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ANATOMICAL AND HISTOCHEMICAL CHARACTERISTICS OF *MORINDA CITRIFOLIA* L. (RUBIACEAE)

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Keywords:

Anatomical, Histochemical, Noni, *Morinda citrifolia*, Rubiaceae

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ABSTRACT: *Morinda citrifolia* (L.) is an important medicinal plant belonging to the family Rubiaceae and commonly known as "Noni" in Tamil was highly distributed in South India. The aim of this research is to understand the concept and techniques of anatomical and histochemical localization of secondary metabolites in *Morinda citrifolia* (L.) as well as take part in the standardization of herbal medicine. Initially the morphological observations were determined by using simple microscope. The shape of leaf, fruit, odor and type of flower were determined. The present study investigated the leaf anatomical characterization of upper and lower epidermis of Noni plant and also characterized the petiole and lamina of leaf. Finally, this study also explained the histochemical localization of various primary and secondary metabolites of stem of *Morinda citrifolia* was carried out with the aim of contributing to its quality control in herbal industries. The results of this study could be useful for correct identification of the plant species and also for the determination of the authenticity of the drug in herbal industry.

INTRODUCTION: *Morinda citrifolia* L. is commonly used in traditional and folklore medicines for treating various diseases, it belongs to the family Rubiaceae. In Tamil, it is commonly called as "Noni". Among the medicinal plants discovered by the ancestors of Polynesians, Noni (*Morinda citrifolia*) is one of the important traditional folk medicinal plants that have been used for over 2000 years in Polynesia. Nowadays, ethnomedicinal practices are preferred largely because medicinal plants are less expensive, readily available, reliable and they are considered to have fewer side effects than modern medicines.

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Earlier to the development of modern medicine, the traditional systems of medicine that have evolved over the centuries within various communities are still maintained as a great traditional knowledge base in herbal medicines [1]. It has been reported to have a broad range of therapeutic and nutritional value. The ancestors of Polynesians are believed to have brought many plants with them, as food and medicine, when they migrated from Southeast Asia 2000 years ago [2]. Of the 12 most common plants they brought, Noni was the second most popular plant used in herbal remedies to treat various common diseases and to maintain overall good health [3, 4].

Noni is commonly referred to as the species *M. citirfolia* and is also called as Indian Mulberry. It is also known in different names locally as Cheese Fruit, Forbidden Fruit, Headache Tree, Hog Apple, Mona, Mora de la India, Nino, Nona, Nono, Nonu, Nuna, Pain Bush, Pain Killer Tree, Pinuela, Wild

Pine, *etc*. in various parts of the world. Noni is an evergreen small shrubby tree. The roots, stems, bark, leaves, flowers, and fruits of the Noni all are involved in various combinations in almost 40 known and recorded herbal remedies [5]. Scientific evidence of the benefits of the Noni fruit juice is limited but there is some anecdotal evidence for successful treatment of colds and influenza [6]. The fruit juice is in high demand in alternative medicine for different kinds of illnesses such as arthritis, diabetes, high blood pressure, muscle aches and pains, menstrual difficulties, headaches, heart disease, AIDS, cancers, gastric ulcer, sprains, mental depression, senility, poor digestion, arteriosclerosis, blood vessel problems and drug addiction.

Morinda is reputed to have antibacterial, antiviral, antifungal, antitumor, antitubercular effect, analgesic activity, immunological activity, mental health and improve high frequency, antihelminthic, analgesic, hypotensive, anti-inflammatory, immune-enhancing *etc*., due to its beneficial effects, the fruit juice of Noni is widely distributed throughout the world as nutraceutical dietary supplement. The leaf of this plant is directly used on skin for ulcerations and minor infections [7, 8].

