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Microscopic and micro chemical evaluation (elemental Analysis) of the medicinal herb, *Lippia nodiflora* (Linn.) Rich (*Phyla nodiflora* Linn. Green)

Sunkara Yashvanth1*, Satla Shobha Rani[2], Avanapu Srinivasa Rao[1] and Sunkara Sakunthala Madhavendra[3]

[1]*Bhaskar Pharmacy College, Yenkapally, Moinabad Mandal, Himayathnagar PO, Hyderabad-500075, AP India*
[2]*Center for Pharmaceutical Sciences, IST, JNTUH, Kukatpally, Hyderabad-500085, AP India*
[3]*Electron Microscopy Center, Indian Institute of Chemical Technology, Hyderabad- 500007, AP India*

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ABSTRACT

Objective: To explore the micro morphology and elemental analysis of *Lippia nodoflora* (*L. nodiflora*) **Methods:** Fresh leaf sections and epidermal peels were used for light microscopy. Glutaraldehyde fixed and dehydrated plant material was used for Scanning Electron Microscopy and Energy Dispersive -ray analysis (EDAX). **Results:** Scanning electron microscopic studies unraveled the characteristic fine details of the plant. These include the mid fixed or malpighigiaceous, rhomboidal, multi cellular leafy hairs, anisocytic or cruciferous stomata, single celled glands. Leaf constants like stomatal number (stomatal frequency/density) and stomatal index were measured. Detailed anatomy of stem, root and leaf was studied. Elemental analysis was carried out in various parts of the plant. **Conclusions:** Microscopic analysis was informative and useful in the sample identification, standardization for quality & purity. Information on micro chemical composition indicates the enrichment of the plant with Si, Ca and Fe. This helps in using the plant in certain herbal preparations that need plants with enriched minerals.

1. Introduction

Lippia nodiflora was a perennial marshy tropical medicinal herb [1, 2]. Its chemical constituents, biological applications, traditional uses and pharmacology are well reviewed [3, 4]. Despite Its immense medicinal uses, very few reports are available on its morphology.. Its morphology was elucidated by Munir [5]. Microscopy (light) is one of the well established techniques for pharmacognostic evaluation and recognized by various pharmacopeias [6-8]. There are certain limitations with the light microscopy in the characterization of surface of thick samples which are of most diagnostic in nature. Scanning electron microscopy is a complementary technique and finding place in pharmacognostic evaluation [9-11]. There are no reports available on the microscopic characterization of L. nodoflora. Hence the present study was taken up to explore the microscopic details of the plant. We report in this paper the light and scanning electron microscopic details of the plant.

Trace elements are considered the "inorganic switches" in various medicinal systems. This concept has gained ground in Ayurveda and the traditional Indian medicinal systems [12-14]. Mineral contents of various medicinal plants were evaluated and correlated with their therapeutic action by numerous workers [15-19]. Atomic absorption Spectrometer (AAS)[20], Energy dispersive x-ray fluorescence (EDXRF) [21], Electro thermal atomic absorption spectrometry (ETAAS)[22], Inductively coupled Plasma -atomic emission spectrometry (ICPAES), Inductively coupled plasma mass spectrometry (ICPMS) [23] , Energy dispersive X-ray analysis/spectroscopy (EDAX/EX/EDS) [24-27] are some of the techniques employed for the elemental analysis of plants. EDAX is a non destructive technique and can be used for multiple sampling in various regions/parts of the plant and can also obtain information from an area of few nanometers. This is very useful to characterize the crystals and inclusions of very minute size. Various plant parts vary in their elemental composition and therapeutical value. Hence EDAX was chosen for the current investigation for studying the elemental composition.

*Corresponding author: Sunkara Yashvanth.Bhaskar Pharmacy College, Yenkapally, Moinabad Mandal, Himayathnagar PO, Hyderabad-500075, AP India
Phone No. 91 9492574008
yashvanth_84@yahoo.co.in

2. Materials and Methods

2.1. Chemicals

Glutaraldehyde, Salts used for buffer preparation (Potassium monhydrogen Phosphate, Potassium dihydrogen Phosphate) and Safranine were obtained from Loba Chemie, India.

2.2. Plant material

L. nodiflora plants were collected from the fields during the month of July 2011 in the coastal area of Andhra Pradesh, India near Yeletipalem. Based on the macro morphological characters, one of the authors (SSM), a botanist identified the plant and a specimen of the plant was preserved for reference.

2.3. Microscopic characterization

Freshly cut hand sections of various plant parts and epidermal peels stained with safranin were used. Olympus BX-51 microscope fitted with CCD camera was employed for recording Light microscopy pictures.

