ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (509-518), Month: July - September 2018, Available at: www.researchpublish.com

Pharmacognosy of an ethnomedicinal plant- *Curculigo orchioides* Gaertn

[1]Rajanikanth Garapati, [2]Rajesh Govindan, [3]Babu Rao Karasala, [4*]Ramesh Narayanaperumal

[1, 2, 3,] Research Scholar, [4]Assistant Professor
[1, 3] Department of Biotechnology, JJ College of Arts and Science, Pudukkottai, Tamil Nadu, India
[2, 4] PG & Research Department of Botany, Government Arts College for Men, Krishnagiri, Tamil Nadu, India

Abstract: ***Curculigo orchioides*** **is a highly medicinal plant. The present study provides detailed microscopic studies of the plant. The rhizome, root and leaves were cut and fixed in FAA. After 24 hrs. of fixing, the specimens were dehydrated with a graded series of tertiary Butyl alcohol as per the schedule. Infiltration of the specimens was carried by gradual addition of paraffin wax until TBA solution attained super saturation. The paraffin-embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the section was 10-12 µm. The section was stained with Toluidine blue. For normal observations bright field was used. Crystals, starch grains and lignified cells, the polarized light was employed. The leaf exhibits circinate vernation of the lamina where the leaf is folded longitudinally into deep grooves and thick ridges; the grooves and ridges are alternate in the horizontal plane. The rhizome which is deep in the soil exhibits certain characteristic features. There is a well-developed periderm all around the rhizome. The periderm is uniformly 150µm thick, comprising six or seven layers of rectangular, thin-walled, homogeneous and suberized phellem cells. The vascular bundles are scanty in this region. The raphide bundles are 25 µm thick and 80 µm long. The variation in the thickness of the midrib, lateral veins, lamina, and vascular strands may be attributed to the difference in the nutrient in the substratum where the plants grow. This anatomical character should be helpful to authentication of the *C. orchioides* sample.**

Keywords: ***Curculigo orchioides*****, Anatomy, Rhizome, Crystals, Starch grains.**

I. INTRODUCTION

Curculigo orchioides Gaertn. (Family - Hypoxidaceae), also called "Kali musli" is a monocot with tuberous rootstocks. The species is a stemless perennial herb of medicinal importance and a native of India. "Kali musli" is reported to have hypoglycemic, spasmolytic and anticancer potential. The rhizome is also prescribed for the treatment of piles, jaundice, asthma, diarrhea [1] and on pimples[2]. It is also used as an antioxidant [3];[4], spermatogenic [5], hepatoprotective [6], immunostimulant [7], anticancer [8], antibacterial[9], antiosteoporotic [10] and hypoglycaemic[11]. This plant species has now become endangered due to depletion in the natural habitat due to over-harvesting, cattle-grazing, poor-seed setting and germination and over-collection of tubers for medicine and food[12];[13].

Raghunathan and Mitra [14] and Nadkarni [15] reported that various vaidyas and traditional tribal's prescribed *Curculigo orchioides* in various systems of medicine, especially in Indian systems, for long periods. Herb-properties in various doses and combinations for the treatment of a number of diseases such as antidiabetic, aphrodisiac, asthma, bronchitis, demulcent, diarrhea, diuretic, dyspnoea, dysuria, gonorrhea, hydrophobia, indigestion, jaundice, leucorrhoea, menorrhagia, menstrual derangements, ophthalmia, pains in the joints, piles, tonic and vomiting have been described [16],[17],[18],[19],[20],[12].

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (509-518), Month: July - September 2018, Available at: www.researchpublish.com

Plant anatomy is one of the essential aspects of the botanical area. Anatomical studies were initiated some 400 years ago. With the help of simple hand lens and crude type of microscope this descriptive study started and during the past decades. After the invention of the sophisticated light microscope and the electrons microscope, the field of botany has contributed immensely to information related to taxonomy, evolution, phylogeny and interrelationships of plants. Anatomy has also donated to pharmacognosy, forensic science, forestry, timber technology and so on.

Realizing the relevance of anatomical studies are concerning other branches of botany, it is decided to make an anatomical analysis of the taxon, *Curculigo orchioides*, a member of Hypoxidaceae. This taxon is said to be one of the endangered plants due to changes in the environmental calamities. In addition to morphological and anatomical studies of *in-vivo* and *in-vitro* plants were also studied to estimate any significant difference between the *in vivo* and *in-vitro* plants and if no essential difference was found to promote *in vitro* method to conserve this interest orchioides species.

II. MATERIALS AND METHODS

2.1 Collection of specimens:

The healthy and disease free *C. orchioides* (Fig. 1 & 2) collected from Sivakasi, Virudhunagar District, Tamil Nadu, India. The rhizome, root, and leaves were cut and fixed in FAA (Formalin - 5ml + Acetic acid - 5ml + 70% Ethyl alcohol - 90ml). After 24 hrs. of fixing, the specimens were dehydrated with a graded series of tertiary Butyl alcohol as per the schedule is given by Sass [21]. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60ºC) until TBA solution attained supersaturation. The specimens were cast into paraffin blocks.

Fig 1: *Curculigo orchioides* Gaertn.;

Fig 2: Rhizome and roots of field grown plant;

2.2 Sectioning:

The paraffin-embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the section was 10-12 µm. The process of the sections was by customary procedure [22]. The section was stained with Toluidine blue as per the method [23] and the toluidine blue is a polychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, and blue to the protein bodies. Wherever necessary sections also stained with safranin and Fast- green and IKI (for starch)

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections were taken parallel to the surface of the leaf) as well as clearing of the leaf with 5% sodium hydroxide or epidermal peeling by partial maceration by employing Jeffrey's maceration fluid [21] were prepared. Glycerin mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerin medium after staining. Different cell component was studied and measured.

2.3 Photomicrographs:

Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic Unit. For normal observations bright field was used. For

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (509-518), Month: July - September 2018, Available at: www.researchpublish.com

the study of crystals, starch grains and lignified cells, the polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against a dark background. Magnifications of the figures are indicated by the scale bars. Descriptive terms of the anatomical feature areas given in the standard Anatomy books [24],[25].

