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Research Article

Pharmacognostical studies on whole plant of *Actiniopteris dichotoma*

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ABSTRACT

Actiniopteris dichotoma Bedd (Family; Polypodiaceae,) is an exceedingly pretty fern, used in traditional folk medicine to treat many diseases and disorders. The plant is bitter, astringent, sweet, cooling, acrid, constipating, a n-thelmintic, haemostatic, antileprotic and febrifuge. This study has analysed the leaf, stem, root and rhizome anatomy with the intention of supply sufficient information to the medicinal plant identification. The thin margins of pinnule curve abaxially and form a protective structure to the sporangia located in the axil of the curved margin and does not undergo any structured changes; it forms a protective covering for the sorus and sporangia, called pseudo indusium. The sporangium has a short stalk. The rachis consists of a thin layer of thick walled, lignified epidermal cells. The rhizome is irregularly lobed with deep furrows and ridges. The stele of the rhizome is dictyostele, consists of broken segments of vascular strands. The root has large squarish epidermal layer of cells with thick walls. The Pharmacognostical characters witnessed in this study could serve as an anatomical tool to document and standardize the much valued medicinal fern, *Actiniopteris dichotoma* Bedd.

Keywords: *Actiniopteris dichotoma;* pinnule; pseudo indusium; sporangium; rachis; root and rhizomes; xylem and phloem elements.

INTRODUCTION

Ferns and fern allies have sustained from Palaeozoic times, and tailored with diverse environmental changes than other primitive vascular plants. Ferns are topmost kind of flowerless plants, having sound developed vascular and tegumentary systems, and revealing a comprehensive distinction into root, stem and leaf. The leaves are big and composite and are branded as fronds. The large assemblage of fern is used as ornamental plant. They live in small cracks and fissures of the most exposed rocks and their fronds dehydrate completely during the dry season. Ferns are expected to have polyphenols; which are likely to reduce the risk factors of chronic disease, remove pathogens and old proteins (Wallace RA et al, 1991).

Actiniopteris dichotoma Bedd, Fam: Polypodiaceae, is an exceedingly pretty fern, synonyms included *Actiniopteris australis* Link and *Actiniopteris radiata* (Sw) Link. From the Greek Aktin 'ray' and pteris a 'fern', fern fronds bear a resemblance to bird's wing. If something's *dichotomous*, it's separated into two diverse parts. It can designate a plant who's leaves pair off in opposing buds or anything. Common names included Peacock's tail in English, Mayursikha in Hindi, Mayurasikha in Sanskrit and Mayiladumsikhai in Tamil. The much valued medicinal fern, *A. dichotoma,* is herbaceous tiny palm - similar fern up to 25 cm high with tightly tufted stipe. Fronds fan – like with frequent dichotomous segments which are rush – like in texture, veins little, sub parallel with diverse midrib, segments of fertile frond lengthier than those of the unfertile one, sori linear, elongate, submarginal and rhizomatous. The fern is found throughout India, especi ally in the Peninsula in dry rocky place, below elevations of 4,000 ft. Very common in the lower hills of Attappady, up to 600 m in Nilgiris. The plant is bitter, astringent, sweet, cooling, acrid, constipating, antileprotic and febrifuge. The whole plant is useful in vitiated situations of *kapha* and *pitta,* diarrhoea, haemoptysis, skin diseases, leprosy, haematemesis, diabetes and fever (Warrier PK et al, 1995). Externally, the plant paste is applied on wounds and expected to have wound healing activity; internally the plant paste is administered to kill intestinal worms. Along with sugar, the plant paste is given to stimulate the sexual desire and used as chilling agent in case of syphilis. In paediatrics, fronds paste can be used to treat Rickets. The paste along with milk can be given in the treatment of piles and leucorrhoea. In the treatment of blood pressure and tuberculosis, the herb is waterlogged overnight,

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administered orally in the morning. One teaspoonful of powdered plant may be administered for cough. Leaves paste with fresh cow's milk, is used to normalize the irregular menstrual cycle. After the menstrual cycle, the ash made from the herb with milk, is given fortnight for conception. Interestingly, the paste of leaves with thin curd is given for birth control (Parihar et al, 2006) (Khare CP, 2004) (Sharma BD and Vyas MS, 1985). The paste of fresh plant been applied externally on temple to cure acute head ache. Pulverized plant been used to stop bleeding, used in the treatment of dysentery, edema, kidney problems and anthelmintic. The fern is used in Goa as alterative in prolonged malarious fevers and is a powerful herb to keep infections away and protect the genital passage area and an important ingredient in Gynecure tablets, a product of Ayurveda research foundation.