In recent time, there has been a great demand for plant-derived products in developed countries [9]. These products are increasingly being sought out as medicinal products, nutraceuticals and cosmetics [10]. The rising popularity of herbal products, both as food and feed supplements and as phytotherapeutic drugs, has also given rise to many reports describing adverse health effects and variable quality, efficacy and contents of herbal products [11]. In biology, morphology is the branch that deals with the form of living organisms. For plants, plant morphology or phytomorphology is the study of the physical form and external structure of plants, whereas plant anatomy is the study of the internal plant structure, mostly at the cellular/microscopic level. The microscopic method is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials [12]. Plant anatomy is now frequently investigated at the cellular level, and often involves the sectioning of tissues and microscopy [13, 14]. Chemical studies performed using histochemical techniques allow a quick and inexpensive preliminary evaluation of bioactive phyto-constituents in medicinally important plant species [15, 16]. Allen reported some information on the ethnobotanical properties of Noni. He said that the fruit is used as deobstruent and emmenagogue. This is one of the earliest articles on the medicinal benefits of Noni [17]. It has been reported to have a broad range of therapeutic and nutritional value [18].

MATERIALS AND METHODS:

Chemicals and Instruments: All the chemicals used were of laboratory grade. Compound microscope, glass slides, coverslips, watch glass and other common glassware were the basic apparatus and instruments used for undertaking the study. Microphotographs of different magnify-cations were taken with Nikon Labphot 2 Microscopic Unit. For normal observations bright field was used. Ethanol solvent and reagents used for staining different sections such as toluidine blue, safranin, and histochemical reagents were procured from The Precision Scientific Co., Coimbatore, India.

Plant Collection and Identification: Fresh leaves and stems of *Morinda citrifolia* were collected from ABS Botanical Gardens, Kaaripatti, Salem **Fig. 1** and **2**. The plants were collected in their flowering and fruiting seasons from the natural habitat. While collecting the study plant, a thorough observation was made regarding the location, natural habitat, distribution pattern, habit, floral and fruit characteristics, *etc*. The plant was identified and authenticated taxonomically with the help of Flora of the Presidency of Madras [19] and The Flora of the Tamil Nadu Carnatic [20] and also confirmed with the help of type specimens available in the herbarium of Botanical Survey of India, TNAU Campus, Southern region, Coimbatore (No. BSI/SRC/5/28/2015-16/Tech. 1300), Tamil Nadu and the type specimens were deposited for further reference.

Anatomical Studies: Freehand section of plant materials were used for the anatomical studies. These sections were mounted in glycerin stained with safranin. The paraffin-embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was done by customary procedure [21].

The specimens were stained with Toluidine blue, Safranin and IKI - Lugol's iodine as per the method of O'Brien *et al*., (1964). Fresh and healthy leaves were separated from the plant and thoroughly washed with running water to remove the adherent impurities for anatomical studies [22]. After clearing the T.S, various microscopical studies were carried out in the study plant [23]. For studying the leaf constants such as stomatal morphology and trichome distribution Jeffrey's maceration fluid was prepared [24]. The bright field was used for normal observations whereas polarized light was employed for the detailed study of crystals and starch grains. Descriptive terms of the anatomical features are taken from the standard anatomy book [25].

Histochemical Localization: The required samples of different organs were cut, removed from the plant and fixed in FAA (Formalin - 5 ml + Acetic acid-5 ml + 70% Ethyl alcohol - 90 ml) in the ratio of 1: 1: 18. After 24 h of fixing, the specimens were dehydrated with a graded series of tertiary – butyl alcohol as per the schedule is given by Sass, 1940. The paraffin-embedded specimens were sectioned with the help of Rotary Microtome. In the present investigation, temporary mounts of sections were taken and treated with different chemical reagents to localize different primary and secondary metabolites [26].

The following standard solutions were employed in the histochemical tests: Molish reagent to reveal carbohydrates [27], Burette reagent to stain proteins [28], Wagner's reagent to stain alkaloids [29], lead acetate which reveals flavonoids [29], ferric chloride and sodium carbonate which stains tannins [30], alcoholic ferric chloride to stain phenols [31], concentrated sulphuric acid which reveals saponins [32] and iodine water for localization of lipids [33]. The results were registered on photomicrograph. Based on the photographs taken, anatomical description and localization of tested chemicals were done.

Photomicrography: Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Trinocular fine vision of 100 µm magnifications. For the study of crystals, starch grains, and lignified cells, polarized light was employed. Under polarized light they appear bright against dark background. Descriptive terms of the anatomical features are as given in the standard anatomy books [25, 34].