III. RESULTS AND DISCUSSION

The plant is a stemless herb. The root-stock is more or less tuberous with crowns of remain old leaves. Leaf is radical, narrow, prominently nerved and plicate.

3.1 Leaf:

The leaf exhibits circinate vernation of the lamina where the leaf is folded longitudinally into deep grooves and thick ridges; the grooves and ridges alternate in the horizontal plane (Fig. 3). Along the median part of the lamina, the leaf is flat and possesses more prominent midrib with the thicker vascular bundle (Fig. 4). The midrib is flat on the adaxial side and broadly conical on the abaxial side. The midrib is 358µm thick and 320 µm wide. The epidermal cells of the midrib are circular or broadly spindle-shaped and wide with thick walls.

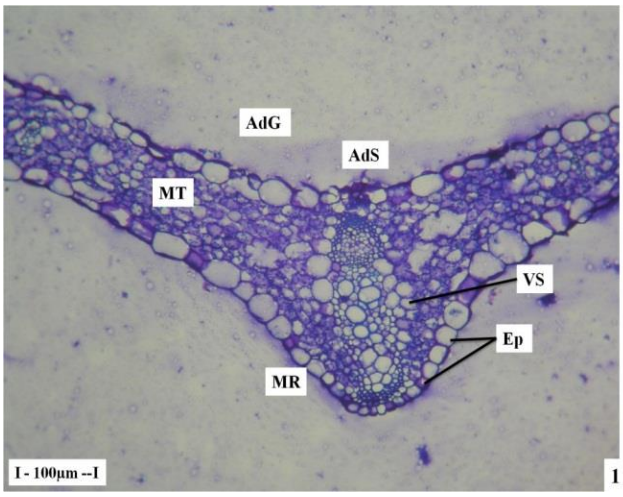


Fig 3: TS of the leaf through midrib

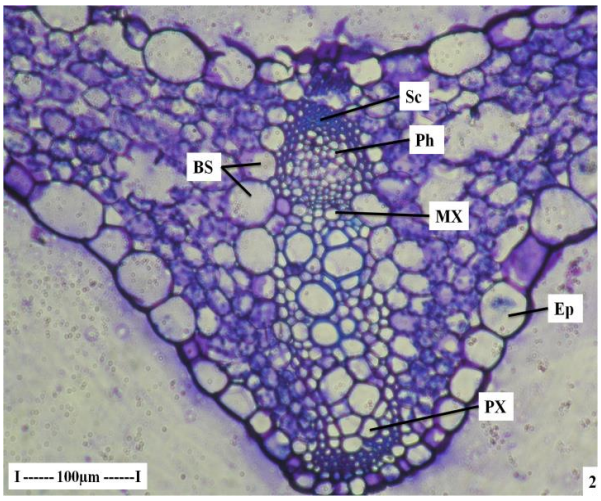


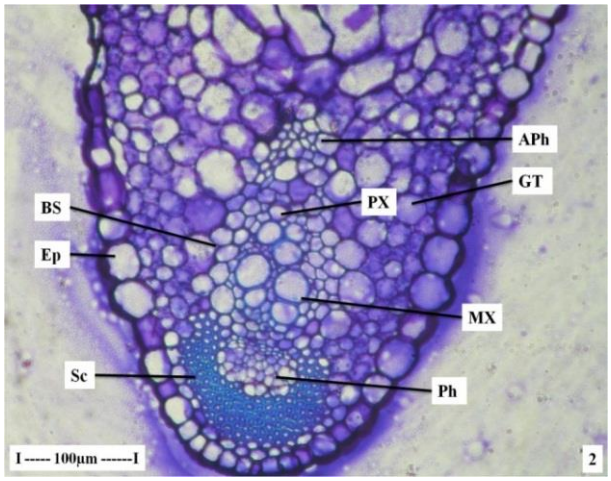
Fig 4: Vascular bundle of the midrib enlarged

AdG - Adaxial Groove; Ads - Adaxial side; BS - Bundle Sheath; Ep - Epidermis; MR - Midrib; MT - Mesophyll Tissue; MX - Metaxylem; Ph - Phloem; PX - Protoxylem; Sc – Sclerenchyma; VS - Vascular Strand

The vascular bundle of the midrib is vertically oblong measuring 300 µm in a vertical plane and 140 µm in breadth. The vascular bundle is collateral with abaxial phloem and adaxial xylem elements. (Fig. 4). The phloem consists of small compact angular darkly stained sieve elements; a thick arc of sclerenchyma elements is abutting the phloem. The xylem elements include two vertical rows of wide, angular, thick-walled cells. The metaxylem elements are 35 µm in diameter. Protoxylem elements are located at the abaxial end of the vascular bundle (Fig.4). Sclerenchyma cap is present along the protoxylem end of the vascular bundle. The entire vascular bundle is surrounded by a single layer of wide, circular thin-walled parenchyma cells. The ground tissue of the midrib includes small, circular parenchyma cells with dark cell contents. Structure of the folded part of the lamina resembles the flat part of the lamina. The folded lamina has a deep, wide adaxial groove and conical abaxial part. The conical structure is 380 µm long and 300 µm thick. The epidermal cells of the conical end are small and angular; the epidermal cells on either side of the conical part are larger, circular and thick walled.

There are two vascular bundles in the conical plicate part. The adaxial bundle is smaller with a small cluster of phloem and a few wide xylem elements (Fig. 5). The abaxial bundle is larger measuring 330 µm in diameter. It consists of two vertical parallel lines of xylem elements which are wide circular and thick walled. The protoxylem elements are towards the adaxial part. A wide circular cluster of sieve elements is located on the lower end of the xylem strand. A circle of bundle sheath parenchyma cells encircles the vascular bundles. A thick deep are of fibers is seen enclosing phloem of the abaxial vascular bundle. (Fig.5).

