Localization of alkaloids and tannins from aerial parts of *Ficus arnottiana* (Miq.) Miq

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Abstract: The medicinally important plant *Ficus arnottiana* (Miq.) Miq. belongs to the family 'Moraceae' have been used extensively by ayurvedic practitioners in India to treat various ailments. The objective of this work was to contribute to the pharmacognostic standardizationof *F.arnottiana*. Microscopic analysis of leaf, petiole and stem showed the presence ofalkaloids, tannins, starch, protein and lipids. The leaf section shows distinct character of double layer of epidermis and a cylinder of vascular bundles in the midrib region which was not observed in the related species of *F. cyclophylla* and *F. elliotiana*. The *F. cyclophylla* and *F. elliotiana* the vascular bundles are discreet. Petiole section shows 4 to 5 distinct crescent shaped vascular bundles. Therefore, from the identified features, it proves useful for the diagnosis of the species which, together with identification of the chemical compounds and its histo-localization, provides support to their quality control.

Key Words: *Ficus arnottiana*, Histochemistry, Leaf, Petiole, Stem.

INTRODUCTION

In many developing countries, traditional medicine is one of the primary healthcare systems. Drugs derived from natural sources play a significant role in the prevention and treatment of human disease and infections (Gurpreet *et.al.,* 2016). The favourable effects of plants are mainly due to the presence of secondary metabolites which provide health promoting properties(Joythisree *et.al.,* 2021).

The genus *Ficus* of the Moraceae family includes a large number of species, which is more than 800 species, mostly found in tropical areas of East Asia. It varies from trees to shrubs often climbers with milky latex distributed throughout India; mostly in rocky hills 1,350 m elevations. About 65 species of *Ficus* occur in India (Berg, 2003; Babu *et.al.,* 2017).

Ficus arnottiana Miq. is a glabrous tree, commonly known as the Indian rock fig with the vernacular name Kodi arasu (or) Kal arasu is a species of fig tree, native to India. It belongs tothe order Ulmaceae (Rosales). One of the distinctive characters of *F.arnottiana* was that the leaves are typical to that of *F. religiosa*, but with wavy margins and the length of the leaf tip is shorter in *F.arnottiana* compared to *F.religiosa*. One of the common ways of recognizing *F. arrnottiana* is to examine the color of the leaf-stalk and the veins which are bright Pink to redin colour. The Leaf tips of *F. religiosa* are tapering, acuminate and long as against the leaf tipsof *F. arnottiana* which are pointed and acuminate but not long.

All parts of *F.arnottiana* are used as medicine in indigenous system of medicine. *Ficus* genusis rich source of tannins, terpenoids, alkaloids, flavonoids, glycosides and many other phenoliccompounds (Kaur *et.al.,* 2016).

Traditionally various parts of different species are used for medicinal purpose. All parts of *Ficus arnottiana* Miq. are used as medicine in indigenous system of medicine; the stem bark is the most potent for medicinal use. The leaves of the plant are used to treat skin diseases. Leaves also have aphrodisiac activity. The roots of the plant are also used as astringent. The bark of the tree was used as astringent, aphrodisiac, demulcent, depurative and emollient. It is also used in inflammation, diarrhoea, purities and diabetes, burning sensation, leprosy, scabies, wound healing and some other skin and vaginal diseases. The latex is used as aphrodisiac in Ayurvedic medicinal system and good for lumbago. The fruit of the plant contain Beta- sitosterol, gluanol acetate, glucose, friedelin. (Gurpreet *et.al.,* 2016).

Considering that the knowledge of the microscopic characteristics is fundamental for the standardization of plants used as medicine. Histochemical analysis is also valuable for standardization processes of quality control of crude drugs especially in locating the

presence of ergastic cell contents in the histological zones of the plant. In the current investigation histochemical characters of the various plant component (i.e.) leaf, petiole and stem of the species *Ficus arnottiana* Miq. was carried out.

MATERIALS AND METHODS

The plant *Ficus arnottiana* was collected from the wilds of Jamunamarathur, Jawathu Hills, Thiruvannamalai district, Tamil Nadu. The plant was identified based on Gamble (1935) and the voucher specimen was deposited in the Department of Botany, Queen Mary's College.

Histochemical studies were performed by the methods described by Johansen (1940), Conn (1977) and Ruthman (1970). For histochemical analysis the various parts of the plants (leaf, petiole and stem) were taken as thin sections and treated with respective reagents to localize components such as alkaloids, tannins, starch, protein and lipids in the tissues.