2.4. SEM & EDAX Analysis

Hand cut sections and small pieces of various plant parts were fixed in 4% glutaraldehyde in phosphate buffer (pH, 0.69, 0.02M), dehydrated in graded alcohol series, mounted on aluminum stubs , coated with gold in Hitachi, HUS-5GB vacuum evaporator and observed in Hitachi S-3000N Scanning Electron Microscope at an acceleration voltage of 10KV and varied magnifications.

EDAX analysis was carried out at an acceleration voltage of 20KV with INCA X-Sight, Oxford detector fitted to Hitachi S-520 SEM. Full screen, window and spot modes were used depending on the size of the plant part/component.

3. Results

3.1. Stem

Stem surface was covered with rhomboidal, multicellular, Malpighious hairs and wax deposits. Wax deposits showed distinct pattern of elongated ridges that run parallel with intermittent bulbous structures. Sessile glands were also noted (Figure 1A). Rosy crystalline deposits were noted on the surface (Figure 1B). In transverse section, stem was distinctly bilobed with deep furrows (Figure 1C, shown with arrow). Epidermis was single layered with sub spherical cells. Cortex was parenchymatous, 7 to 8 layers with inclusions (indicated by arrows, Figure 1D) in some cells. Cambium was distinct, with phloem towards the exterior and endarch xylem towards the center. Medulla was parenchymatous with few cells filled with phenolic contents Figure 1E depicts a sessile gland with enlarged basal cell embedded in the epidermis and protruding into the cortex.

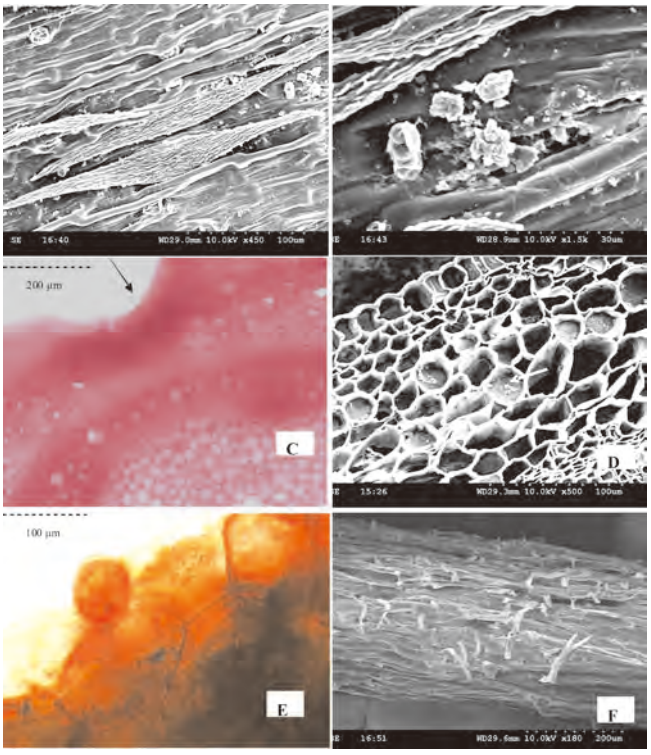

Figure 1.
A: Stem Surface with hairs and gland (at arrow); B: Crystals on stem surface (at arrow); C: Part of stem Cross Section with furrow (at arrow); D: Part of stem Cross Section, inclusions at arrow; E: Enlarged view of glandular hair in stem cross section; F: Root surface.

3.2. Root

Root surface was rough with scanty root hairs (Figure 1F). Root was spherical in cross section (Figure 2A). Epidermis was not coherent. Cells were elongated radially with undulated walls and lie loose and showed a rough texture. Cortex was not distinct. Phloem and cambial layers were clear. Medulla was fibrous and stellate with arms extending in to the xylem (Figures 2A, B).

