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Elemental composition and X-ray diffraction studies of *Strobilanthes* species

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Strobilanthes Blume is a member of family Acanthaceae. Energy Dispersive X-ray Spectroscopy (EDS) and X-ray diffraction (XRD) analysis were carried out on leaf and stem of ten species of *Strobilanthes* collected from the Northern Western Ghats (Maharashtra and Goa), India. The overall elemental composition of leaf and stem showed the presence of 10 elements (C, O, Mg, Cl, Si, Ca, K, S, Al and Fe). Al and Fe were detected only in *S. callosa, S. integrifolia* and *S. ixiocephala*. Elemental composition (atom percentage) varied among the species of *Strobilanthes*. Cystoliths constitute the characteristic feature of this family which also possesses different forms of Calcium Oxalate (CaOx) crystals such as raphides (needle-shaped) and styloids (prismatic). XRD analysis of leaf and stem samples revealed similar patterns of diffraction with two broad maxima arising from amorphous forms of; silicon oxycarbide & silica and calcium oxalate; along with a calcite peak and minor reflections from calcium oxalate crystals, suggesting the co-existence of both amorphous and crystalline mineral phases which could be of taxonomic importance. Although the functions of cystoliths and Calcium Oxalate crystals are not completely known, they play an important role in physical protection against grazing, biochemical functioning of plants including the photosynthetic process.

Keywords: Cystoliths, EDS, Mineral phase, Raphides, *Strobilanthes*, XRD

Strobilanthes Blume belongs to Acanthaceae family and it is the second largest genus after *Justicia* L. It constitutes approximately 450 species in the tropical regions of Asia[1]. One of the most important anatomical features of this family is the occurrence of diversely produced ‘cystoliths’ which may or may not be enclosed in a lithocyst and are present in both stem and leaf of species[2,3]. They are also found in various sizes, colours and types. These micromorphological characters are employed when exomorphic characters are insufficient[4]. CaOx crystals are secreted in the tissues as raphides, styloids and other shapes[2].

The function of these bio-minerals is not completely known. But research suggests that their utility is based upon their shape, size, abundance and chemical composition, thus regulates the biochemical functioning in plants[5] and protects against the herbivores[6].

Apart from the occurrence of the bio-minerals in *Strobilanthes*, the understanding of the mineral composition of the species is necessary. EDS can be used as a versatile, accurate and reproducible technique to quantify the major elements[7]. The plant physiological conditions required for the metabolic regulation is highly controlled by the macro and micro elements. Also, the plants are used for various purposes mainly as food and medicine. In this genus, *S. auriculata* is one species reported from Manipur, Northeast India consumed as vegetable. The presence of some of the major elements makes the species rich in the nutrients and used by the local people to increase their stamina and immunity towards the diseases[8].

Here we examined the samples by XRD to study the amorphous and crystalline phases that are present in leaf and stem of *Strobilanthes* species. The variation of the elemental composition within the species using EDS helped to support the findings of XRD.

Materials and Methods

Plant material

Ten *Strobilanthes* species *viz*. *S. callosa* Nees, *S. ciliata* Nees, *S. integrifolia* Kuntze, *S. ixiocephala* Benth., *S. heyneana* Nees, *S. lupulina* Nees, *Strobilanthes* Blume sp., *S. barbata* Nees, *S. reticulata* Stapf and *S. sessilis* Nees var. *ritchiei* were collected from Northern Western Ghats regions. Fresh and healthy plants were collected and correct taxonomic identification of species was done using standard floras, monographs, herbarium records,

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etc.[9-13]. The herbarium of all the collected species were prepared and deposited in Botany Department Herbarium at Goa University, Goa, India.

SEM-EDS and XRD analysis

Dried samples of mature stem and leaf were finely powdered for EDS characterization and XRD analysis. A scanning electron microscope-Vega3, Tescan (Czech Republic) was used to capture the images of the powdered sample followed by elemental composition, was carried out using an energy dispersive X-ray spectroscopy (EDS Bruker Nano, GmbH, D-12489, Germany). The mineral phases were studied using Philips diffractometer (PW1840), HT generator (PW1729) and Chart recorder (PW8203A). Samples were run from 10 to 80° 2θ at 3°/min scan speed with Cu Kα radiation (λ = 1.541838A).