Figure 1: Actiniopteris dichotoma Bedd

The main bioactive components are Hentriacontane, Hentriacontanol, β-sitosterol, Quercetin-3-rutinoside, β-sitosterol palmitate, β–sitosterol-D-glucoside, glucose, fructose alkaloids, glycosides, flavanoids, tannins and phenols (Taneja and Tiwari, 1974) (Taneja and Tiwari, 1972). Ethanolic and aqueous extract reveals potent peripheral facilitated analgesic activity and prevents mostly peripheral pain mechanism. Ethanolic extract exhibits good wound healing activity on excision and incision wound models and it also effect on granulation tissues and hydroxyproline content in a dose dependent manner. (P. Ramalingeswara Redyy et al, 2012). Ethanolic and ethyl acetate extract exhibits significant anti hyperglycaemic activity at a dose of 100 mg/kg in glucose loaded hyperglycaemic model (Lakshminarasimhaiah et al, 2012). The broad spectrum of anti-bacterial activity exhibited by *A.radiata* may be attributed to the various active constituents presents in it which either due to their individual or combined action. The supplementation of ethanolic extract of *A.radiata* results in hepatoprotective effect against the oxidative damage induced by CCl_4 in albino rats. CCl_4 increased the hepatic malondialdehyde levels significantly, which was inhibited by the extract. The anti - oxidant enzymes SOD, CAT, GPx and non-enzymatic antioxidant, GSH were restored near the control levels after treatment with ethanolic extract of *A.radiata*. The hepatoprotective activity of *A.radiata* has been attained due to the presence of flavonoids and phenolic compounds (Manjunath et al, 2014)

Chloroform extract revealed the presence of flavonoids & sterols, while hexane extract has wax, mainly glycosides and saponins. Ethyl acetate extract has both flavonoids and glycosides. The flavonoids, glycosides and phenols present in ethanolic extract, exhibi ts the highest anti-bacterial activity (M Manjunath, et al 2008). Flavonoids are an effective antimicrobial substance due to their ability to complex with extra cellular and soluble proteins and to complex with bacterial cell wall. The more lipophilic flavonoids may disrupt the microbial membrane of the micro-organisms (Tsuchiya et al, 1996). Phenolics and polyphenols are known to be toxic to micro-organisms (Mayson and Wasesrman, 1987). Tannin has the ability to inactivate microbial adhesions, enzymes and cell envelope transport proteins, and complex with polysaccharides pay the antibacterial activity (Ya C et al, 1988).

The HPLC investigation of ethanolic extract of *Actiniopteris dichotoma* shows the presence of Gallic acid 0.5 µg/gm, Caffeic acid 0.90 µg/gm, Rutin 2.0 µg/gm, Quercitin 2.0 µg/gm, and Ferulic acid 0.1 µg/gm (R. Manonmani and S. Catharin Sara, 2016). The Gallic acid is used to treat albuminaris, psoriasis, diabetes and external haemarroids. Caffeic acid is a promising compound for dermal diseases. Rutin is an important compound to prevent blood clots, heart attacks, and strokes. Quercetin is an effective bronchodilator and acts against allergic or inflammatory chemicals; exerts cytotoxic properties against the human cancer cell line (Mario A et al, 2006). Together, rutin and quercetin frequently been used in allergic conditions. Ferulic acid was found to have antitumor activity against breast and liver cancer (Ambrose et al, 1947). Flavonoids are extensively used in food production for the cons ervation of food to stretch the shelf life by inhibiting or postponing the oxidation course. (D Szostak et al, 1999). GC-MS investigation of ethanolic extract of Actiniopteris dichotoma shows the occurrence of hexadecanoic acid ethyl ester (palmitic acid ester in nature), 9, 12–octadecadienoic acid methyl ester (linoleic acid ester in nature), (E)-9-octadecenoic acid ethyl ester (oleic acid ester in nature), docosanoic acid ethyl ester (fatty acid ester in nature) and heptadecanoic acid heptadecyl ester (fatty acid ester in nature) (R. Manonmani and S. Catharin Sara, 2015). Hexadecanoic acid ethyl ester exhibit antioxidant, hypocholesterolemic, lubricant, anti-androgenic, haemolytic, 5–alpha reductase inhibitor activity. 9, 12–Octadecadienoic acid methyl ester exhibits cancer preventive, hypocholesterolemic, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, anti-acne, alpha reductase inhibitor, antiandrogenic, antiarthritic and anti - inflammatory activity. (E)-9-Octadecenoic acid ethyl ester exhibits anti-inflammatory, dermatitigenic, cancer preventive, hypocholesterolemic, 5-alpha reduc-

