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Macroscopic And Microscopic Diagnostic Features Of Erythrina Subumbrans (Hassk.) Merr.

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ABSTRACT

This study provides a deep insite on thePhytomorphology and Plant anatomy of *Erythrina subumbrans (Hassk.) Merr* family- Fabaceae. Phytomorphology deals with external shape, structure and physical form, while plant anatomy deals with internal structure mostly at microscopic/cell level. Both Anatomy and morphology play a vital role in that physical characteristic of an internal and external plant during different ages of plants. The purpose of this study is to understand the techniques and basic principal of histochemical and anatomical localization of metabolites in *Erythrina subumbrans (Hassk.) Merr*- a widely distributed plant in Western Ghats of India. Using a simple microscope, the initial phase of Phytomorphologyand shape of the vegetative part of leaf were determined which are collected during phenological phase of the flowering period. The current study investigated the anatomical characterization of the lower and upper epidermis and characterized the lamina and petiole of the vegetative part of a leaf. Primary and secondary metabolites of a leaf of Erythrina *subumbrans (Hassk.) Merr* was carried out with the aim in improving the Quality, Safety and Efficacy of the herbal medicine. Established macroscopical and microscopical diagnostic features, which will prevent misuse and to avoid process adulteration. The information about this species and this manuscript brings additional data which are studies for the first time according to the author's knowledge. Considerable macroscopical and microscopical, Quantitive and powder microscopic features of the vegetative part of *Erythrina subumbrans (Hassk.) Merr* were described in detail.

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INTRODUCTION

Currently, the interest in the usage of drugs of natural origin is significantly increased and it is estimated to have $\frac{1}{4}$ of the prescribed medicines in the global market (Sasidharan *et al.*, 2011). Traditional medicine is the first to first choice for treatment of any disease. Per a statement by the World health organization, 80% of the globe population prefers the traditional way of medication (Sahoo *et al.*, 2010). In Human body, phytomedicines are easily assimilated and possess less toxicological side

effects compared to synthetic medicine. Medicines taken from plants origin were available in less cost, easily accessible, however susceptible to being adulterated (Handa, 2004). A branch that deals with the form of living organisms is known as Morphology. The study which deals with physical appearance and form is recognized as Phytomorphology or plant morphology, whereas the internal structure of plant likely at macroscopical and cellular level is known to be plant anatomy. Most plant species are vascular plants and are known to be higher plants totrichophytes., which are prone to have ducts for fluid circulation, such as Phloem and Xylem. The key focus will be the phytomorphology and anatomy of a vegetative part, as they possess a significant role in the internal and external plant. Substitution of material from plant-related origin or by addition of less quality substance are the most possible way of adulteration. Due to the presence of biologically compounds which are active, exhibits a therapeutic effect of the medicinal plants (Sumitra, 2014). Error in the identification of botanical species results in the misuse of drugs from herbal origin. By accurate Pharmacognostical specification for herbal origin, all above problems can be resolved. Reproducible quality, efficacy and safety can be ensured by authentication of herbal drugs and Pharmacognostical studies (Handa, 2004). Microscopical, macroscopically, quantitative and power microscopy is one of the Pharmacognostical indices to ensure the quality of herbal medicines (Alam and Saqib, 2015).

The aim of this research activity is to present the results of microscopic, macroscopic, quantitative and powder microscopy assessments on a leaf of *Erythrina subumbrans (Hassk). Merr* as a part of extended Pharmacognosy studies. The purpose of the indicative morphology studies is to create a reliable acceptance criterion for evaluating the efficacy, quality and the safety of herbal medicine which is of natural origin.

MATERIALS AND METHODS

Plant Material

The leaves of *Erythrina subumbrans (Hassk.) Merr* were collected from western Ghats of India and authenticated at Siddha central research institute (Ministry of AYUSH, Government of India), Chennai by Research officer and Head of a pharmacognosy department Dr K.N. Sunil Kumar and confirmed by Assistant Director in-charge Dr P. Sathiyarajeswaran (Authentication certificate 112.04011901 dated 04 Apr 2019). The harvested vegetative part was dried in a nominal condition and stored in a container made of Kraft paper.