Results and Discussion

Elemental composition

The elemental composition in 10 *Strobilanthes* Blume species was calculated for both leaf and stem and representative spectra for few species is shown in (Fig. 1). The elemental concentration was determined from the atomic percentage (Table 1). Leaf samples showed the presence of 10 elements such as Carbon (C), Oxygen (O), Magnesium (Mg), Chlorine (Cl), Silicon (Si), Calcium (Ca), Potassium (K), Sulphur (S), Aluminium (Al) and Iron (Fe). Stem revealed almost all elements reported for leaf except Aluminium and Iron. The analysis has shown that the number and concentration of elements varied among the species.

EDS spectra of *Strobilanthes ciliata* stem and leaf showed large peak of carbon, oxygen and small peak of magnesium, chlorine, silicon, potassium, sulphur except sulphur which found to be absent in leaf (Fig. 1A & B). All the 10 elements were seen in the leaf of *S. callosa*, *S. integrifolia* and *S. ixiocephala*; whereas stem with carbon, oxygen, calcium, potassium, chlorine and magnesium peaks with differing elemental concentration (Figs. 1C & D). *S. heyneana* leaf and stem showed carbon, oxygen, calcium, magnesium, potassium except chorine present in leaf.

Elements such as carbon, oxygen, magnesium, calcium, chlorine in varying concentration were seen in both leaf and stem of *S. reticulata* and *S. sessilis* var. *ritchiei*. *Strobilanthes* sp. exhibited elements *viz.* carbon, oxygen, calcium, chlorine in both parts except magnesium in leaf and potassium only in stem. *S. barbata* showed the presence of elements like carbon, oxygen, calcium, magnesium and chlorine in both the plant parts under study. Carbon, oxygen, magnesium and calcium were seen with differing concentration in *S. lupulina* in both parts except chlorine in leaf and potassium in stem.

Energy Dispersive X-ray Spectroscopy spectra from both the parts (leaf and stem) of *Strobilanthes*

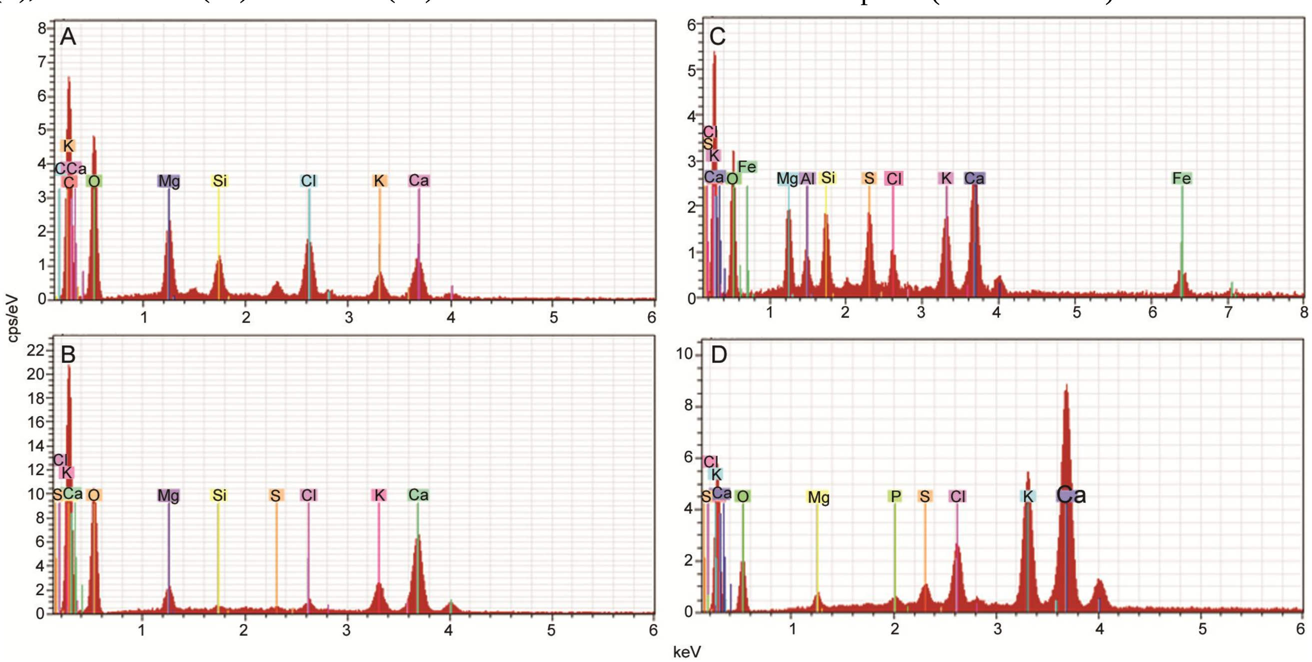

Fig. 1 — EDS spectra of leaf and stem (A & B) *S. ciliata*; and (C & D) *S. callosa*

Table 1 — Atom percentage of elements of leaf and stem of *Strobilanthes* spp.