tase inhibitor and antiandrogenic acitivity (Duke, 1992).

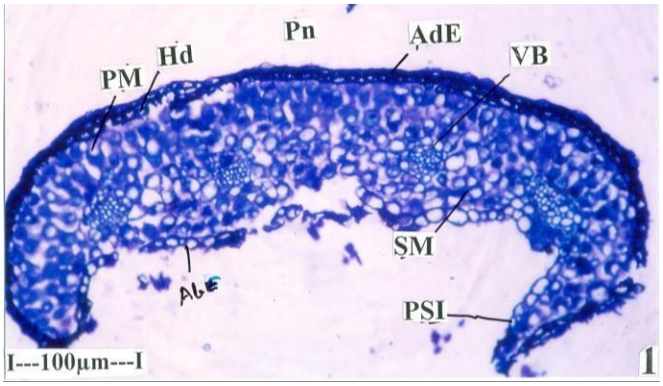

Figure 2: TS of lamina of the Pinnule

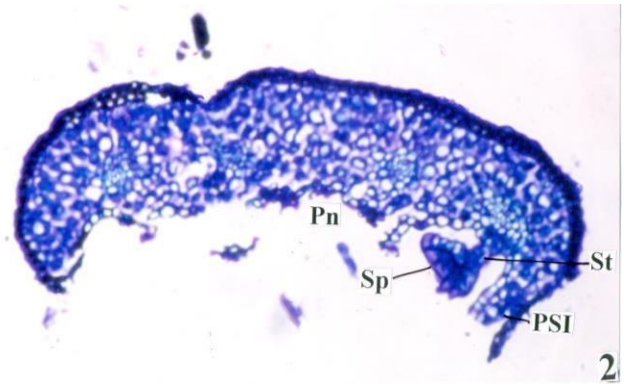

Figure 3: TS of Pinnule with sporangium in the axil of the pseudo indusium

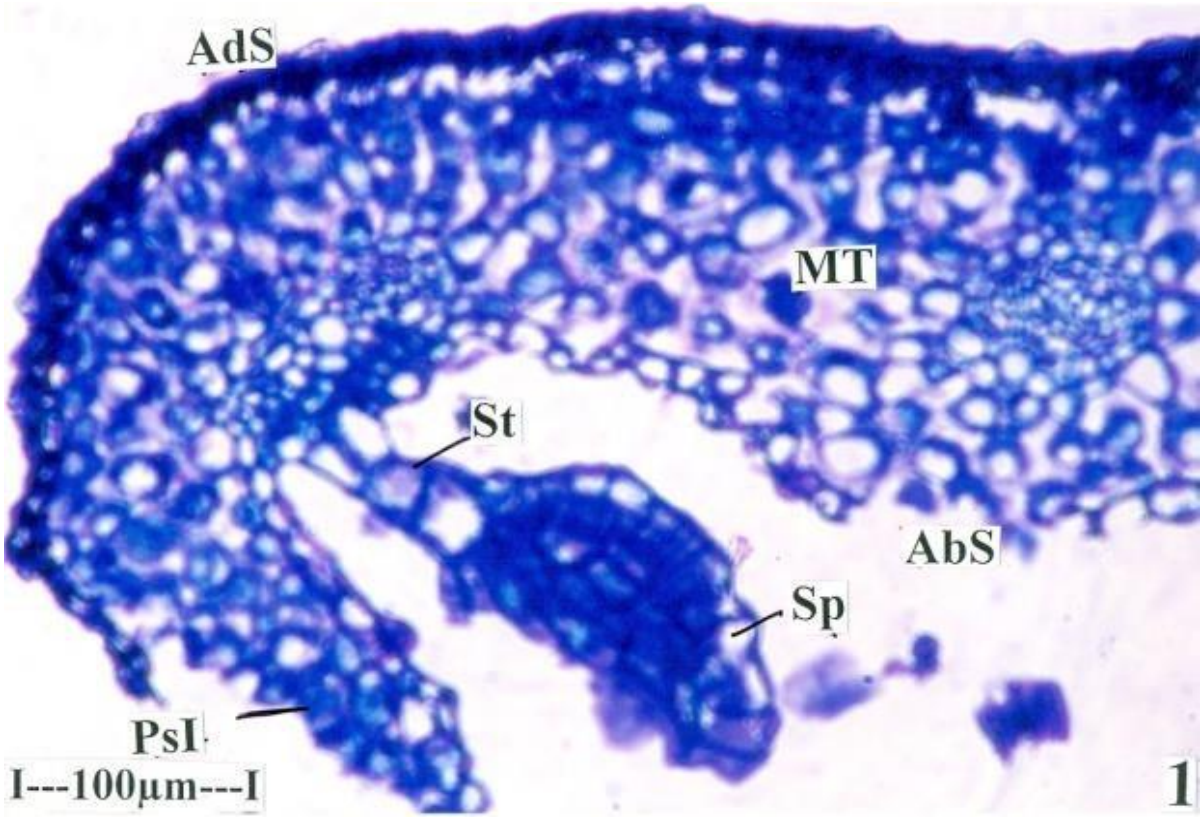

Figure 4: TS of Pinnule showing a sporangium in the axil of the pseudo indusium

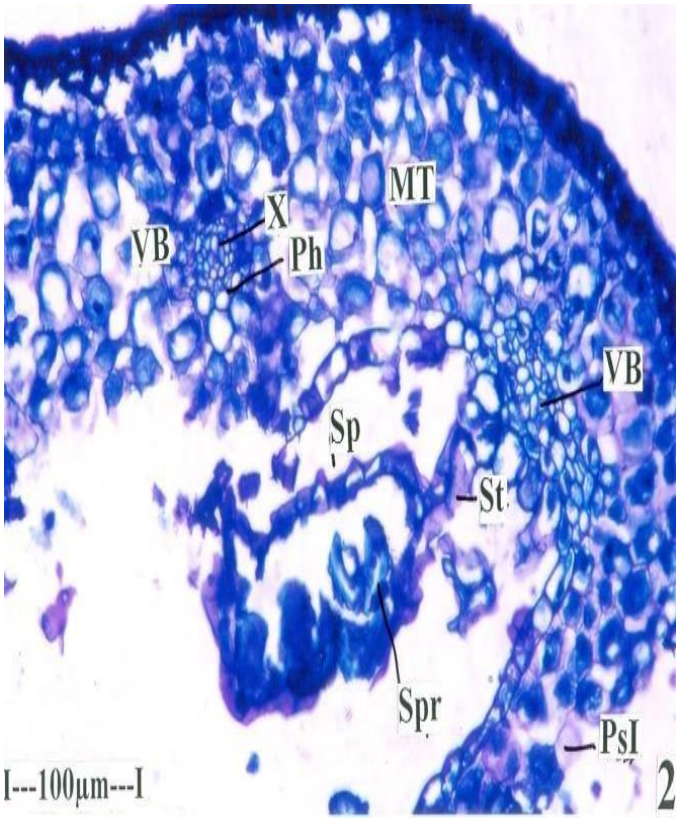

Figure 5: Sectional view of the sporangium with a spore

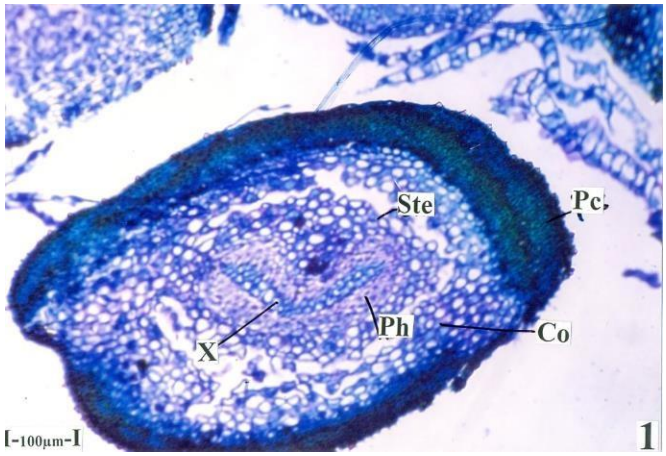

Figure 6: TS of Rachis

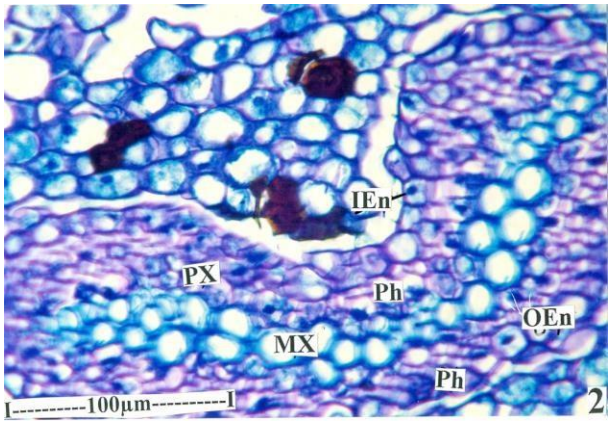

Figure 7: Stele of the Rachis – enlarged

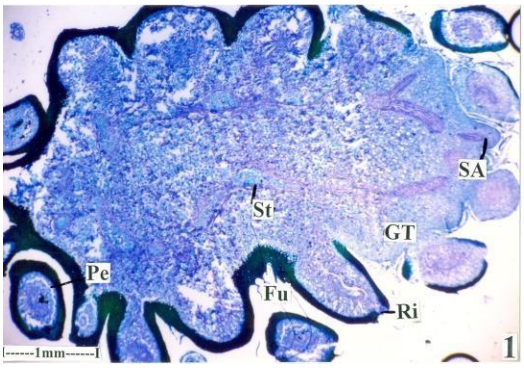

Figure 8: TS of Rhizome - entire view

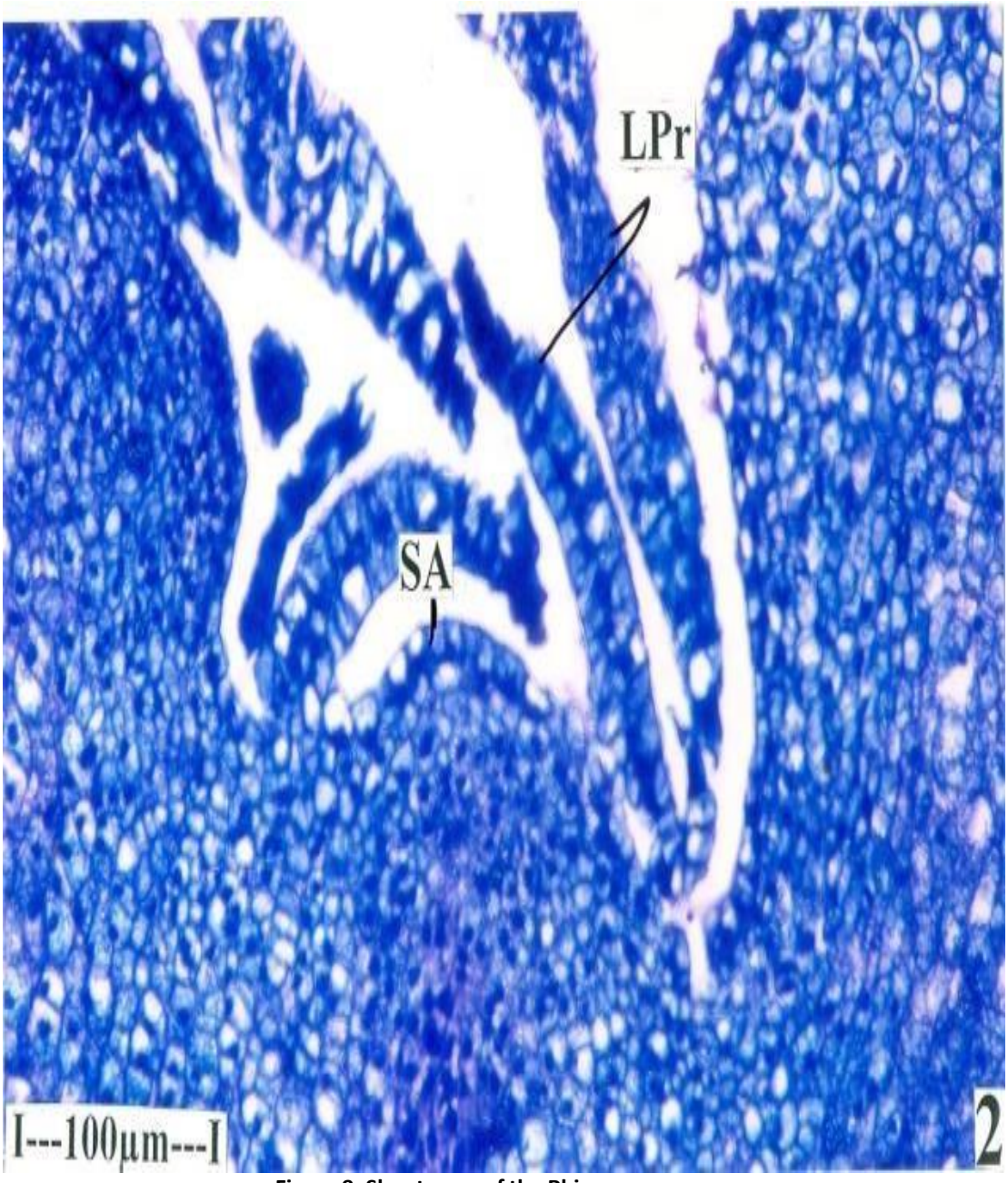

