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Macro-microscopy and TLC atlas of leaves of *Costus igneus* Nak.

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ABSTRACT

Introduction: *Costus igneus* Nak., commonly known as Spiral flag, is a member of Costaceae and a newly introduced plant in India from South and Central America. In southern India, it usually is grown as an ornamental plant and its leaves are used as a dietary supplement in the treatment of diabetes mellitus. Recently, a number of researches have been carried out and it has been proven to possess various pharmacological activities like antidiabetic, hypolipidemic, diuretic, antioxidant, anti-microbial and anti-cancer. **Methods:** Macro-microscopy and TLC investigation for the leaves of *C. igneus* were done using standard methodology. **Results:** The chief microscopic characters of leaves include epidermis with anomocytic stomata, mesophyll cells with calcium oxalate crystals and fibres associated with parenchyma cells. TLC of ethanolic extract showed 7 and 12 spots at short UV nm and long UV respectively. **Conclusion:** This study will serve as a standard reference for identification of *Costus igneus* Nak. leaf.

KEYWORDS

Costus igneus Nak, HPTLC, Insulin plant, Pharmacognosy, Quality control, Standardisation

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Costus igneus Nak (syn. *Costus pictus* D. Don, *Costus mexicanus* Liebm ex Petersen or *Costus congenitus* Rowle), commonly known as fiery costus, Step ladder or Spiral flag or Insulin plant, is native to South and Central America. It is a recent introduction to India from America as an herbal cure for diabetes and hence commonly called as 'insulin plant'.^[1] It is widely grown in gardens as ornamental plant in South India and also run wild in many places.^[2] It is used in India to control diabetes, and it is known that diabetic people eat one leaf daily to keep their blood glucose low.^[3] Leaves of *C. igneus* were one among the plants known to be effectively used for treating diabetes by the tribal people of Kolli hills of Namakkal district, Tamilnadu.^[4] In Mexican folk medicine, the aerial part of *C. pictus* D. Don is used as an infusion in the treatment of renal disorders.^[5]

It is a perennial, upright, spreading plant reaching about two feet tall, with the tallest stems falling over and lying on the ground. Leaves are simple, alternate, entire, oblong, evergreen, 4-8 inches in length with parallel venation. The large, smooth, dark green leaves of this tropical evergreen have light purple undersides and are spirally arranged around stems, forming attractive, arching clumps arising from underground rootstocks. Beautiful, 1.5-inch diameter, orange flowers are produced in the warm months, appearing on cone-like heads at the tips of branches.^[6] The plant is a rich source of various phytochemicals viz., the leaves are reported to contain protein, iron, ascorbic acid, α -tocopherol, β -carotene, terpinoids, steroids, and flavonoids.^[7,8] The essential oil obtained by steam distillation of leaves revealed the presence of 6 phyto constituents.^[1] Recent researches on the pharmacological activities of the leaves of *C.igneus* Nak reveals its potential as a antidiabetic, hypolipidemic, diuretic, antioxidant, ameliorative, antimicrobial, anticancer and putative agent.^[9] The present study was planned to study the detail macroscopic, microscopical and chromatographic characteristics of the leaves of *C.igneus* Nak which would serve as a reference standard for identification, authentication and for distinguishing the plant from potential adulterants.

MATERIALS AND METHODS

Plant Material

The fresh leaves of *C. igneus* Nak was collected from the herbal garden of Sri Dharmasthala Manjunatheshwara College of Ayurveda & hospital, Hassan. The authentication was done at S.D.M Research Center for Ayurveda and Allied Sciences, Udupi and a voucher specimen maintained in the same laboratory. The collected leaves were shade dried and finally pulverized into coarse powder. It was stored in a well closed container free from environmental climatic changes or any other contamination till usage for further studies.

Macroscopy

Macroscopic features of the leaves of *C.igneus* Nak were observed under Stereo microscope (Zeiss Stemi) and the characters recorded with reference to leaf characters reported in literature.