Methods

Macroscopic Studies

The Macroscopic studies are the external features of the plants morphology parts which was demonstrated by a naked eye by placing the leaf on a white background and documented using Nikon COOLPIX 5400 digital camera (Wallis, 1965).

Microscopical Study

The microscopic study is the part of anatomical studies which was carried out by selecting the appropriate sections of the vegetative parts. Robust standardized methods in evaluation of anatomical plants were applied during preparation and describing the preparations. The vegetative parts which was previously dried were preserved in a fixative solution. The fixative solution used was FAA ((Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). The materials were left in the FAA for more than 48 hours. The preserved specimens were placed on microscopical slide. By using dissecting needle, leaf parts were divided into pieces in the form of thin transverse section and the sections were stained with safranin. The thickness of the material was 10 to 15 μm. The microscopic slides were covered by coverslip and then observed from both the sides under the microscope attached with Zeiss AxioCam Erc5s digital camera under bright field light. Transverse sections were photographed simultaneously using Nikon ECLIPSE E200 trinocular microscope. Magnifications of the figures are indicated by the scale-bars (Fahn and Plant, 1982).

Quantitative Microscopy

The leaf fragments of about 5×5 mm in size were taken in a test tube containing about 5 ml of 4% potassium hydroxide solution and were heated for about 15 minutes or until the fragments became transparent. Transferred a fragment to a microscopic slide after staining with safranin and prepared the mount in Glycerol solution. Examined with a 40x objective and a 10x eyepiece, to which a microscopic apparatus (Camera lucida – Prism type). Drawn a line representing 1mm on a sheet of paper with the help of stage micrometre placed under 40x and constructed a square on this line representing an area of 1 square millimetre. Marked on the drawing paper a cross (x) for each epidermal cell and dot (.) for each stoma and calculated the average number of epidermal cells and stomata per square millimetre for each surface of the leaf and calculated the stomatal index. For determination of palisade ratio, traced four adjacent epidermal cells on a paper through the Camera lucida, focussed the palisade cells and traced sufficient palisade cells to

cover the area of the outlines of the four epidermal cells.

