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Insulin Plant (*Costus pictus*) Leaves: Pharmacognostical Standardization and Phytochemical Evaluation

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ABSTRACT

To explore the micro morphology and physicochemical parameters of the leaves of *Costus pictus* D. Don (Costaceae). Macroscopy, microscopy, physicochemical analysis, preliminary phytochemical screening and other WHO recommended parameters for standardizations were performed. *Costus pictus* D. Don is a perennial herb and hardy vigorous growing ginger. The leaves are simple, spirally arranged, oblong-lanceolate with the length 10 to 25cm and width 2.5 to 6cm. The midrib consists of thin epidermal layer of small squarish cells on the adaxial side and fairly thick cylindrical epidermal cells on the abaxial side. The vascular system of the midrib includes an abaxial band of three vascular bundles and adaxial single median bundle. The adaxial epidermal layers of the lamina consist of thick, cylindrical cells. The abaxial epidermis is thin which consists of narrow tubular cells. The adaxial epidermis is apostomatic and abaxial side is stomatiferous. Calcium oxalate crystals of minute particle are aggregated into large masses in the mesophyll cells. Hexacytic type stomata are present. Vein islet numbers, vein termination numbers, stomatal number, stomatal index and other physico chemical tests like ash values, loss on drying and extractive values were determined. Preliminary phytochemical screening showed the presence of carbohydrates, triterpenoids, proteins, alkaloids, tannins, saponins, flavonoids, sterols and volatile oil. Powder microscopy of the leaf showed fragments of unicellular trichomes, hexacytic stomata, abaxial solitary bundle and calcium oxalate crystals. Microscopic analysis was informative and provides useful information in the botanical identification, standardization for purity & quality and immense value in authentication of the leaf.

Keywords: *Costus pictus*, Insulin plant, Costaceae, Microscopical evaluation, Physico chemical analysis.

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INTRODUCTION

Costus pictus D. Don is commonly known as spiral ginger, belonging to the family Costaceae. It is a magical cure of diabetes. Its leaf helps to build up insulin the human body. So, it is commonly known as Insulin plant [1, 2]. Insulin plant was grown in America and is becoming popular in India because of its medicinal value [3]. It is now accepted and used widely as an Ayurvedic medicinal herb. Leaf is traditionally used as antidiabetic[4-12], Antioxidant[13], Antibacterial [14, 15], Anti-cancer [16] and diuretic [17, 18].

It was reported that fresh leaves contains 18 chemical compounds were identified by using GC-MS. From the chromatogram, it was evident that the major component in the ether fraction is bis (2'- ethyl hexyl)-1, 2-benzene dicarboxylate (59.04%). The major component in the acid fraction are haxadecanoic acid (44.53%) and 4, 8, 12, 16- tetra methyl hepta decan 4-olide (27.86%) [19]. Trace elemental analysis showed that the leaves of *C. pictus* contains appreciable amounts of the elements K, Ca, Cr, Mn, Cu, and Zn [4]. Steam distillation of leaves of *C. pictus* D. Don yielded clear and yellowish essential oils. The major constituents were Dodecanoic acid, Hexadecanoic acid, 1, 1-diethoxy Ethane, cis-3-Hexenol, 2-ethoxy Butane, 2-Pentanol, Tetradecane, β-Ionone, α-Ionone, n-Nonadecane, Farnesyl acetone identified [20]. In spite of the numerous medicinal uses attributed to this plant, limited report available on its pharmacognostical information. These standards are of utmost importance not only in finding out genuity, but also in detection of adulterants in marketed drug [21]. The objective of the present study was to establish various Pharmacognostic standards, important microscopical feature (Midrib and Lamina) and to evaluate preliminary phytochemical and physicochemical analysis of *Costus pictus* that can facilitate identification and assist in the preparation of monograph of this plant.

MATERIALS AND METHODS

Chemicals

Formalin, acetic acid, ethyl alcohol, chloral hydrate, toludine blue, phloroglucinol, glycerin, hydrochloric acid and all other chemicals used in this study were of analytical grade.

Plant collection and authentication

The leaves of the *Costus pictus* selected for our study was collected from Dr. Gour, Rock Garden, G. Amsapuram, Theni District, Tamil Nadu, India during the month of August 2013 (Figure 1). The plant specimen was identified and authenticated as *'Costus pictus'* (Costaceae) by Dr. Stephen, Taxonomist, Dept. of Botany, The American College, Madurai, Tamil Nadu, India.

Microscopical work was done by Prof. Dr. P. Jayaraman, Director of Plant Anatomy Research Institute, Tambaram, Chennai, Tamil Nadu, India.

Figure 1: Habit of *C.pictus*

Macroscopic analysis

Macroscopic observation of the plant was done. The shape, size, surface characters, texture, colour, odour, taste etc was noted. It is a technique of qualitative evaluation based on the study of morphological and sensory profiles of herbs [22].