		Elements									
Species		Al	C	Ca	Cl	Fe	K	Mg	O	S	Si
Strobilanthes ciliata	Leaf	–	55.79	0.48	0.58	–	0.24	1.83	40.58	–	0.50
	Stem	–	58.37	1.08	0.09	–	0.32	0.72	39.36	0.02	0.05
Strobilanthes callosa	Leaf	0.53	59.90	1.31	0.27	0.39	0.71	1.64	33.82	0.62	0.82
	Stem	–	60.19	4.82	0.90	–	2.14	–	31.24	–	–
Strobilanthes integrifolia	Leaf	0.16	59.05	2.12	1.66	0.03	1.06	0.87	34.86	0.11	0.08
	Stem	–	53.09	7.73	0.96	–	1.12	–	36.50	–	–
Strobilanthes ixiocephala	Leaf	0.15	50.07	13.46	2.30	0.00	0.83	0.46	32.55	0.10	0.08
	Stem	–	65.96	0.86	–	–	0.30	0.43	32.23	–	–
Strobilanthes heyneana	Leaf	–	66.48	1.27	0.56	–	–	0.59	30.86	–	–
	Stem	–	62.14	0.65	–	–	–	0.4	36.33	–	–
Strobilanthes reticulata	Leaf	–	68.09	0.64	0.17	–	–	0.66	30.33	–	–
	Stem	–	59.83	0.86	0.21	–	–	0.69	37.97	–	–
Strobilanthes sessilis var. *ritchiei*	Leaf	–	45.19	11.68	0.68	–	–	1.38	40.75	–	–
	Stem	–	33.28	1.56	1.12	–	–	0.64	63.93	–	–
Strobilanthes sp.	Leaf	–	67.77	0.50	0.42	–	–	0.53	30.32	–	–
	Stem	–	68.30	0.62	0.35	–	0.29	–	30.32	–	–
Strobilanthes barbata	Leaf	–	59.84	2.99	0.63	–	–	0.80	34.33	–	–
	Stem	–	63.91	3.07	1.00	–	–	1.30	30.36	–	–
Strobilanthes lupulina	Leaf	–	61.65	0.53	0.42	–	–	0.80	36.03	–	–
	Stem	–	56.88	3.29	–	–	0.87	0.80	37.85	–	–

species revealed large peaks of carbon, oxygen, calcium, magnesium and indeed small amount of chlorine, potassium, aluminium, sulphur, silicon and iron. In all species, peaks of calcium and magnesium was also seen in varying concentration which are involved in distinct phases of cystolith formation (Table 1), confirming their role in formation of cystoliths and other crystals in *Strobilanthes* species.

XRD analysis

The X-ray diffraction studies of leaf and stem of *Strobilanthes* species were carried out. All the 10 samples showed similar diffraction pattern (Fig. 2A & B). The first broad maxima is likely to be due to amorphous silicon oxycarbide[14] at 15° together with amorphous Calcium Oxalate[15,16], while the broad maxima at approximately at 22° may be due to both amorphous silica[17] and amorphous calcium carbonate (ACC)[18,19]. Secondly, the crystalline peak due to calcite (104) ($2\theta = 29.8$) is prominent and appears in most of the samples (Fig. 3). Also, a few minor Calcium Oxalate reflections have also been observed. In addition, cystoliths are found distributed in various plant parts of *Strobilanthes* and they constitute the characteristic feature of this family. Anatomy of leaf, stem and petiole revealed the presence of cystoliths and different forms of Calcium Oxalate crystals such as raphides (needle shaped) and styloids (prismatic) (Fig. 4).

EDS analysis exhibited the varied amount of silicon, aluminium, potassium, sulphur and chlorine in all species based on its atomic percentage. *S. auriculata* is restricted to the North East regions of India. As reported in the previous study, members of some tribal groups from Manipur, India use *S. auriculata* as vegetable due to its importance as a source of minerals that protects the people from several ailments[8]. The elemental content makes the plant to be rich in curing many diseases and ailments[20]. *Strobilanthes crispus* is widely used as herbal tea containing calcium, iron, phosphorous, sodium, potassium elements in combination with other