Microscopy

Transverse section of the leaf through midrib and lamina was taken and observed for their characteristic features.^[10]

Powder characteristics

Minimum quantity of leaf powder was mounted on a microscopic slide, stained with safranin, characters were observed under trinocular microscope (Zeiss AXIO).^[11]

High Performance Thin Layer Chromatography

Extract preparation

1 g of the sample was soaked in 20 ml ethanol for 24 hours. The extract was filtered and made upto 20 ml with ethanol. 10, 20 and 30 µl of the above extract was applied on a precoated silica gel F254 on aluminum plates to a band width of 8 mm using Linomat 5 TLC applicator. The plate was developed in Toluene : Ethyl acetate (7 : 2) and the developed plates were visualized and scanned under UV 254, 366, and after derivatisation in vanillin-sulphuric acid spray reagent at 620 nm.^[12] R_f, colour of the spots, densitometric scan and superimposability of densitogram were recorded.^[13-14]

RESULTS AND DISCUSSION

Macroscopy

The leaves were simple, alternate, entire, oblong, smooth, parallel venation and spirally arranged around stem (Figure 1.1).

Microscopy

Transverse section of leaf shows upper and lower epidermii embedding wide mesophyll tissue consisting of parenchyma embedding continuous strands of fibro vascular bundles. The mesophyll tissue is majorly composed of large parenchyma cells. Cells of the upper epidermis are comparatively smaller than the lower epidermal cells. Lower epidermis is embedded with stomata and the epidermal cells are often beaded. The vascular bundles are embedded in continuous strands of chlorenchyma, cell of which are either elongated or spherical, and the vascular bundles are comprised of large vessels in the centre, surrounded by phloem tissue. The bundles are fibrous and the parenchymas adjacent to them are loaded with rosette crystals of calcium oxalate. Fibres are thin-walled, forming two arc-like patches protecting the vascular tissue from either side. The extra vascular bundles in the mid-rib has 'U' shaped fibrous sheath in the lower side. TS through the lamina show similar anatomy without extra-vascular bundles (Figure 1.2, 1.3 and 2)

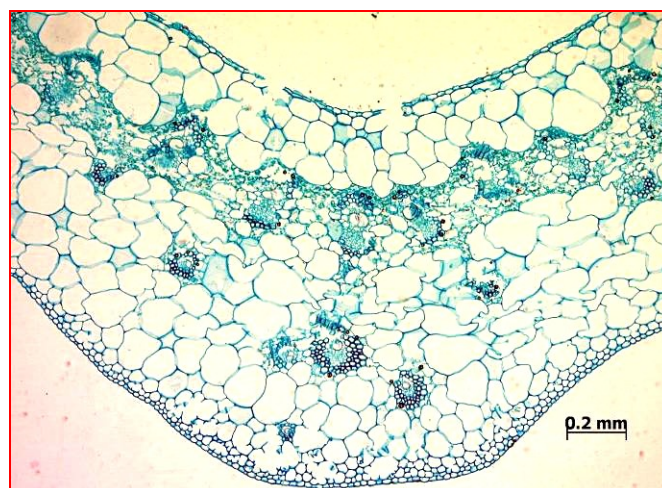
Powder microscopy

The powder shows fragments of upper epidermis composed of polygonal cells without stomata, often with underlying large mesophyll cells; fragments of lower epidermis with anomocytic stomata and beaded walls; fragments of mesophyll region with cells embedding chloroplasts and plenty of druses of calcium oxalate; bundles of thin-walled fibres associated with parenchyma containing druses forming crystal fibres; fragments or entire vessels with spiral thickening; few fibres isolated and pittedly walled, often associated with parenchyma containing druses often associated with phloem elements; druses and sandy crystals are scattered throughout the powder (Figure 3).