RESULTS AND DISCUSSION:

Anatomy of the Leaf: Macroscopical investigation of *Morinda citrifolia* revealed that the present findings may facilitate to identify certain novel compounds in the plant material. The upper and lower epidermis contains paracytic stomata and glandular trichomes. The ground tissue is differentiated into two to four layers of outer, smaller, collenchymatous cells and remaining portion with large, circular, thin-walled less compact, parenchyma cells. The transverse section of leaf exhibits bilateral symmetry with prominently projecting midrib **Fig. 3** the vascular system consists of a wide bowl-shaped strand with adaxial bent loop-like structure **Fig. 3** and **4**. The bowl-shaped strand is made up of several, radially – oblong, wedge-shaped segments which are very closely arranged **Fig. 4, 5** and **6**. The segments are collateral with fairly long, parallel lines of wide, thick-walled, angular or circular xylem elements. The outer part of the segments occurs prominent masses of phloem **Fig. 4** and **5**.

FIG. 1: FRUITING TWIG OF *MORINDA CITRIFOLIA*

FIG. 2: ENLARGED PORTION OF FRUIT

The adaxial loop of the vascular strand has a thickness of the midrib is 2.75 mm; the breadth is 3.1 mm. The epidermal layer consists of single, narrow, rectangular cells with prominent cuticle **Fig. 6**.