Microscopic analysis

Transverse section midrib region of fresh leaf pieces were cut and fixed in FAA and then dehydrated by employing graded series of ethyl alcohol and tertiary butyl alcohol [23]. Sections were taken using microtome. The sections were then stained with toludine blue as per standard procedure [24]. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. The sections were also stained with saffranin and fast-green and iodine wherever necessary. In order to supplement the descriptive part the photomicrographs in different magnifications of all necessary cells and tissues were taken with NIKON Coolpix digital camera and Labphot2 microscopic unit.

Physicochemical analysis

Total ash, acid insoluble ash, water soluble ash, loss on drying, extractive values, foaming index, swelling index and moisture content were performed as per standard procedure [25-27].

Leaf constants

Quantitative analytical microscopy is useful for the measurement of cell contents of the crude drug and thus helps in their identification, characterization and standardization. Leaf constants

such as vein islet numbers, vein terminal number, stomatal number, stomatal index and palisade ratio were determined [28, 29].

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out to find out the presence of various phytoconstituents using standard procedure [30, 31].

Powder microscopy

Chloral hydrate, glycerin, iodine, phloroglucinol, hydrochloric acid (1:1), lacto phenol etc. were employed as mounting medium.

RESULTS AND DISCUSSION

Macroscopy:

Sensory evaluation plays a key role in determining the denunciation of a crude drug. Organoleptic testing of a crude drug is mainly for qualitative evaluation based on the observation of morphological and sensory profile [32]. In this report, various morphological, microscopical, physicochemical standards have been developed. Hence we have undertaken this study to serve as a tool for developing standards for identification, quality and purity of *Costus pictus* leaves.

Costus pictus D. Don is a perennial herb. The plant is a hardy vigorous growing ginger. The stem is red with spiral light leaves and airy, the tissue paper like flowers (Figure 1). The leaves are simple, large fresh looking spirally arranged, oblong-lanceolate being dark green above and lighter green below. The shape of the leaf is narrowly elliptic with the length 10 to 25cm and width 2.5 to 6cm (Figure 2). Leaf is green in colour with characteristic taste and odour.

Figure 2: Dorsal and Ventral view of *Costus pictus* D. Don Leaves

Microscopy of the leaf:

The initial step in quality control of medicinal plants is ensuring the authenticity of the desired species for the intended use. It can be conducted via a variety of techniques, namely macro and microscopic identification and chemical analysis especially description of microscopic botanical aspects to determine definitively the proper species of plant material while it is still in its non extracted form [33]. The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs. Microscopic evaluation is one of the simplest and cheapest methods for the correct identification of the source of the materials [35]. In the previous report [36, 37] some important observations like type of stomata, adaxial epidermis apostomatic, calcium oxalate crystal distribution have been not mentioned. All the observations (Midrib and Lamina) are included in this study and moreover clear microscopical plates are displayed.

T.S. of Leaf through adaxially depressed Midrib

Leaf is shallowly boat shaped, having a group of vascular bundles. The midrib is 750µm thick. It consists of thin epidermal layer of small squarish cells on the adaxial side and fairly thick cylindrical epidermal cells on the abaxial side (Figure 3).