Figure 1: Macroscopy of Leaf

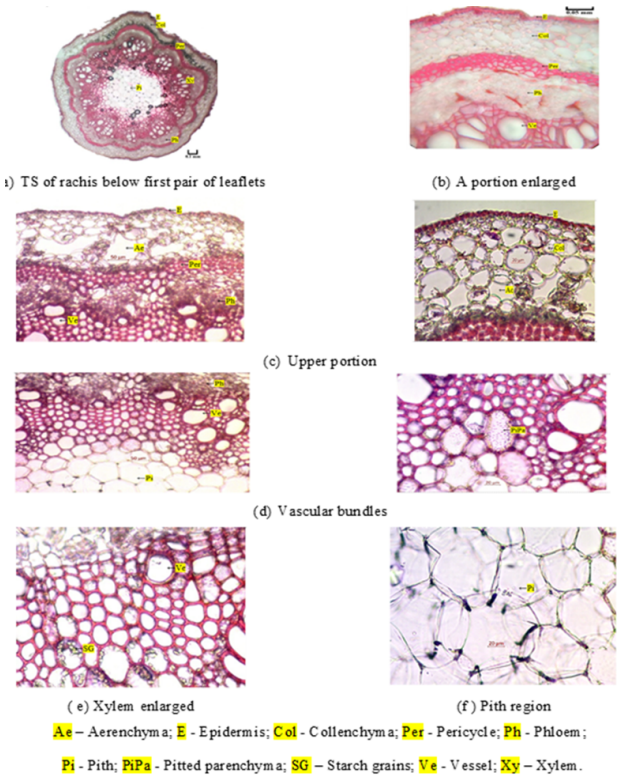

Figure 2: Erythrina subumbrans leaves

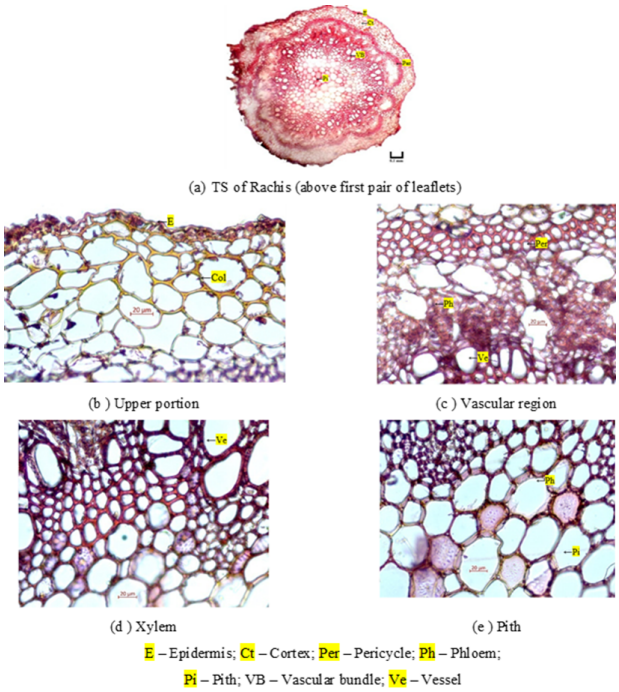

Figure 3: TS of Rachis above first pair of leaflet

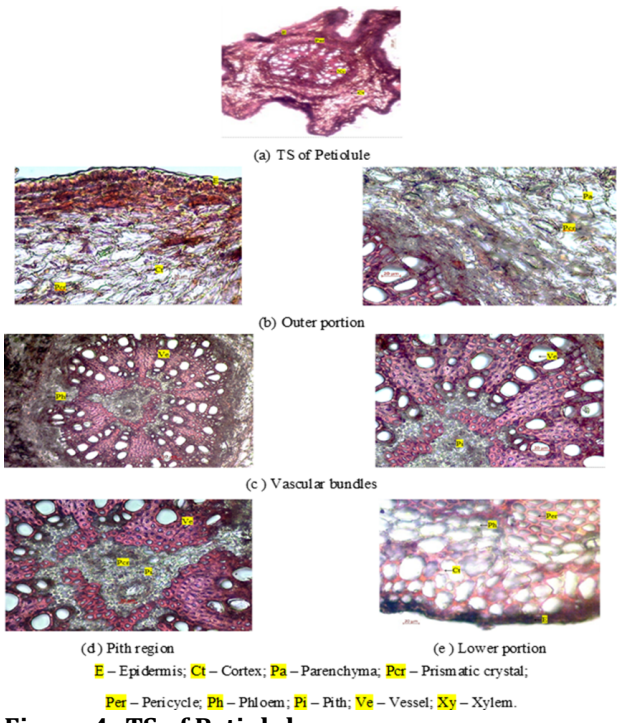

Figure 4: TS of Petiolule

Calculated the average number of palisades beneath one epidermal cell. The vein-islet number and vein

Table 1: Quantitative parameters of leaflet

Parameters	Upper epidermis	Lower epidermis
Epidermal Number	800 - 1000	900 - 1500
Stomatal Number	20 – 50	100 - 156
Stomatal Index	2.44 – 4.76	9.42 – 10
Palisade Ratio	5 to 8	
Vein islets	12 to 25	
Vein termination	8 to 10	