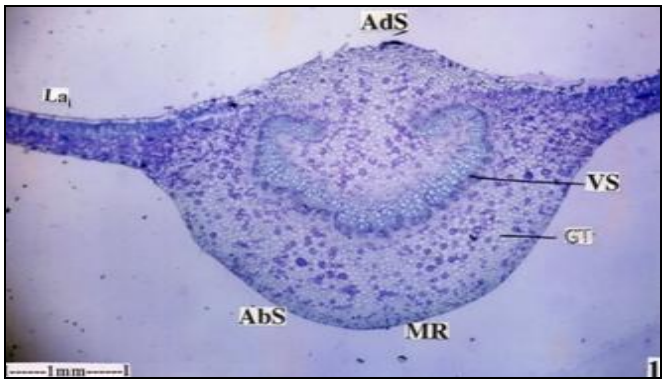

FIG. 3: T.S OF LEAF THROUGH MIDRIB WITH LAMINA

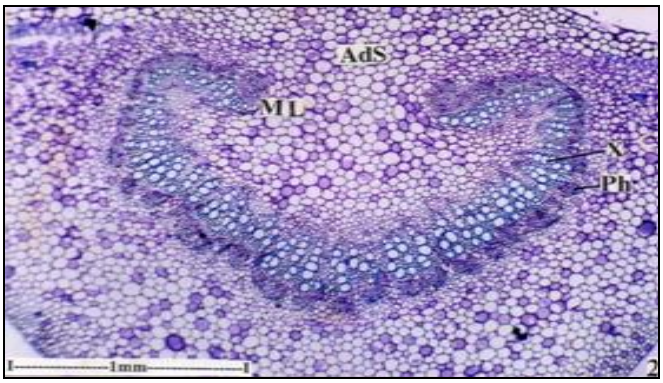

FIG. 4: T.S OF MIDRIB – VASCULAR BUNDLE MAGNIFIED

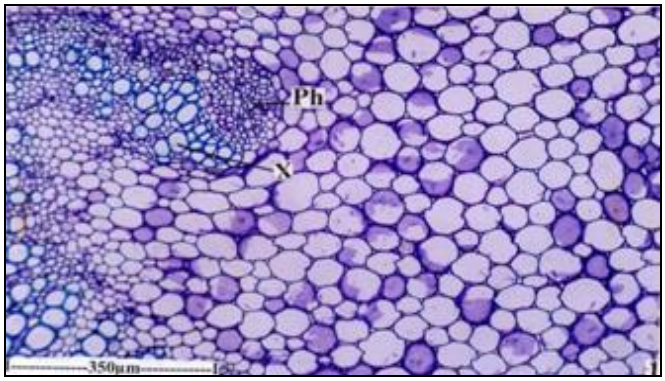

FIG. 5: T.S OF MIDRIB VASCULAR BUNDLE – MARGINAL LOOP MAGNIFIED

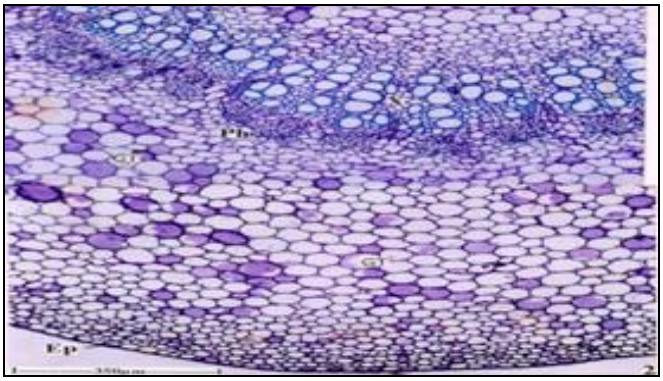

FIG. 6: T.S OF MIDRIB MEDIAN VASCULAR STRAND WITH GROUND TISSUE ENLARGED

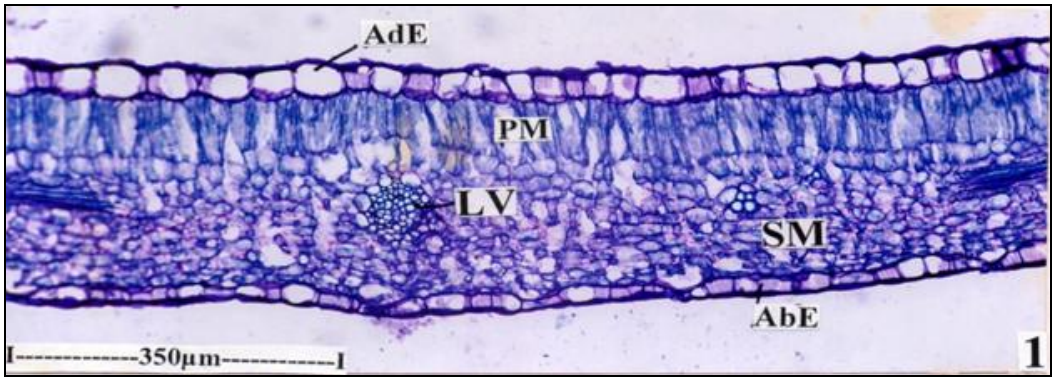

FIG. 7: T.S OF LAMINA THROUGH LATERAL VEIN

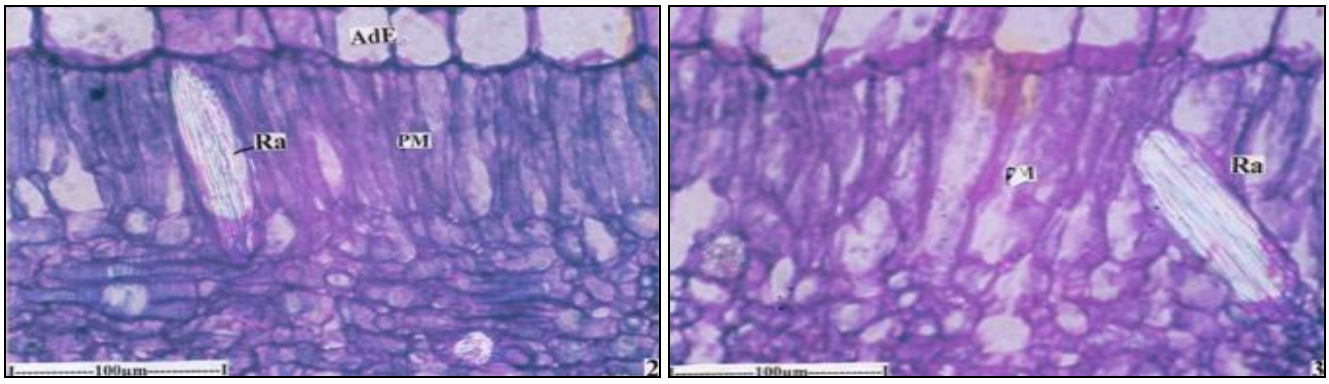

FIG. 8: RAPHIDES IN THE MESOPHYLL TISSUE (UNDER POLARIZED LIGHT MICROSCOPE)