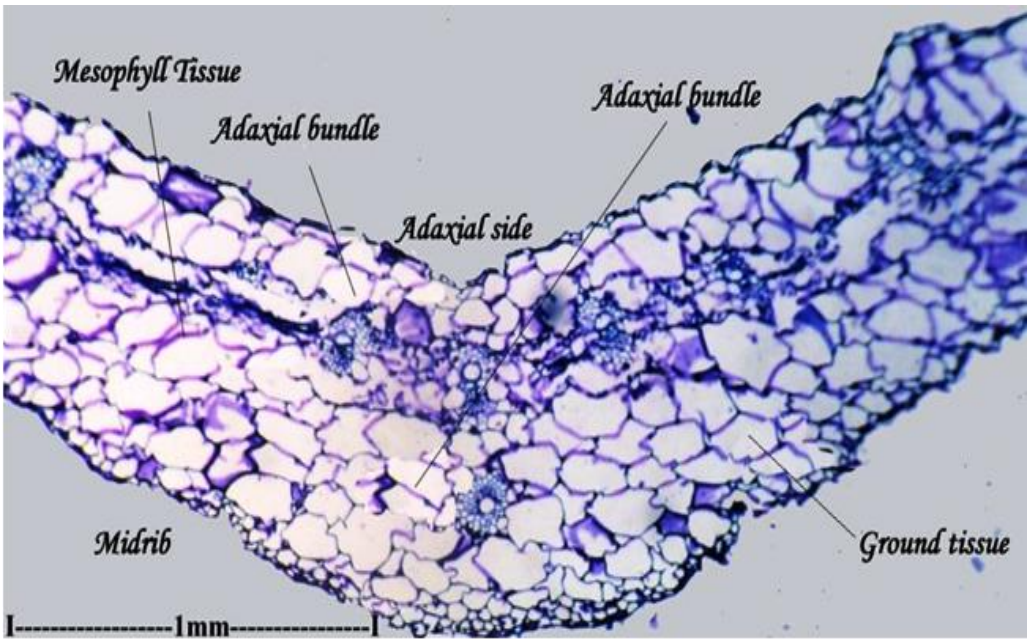

Figure 3: T.S. of Leaf through Adaxially depressed Midrib

The ground tissue includes, wide, thin walled compact parenchyma cells with shrunken walls. The vascular system of the midrib includes an abaxial band of three vascular bundles and adaxial single median bundle. The adaxial vascular bundles have only a few xylem elements; these are two or three wide circular xylem elements, but the phloem is a large mass of sieve elements (Figure 4).

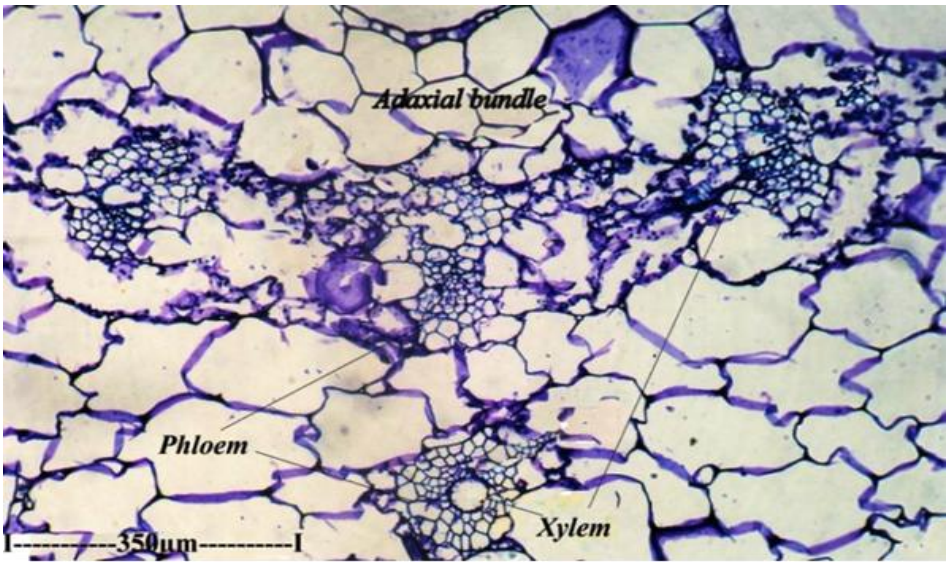

Figure 4: Midrib- Vascular Bundle enlarged

The abaxial solitary bundle has a single, wide, circular thin walled xylem elements and a small clusters of phloem elements located on the upper side**.** The vascular bundle is surrounded by wide layer of parenchymatous bundle sheath (Figure 5).

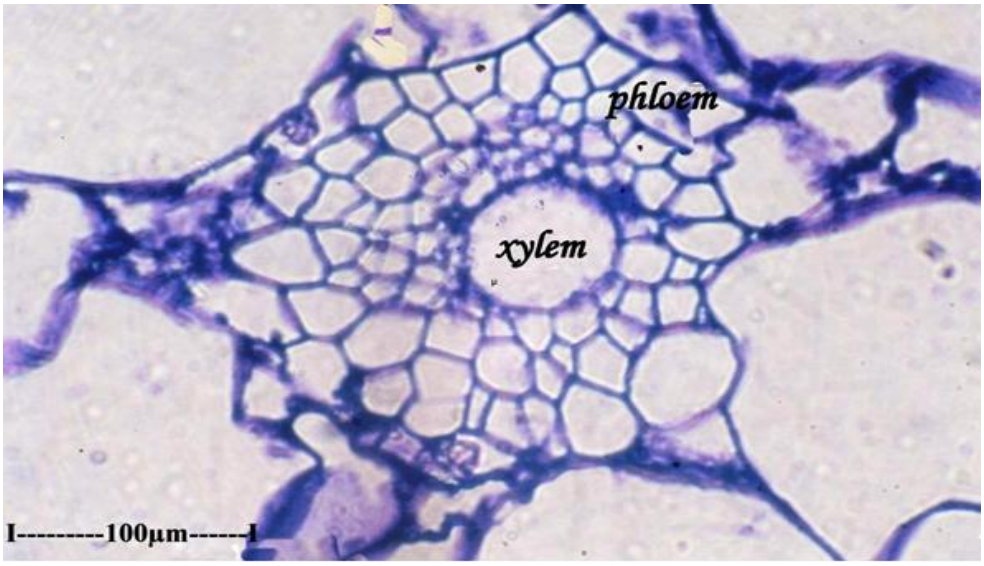

Figure 5: The Adaxial Median Bundle enlarged

T.S. of Leaf through Lamina

The vascular bundles of the lamina occur in a single horizontal row of the median part of the lamina. The lamina- bundles also have one or two wide circular xylem elements and thick clusters of phloem and parenchymatous bundle sheath. The adaxial epidermal layers of the lamina consist of thick, cylindrical cells. The abaxial epidermis is thin and it consists of narrow tubular cells. The adaxial epidermis is apostomatic. The adaxial epidermis is stomatiferous (Figure 6, 7).