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (509-518), Month: July - September 2018, Available at: www.researchpublish.com



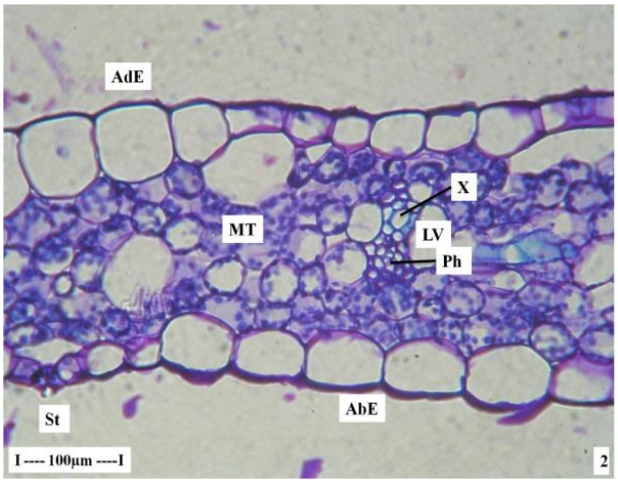
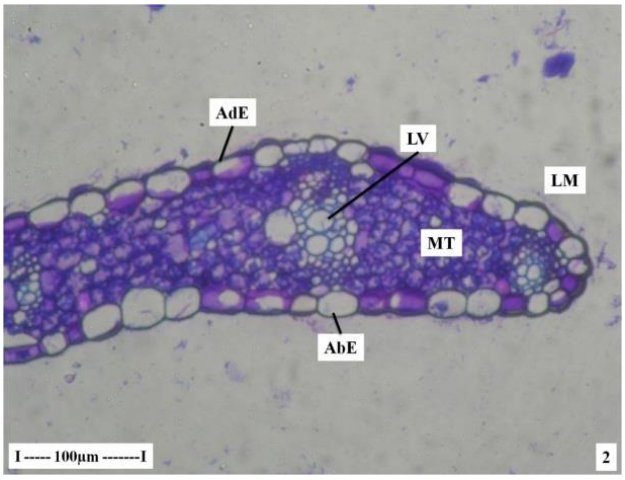


Fig 5: Vascular bundle of plicate region enlarge **Fig 6: TS of leaf showing smaller (Minor) vein.**

AbE - Abaxial Epidermis; AdE - Adaxial epidermis; APh - Adaxial phloem; BS - Bundle Sheath; Ep - Epidermis; GT - Ground Tissue; LV - Lateral Vein; MT - Mesophyll Tissue; MX - Metaxylem; Ph - Phloem; PX - Protoxylem; Sc – Sclerenchyma; St – Stomata; X –Xylem

3.2 Lamina:

Lateral flat lamina part of the leaf is 340 µm thick. It consists of highly dilated rectangular thin-walled epidermal cells which are 50-60 µm thick. The mesophyll tissue is undifferentiated into palisade and spongy parenchyma cells. The tissue consists of about six layers of spherical, less compact darkly stained parenchyma cells. There are small vascular strands possessing collateral vascular bundles (Fig.6). Alternating with the smaller vascular bundles, these are larger vascular bundles, located in between smaller bundles. The smaller bundles have a group of four or five xylem elements and small units of phloem elements (Fig.6). The larger bundle consists of a vertical row of wide, circular thick-walled xylem elements and a wide circle of phloem elements. The vascular bundle is lined by a layer of parenchymatous bundle sheath (Fig. 7).



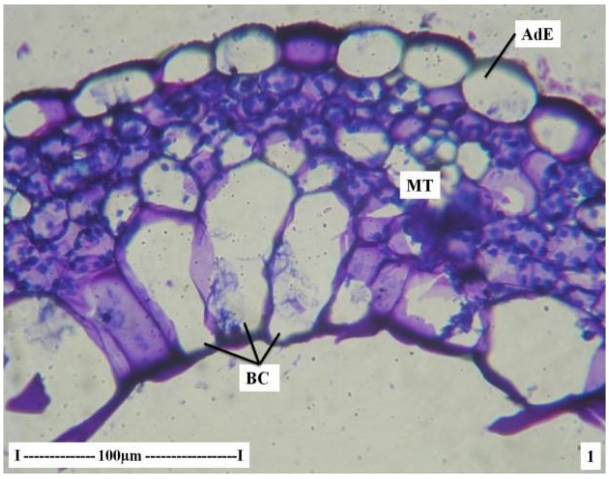


Fig 7: TS of the leaf through the marginal part; **Fig 8: TS of the leaf through bulliform cells;**

AbE - Abaxial Epidermis; AdE - Adaxial epidermis; BC- Bulliform Cells LM - Leaf Margin; LV - Lateral Vein; MT - Mesophyll Tissue;

3.3 Bulliform cells or Motor cells:

(Fig. 8): Certain regions of the abaxial epidermis, a row of three or four epidermal cells are highly dilated and become radially elongated. These larger cells are called bulliform cells or motor cells. These cells shrink when the atmosphere is dry and make the leaf fold abaxially to cover the stomata and to prevent excessive evaporations of water from the leaf cells. During moist conditions the bulliform cells expand rendering the lamina to become flat.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (509-518), Month: July - September 2018, Available at: www.researchpublish.com