3.3. Leaf

Hairs similar to that were observed on the stem were found on the leaf. Hairs on the upper surface of the leaf were smooth, but they were rough on the lower surface. Figure 2C depicts the ventral view of a detached scale from the lower surface of the leaf with 4 flaps, 2 short ones on the ventral side and 2 large ones on the dorsal side. However, the frequency of hairs and waxy deposits was low on the upper surface, whereas, the stomatal frequency/density was high

– 320/mm2 (Figures 2D, 2E) and the stomatal index was 30. Stomata were diacytic and anisocytic (cruciferous). Five to six elongated epidermal cells formed a whorl around sessile glands (arrow in Figure 2F). On lower leaf surface Stomatal frequency/density was low – 200/mm2 (Figures 3A, B) and the stomata index was 22. Stomata showed dense ridges on the rim of the guard cells and subsidiary cells (Figure 3C) and the Leaf has a distinct midrib. Transverse section of the leaf in the mid rib region is shown in Figure 3D. Single, arc like vascular bundle was noted in the midrib. Single layered bundle sheath was distinct with

phloem on the periphery and the xylem in the center. Leaf blade in Transverse section showed distinct upper epidermis with barrel shaped cells, followed by one or two layers of palisade cells and 4–6 layers of compact mesenchyma and lower epidermis (Figure 3E).

3.4. Flower

Surface of the calyx was covered with wax in undulated pattern with rudimentary single celled hairs (arrow, Figure 3F). Corollary surface showed rosy pattern with parellel striations on the petaloid wax coating and devoid of any hairs (Figure 4A). Figure 4B depicts the gynoecium with a slightly rough texture, short style and peg type papillate stigma.

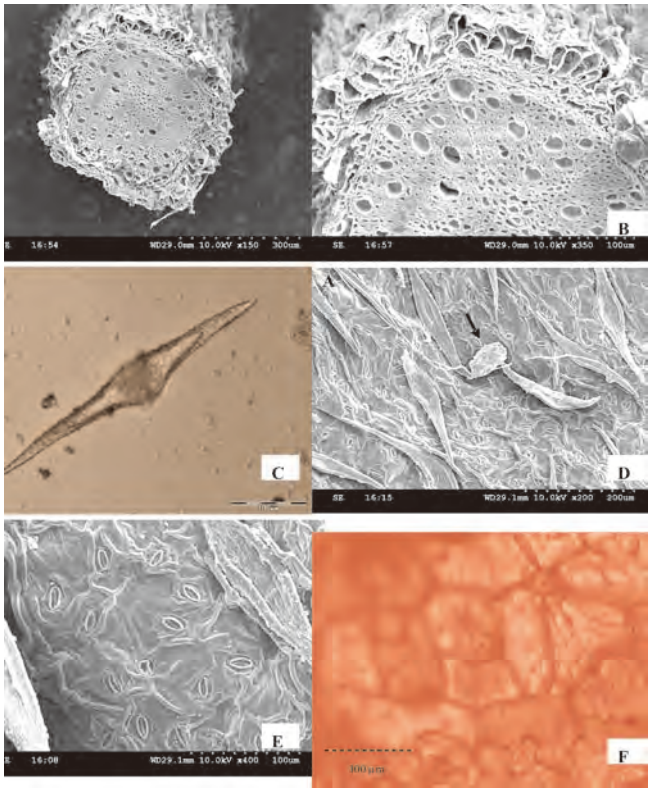

Figure 2. A: Root Cross Section; B: Enlarged view of root Cross Section. C: Malpighiaceous hair; D: Leaf upper surface; E: Enlarged view of leaf upper surface; F: Epidermal peel from leaf upper surface.

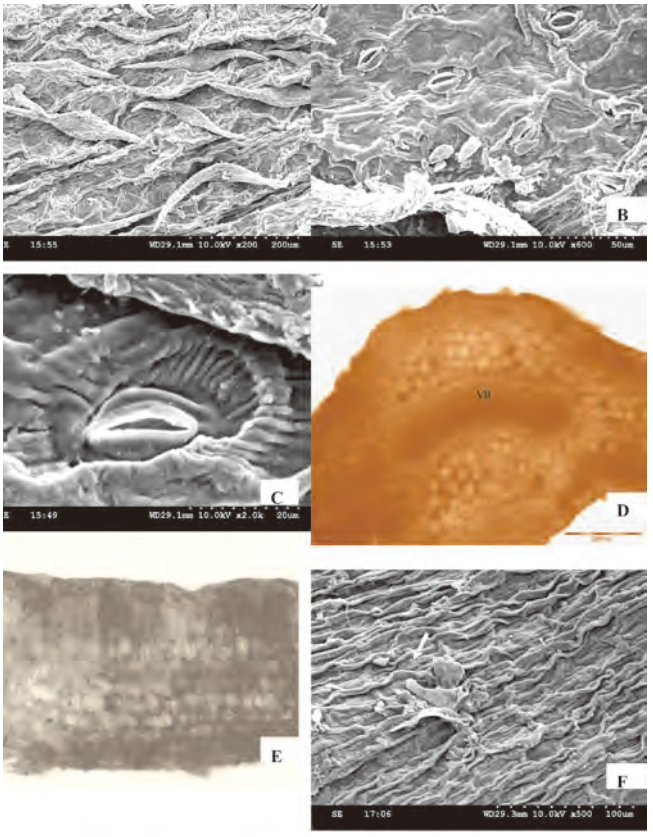

Figure 3. A: Leaf lower surface; B: Enlarged view of leaf lower surface; C: Stomata on leaf lower surface; D: Cross section of leaf at mid rib, VB – Vascular Bundle; E: Cross section of leaf blade; F: Surface of Calyx, single celled hair at the arrow.