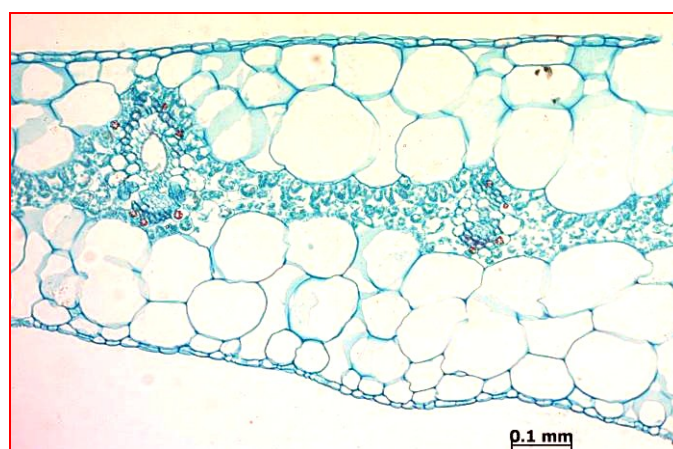
Figure 1. Macro-microscopic features of leaf of *Costus igneus* Nak



1.1. Photograph from natural habitat



1.2. Detailed TS of leaf passing through midrib



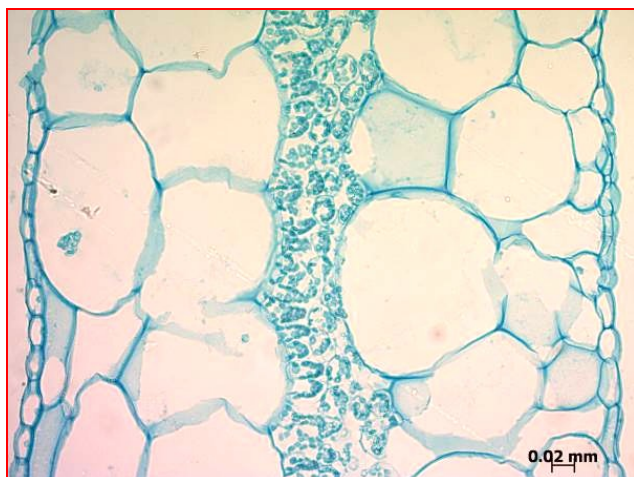
1.3. Detailed TS of leaf passing through lamina

Table 1. Rf values of ethanolic extract of *Costus igneus*

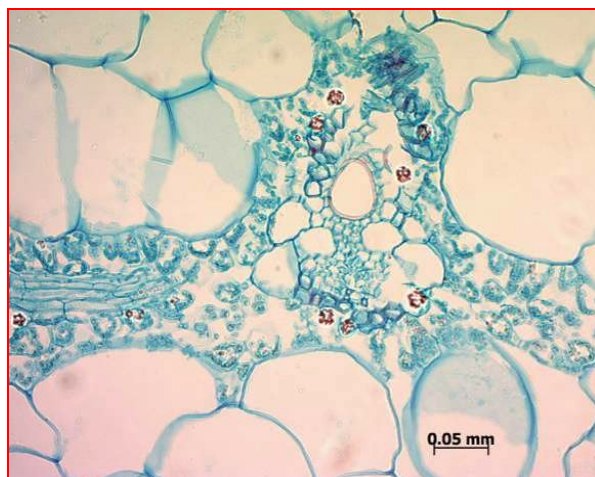
Short UV	Long UV	After derivatisation
0.06 L Green	--	--
--	--	0.10 Blue
--	0.11 FL Pink	--
--	0.14 FL Pink	--
0.21 L Green	--	--
--	0.23 FL Pink	--
0.25 L Green	--	--
--	--	0.27 Blue
--	0.30 FL Pink	--
--	0.40 F Pink	--
--	--	0.41 Blue
--	0.42 F Pink	--
--	--	0.51 Blue
--	0.54 FL Pink	--
--	--	0.62 Blue
--	0.66 F Blue	0.67 Blue
0.71 L Green	0.71 F Pink	--
0.76 L Green	0.76 F Pink	--
--	0.83 F Pink	--
0.87 Green	--	--
--	--	0.90 Pink
0.98 L Green	0.98 FL Yellow	--