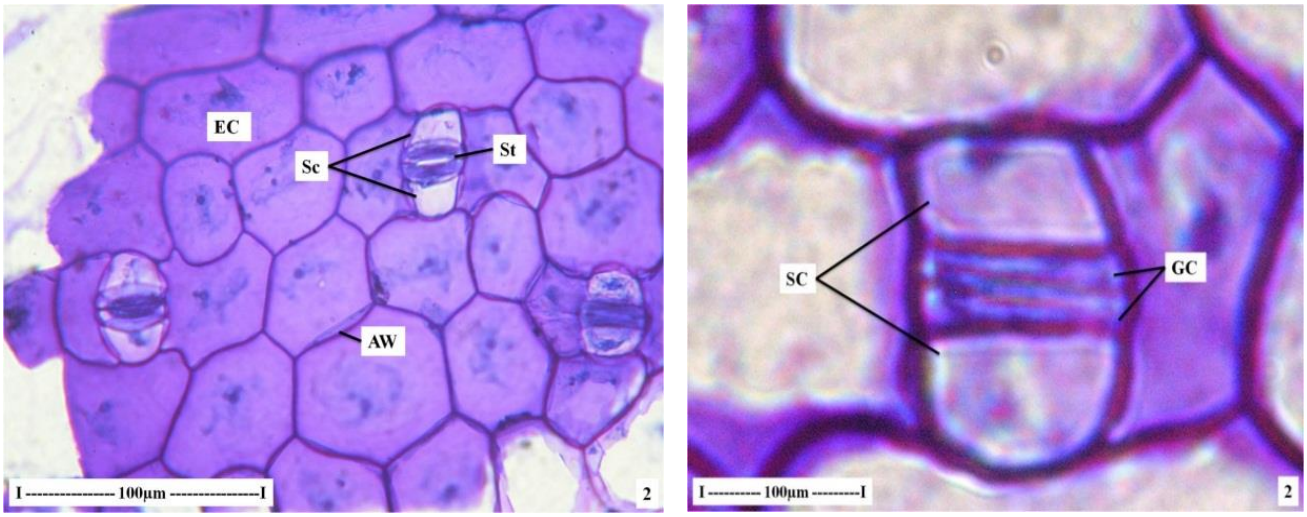


Fig 9: Paradermal sections of the lamina showing surface vein of the cells and stomata; Fig 10: A stoma enlarged;

AW - Anticlinal walls; EC - Epidermal Cells; GC – Guard Cells; St - Stoma; SC - Subsidiary cells;

3.4 Leaf-margin:

(Fig. 7): The marginal part of the lamina is slightly thin and bluntly conical. The marginal leaf is 100 µm thick. The epidermal cells of the leaf margin are slightly smaller and thin walled. A small circular vascular bundle is seen at the extreme margin of the lamina. The mesophyll tissue consists of smaller, compact, darkly stained parenchyma cells.

3.5 Stomata:

(Fig. 9): The lamina is amphistomatic, ie, stomata are present on both the adaxial and abaxial sides of the lamina. The guard cells are at the surface level of the epidermis. The guard's cells have characteristic beaklike outgrowths called stomatal ledges (Fig.10). The stomatal chamber is wide and prominent. Surface view of the epidermal tissue exhibits shape and size of the epidermal cells and the stomatal morphology (Fig. 9). The stomata are broadly elliptical in outline measuring of 15 x 25 µm in size. The stomatal aperture is narrowly slit-like (Fig. 8). The stomata are the exclusively paracytic type. Each stoma has two, semicircular subsidiary cells, one subsidiary on the lateral side of the stoma. The subsidiary cells do not stain as compared with the epidermal cells (Fig. 10). The epidermal cells are pentagonal or hexagonal in outline with straight thin walls.

3.6 Rhizome:

The rhizome, lying just beneath substratum consists of rhizome ensheathed by successive rings of the basal part of the leaf (leaf-sheath). The leaf sheath consists of spindle-shaped portions alternating with thin and flat portions (Fig. 11,12). The thick spindle-shaped portions possess collateral vascular bundles which consist of thick sclerenchyma bundle cap, small clusters of xylem and phloem. Alternating with vascular bundles are small clusters of sclerenchyma cells.

The rhizome which is unsheathed by the leaf sheaths is thick cylindrical with an even outline. It consists of fairly prominent squarish thin walled epidermal cells with thick cuticle. The ground tissue is homogeneous and parenchymatous. The cells are circular to angular thin walled and compact (Fig. – 11,12). There are sparsely distributed small vascular strands in the outer zone of the rhizome. The vascular bundles have a small group of xylem and phloem elements.

Towards the interior of the rhizome the number and size of the vascular bundle's increase. The vascular bundles are circular and collateral. Each bundle consists of a thick arc of xylem elements enclosing a mass of phloem elements. The xylem elements are angular, fairly wide, thick-walled and lignified (Fig. 11). The ground parenchyma cells are slightly larger in size and thin walled.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (509-518), Month: July - September 2018, Available at: www.researchpublish.com

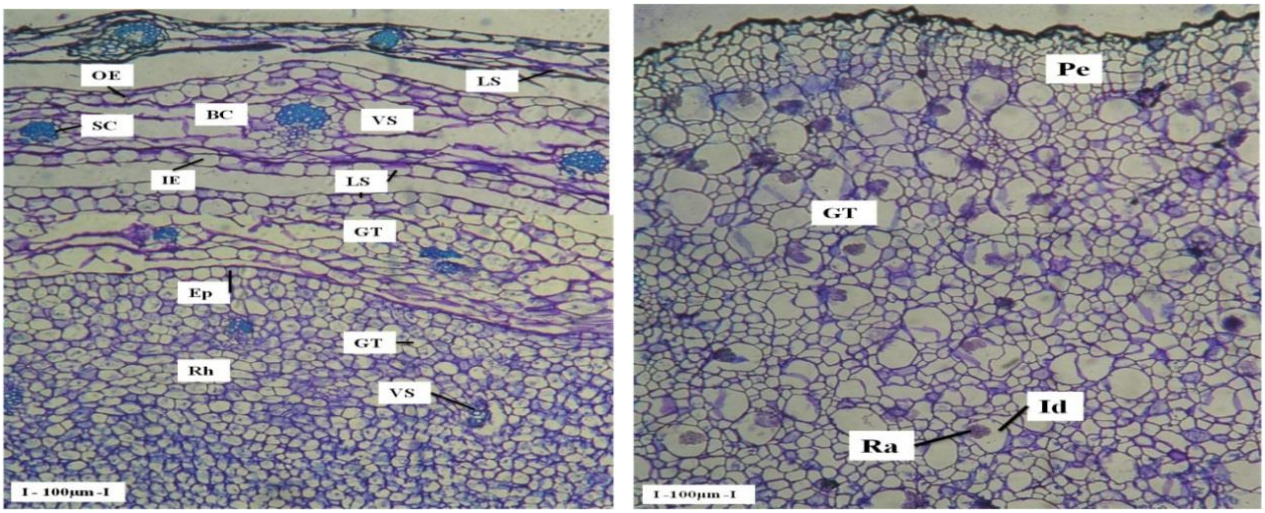


Fig 11: TS of the upper portion of the rhizome showing leaf sheaths; Fig 12: The lower portion of the rhizome;