Figure 9: Shoot apex of the Rhizome

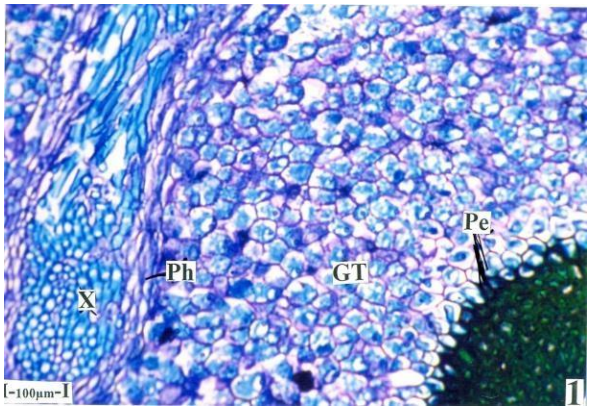

Figure 10: A portion of rhizome enlarged

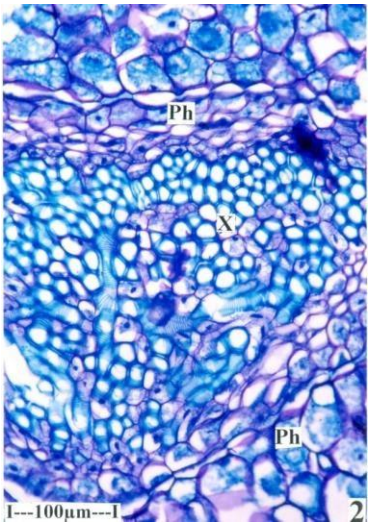

Figure 11: Vascular tissue of Stele

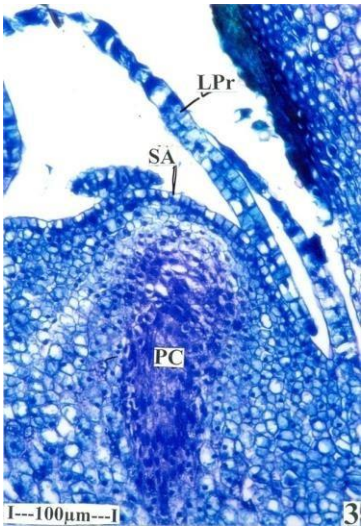

Figure 12: Short apical portion of the rhizome

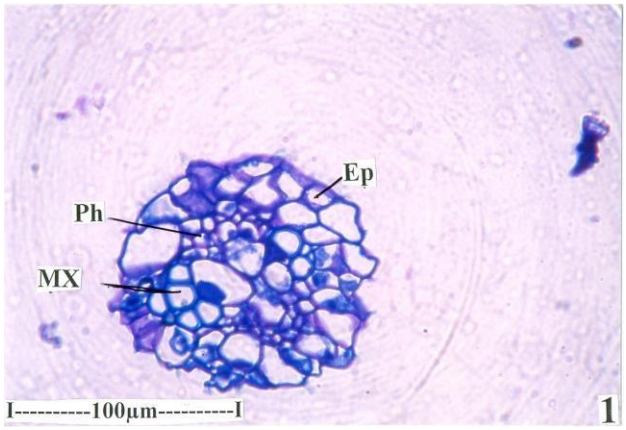

Figure 1: TS of thin root

Actiniopteris is identified as potential Arsenic accuulator (Srivastava M et al., 2009) and considered as amn efficient selenium accumulator and translocator to the fronds (Srivastava M et al, 2005). In 1992, Botswana issued a postage stamp of *A.radiata* by Gillian Condy, a South African botanical artist depicting this species. The secondary metabolites are