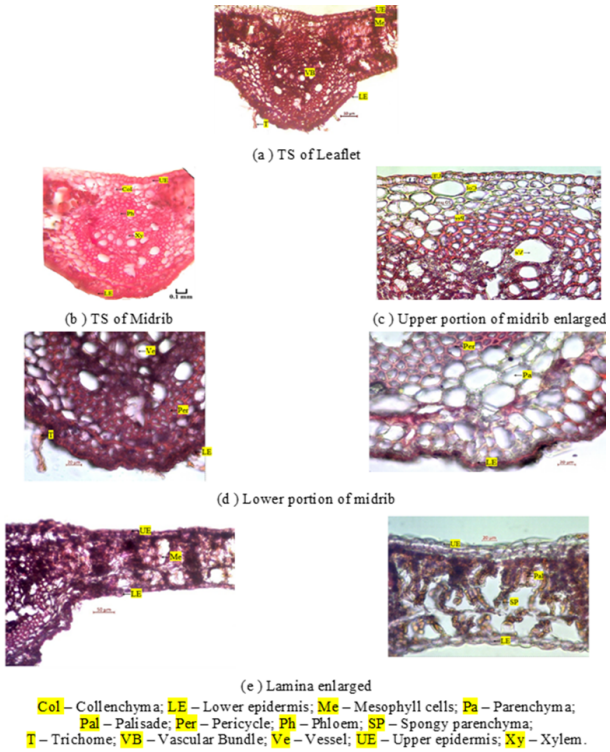

Figure 5: TS of leaflet

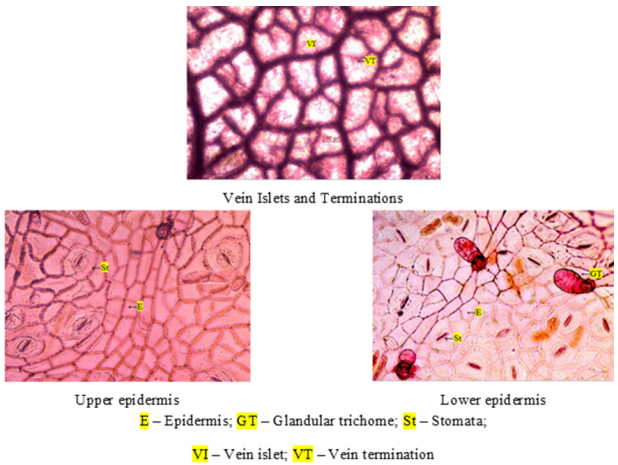

Figure 6: Quantitative Microscopy

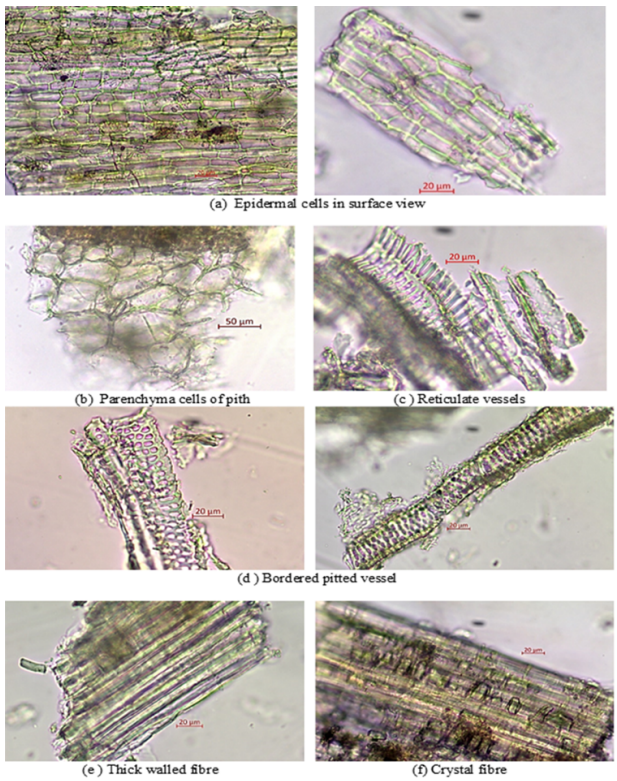

Figure 7: Powder Microscopy-Rachis

termination number were also calculated by using camera lucida (Siddha pharmacopoeia, 2008).