BC - Bundle Cap fibers**;** Ep – Epidermis; GT – Ground Tissue (Parenchyma); Id- Idioblast; IE - Inner Epidermis; LS - Leaf Sheath; OE - Outer Epidermis; Pe - Periderm; Ra – Raphide; Rh – Rhizome; Sc – Sclerenchyma strand chamber; VS-Vascular Strand;

The rhizome which is deep in the soil exhibits certain characteristic features (Fig. – 13). There is a well-developed periderm all around the rhizome. The epidermis is crushed into the thin dark layer. The periderm is uniformly 150µm thick, comprising six or seven layers of rectangular, thin-walled, homogeneous and suberized phellem cells. The ground tissue consists of small, angular and thin-walled parenchyma cells. The vascular bundles are scanty in this region. There are wide, circular cavities, diffusely distributed in the ground tissue. These cavities are idioblasts or specialized cells possessing calcium oxalate bundle of thin pointed needles of raphides (Fig. 13). The raphide bundles are 25 µm thick and 80 µm long. Hundreds of these pointed needles are aggregated into a raphide bundle (Fig. 13 & 14)

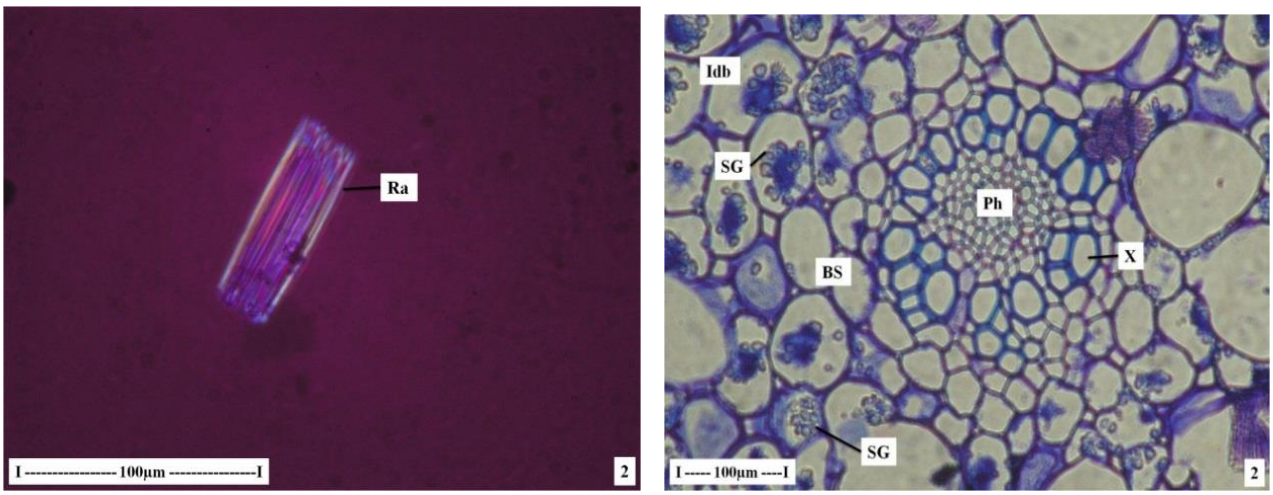


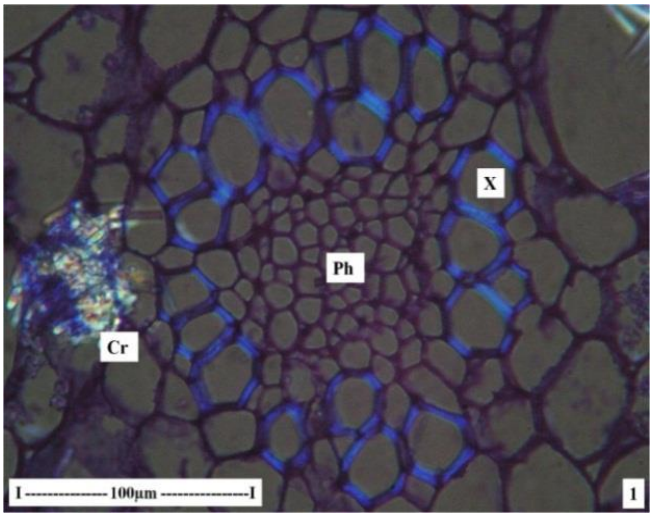
Fig 13: One raphide bundle enlarged; Fig 14: Rhizome showing circular vascular bundles which are of amphivasal type (Phloem surrounded by xylem) TS of rhizome showing vascular bundles and ground cells with raphides and starch grains

BS – Bundle Sheath; Idb – Idioblast; Ph – Phloem; Ra – Raphide bundle; SG – Starch Grain; X- Xylem

3.7 Central portion of the rhizome:

The central part of the rhizome has a number of large concentric vascular bundles (Fig. 14). The ground parenchyma cells have raphide crystal bundles and starch grains (Fig. – 14). The vascular bundles are amphicribral type in which phloem occurs in the center and xylem surrounds the phloem (Fig. 14). The xylem elements are angular, thick walled and wide. The elements are mostly in a single ring. The xylem elements have wide, polygonal cells with dark cell contents (Fig. 15). Raphide type of crystals are seen as an intact bundle or broken into individual needles (Fig. 16).