showing more drug similarity and biologically responsiveness. It is recommended as a plant of pharmaceutical importance. However, little or no information is available pertaining to the microscopical studies of *Actiniopteris dichotoma* Bedd. It was therefore interesting to investigate the anatomical characters of leaves, stem, root and rhizomes of *A. dichotoma*.

MATERIALS AND METHODS

Collection

The whole plant of *Actiniopteris dichotoma* Bedd (Family; Polypodiaceae) were collected from Coonoor, The Nilgiris (Dt), Tamilnadu, during the rainy season of 2015 and authenticated by Dr. P. Jayaraman, Botanist, PARC, Chennai (S.No: PARC/2015/114)

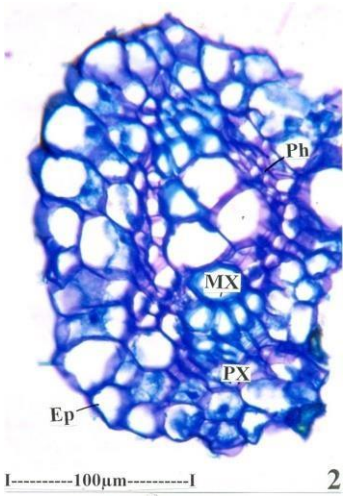

Figure 14: TS of thick root