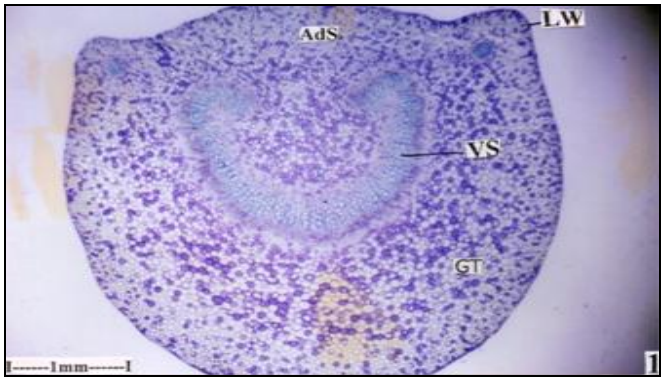

FIG. 9: T.S OF PETIOLE – ENTIRE VIEW

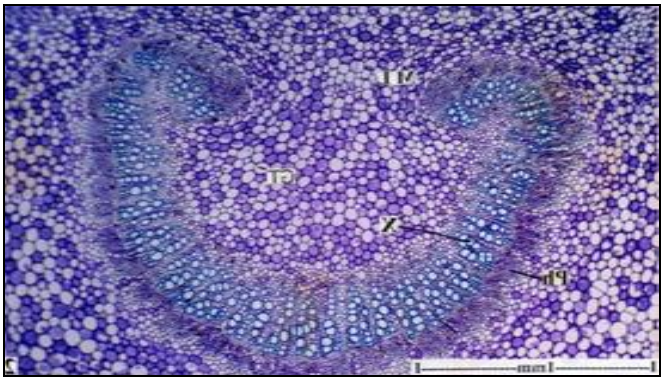

FIG. 10: T.S OF PETIOLE – VASCULAR BUNDLE MAGNIFIED

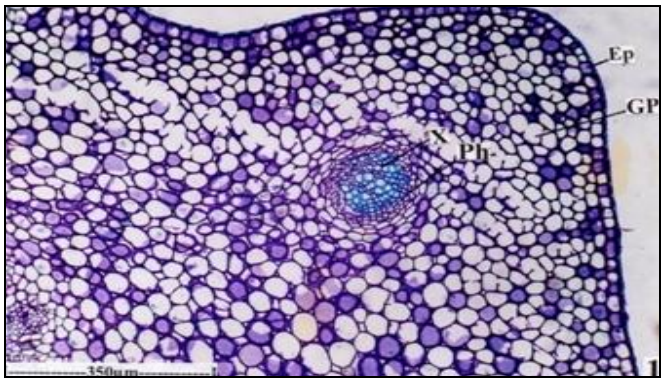

FIG. 11: T.S OF PETIOLE – WING VASCULAR BUNDLE ENLARGED

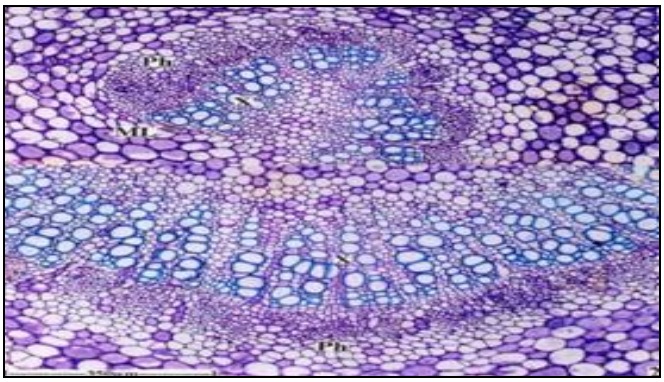

FIG. 12: T.S OF PETIOLE – MARGINAL LOOP MAGNIFIED & MEDIAN VASCULAR BUNDLE MAGNIFIED

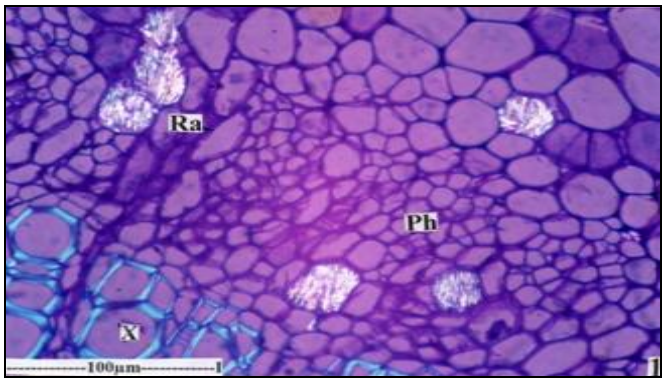

FIG. 13: T.S OF PETIOLE SHOWING RAPHIDES IN THE PHLOEM TISSUE AND GROUND TISSUE (UNDER POLARIZED LIGHT MICROSCOPE)