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (509-518), Month: July - September 2018, Available at: www.researchpublish.com



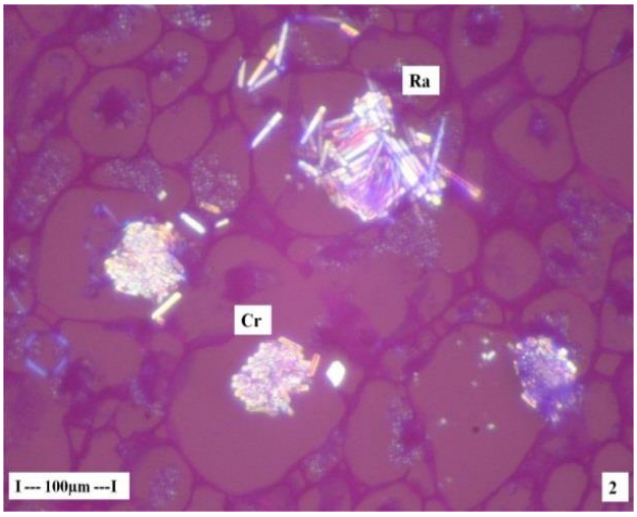
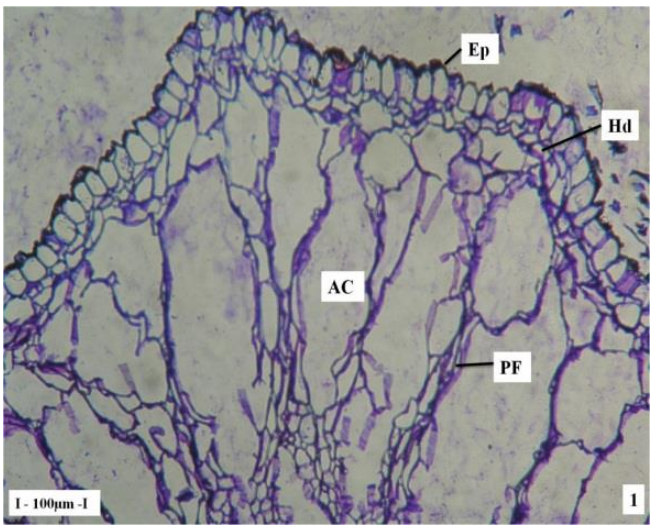


Fig 15: A vascular bundle as seen under polarized light (Xylem elements shining); Fig 16: Calcium oxalate crystals as seen under polarized light;

Cr. Crystals; Ph – Phloem; Ra – Raphide bundle; X- Xylem

3.8 Root:

The root consists radially of long large epidermal cells with prominent cuticle. The epidermis is followed by a single layer of hypodermal cells (Fig. 17). The cortex is wide and is differentiated into outer zone of aerenchyma and an inner zone of compact parenchyma. The aerenhymatous zone consists of wide, radially stretched air-chambers separated laterally from each other by thin radical filaments (Fig. 17). The inner cortex is narrow and the cells are angular, fairly thick walled and compact (Fig. 18). The vascular strand consists of a central core of wide, angular, thin-walled metaxylem elements and center four points of protoxylem elements. The metaxylem elements are 30µm wide.



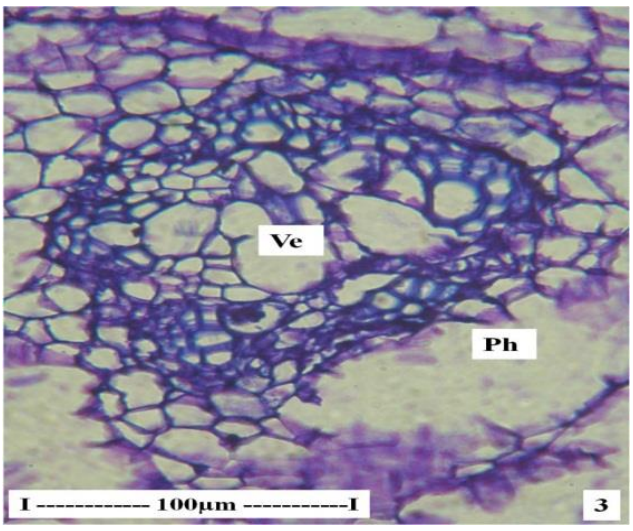


Fig 17: TS of root – showing cortical aerenchyma zone; Fig 18: Vascular strand - enlarged

AC- Air Chambers; Ep; Epidermis; Hd- Hypodermis; PF-Partition filaments; Ph - Phloem; Ve- Vessels;

The bulliform cells are more in number and larger in the leaves. This increase and number of bulliform cells in the leaves may be attributed to the safety measures of the plants. The marginal part of the lamina is thicker (Fig 7,8). In the submarginal region of the lamina, the mesophyll tissues are thick walled and appear dark compact mass. The mesophyll cells and the vascular tissue are larger. (Fig. 7). Epidermal and stomata: The leaf epidermal cells are wide, polygonal with four or five sides (Fig. 9 &10). The outline and arrangement of epidermal cells of the leaf of the plant are dicot type (Fig. 9). Calcium oxalate crystals of raphide type are found in the leaf mesophyll and ground parenchyma of rhizome and root (Fig. 12 & 13)

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (509-518), Month: July - September 2018, Available at: www.researchpublish.com

3.9 Powder Microscopy:

3.9.1 Fibers:

long narrow thick-walled fibers are common in powder .the fibers are 500 µm long and 10 µm thick (Fig. 19)

3.9.2 Tracheid's:

Xylem elements of tracheids are seen in the powder (Fig. 20). The Tracheids are long; cylindrical thick wall cell pits closed spiral and scalariform lateral wall thickening. Some of the tracheids have two vertical rows of rectangular scalariform thickening. The tracheids are 60µm thick.

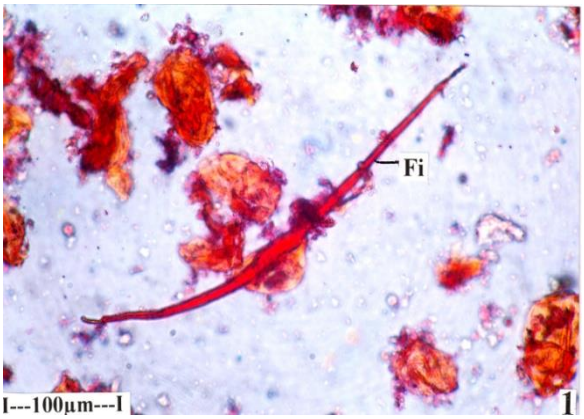


Fig 19: Long narrow thick-walled fibers of rhizome

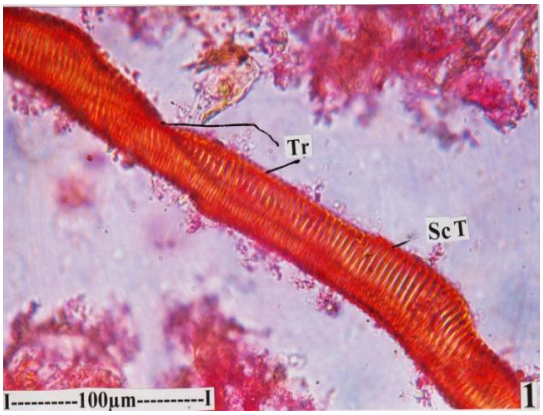


Fig 20: Xylem element of tracheids of rhizome

Fi- Fiber; ScT – Scalariform thickening; Tr – Tracheid;

3.9.3 Epidermal cell of the rhizome:

The epidermal cells are seen in the surface view of the epidermal peeling. The epidermal cells are angular with the thick straight anticlinal wall (Fig. 21).