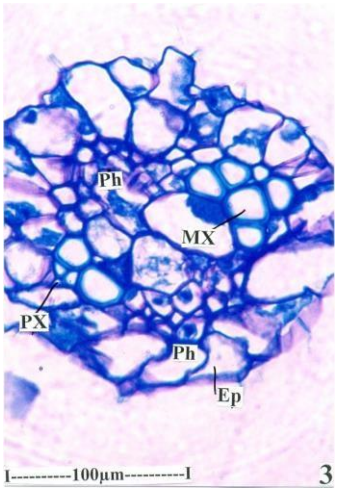

Figure 15: TS of thick root

Sectioning

Healthy *Actiniopteris dichotoma* plant and normal organs were selected cautiously. Sufficient quanti ty of leaf, stem, root and rhizomes were cut and removed from the plant and fixed in FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hrs, the specimens were dehydrated with graded series of tertiary-butyl alcohol (Sass, 1940). The specimens were infiltered by gradual addition of paraffin wax (melting point 58-60 ºC) until TBA solution attained super saturation. The specimens were cast into paraffin blocks and sectioned by Rotary Microtome. The thickness of the sections was 10-12 µm. The sections were dewaxed (Johansen, 1940) and stained with toluidine blue (O'Brien et al 1964). The staining results were remarkably good; as because of the polychromatic nature of toluidine blue. The phytochemical reaction turns cellulose walls into pink colour, lignified cells into blue, suberin to dark green, mucilage into violet, protein bodies into blue colour. Sections were also stained with safranin and Fast-green and IKI (for Starch), if needed.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs. Photographs of different magnifications were taken with Nikon Labphoto 2 microscopic unit. Bright field was used for normal observations. Polarized light was employed for crystals, starch grains and lignified cells; they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are used (Esau, 1964).