Powder Microscopy

The powder of the sample was treated with 4% potassium hydroxide solution and mounted in glycerine on clean slides and the powder characters were photographed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss AxioCam Erc5s digital camera under bright field light.

RESULTS AND DISCUSSION

Macroscopy of Leaf

When examining the external features of Erythrina subumbrans (Hassk.) Merr leaves, it was possible to observe the specific characteristic; leaves are trifoliate, alternate, stipulate, with large terminal leaflet

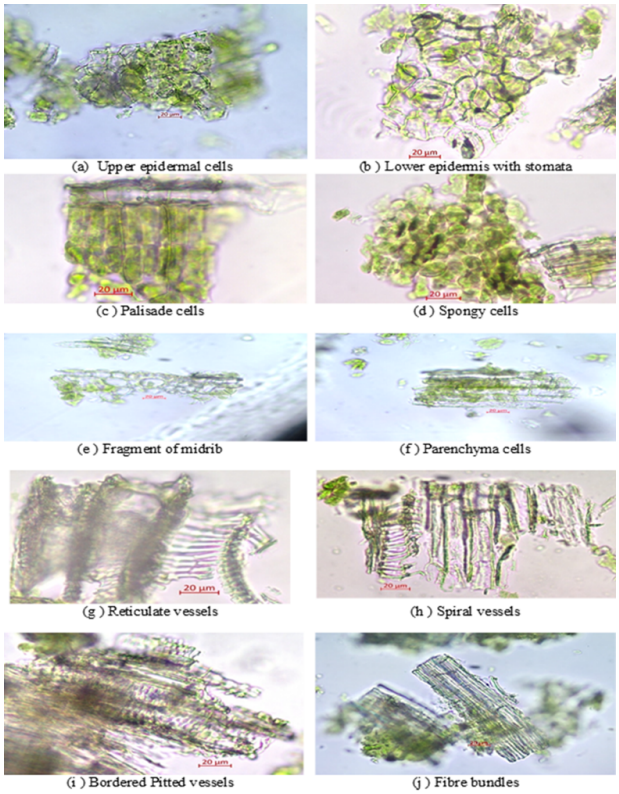

Figure 8: Powder Microscopy-Lamina

and small lateral leaflets, 10 to 15 × 4.5 to 9.5 cm, gland-like stipels are present. The stipels are ovate to triangular to rhomboidal in shape, apex acuminate, base cordate in terminal leaflets, elliptic-ovate in lateral leaflets, glabrous, margin entire, 5 to 8 pairs lateral veins can be seen. Leaves exhibits taste and smell characteristic. The detailed macroscopy structure of the leaf was presented in Figure 1.

Microscopy of Leaf

Rachis Below First Pair of Leaflets

Erythrina subumbrans (Hassk.) Merr leaves possess a deeply located petiole and deepened parenchyma and aerenchyma cells. Upon carrying the microscopical studies, the following anatomical characteristics were observed. Transverse section of petiole is circular with the wavy outline. The epidermis found is formed of single-layered covered by cuticle; 3 to 4 layers of parenchyma followed by aerenchyma cells forms the cortex; a ring formed by 10 to 12 collateral vascular bundles are arranged in the inner cortex. There is a continuous layer of pericyclic fibres followed by outer phloem and inner xylem consisting of vessels, xylem parenchyma and fibres. Pith is composed of parenchyma cells. The detailed microscopic pictures (a) Transverse section below the first pair of leaflets, (b) Enlarged portion (c) upper portion (d) Vascular bundles(e) Xylem enlarged (f) Pith region were presented under TS of Rachis below the first pair of leaflets from Figure 2.