3.9.4 Oil bodies:

Spherical, shinning large and small oil bodies are abundant in the powder. They are scattered diffusely (Fig. 22). The oil bodies areare 20 µm in diameter.

3.9.5 Epidermal cells of the leaf:

Small fragment of epidermal peeling are often seen in the powder. The epidermal cells are rectangular, squarish are polygonal in shape. There anticlinal walls of thick and straight. All the cells have a distinct nucleus. Stomata are also seen in the epidermal cell. The guard cells are elliptical in shape. This stroma two lateral and two polar subsidiary cells (Fig. 23). Starch grain of different sizes are visible in the powder and when stained with IKI this starch grain turned in to dark blue.

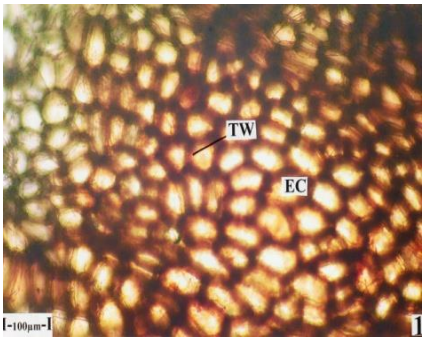


Fig 21: Epidermal cells with anticlinal wall

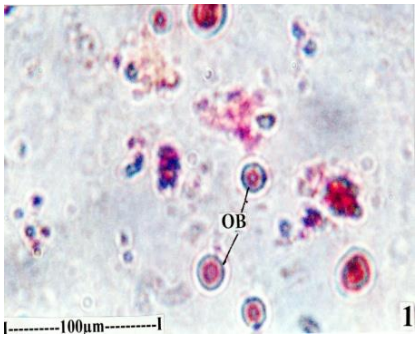


Fig 22: oil bodies in rhizome

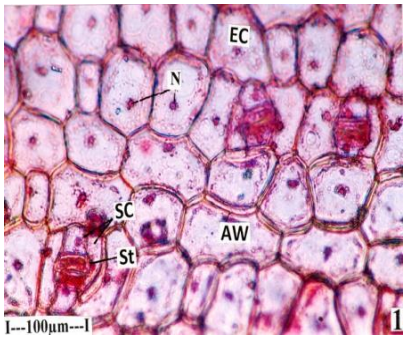


Fig 23: Stroma with two lateral and two polar subsidiary cells

AW – Anticlinal wall; EC - Epidermal cell; N – Nucleus; OB – Oil bodies; Sc – Stomatal chamber; St – Stomata; TW – Thick wall ;

Presence or absence of calcium oxalate crystals in plants is perhaps determined by chemical and physiological parameters of the plant tissues. Present or absent of different types crystal in plants may represent a useful taxonomic character in some groups. For example, on the basis of both morphological and molecular characters, Rudall and Chase [26] demonstrated that the genera formerly included in xanthosrhoeaceae represented three different and distinct families. In *C. orchioides* the styloid crystals are present either in leaf or rhizomes and thereby it proves that the taxon finds a correct place in Hypoxidaceae.

The detailed studies on anatomical features of *Curculig*o, and highlighted certain microscopic features of the taxon as common linking different species of *Curculigo* [27], [28], [29] [30]. Bulliform cells, styloid crystals, stomatal type, mucilage canals, vascular tissues of the leaf, stem, rhizome and root have been found to be similar features of different species in Curculigo of Hypoxidaceae. However, studies of other species of Curculigo with reference to anatomical features may provide certain guidelines to differentiate them anatomically**.**

An anatomical feature of plants plays a potential role in various branches of plant science. For interspecific and intergeneric circumscription of plant anatomical studies have contributed immense of data. In the fields of pharmacognosy, forensic science, forestry and wood science microscopic studies have played a most valuable role. The surface feature of the leaf was found to be of little diagnostic value in distinguishing different species of Hypoxidacae. However, internal anatomy of the leaf was more useful and provided many charters of diagnostic value. Rudall et al. [29] have published the view that both morphological and molecular analyses consistently supported the view of Hypoxidaceae as a monophyletic clade. The characters such as the presence of bulliform cells, successive microsporogenesis, ten uninucleate ovules and mucilage canals are believed as the evidence as for the systematic affinities of the Hypoxidaceae. Curculigo orchioides possesses the above-said features and so the taxon justifies the inclusion in the family Hypoxidaceae.

The morphology of the epidermal cells and presence or absence of cystolith crystals in the plants need some explanation. The stomata have narrowly oblong guard cells. The cystolith is absent in the leaf, but they are abundant in the ground tissue of rhizome (Fig. 14). These differences may be attributed to the substratum where the plants grow. The synthetic medium may provide enough nutrients to the plants to maintain the morphology of the epidermal cells and presence of cystolith crystals in the leaf and not in the rhizome. The soil substratum may be in the presence of crystals in the rhizome, whereas the crystals are not found leaves.

IV. CONCLUSION

The variation in the thickness of the midrib, lateral veins, lamina and vascular strands may be attributed to the difference in the nutrient in the substratum where the plants grow. The soil substratum provides richer and natural nutrients to the plants leading to an increase in the thickness of the plant's parts. This anatomical character should be helpful to authentication of the *C. orchioides* sample.

ACKNOWLEDGMENT

The authors express sincere thanks to Dr. P. Jayaraman, Director, Plant Anatomical Research Centre, Chennai for valuable guidance and support to anatomical work.