RESULTS AND DISCUSSION

Anatomy of Pinnule

The pinnule is dorsiventral in symmetry. It is flat with the two margins being curved abaxially to form pseudo indusium. The pinnule is 140µm thick. The adaxial epidermis has small cubical thick walled cells. The abaxial epidermis has small circular thin walled cells. Beneath the adaxial epidermis there is fairly large thick walled hypodermal layer of cells (Figure 2). The mesophyll cells are differentiated into adaxial two short layers of palisade cells and abaxial zone of six or seven layers of spongy parenchyma cells which are small spherical or lobed and loosely arranged. The vascular system of the pinnule consists of four vascular bundles which are equal in size and shaped and are arranged in horizontal row (Figure 2). The two margins of the pinnule become thin measuring of 40 µm in thickness. The thin margins curve abaxially and form a protective structure to the sporangia located in the axil of the curved margin. Thus, the marginal portion does not undergo any structured changes; it forms a protective covering for the sorus and sporangia. So it is called pseudo indusium (Figure 4). The sporangium has a short stalk which consists of basal two celled stalk and two cells lying one above other. The stalk is 100 µm long. The sporangium is elongated and cylindrical. It is 160µm long 80µm thick (Figure 4). The sporangial wall is one cell thick; the cells are rectangular narrow and thick walled. The spores are spherical and thick walled. (Figure 5)

Anatomy of Rachis (petiole)

The rachis is elliptical in cross sectional outline. It is 800µm in horizontal plane and 500 µm in vertical plane (Figure 5). It consists of a thin layer of thick walled and lignified epidermal cells. Inner to the epidermis is a wide, dark zone of thick walled tannin filled periderm. The stele is somewhat elliptical in outline. It consists of wide squarish inner and outer epidermal layers. The xylem is in the form of wide shallow arc. The xylem elements include wide thick walled circular meta xylem elements arranged in horizontal row. The protoxylem elements are limited in number and they are found on the inner part of the xylem arc (Figure 7). Phloem is distributed all around the xylem arc.

Anatomy of Rhizome

The rhizome is irregularly lobed with deep furrows and ridges. There is an outermost epidermal of thick ligni-

fied cells followed by thick dark cylinder of periderm. Inner to the periderm is the ground parenchyma cells. The stele of the rhizome is dictyostele which consists of broken segments of vascular strands. The vascular segments include central xylem elements which are surrounded by phloem all around. (Figure 8, 10, 11). The xylem elements are small circular and thick walled lignified. Shoot apex is seen along the periphery of the rhizome (Figure 9, 12). The shoot apex has a thick apical layer of protoderm and central core of procambium.

Anatomy of root

The root varies in thickness from 110 – 280µm. The root has large circular or squarish epidermal layer of cells with thick walls. The cortex consists of three layers of cells. These are two ex arc xylem strands alternating two phloem strands. The xylem elements have wide angular highly thick walled cells. The meta xylem elements are 30µm in diameter. Phloem consists of a dense cluster of wide, angular cells with dense cytoplasmic contents. (Figure 13, 14, 15).

Figure 2 and 3: (AbE: Abaxial Epidermis; AdE: Adaxial Epidermis; Hd: Hypodermis; PM; Palisade Mesophyll; Pn: Pinnule; PSI: Pseudo indusium; SM: Spongy Mesophyll; Sp: Sporangium; St: Stalk; VB: Vascular Bundles)

Figure 4 and 5: (AbS: Abaxial side; AdS: Adaxial side; MT: Mesophyll Tissue; Ph: Phloem; PSI: Pseudo indusium; Sp: Sporangium; Spr: Spore; St: Stalk; VB: Vascular Bundles; X: ylem)

Figure 6 and 7: (Co: Cortex; IEn: Inner Endodermis; MX: Meta Xylem; Ph: Phloem; OEn: Outer Endodermis; PX: PrortoXylem; X: Xylem)

Figure 8 and 9: (Fu: Furrow; GT: Ground Parenchyma Tissue; Ri: Ridge; SA: Short Apex; St: Stele)

Figure 10, 11 and 12: (SA: Short Apex; GT: Ground Tissue; PC: Pro cambium; Ph: Phloem; Pe: Periderm; X: Xylem)

Figure 13, 14 and 15: (Ep: Epidermis; MX: MetaXylem; Px: Protoxylem; Ph: Phloem)

CONCLUSION

The anatomical characters observed in the present study may provide referential information for the future researchers to authenticate & validate the drug. This detailed anatomical study and the microcopical parameters may add value to the existing knowledge of *Actiniopteris dichotoma.*

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