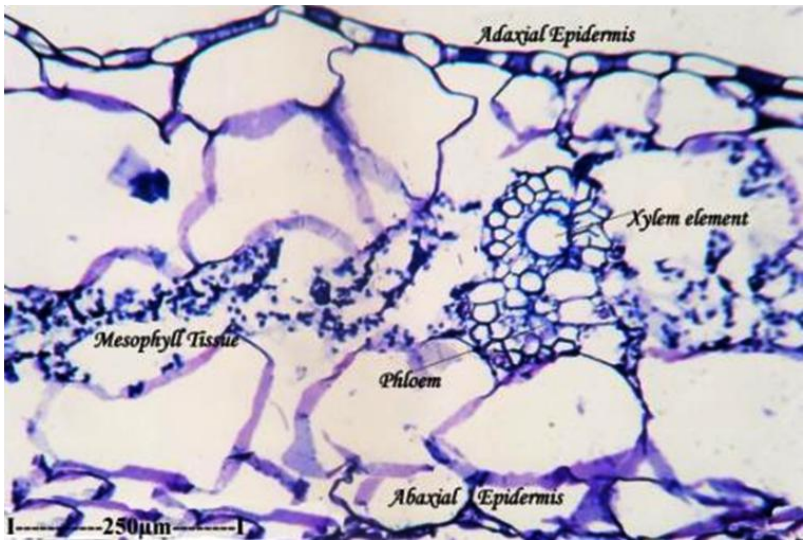

Figure 6: T.S. of Lamina

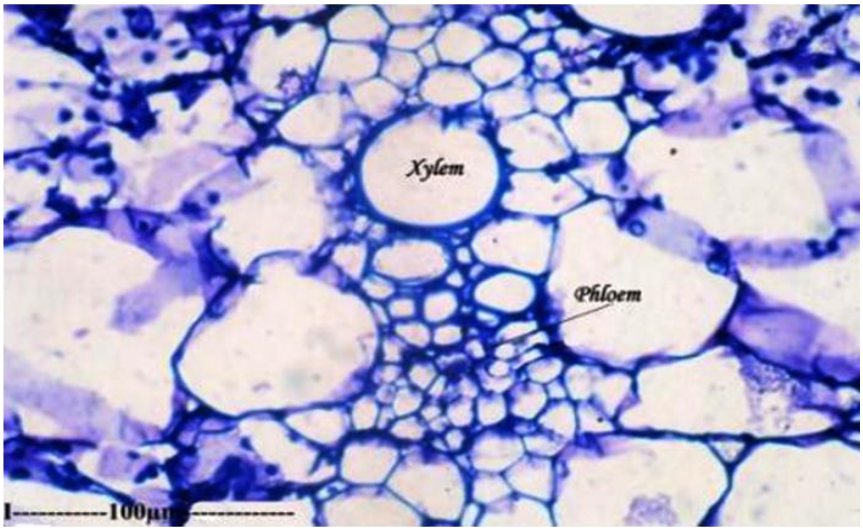

Figure 7: Vascular Bundle of Lamina enlarged

Crystal distribution

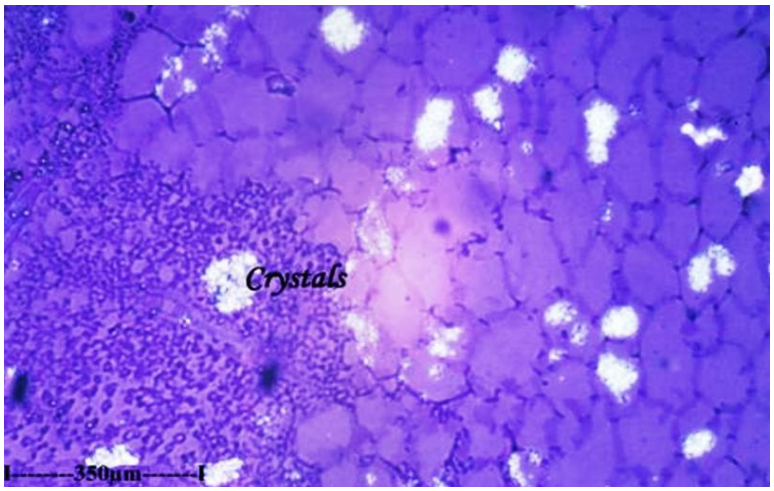

Figure 8: Crystal distribution in the Mesophyll

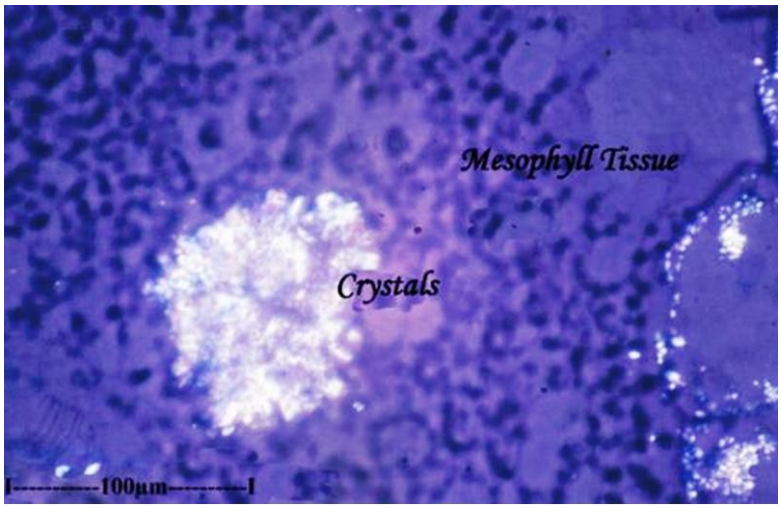

Figure 9: One crystal enlarged

Calcium oxalate crystals of minute particle are aggregated into large masses in the leaf mesophyll cells. These microcrystal masses are random and diffuse in distribution. The crystals are located in ordinary unspecialized cells (Figure 8 & 9).

Epidermal cells and stomatal morphology

The epidermal cells and stomata were observed in the paradermal sections**.** The epidermal cells are rectangular or polygonal; the anticlinal walls are thick and straight. The stomata are diffuse in distribution. They are surrounded by the two pairs of lateral subsidiary cells. This type of stomata is called hexacytic type. The guard cells are 15 X 30µm in size (Figure 10 & 11).