REFERENCES

[1] Kirtikar, K.R. and Basu, B.D. 1935. Indian Medicinal Plants, Vol. IV, Blatter E, Caius JF and Mhaekar KS (Eds.), Bishen Singh Mahendra Pal Singh, Delhi, India, p 2469-2470.

[2] Bhamare, P.B. 1998. Traditional knowledge of plants for skin ailments of Dhule and Nandurbar districts, Maharashtra (India). *Journal of Phytological Research* 11: 196-198.

[3] Venukumar, M.R. and Latha, M.S. 2002a. Antioxidant activity of Curculigo orchioides in carbon tetrachloride-induced hepatopathy in rats. *Indian Journal of Clinical Biochemistry* 17 (2): 80-87.

[4] Nagesh, K.S., Nayaka, H.M.A., Dharmesh, S.A., Shanthamma, C. and Pullaiah, T. 2008. In vitro propagation and antioxidant activity of Curculigo orchioides. *J. Trop. Med. Plants* 9(2): 405-410.

[5] Chauhan, N.S. and Dixit, V.K. 2008. Spermatogenic activity of rhizomes of Curculigo orchioides Gaertn in male rats. *International Journal of Applied Research in Natural Products* 1(2): 26-31.

[6] Venukumar, M.R. and Latha, M. S. 2002b. Hepatoprotective effect of the methanolic extract of Curculigo orchioides in carbon tetrachloride treated male rats. *Indian Journal of Pharmacology* 34: 269-275.

[7] Bafna, A.R. and Mishra, S.H. 2006. Immunostimulatory effect of methanol extract of Curculigo orchioides on immunosuppressed mice. *J Ethnopharmacol*. 104: 1–4.

[8] Raaman, N., Selvarajan, S., Gunasekaran, C. P., Ilango, V., Iiliyas, M. and Balamurugan. G. 2009. In vitro cytotoxic and anticancer activities of Curculigo orchioides Gaertn. rhizomes. *Journal of Pharmacy Research* 2(8): 1272-1273.

[9] Nagesh, K.S. and Shanthamma, C. 2009. Antibacterial activity of Curculigoorchioides rhizome extract on pathogenic bacteria. *African Journal of Microbiology Research* 3(1): 5-9.

[10] Jiao, L., Peng cao, D., Qin, L.P., Han, T., Zhang, Q. Y., Zhu, Z. and Yan, F. 2009. Antiosteoporotic Activity of phenolic compounds from Curculigo orchioides, Phytomedicine 16: 874–881.

[11] Jain, A. K., Sharma, N., Upadhyay, A. and Dixit, A. 2010. Hypoglycemic activity of Curculigo orchioides Gaertn in normal and streptozotocin induced diabetic rats, *Research J. Pharm. and Tech.* 3(1): 558

[12] Chauhan, N.S., Sharma, V., Thakur, M., and Dixit, V.K. 2010. Curculigo orchioides: the black gold with numerous health benefits. Zhong Xi Yi Jie He Xue Bao. 8:613-623.

[13] Gupta, R. and Chadha, K.L.1995. Medicinal and Aromatic Plants in India. In: Advances in Horticulture, Medicinal and Aromatic Plants. eds. Chadha, K.L. and Gupta, R. Malhotra Publishing House, New Delhi. pp 1-44.

[14] Raghunathan, K. and Mitra, R. 2001. Pharmacognosy of Indigenous drugs, Central Council for Research in Ayurveda and Siddha publication, New Delhi, Vol. II,: pp 667-670.

[15] Nadkarni, K.M. 2002. The Indian Materia Medica, 2nd Edition, Vol.I, Bombay Popular Prakashan, Mumbai, p. 410-413.

[16] Atal, C.K. and Kapoor, B.M. 1982. Cultivation and utilization of medicinal plants. Jammu-Tawi, India: Regional research laboratory, *Council of Scientific and Industrial Research*.

[17] Bhattacharjee, S.K. 1998. Handbook of Medicinal Plants, Pointer Publishers, Jaipur, India.pp. 118.

[18] Chopra, R.N., Nayar, S.L. and Chopra, I.C. 1956. Glossary of medicinal plants CSIR New Delhi. p. 84

[19] Parrotta. J.A. 2001. Healing Plants of peninsular India, Cabi Publishing, Newyork, pp58-59.

[20] Dash, S.K. and Padhy, S. 2006. Review on ethnomedicines for diarrhea diseases from Orissa: prevalence versus culture. *J. Hum. Ecol.* 20(1): 59-64

[21] Sass, J .E. 1940. Elements of Botanical microtechnique. McGraw Hill Book Co, New York. pp. 222.

[22] Johansen, D.A. 1940. Plant Micro technique. Me Graw Hill Book co, New York. Pp.523.

[23] O'Brien, T.P., Feder, N. and Mc Cull, M.E. 1964.Polychromatic Staining of plant Cell walls by toluidine blue – O.Protoplasma, 59:364-373.

[24] Esau, K. 1964. Planty Anatomy. John Whiley and Sons. New York. Pp.1-767.

[25] Esau, K. 1977. Anatomy of the Seed Plants. 2nd Edition, John Wiley & Sons Ltd, New York

[26] Rudall, P.J. and Chase, M.W. 1996. Systematics of Xanthorrhoeaceae sensu lato: evidence for polyphyly. Telopea 6: 629–647.

[27] Schnarf. W.1892. Beitrage Zur Anatomie der Hypoxideen und einiger verwandter pflanzen. Botanische Zentralblatt, 52:152-327.

[28] Schulze, R.1893. Beitrage Zur vergleichenden Anatomie der Liliaceen, Haemodoraceen, Hypoxidoideen und Velloziaceen. *Botanische Fahrbilcher* 17:295-394

[29] Rudall, P.J., Chase, M.W., Cutler, D.F., Janet Rusby and De Bruijn, A.Y. 1998. Anatomical and Molecular Systematics of Asteliaceae and Hypoxidaceae. *Botanical Journal of the Linnean Society* 127: 1-42.

[30] Arber, A. 1925. Monocotyledons a morphological study. Cambridge University Press, London.