F: Fluorescent; L: Light

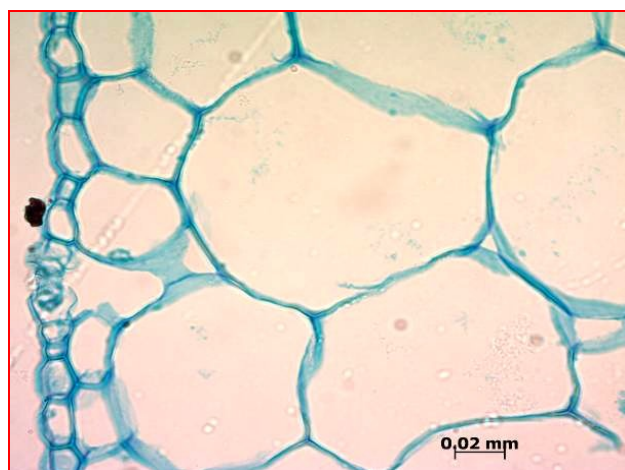
Figure 2. Detailed microscopic features of leaf of *Costus igneus* Nak



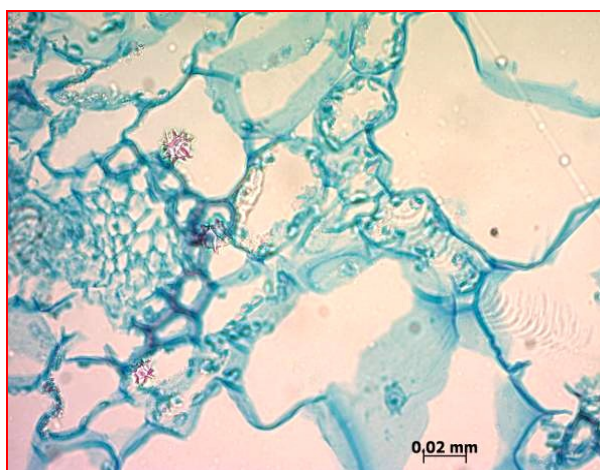
TS through lamina showing mesophyll



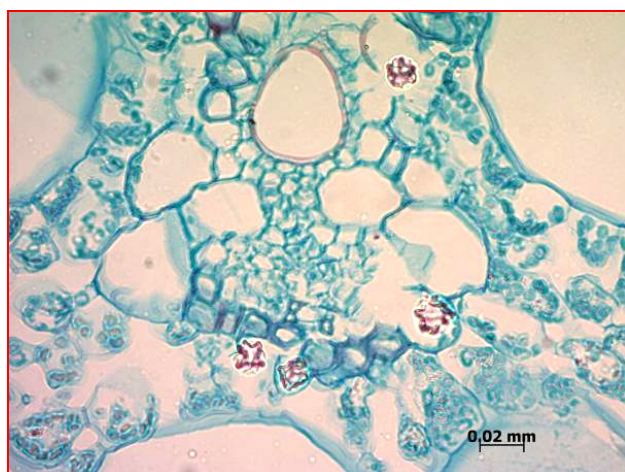
A vascular bundle enlarged



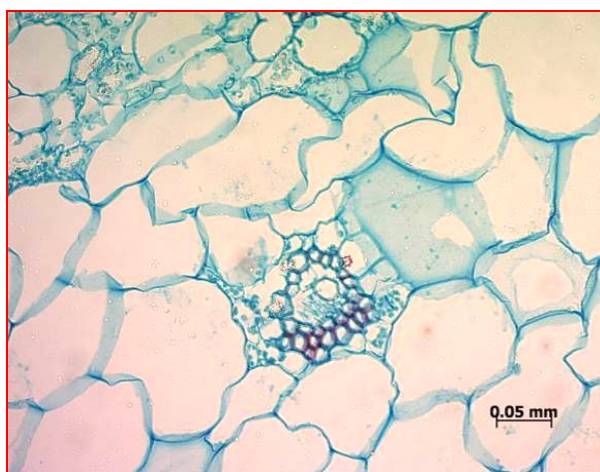
Stoma in lower epidermis



Rosette crystals of calcium oxalate



Crystal idioblasts with fibres



Fibro vascular bundle

HPTLC

Thin layer chromatography of ethanolic extract of *Costus igneus* Nak leaves showed presence of various compounds as represented in photo-documentation and densitometric scan at various wavelengths (Figure 4). Photo-documentation of 30µl ethanolic extract under short UV showed 7 spots with R_f values 0.06, 0.21, 0.25, 0.71, 0.76, 0.87, 0.98. Under long UV, 12 spots were visible with R_f values 0.11, 0.14, 0.23, 0.30, 0.40, 0.42, 0.54, 0.66, 0.71, 0.76, 0.83, 0.98. Under white light after derivatisation with vanillin sulphuric acid, 7 spots with R_f values 0.10, 0.27, 0.41, 0.51, 0.62, 0.67, 0.90 were visualized (Table 1.)

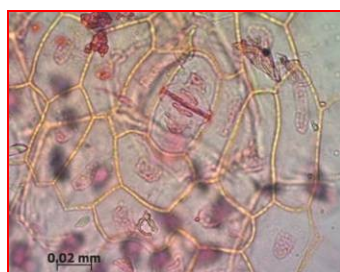
The major problem faced in herbal formulation industry is the identification of authenticated raw material, the absence of which leads to adulteration. The detailed systematic pharmacognostic evaluation of plant and plant material provides means of

standardization of an herb.^[15] The morphological studies reported herein established the macroscopic and microscopic parameters that characterize the genuine plant drug *Costus igneus* Nak (Family: Costaceae). These morphological characters can be utilized for quick identification of the drug. The microscopical characteristics are particularly useful in case of powdered drug. HPTLC fingerprinting for various phytoconstituents in the extract serve as specific tool to differentiate various extracts from raw material of different species of herbs.^[16] The results obtained from HPTLC may serve as identification for *Costus igneus* Nak leaves.