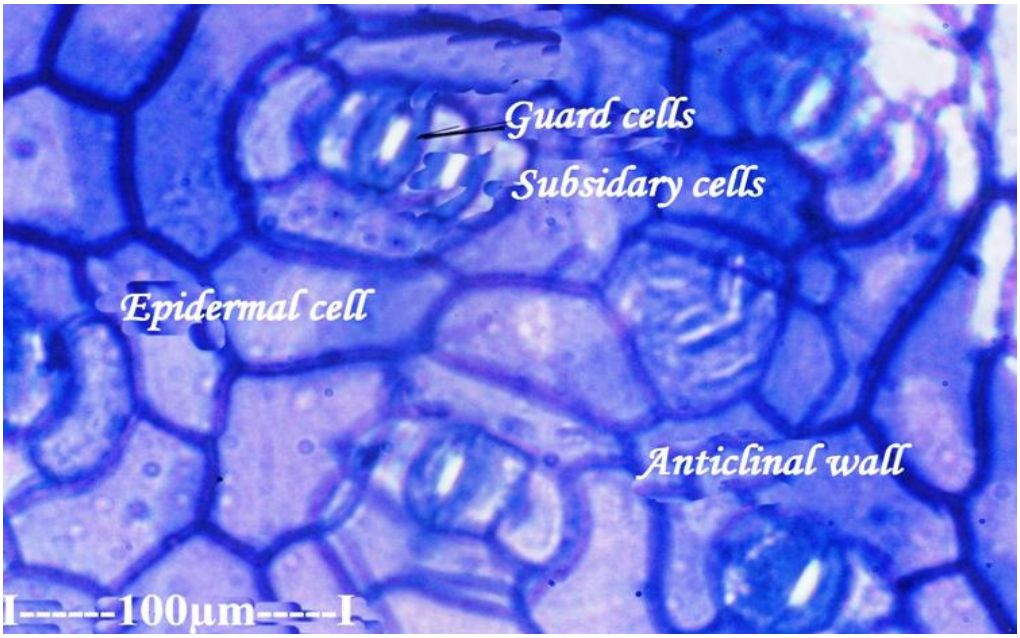

Figure 10: Paradermal section showing Stomata on the Abaxial Epidermis

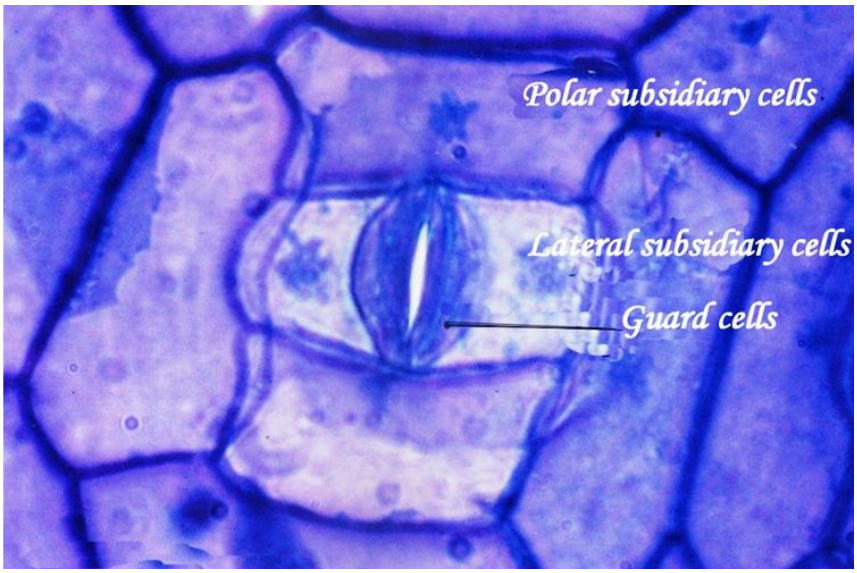

Figure 11: One Stoma enlarged

Venation pattern

The leaf exhibits fairly prominent parallel main veins. The main parallel veins are interconnected by thin, less prominent horizontal veins (Figure 12).

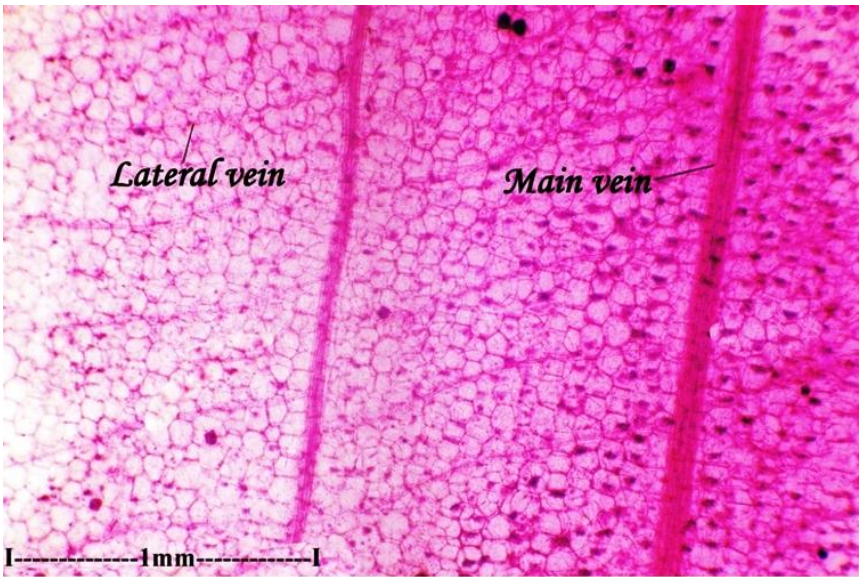

Figure 12: Venation pattern of the Lamina

Physicochemical analysis

The ash values are particularly important to find out the presence or absence of foreign inorganic matter such as metallic salts and or silica (earthy matter) [38]. Acid insoluble ash provides information about non-physiological ash produced due do adherence of inorganic dirt, dust to the crude drug. Increased acid insoluble ash indicates adulteration due do dirt, sand (or) soil. The

extractive values are primarily useful for the determination of exhausted or adulterated drug and helpful in the detection of adulteration [39].

Total ash, acid insoluble ash, water soluble ash, loss on drying, extractive values, foaming index, swelling index and moisture content were determined and tabulated (Table 1).

Table 1: Standardization parameters of leaves of *Costus pictus* D. Don

S.NO	Parameters*	Values*expressed as
1	**Moisture content**	12.38±0.033
	Ash value	
	Total ash	15.28±0.140
2	Acid insoluble ash	3.17±0.080
	Water soluble ash	9.70±0.110
3	**Foreign organic matter**	0.06±0.080
	Extractive values	
	Petroleum ether	25.74±0.150
	Chloroform	7.20±0.300
4	Ethyl acetate	8.60±0.030
	Ethanol	5.10±0.110
	Methanol	16.25±0.040
	Water	11.12±0.600
	Benzene	5.76±0.090
5	**Foaming index**	Less than100
	Swelling index	**expressed as ml**
6	Initial volume	3.2±0.10
	Final volume	7.6±0.140

Leaf constants

Quantitative analytical microscopy is useful in measuring the cell contents of the crude drugs and help in their identification, characterization and standardization. A clear idea about the identity and characteristic features of the drug can be obtained after several numbers of determinations. Quantitative analytical microscopy is useful for the measurement of cell contents of the crude drug and thus helps in their identification, characterization and standardization. Leaf constants such as vein islet numbers, vein terminal number, stomatal number and stomatal index were determined and tabulated (Table 2).