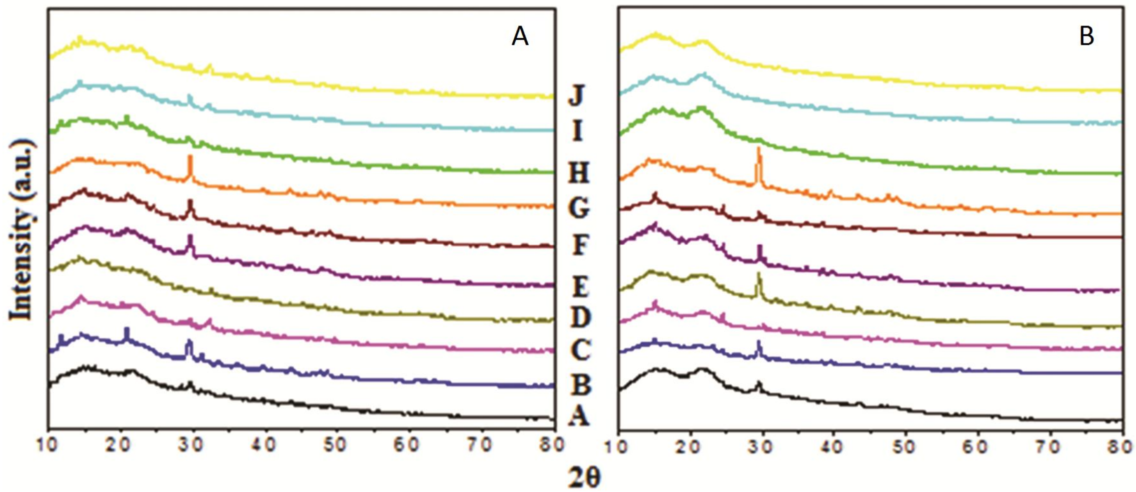

Fig. 2 — XRD spectra of leaf & stem samples of *Strobilanthes* spp. (A) *S. integrifolia*; (B) *S. ixiocephala*; (C) *S. ciliata*; (D) *S. callosa*; (E) *S. heyneana*; (F) *S. lupulina*; (G) *S. barbata*; (H) *S.* sp.; (I) *S. sessilis* var. *ritchiei*; and (J) *S. reticulata*

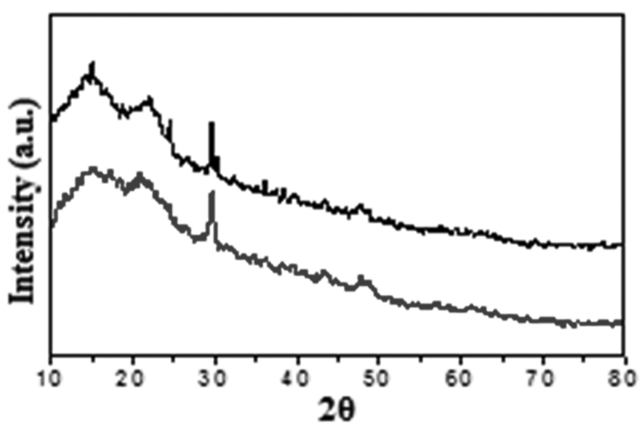

Fig. 3 — XRD spectra of *S. heyneana* showing prominent calcite reflection (104)

chemical composition[21]. *S. callosa*, *S. integrifolia* and *S. ixiocephala* showed iron (Fe) in sparse amount in leaf samples which may have come from the soil. The different geographical regions could determine the elemental composition of plants. In the present study, most of the *Strobilanthes* studied are endemic and restricted to a particular geographical region/area, hence, the elemental compositions of these species were studied. The concentration of bio-minerals in plant species may add to tolerance of stresses of biotic and abiotic origin[22].

With stable oxygen isotope analysis of plant it is possible to estimate the composition of plant organic matter with the help of process-based models[23]. Carbon fixation is an important process in plants leading to the uptake of inorganic carbon into organic compounds particularly by photosynthesis. In all species of *Strobilanthes*, carbon and oxygen was found to be dominant elements as the major plant components (biogenic) necessary for plant system (Table 1).

The process of mineralization of cystoliths ACC phase is under biological control[24]. In the present study, cystoliths consist of crystalline calcium carbonate as a mineral and an amorphous phase. This has been confirmed in the present study as shown (Figs. 3 & 4). Studies have shown that formation of cystoliths is as four distinct phases: A pure silica phase; a silica phase which becomes an Mg-rich silica phase; a silica phase superimposed with a relatively stable ACC phase; and a bulky and less stable ACC phase which over lays the first stable ACC phase[25]. *Strobilanthes* species in the present study have shown evidence for these phases through XRD and EDS data. It is evident that using EDS the mineral composition and the purity of crystalline structure was confirmed by X-ray Diffraction analysis[26].