RESULTS AND DISCUSSION:

HISTOCHEMICAL ANALYSIS:

The epidermal surface of leaf of *F.arnottiana* was covered with thin cuticle layer, whereas in *F.cyclophylla* and *F. elliotiana* were covered with a thick cuticle (Nathalia *et.al.,* 2013). In boththe species, the cystoliths were observed on the entire leaf lamina, on the contraey, cystoliths are absent in *F.arnottiana*. In *F.arnottiana*, the epidermis was uniseriate with tabular cells, hypodermal layers of irregular cells which are biseriate and larger than the epidermal cells. *F.cyclophylla* have uniseriate hypodermal layer. The palisade was uniseriate in *F.arnottiana* whereas biseriate in *F.elliotiana* and *F.caatingae*. In *F.cyclophylla* and *F. elliotiana* the spongyparenchyma cells showed different formats with a tendency to be braciform giving a miceliform pattern to the tissues. While, in *F.arnottiana* the spongy parenchyma was compact with smaller cells with less intercellular space. The angular collenchyma was generally lignified, with five to seven in *F.cyclophylla*, whereas in *F.arnottiana* the collenchymaa cells was five to nine in the leaf midrib (Nathalia *et.al.,* 2013). The vascular system of mid rib consists of several collateral vascular bundles coalesced together to form a single circle of xylem and phloem. Primary and secondary phloem are seen in the inner portion of the vascular tissues in *F.arnottiana.* The presence of discontinuous sclerenchyma ring surrounding the vascular bundles are seen in *F.cyclophylla* and *F.caatingae*, whereas it was not observed in *F.arnottiana*. The petiole in cross-section, was circular to semi-circular in shape in *F.arnottiana.* In *F.caatingae*, the petiole was reniform. In all the species studied, the epidermis was uniseriate or biseriate depending on the section observed and covered by a thick cuticle. The cortex beneath the epidermis comprised of continuous layer of angular collenchyma cells similar to the midrib. The cortex consisted of parenchyma cells organized in well-developed trabeculae,which enclosed the empty space in *F.caatingae,* which was absent in *F.arnottiana*. Phloem strings was present in the inner portion of the vascular cylinder. The perivascular region was marked by the presence of discontinuous sclerenchymatic ring around the vascular bundle in *F.cyclophylla* and *F. caatingae*. Whereas in *F.arnottiana* it is differing from the other species,by having perivascular region with continuous sclerenchymatic ring around the vascular bundle.

The transverse section of the young stem in *F.arnottiana* is almost circular (or) rhomboidal in outline. It shows an outer epidermis followed by a row of hypodermis. The cortex of young stem is formed of an outer collenchyma consist of 3-5 rows of thick-walled cellulosic cells and an inner parenchyma composed of 6-8 rows of thin-walled cellulosic cells. The pericycle consist of group of fibres interrupted by parenchyma. The vascular tissues consist of a ring of 3 or 4 strands of collateral vascular bundle surrounding a wide parenchymatous pith. Each vascular strand is crowned by a group of pericyclic fibres and formed of an outer phloem and inner xylem. The cambium is not well distinct. The xylem is wide and formed of alternate bands of xylem parenchyma and xylem fibres giving false annular rings appearance. Xylem vessels are diffused single (or) in small groups of 4-5 vessels being of annular, spiral and pittedtypes. The xylem parenchyma is of meta tracheal type, formed of tangential bands of 4-7 rows of polygonal thick-walled axially elongated lignified cells. Xylem fibres bands are formed of 4-8 rows of lignified fibres with straight and thick pitted walls. In phloem, the cells are sub rectangular and cellulosic. The pith is formed of rounded, nearly isodiametric, thick-walled, pitted and lignified parenchyma with narrow intercellular space.

Histochemistry is a powerful technique for localization of trace quantities of substances presentin biological tissues. Histochemical techniques have been employed to characterize structure and development and to study time course of deposition and distribution of major storage compounds such as alkaloids, tannins, starch, protein and lipids.

ALKALOIDS:

Alkaloids are degradation of protein; they were investigated by two methods, namely Mayer's and Wagner's method. In Mayer's method, the presences of alkaloids were observed in leaf tissues of *F.arnottiana* in the upper and lower epidermis, spongy parenchyma, collenchyma, mid-rib pith parenchyma, xylem and phloem cells, sclerenchyma (Plate- 1A and 1B). In petioleof cortical parenchyma, pericyclic fibre, xylem and pith parenchyma cells (1C and 1D). Alkaloids are present in stem sections mostly in the epidermis, medullary rays, xylem vessels, phloem, wood parenchyma, wood fibres and pith parenchyma (1E and 1F).

In Wagner's method, the alkaloids were observed in leaf in both upper and lower epidermis, infew cells of mesophyll, sclerenchyma, xylem and phloem cells (Plate- 2A and 2B). In petiole it was observed in epidermis, collenchyma, pericyclic fibre, xylem and phloem cells, pith parenchyma (2C and 2D). In stem the alkaloids were found in collenchyma, pericyclic fibre, medullary rays, wood parenchyma, wood fibres and xylem vessels (2E and 2F). In both the methods followed for alkaloids, the presence of phenolic idioblast in the tissues was observed.

TANNIN:

In the leaf section *F.arnottiana* the tannin was present in the leaf tissues of scattered cells of mesophyll, collenchyma, xylem and phloem cells (Plate- 3A and 3B). In petiole of epidermis,collenchyma, pericyclic fibre, xylem cells and pith parenchyma (3C and 3D). Tannin was observed in stem of collenchyma, parenchyma, phloem cells, medullary rays, xylem vessels, wood parenchyma, wood fibres and pith parenchyma (3E and 3F). In *F.arnottiana* the tannin was observed as phenolic idioblast in the tissues.