3.5. Seed

Figure 4C and D depict the dorsal and ventral views respectively of the seed. Seed appears heart shaped. Dorsal side had a convex surface while the ventral side showed a rim like structure on the periphery followed by a slight groove and a convex central area. Figure 4E and F show the enlarged views of the dorsal and ventral seed surfaces respectively. On dorsal side, the cells showed undulated outline and free from any deposits. Whereas the ventral side showed cells with relatively smooth outline and deposits of varied size and shape were noted.

3.6. Elemental (EDAX) Analysis

Carbon, Oxygen, Silica and calcium were detected in all the parts studied. However, there was a variation in their percentages. Carbon & Oxygen ranges were 36.59–53.82 and 33.56–54.90 respectively. Highest carbon content was from hairs on stem surface and the lowest was from crystal on leaf. Highest oxygen content was from root cross section and the lowest was from crystal the leaf surface. Aluminum was detected in stem inclusion, root cross section, root hair, leaf lower surface, crystal on leaf and corolla. Highest concentration was in the crystal on leaf, followed by stem inclusion. Chlorine was restricted to leaf lower surface and

ventral side of the seed. Iron was high in the crystals on leaf, followed by root hair, leaf lower surface and root surface. Potassium and Titanium were detected in the crystal on leaf surface. Seed ventral side showed magnesium (Table 1&2).

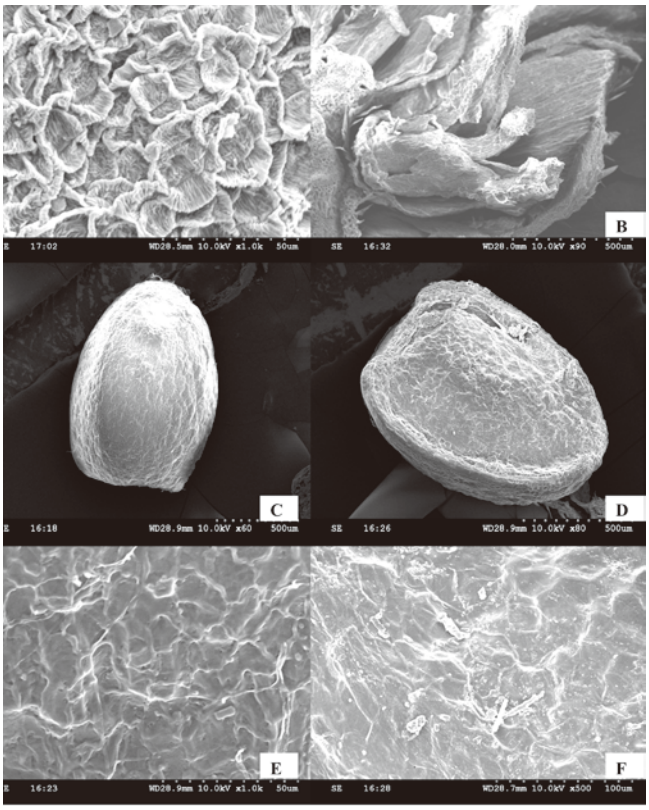

Figure 4. A: Surface of corolla; B: Gynoecium; C: Dorsal view of seed; D: Ventral view of seed; E: Enlarged view of seed on dorsal side F: Enlarged view of seed on ventral side.