In the present study, cystoliths have been observed in stem, leaf and petiole. The function of cystoliths is not completely known. Metcalfe & Chalk (1950)[2] reported the occurrence of variously shaped cystoliths in stem and leaf tissues of this family. Their presence, shape and size in the leaf of some taxa is a character for identification[4,27,28] which has been elucidated in the XRD pattern during this study. Also there are varied forms of CaOx crystals and are known to protect the plants from herbivore[29,30] and it is reported that raphides reduces the metal toxicity in plants because of the presence of divalent[31,32]. Calcium oxalate crystal occurrences may impart light scattering and enhance the rate of photosynthesis[33,34].

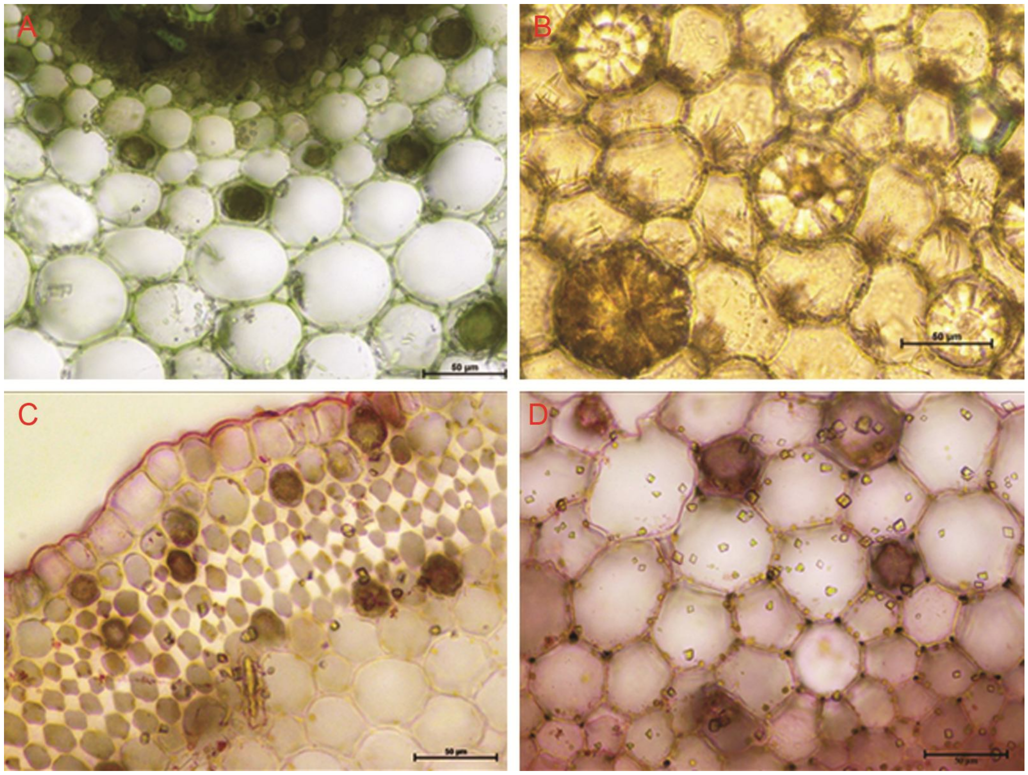

Fig. 4 — Distribution of cystoliths and CaOx crystals in different plant parts of *Strobilanthes* Blume species. (A) Cystoliths in leaf midrib (abaxial region) of *S. callosa* (X400); (B) CaOx crystals as raphides in the cortex of *S. callosa* (stem) and cystoliths (X400); (C) Cystoliths and styloid CaOx crystals in petiole of *S. heyneana* (X400); and (D) CaOx crystals present in petiole of *S. barbata* as styloids and cystoliths (X400)

Conclusion

Minerals play an important role in the biochemical functioning and the systemic integrity of the plants. Elemental composition of leaf and stem showed the presence of 10 elements (C, O, Mg, Cl, Si, Ca, K, S, Al and Fe). Al and Fe were detected only in *Strobilanthes callosa*, *S. integrifolia* and *S. ixiocephala*. The percentage of various elements reported differs among the ten *Strobilanthes* species studied. All samples in the study show phases of amorphous silicon oxycarbide, amorphous silica, ACC, calcite and minor reflections from calcium oxalate crystals which are co-existing in both leaf and stem of these *Strobilanthes* species. Presence of different forms of calcium crystals can be of taxonomic importance. Also, these biominerals play an important role in gathering and scattering of light to manage photosynthetic process and protection against herbivores.

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