. plant. J. Pharm. Res. 2010; 3(4): 669-670.
36. Reddy SJ, Mauerhofer E, Porte N, Damodar J, Denschlag HO. Determination of elemental levels in medicinally important Indian leaves by instrumental neutron activation analysis. J. of Radioanalytical and Nucl. Chem. 1998; 238(1-2): 83-89.
37. Devi K, Sarma N, Nandakumar H, Kumar S. Particle induced x-ray emission studies of some Indian medicinal plants. Intl. J. of PIXE 2007; 17(3&4): 169-176.
38. Borchert R. Calcium acetate induces calcium uptake and formation of calcium-oxalate crystals in isolated leaflets of *Gleditsia tricanthos* L. Planta 1986; 168: 571-578.
39. Madhur RK, Dhara MK, Rajaraz CS. Geobotanical study of *Leucas aspera* in the Rajpura-Daria mineralized area, Udaipur district, Rajasthan. Special Publication Series- Geological Survey of India. 1982; 8: 179-181.
40. Veeranjaneyulu K, Das VSR. Heavy metal tolerance in normal populations in some weeds species. Indian J. Pl. Physiol. 1982; 25(4): 400-406.
41. RAI V, Agarwal M, Khatoon S, Rawat AKS, Meharotra S. Estimation of Co and Mn in some medicinal plants. Bulletin of Environ. Contamin. Toxicol. 2001a; 66(4): 427-432.
42. Rai V, Kakkar P, Khatoon S, Rawat AKS, Meharotra S. Heavy metal accumulation in some herbal drugs. Pharmaceutical Biol. 2001b; 39: 384-387.
43. Kannan SK, Das B, Prabhu B, Devarajan P, Periakali P, Krishnamoorthy R, Rao NR, Sridhar, SGD, Ballukrya PN, Sharma KK, Jayaprakash MA. Preliminary study on herbal resources and their contamination with toxic heavy metals around Pulicat Lake, north Chennai, South East coast of India. Res. J. Environ. Toxicol. 2007; 1(2): 78-84.
44. Rai V, Agnihotri AK, Khatoon S, Rawat AKS, Meharotra S. Chromium in some herbal drugs. Bull. Environ. Contamin. and Toxicol. 2007; 74(3): 464-469.
45. Patil UH, Gaikwad DK. Effect of varying environmental conditions of mineral status of stem bark of *Anogeissus latifolia*. J. Pharma. Res. 2012; 5(2): 1140-1143.