Figure 3. Microscopic features of macerate of leaf of *Costus igneus* Nak



Upper epidermis & mesophyll



Lower epidermis & stomata



Beaded epidermal cells



Chlorenchyma



Idioblasts with rosettes



Crystal fibre bundle



Phloem elements



Fibre and vessel



Parenchyma



Isolated crystal fibre



Fibre & phloem parenchyma



Pitted fibres



Prismatic crystal and spiral vessel



Vessel magnified

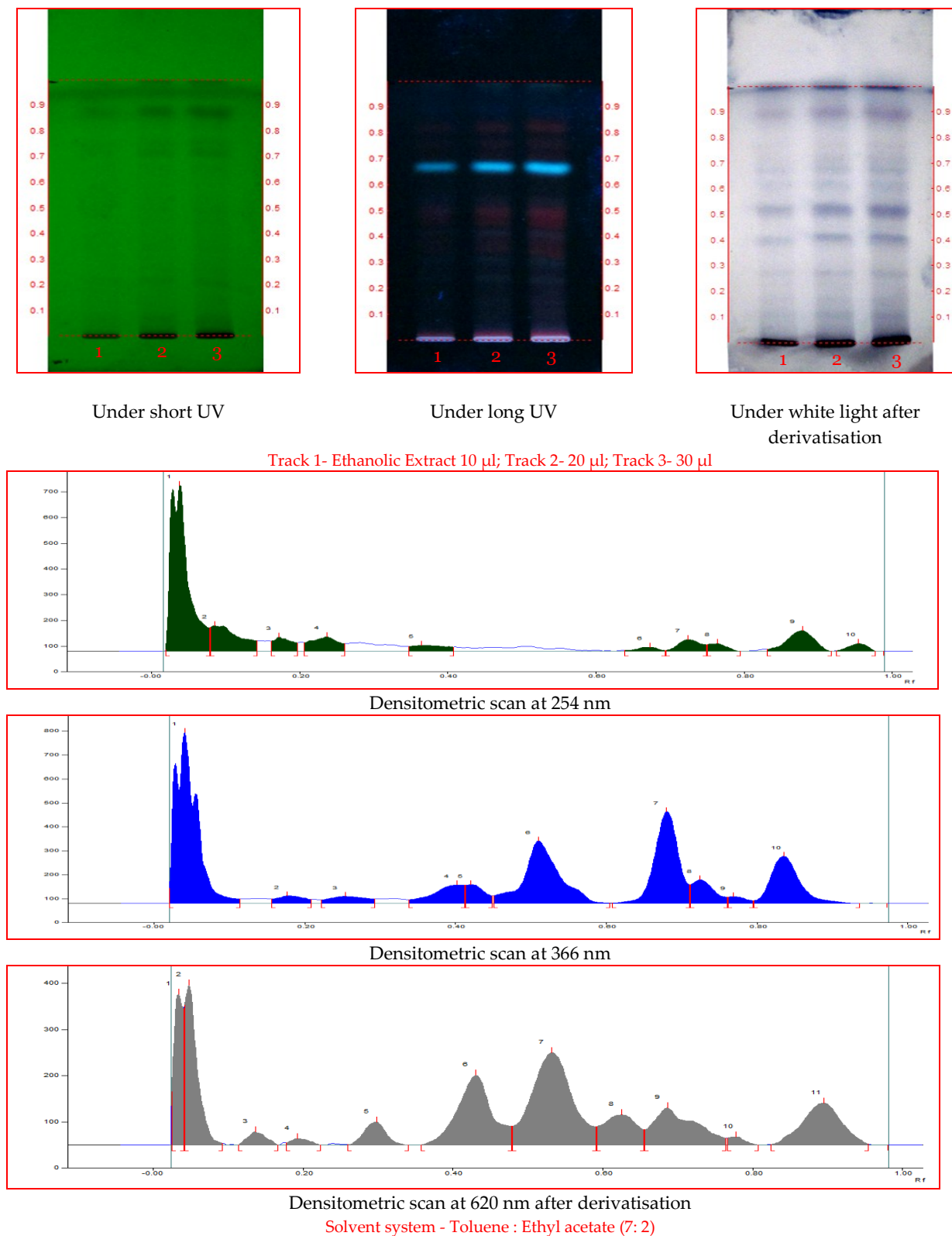


Fibres and crystals

CONCLUSION

The microscopic and chromatographic fingerprinting can be used to judge the adulteration and purity of the drug. The plant *Costus igneus* Nak exhibits a set of diagnostic characteristics like epidermis with anomocytic stomata, calcium oxalate crystals which will help for identification. HPTLC fingerprinting will help to supplement information in regard to its identification and standardization.