STARCH:

In the present study in *Ficus arnottiana* the starch was present in leaf tissues of epidermis, scattered cells of mesophyll and mid-rib pith parenchyma (Plate- 4A and 4B). Starch was present in petiole of collenchyma, pericyclic fibre, xylem and phloem cells, pith parenchyma (4C and 4D). In stem of epidermis, pericyclic fibre, xylem vessels, medullary rays, phloem cells, wood parenchyma, wood fibres and pith parenchyma (4E and 4F). In this plant the phenolic idioblast was observed along with that of starch.

PROTEIN:

In *F,arnottiana* the protein was observed in leaf tissues of both upper and lower epidermis, scattered cells of mesophyll and mid-rib pith parenchyma, xylem and phloem cells (Plate- 5Aand 5B). In petiole the protein was present in epidermis, collenchyma, pericyclic fibre, xylemcells, phloem and pith parenchyma (5C and 5D). In stem section, the epidermis, pericyclic fibre, xylem vessels, medullary rays, wood parenchyma, wood fibres and pith parenchyma showed the presence of starch (5E and 5F). In histochemical analysis of protein, the reagent was also absorbed by phenolic idioblast of all tissues.

LIPIDS:

Lipids are the major component of biological membranes. They were investigated by two methods, namely Sudan black B and Sudan red III. In Sudan black B method, the lipids were present in leaf of upper and lower epidermis, scattered cells of mesophyll tissues, xylem and phloem cells, mid- rib pith parenchyma (Plate- 6A and 6B). In petiole, lipids are present in cells of epidermis, collenchyma, pericyclic fibre, xylem and pith parenchyma (6C and 6D). Instem, lipids are seen in the cells of epidermis, collenchyma, medullary rays, xylem vessels, phloem cells, wood parenchyma, wood fibres and pith parenchyma (6E and 6F).

In Sudan red III method, lipids were present in both upper and lower epidermis of leaves. It was also observed in the cells of mesophyll, xylem and phloem cells, mid-rib pith parenchyma(Plate- 7A and 7B). In petiole it is present in epidermis, collenchyma, xylem cells and pith parenchyma (7C and 7D). Lipids was observed in epidermis, collenchyma, pericyclic fibre, xylem cells, wood parenchyma, wood fibres and pith parenchyma of stem (7E and 7F). Phenolic idioblast was observed in sections of leaf, petiole and stem while studying for lipids by both the methods.

PLATE 1 - Histochemical Analysis of *Ficus arnottiana*

1A: T.S. of Leaf (Mid-rib); 1B: T.S. of Leaf (Mesophyll); 1C and 1D: T.S. of Petiole; 1E and 1F: T.S. of Stem.
2A: T.S. of Leaf (Mid-rib); 2B: T.S. of Leaf (Mesophyll); 2C and 2D: T.S. of Petiole; 2E and 2F: T.S. of Stem.
3A: T.S. of Leaf (Mid-rib); 3B: T.S. of Leaf (Mesophyll); 3C and 3D: T.S. of Petiole; 3E and 3F: T.S. of Stem.
4A: T.S. of Leaf (Mid-rib); 4B: T.S. of Leaf (Mesophyll); 4C and 4D: T.S. of Petiole; 4E and 4F: T.S. of Stem.
5A: T.S. of Leaf (Mid-rib); 5B: T.S. of Leaf (Mesophyll); 5C and 5D: T.S. of Petiole; 5E and 5F: T.S. of Stem.
6A: T.S. of Leaf (Mid-rib); 6B: T.S. of Leaf (Mesophyll); 6C and 6D: T.S. of Petiole; 6E and 6F: T.S. of Stem.
7A: T.S. of Leaf (Mid-rib); 7B: T.S. of Leaf (Mesophyll); 7C and 7D: T.S. of Petiole; 7E and 7F: T.S. of Stem.

Co: Collenchyma; Ep: Epidermis; U-Ep: Upper epidermis; L-Ep: Lower epidermis; Fp: Fundamental parenchyma; Mr: Medullary; P: Parenchyma; Pf: Pericyclic fibre; Ph: Phloem; P-Ph: Primary phloem; S-Ph: Secondary phloem; Pi: Phenolic idioblast; Pp: Palisade parenchyma; Pp: Pith parenchyma; Sc: Sclerenchyma; Sp: Spongy parenchyma; S-Ph: Secondary phloem; Vb- Vascular Bundle; Wf: Wood fibre; Wp: Wood parenchyma; Xv: Xylem vessels; Xy: Xylem.

CONCLUSION

The present study portraits the presence of secondary metabolites in leaves and bark of *Ficus arnottiana* tree that may contribute, in many significant ways, for the pharmaceutical properties of the plant. Histochemical standardization of *Ficus* species could be useful to identify and determine the authenticity of the drug in the herbal industry. The observed histochemical analysis provides distinctive characters to separate them, which can be used as an additional resource to improve the identification of *Ficus* sp.

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