Table 2: Quantitative analytical microscopical parameters of the leaves of *Costus pictus* D. Don

S. No.	Parameters*	Values obtained
1	Stomatal number (lower epidermis)	13±0.28
2	Stomatal index (lower epidermis)	10.38±0.23
3	Vein islet number	4.1±0.057
4	Vein termination number	3.9±0.088

*** mean of 6 readings ± SEM**

Preliminary phytochemical screening

Phytochemical evaluation and molecular characterization of plants is an important task in medicinal botany and drug discovery [40].

Preliminary phytochemical screening showed the presence of carbohydrates, triterpenoids, proteins, alkaloids, tannins, saponins, flavonoids, sterols and volatile oil.

Powder microscopy

The microscopic analysis of the powder of the leaf showed fragments of simple unicellular trichomes, hexacytic stomata; abaxial solitary bundle has single, wide, circular thin walled xylem elements and small clusters of phloem elements located on the upper side. The vascular bundle is surrounded by wide layer of parenchymatous bundle sheath, mesophyll cells containing starch grains, Calcium oxalate crystals of minute particle are aggregated in to large masses in the leaf mesophyll cells, Tracheids with spiral thickenings and epidermal cells are observed.

CONCLUSION

The pharmacognostical studies of leaves of *Costus pictus* D. Don gave the valuable information regarding the morphology of crude drugs. They can be useful for the authentication of this plant among all species of *Costus*. The microscopic character, leaf constants, quantitative analysis and physico-chemical parameters studied are useful for setting standards for crude drug and to judge the adulteration and purity of this drug. Since the parameters are constant and any change in these values are indicative of substitution and adulteration of the plant materials.

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REFERENCES

1. Jose B, Reddy LJ. 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 (Suppl 2): 100–1.
2. Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam. Indian Herbs and Herbal Drugs used for the treatment of Diabetes. J Clin Biochem Nutr. 2007 May; 40(3): 163-73.
3. Martinez M. Las Plantas Medicinales De Mexico. 6th ed., Mexico: Andres Botas; 1996.

4. Jayasri MA, Gunasekaran S, Radha A, Mathew TL. Anti-diabetic effect of *Costus pictus* leaves in normal and streptozotocin-induced diabetic rats. Int J Diabetes and Metabolism. 2008; 16: 117–22.
5. Gireesh G, Thomas SK, Joseph B, Paulose CS. Antihyperglycemic and insulin secretory activity of *Costus pictus* leaf extract in streptozotocin induced diabetic rats and in in vitro pancreatic islet culture. J Ethno Pharmacol. 2009; 123: 470–4.
6. Jothivel N, Ponnusamy SP, Appachi M, Singaravel S, Rasilingam D, Deivasigamani K et al. Anti-diabetic activity of methanol leaf extract of *Costus pictus* D. Don in alloxan-induced diabetic rats. J Health Sci. 2007; 53: 655–63.
7. Sethumathi PP, Nandhakumar J, Sengottuvelu S, Duraisamy R, Karthikeyan D, Ravikumar VR et al. Anti diabetic and antioxidant activity of methanolic leaf extracts of *Costus pictus* D. Don in alloxan induced diabetic rats. Pharmacology online. 2009; 1: 1200–13.
8. Mani P, Kumar AR, Bastin TM, Jenifer S, Arumugam M. Comparative evaluation of extracts of C. igneus (or C. pictus) for hypoglycemic and hypolipidemic activity in alloxan diabetic rats. Int J Pharm Tech. 2010; 2: 183–95.
9. Pareek A, Suthar M, Godavarthi A, Goyal M, Bansal V. Negative regulation of glucose uptake by *Costus pictus* in L6 myotube cell line. J Pharm Negative. 2010; 1: 24–6.
10. Al-Romaiyan A, Jayasri MA, Mathew TL, Huang GC, Amiel S, Jones PM et al. *Costus pictus* extracts stimulate Insulin secretion from mouse and human islets of Langerhans in vitro. Cell Physiol Biochem. 2010; 26: 1051–8.
11. Isaac ST, Alphonse JK. Comparative study of hypoglycemic activity of *Costus pictus* and *Costus igneus* in streptozotocin induced diabetic rat. J Pharm Res. 2011; 4: 3628–9.
12. Suganya S, Narmadha R, Gopalakrishnan VK, Devaki K. Hypoglycemic effect of *Costus pictus* D. Don on alloxan induced type 2 diabetes mellitus in albino rats. Asian Pac J Trop Disease. 2012; 2: 117–23.
13. Jayasri MA, Mathew L, Radha A. A report on the antioxidant activity of leaves and rhizomes of *Costus pictus* D. Don. Int J Integr Biol. 2009; 5 (1): 20–6.
14. Reddy LJ, Jose B. Evaluation of antibacterial activity of the essential oil of *Costus pictus* D. Don from South India. International Journal of Current Pharmaceutical Research. 2010; 2(3): 68-70.
15. Majumdar M, Parihar PS. Antibacterial, antioxidant and antiglycation potential of *Costus pictus* from Southern region, India. Asian J Plant Sci Res. 2012; 2: 95–101.