Figure 4. HPTLC of ethanolic extract of leaf of *Costus igneus* Nak



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REFERENCES

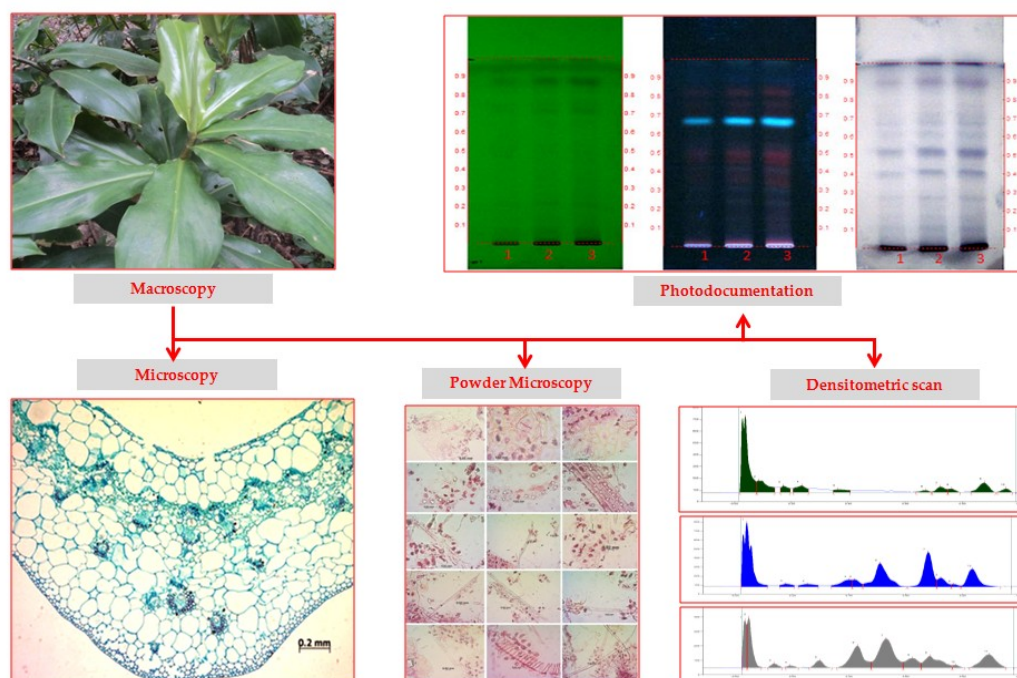
1. Jose B, Reddy L J. Analysis of the essential oils of the stems, leaves and rhizomes of the medicinal plant *Costus pictus* from southern India. *Int J Pharmacy Pharm Sci* 2010;2(2):100-1.
2. Benny M. Insulin plant in gardens. *Natural Product Radiance* 2004;3(5):349-50.
3. Devi VD, Urooj A. Hypoglycemic potential of *Morus indica* L. and *Costus igneus* Nak.: A preliminary study. *Indian J Exp Biol* 2008;46:614-6.
4. Elavarasi S, Saravanan K. Ethnobotanical study of plants used to treat diabetes by tribal people of Kolli Hills, Namakkal District, Tamilnadu, Southern India. *Int J Pharm Tech Res* 2012;4(1):404-11.
5. Meléndez-Camargo ME, Castillo-Nájera R, Silva-Torres R, Campos-Aldrete ME. Evaluation of the diuretic effect of the aqueous extract of *Costus pictus* D. Don in rat. *Proc West Pharmacol Soc* 2006;49:72-4.
6. Gilman EF. *Costus igneus*. Fact sheet. FPS-151. EDIS-Electronic Data Information Source-UF/IFAS Extension. Florida: University of Florida, Inc.; c2012 (cited 2012 Dec 28), Available from <http://edis.ifas.ufl.edu/fp151>
7. Devi VD, Urooj A. Nutrient profile and antioxidant components of *Costus speciosus* Sm. and *Costus igneus* Nak. *Indian J Nat Prod Resour* 2010;1:116-8.
8. Shankarappa L, Gopalakrishna B, Jagadish NR, Siddalingappa GS. Pharmacognostic and phytochemical analysis of *Costus ignitus*. *Internationale Pharmaceutica Scientia* 2011;1(2):36-41.
9. Hegde PL, Rao HA, Rao PN. A review on Insulin plant (*Costus igneus* Nak). *Phcog Rev* 2014;8:67-72.
10. O'Brein TP, Feder N, Mc Cull ME. Polychromatic staining of plant cell walls by toluidine blue-O. *Protoplasma* 1964;59(2):368-73.
11. Fahn A. *Plant Anatomy*. 3rd ed. New York: Pergamon Press; 1987; p554.
12. Waksmundzka HM, Sherma J, Kowalska T. *Thin layer chromatography in phytochemistry*. London: CRC Press, Taylor & Francis Group; p595.
13. Stahl I. *Thin layer chromatography. A Laboratory Hand Book* (student edition). Berlin: Springer-Verlag; 1969; p52-86.
14. Sethi PD. *High Performance Thin Layer Chromatography*. 1st ed. New Delhi: CBS Publishers and Distributors; 1996.
15. Trivedi Ashish, Sethiya Neeraj K, Mishra S H. Preliminary pharmacognostic and phytochemical analysis of Granthika (*Leonotis nepetefolia*): An Ayurvedic herb. *Indian Journal of Traditional knowledge* 2011;10(4):682-8.
16. Bollywar AD, Sushma GS, Sharaish P, Bharathi P, Rama Devi M and Siva Subramanian N. Phytochemical screening and HPTLC fingerprint analysis of bark extracts of *Ficus nervosa* Heyne Ex Roth. *Int. J. of Pharm. & Life Sci.* 2013;4(3):2432-6.

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GRAPHICAL ABSTRACT



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