4. Discussion

Both light and scanning electron microscopy techniques are proven techniques for the evaluation of the plant drugs [6-11]. Sandipkumar *et al* [27] emphasized the need for enhanced use of electron microscopy in the characterization of plant drugs. Present authors fully endorse it and the current investigation, employing SEM unfolded several micro morphological features of *L. nodiflora*, which would not have been possible only with light microscope, *L. nodiflora* exhibited typical diagnostic characteristic viz., midfixed malpighiaceous rhomboidal, multicellular hairs, bilobed stem with distinct furrows, fibrous and stellar medulla of the root, high stomatal frequency and stomatal index on leaf upper surface, distinct surface patterns of calyx, corolla and the seed surfaces. Of the distinct characteristics reported herein only one feature, malpighiaceous hairs on leaves was reported earlier by Munir 1993 [5] and the current paper stands unique in bringing out the salient ultramicroscopic, diagnostic features of the plant *L. nodiflora*. EDAX analysis indicated the enrichment of Silica and calcium in all the parts of the plant. Plant crystals are believed to be of either calcium oxalate or calcium carbonate. Present study indicate that the crystals apart from calcium carbonate and oxalate, contain iron, potassium and titanium in this plant. Aluminum was found in stem inclusions, on the lower surface of the leaf and corolla. Seeds showed magnesium and its presence was noted on the ventral surface. Pirzada *et al*., 2000 [16] have studied the elemental composition of *L. nodiflora* using Atomic absorption spectrophotometer and UV spectrometer and reported high concentrations of Calcium and presence of Aluminum, Magnesium and Iron. These elements may have role in the therapeutic action of the plant. Elemental composition of the plant varies from part to part. Many species were also found to exhibit phylogenic variation [27]. Patil *et al* [28] have discussed the significance of various elements with respect to human physiology. It can be concluded that the present investigating explored several diagnostic features of the plant *L. nodiflora* that might be of useful to authenticate and standardize.

Table1

Elemental composition (weight & atomic weight* %) of various regions in stem and root of *L. nodiflora*

S. No.	Element	Stem Surface	Stem Cross section	Stem inclusion	Scale on Stem	Root Surface	RootCross section	Xylem From Root	Root Hair
1	Carbon	48.9	40.71	37.77	53.82	40.94	40.45	44.41	45.20
		357.17	49.47	46.99	62.30	49.03	48.48	52.08	55.23
2	Oxygen	47.07	52.51	51.34	41.40	54.43	54.90	53.48	44.2
		41.27	47.90	47.94	35.97	48.93	49.40	47.05	240.57
3	Silica	1.01	1.04	2.67	0.45	2.29	2.03	0.99	3.39
		0.50	0.54	1.42	0.22	1.17	1.04	0.49	1.77
4	Calcium	3.01	5.73	4.94	4.33	1.78	1.81	1.10	3.03
		1.06	2.09	1.84	1.50	0.64	0.65	0.39	1.11
5	Aluminum	–	–	3.27	–	–	0.81	–	0.83
				1.81			0.43		0.45
6	Iron	–	–	–	–	0.55	–	–	3.33
						0.22			0.87

* Values given in the bottom in each row; $P < 0.001$ for all the values

Table 2

Elemental composition (weight & Atomic weight* %) of various parts of *L. nodiflora*

S. No.	Element	Leaf Upper Surface	Leaf Lower Surface	Crystal on Leaf Upper Surface	Creston Leaf Upper surface	Scale on leaf Upper surface	Inflore-scence Cross Section	Calyx	Corolla	Seed Dorsal	Seed Ventral
1.	Carbon	42.09	37.95	36.59	27.74	43.93	41.21	46.12	44.31	45.71	42.08
		50.41	47.16	50.51	40.28	53.71	49.28	53.84	52.44	53.20	49.96
2.	Oxygen	52.90	52.23	33.56	42.54	45.29	54.82	51.69	51.43	52.95	54.50
		47.57	48.72	34.78	46.39	41.57	49.21	45.29	45.70	46.27	48.58
3.	Silica	1.45	2.66	13.60	1.23	4.91	0.63	0.71	0.98	0.42	0.45
		0.74	1.41	8.03	0.76	2.57	0.32	0.36	0.50	0.21	0.23
4.	Calcium	3.56	4.69	1.69	25.53	5.88	3.34	1.48	2.06	0.92	1.43
		1.28	1.75	0.70	11.11	2.15	1.20	0.52	0.73	0.32	0.51
5	Aluminum	–	0.82	4.70	–	–	–	–	1.22	–	–
			0.45	2.89					0.64		
6.	Chlorine	–	0.39	–	2.97	–	–	–	–	–	0.99
			0.16		1.46						0.40
7.	Iron	–	1.26	7.90	–	–	–	–	–	–	–
			0.34	2.34							
8.	Potassium	–	–	0.99	–	–	–	–	–	–	–
				0.42							
9.	Titanium	–	–	0.97	–	–	–	–	–	–	–
				0.34							
10.	Magnesium	–	–	–	–	–	–	–	–	–	0.55
											0.32

Values given in the bottom in each row; $P < 0.001$ for all the values

Conflict of interest statement

We declare that there are no conflict of interest.

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