Rachis After First Pair of Leaflets

Rachis is circular and wavy; the epidermis is single-layered, cuticularized; followed by 4 to 5 layers of outer cortical region formed of collenchyma cells; 12 to 14 vascular bundles are arranged radially in the inner cortex; a continuous wavy band of pericycle which surrounds the vascular bundles. Xylem consists of vessels, xylem parenchyma and fibres; pith is composed of large thin walled parenchyma cells and few pitted parenchyma cells at random. The detailed microscopic pictures (a) Transverse section of Rachis (above first pair of leaflets), (b) Upper portion (c) vascular region (d) Xylem enlarged (f) Pith region were presented under TS of Rachis above the first pair of leaflets from Figure 3.

Petiolule

Transverse section of Petiolule is irregular shaped with ridges and furrows; the single-layered, cuticularized epidermis is followed by 6 to 7 layers of the parenchymatous broad cortex. The inner cortex of Petiolule consists of a ring of vascular bundles, covered by sclerenchymatous pericyclic layer. The central portion of Petiolule is occupied by parenchymatous pith. Prismatic crystals are distributed in the cortex and pith region. The detailed microscopic pictures (a) Transverse section petiolule, (b) Upper portion (c) vascular region (d) Pith region (e) Lower portion were presented under TS of Petiolule from Figure 4.

Lamina

The leaf is amphistomatic and dorsiventral; epidermis present in the lamina is single-layered covered by cuticle; followed by 3 to 4 layers of compactly arranged collenchyma cells in the midrib region. A collateral vascular bundle is present at the centre of the lamina, covered by a pericyclic sheath. The lamina shows undifferentiated loosely arranged mesophyll tissues and the stomata inside lamina is anomocytic type. Glandular trichomes are seen on lower epidermis of a lamina. The detailed microscopic pictures (a) Transverse section of Leaflet, (b) Transverse section of midrib(c) Upper portion of midrib enlarged (d) Lower portion of midrib (e) Lamina enlarged were presented under TS of Leaflet from Figure 5.

Quantitative Microscopy

It revealed the presence of anomocytic stomata on both the surfaces of *Erythrina subumbrans (Hassk.) Merr* leaves. Both upper and lower epidermis were formed of isodiametric cells. The lower epidermis showed the presence of glandular trichomes. The detailed Quantitative microscopic pictures (a) Vein

islets and Terminations, (b) Upper epidermis and (c) Lower epidermis were presented under Quantitative microscopy from Figure 6. Other quantitative microscopic features were used to clearly differentiate upper and lower epidermis under a microscope were presented in Table 1.

Powder Microscopy

Erythrina subumbrans (Hassk.) Merr leaf showed the presence of epidermal fragments with anomocytic stomata, mesophyll cells, parenchyma cells, pitted parenchyma cells, reticulate vessels, bordered pitted vessels and spiral vessels. Fibre bundles and crystal fibres were observed in petiole. The detailed powder microscopic pictures (a) Epidermal cells in surface view (b) Parenchyma cells of pith (c) Reticulate vessels (d) Bordered pitted vessels (e) Thick-Walled fibres (f) Crystal fibres (g) Pitted parenchyma were presented under Powder Microscopy-Rachis from Figure 7. Further, (a) Upper epidermal cells (b) Lower epidermal with stomata (c) Palisade cells (d) Spongy cells (e) Fragment of Midrib (f) Parenchyma cells (g) Reticulate vessels (h) Spiral vessels (i) Bordered pitted vessels and (j) Fibre bundles were presented under power microscopy -lamina from Figure 8. The powder is green coloured, with characteristic taste and odor.

CONCLUSIONS

In order to avoid potential adulteration and misuse of wrong drugs, authentication of any herbal origin needs to be consistent using pre-approved procedures. Macroscopic, microscopic, Quantitative and powder microscopic studies are one of the vital tools composing the botanical identification of a herbal drug. *Erythrina subumbrans (Hassk.) Merr* is a herbaceous tree grown in Western Ghats of India and as no detailed standardized work has been reported in the literature for this plant so far, this work provided unique authenticity features, which will be useful for authentication of this species.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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