16. Nadumane VK, Rajashekar S, Narayana P, Adinarayana S, Vijayan S, Prakash S et al. Evaluation of the anticancer potential of *Costus pictus* on fibrosarcoma (HT-1080) cell line. J Nat Pharm. 2011; 2: 72–6.
17. Caceres A, Grion LM, Martinez AM. Diuretic activity of plants used for the treatment of urinary ailments in Guatemala. J Ethnopharmacol.1987; 19: 233-45.
18. Comargo MEM, Najera CR, Torres RS, Alderate MEC. Evaluation of the diuretic effect of the aqueous extract of *Costus pictus* D. Don in rat. Poc West Pharmacol Soc. 2006; 49: 72-4.
19. George A, Thankamma A, Remadevi VK, Fernande IA. Phytochemical investigation of Insulin plant (*Costus pictus*). Asian J Chem. 2007; 19(5): 3427-30.
20. Hegde PK, Rao HA, Rao PN. A review on Insulin plant (*Costus igneus* Nak). Pharmacogn Rev. 2014; 8(15): 67–72.
21. Johanson DAO. Plant Micro technique. New York: Mc. Grew Hill Book Co; 1940.
22. Agrawal SK, Karthikeyan V, Parthiban P. Pharmacognostic and physiochemical standardization of the leaves of *Scoparia dulcis* L. Int J Univers Pharm Bio Sci. 2014; 3(2): 131-44.
23. Sass JE. Elements of Botanical Micro technique. New York: Mc. Grew Hill Book Co; 1940.
24. O' Brien TP, Feder N, Mc Cull ME. Polychromatic staining of plant cell walls by Toludine blue-O. Protoplasma 1964; 59: 364-73.
25. Agrawal SS, Paridhavi M. Herbal Drug Technology. 2nd ed., Hyderabad, India: Universities press Pvt. Ltd; 2007; 626-644.
26. Ahmad I, Aqil F, Owais M. Modern Phytomedicine. Turning medicinal plants into drugs. Weinheim: John Wiley and Sons; 2007; 25-57.
27. Mukherjee PK. Quality Control of Herbal drugs: An approach to evaluation of botanicals. 1stedn. Kolkata: Business Horizons Pharmaceutical Publishers; 2002; 132- 133, 161, 173, 177, 186- 187, 214, 287 – 288.
28. Kokate CK, Purohit R, Gokhale SB. Pharmacognosy. 36th ed., Pune: Nirali Prakashan; 2006; 318.
29. Trease and Evans. Text Book of Pharmacognosy. 15th ed., New Delhi: Elsevier Publishers; 523.
30. Rangari VD. Pharmacognosy and Phytochemistry. 1st ed., Nasik: Career Publication; 2003; 224.

31. Khandelwal KR. Practical Pharmacognostic Techniques and Experiments. 19th ed., Pune: Nirali Prakashan; 2008; 149-156.
32. Periyanayagam K, Karthikeyan V. Pharmacognostical, SEM and XRF profile of the leaves of *Artocarpus heterophyllus* L. (Moraceae) – A contribution to combat the NTD. Innovare Journal of Life sciences 2013; 1(1): 23-28.
33. WHO. Quality control methods for Medicinal Plant Materials. World Health Organization; Geneva: 1998.
34. Kumar S, Kumar V, Om Prakash. Microscopic evaluation and physiochemical analysis of *Dillenia indica* leaf. Asian Pac J Trop Biomed 2011; 1(5): 337-40.
35. Remya R, Daniel M. Phytochemical and pharmacognostic investigation of anti diabetic *Costus pictus*. D. Don. Int J Pharm Biomed Res. 2012; 3: 30–9.
36. Kumawat U, Shimpi S. Pharmacognostic studies of the *Costus pictus* D. Don. J Herb Med Toxicol, 2009; 3(1): 127-30.
37. Nayak BS, Patel KN. Pharmacognostic studies of the *Jatropha curcas* leaves. Int J Pharm Tech Res. 2010; 2(1): 140-3.
38. Thomas S, Patil DA, Patil AG, Chandra N. Pharmacognostic evaluation and physicochemical analysis of *Averrhoa carambola* L. fruit. J Herb Toxicol. 2008; 2(2): 51-4.
39. Pimple BP, Patel AN, Kadam PV, Patil MJ. Microscopic evaluation and physicochemical analysis of *Origanum majorana* Linn leaves. Asian Pac J Trop Diseases 2012; 2(2): S897-903.
40. Agrawal SK, Karthikeyan V. Quality assessment profile of the leaves of *Tecoma stans* L. International Journal of Pharmaceutical Research, 2014; 6(2): 94-9.