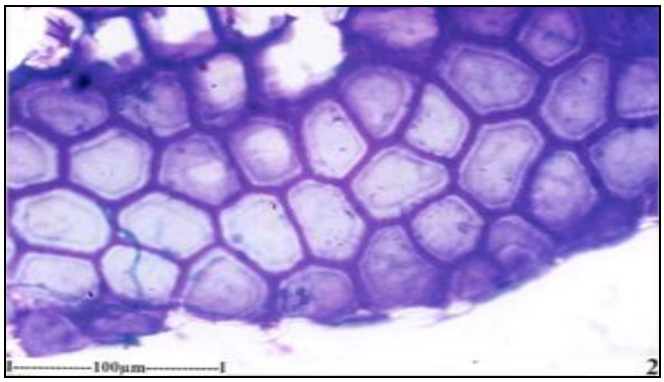

FIG. 14: ADAXIAL EPIDERMIS

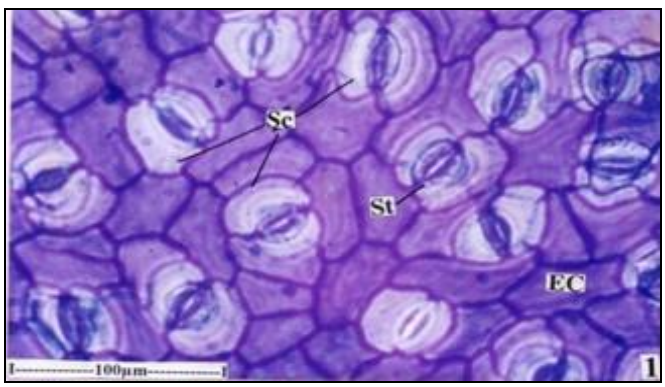

FIG. 15: ABAXIAL EPIDERMIS SURFACE VIEW SHOWING STOMATA

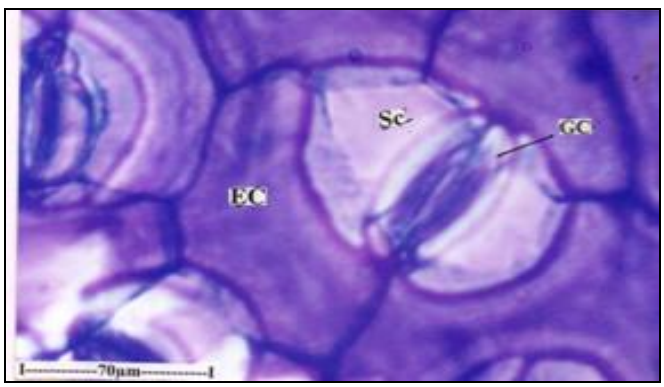

FIG. 16: ABAXIAL EPIDERMIS WITH PARACYTIC STOMATA

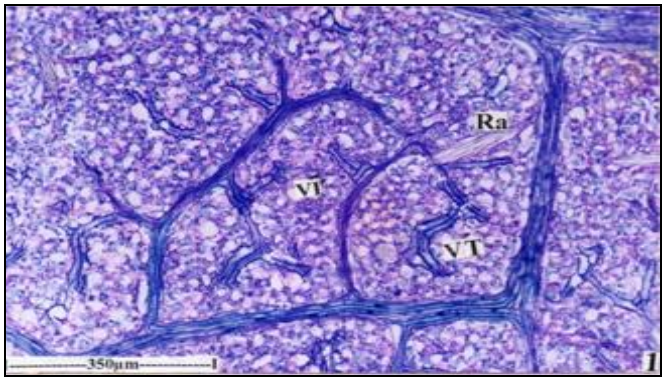

FIG. 17: PARADERMAL SECTION SHOWING VEIN – ISLETS, AND VEIN – TERMINATION

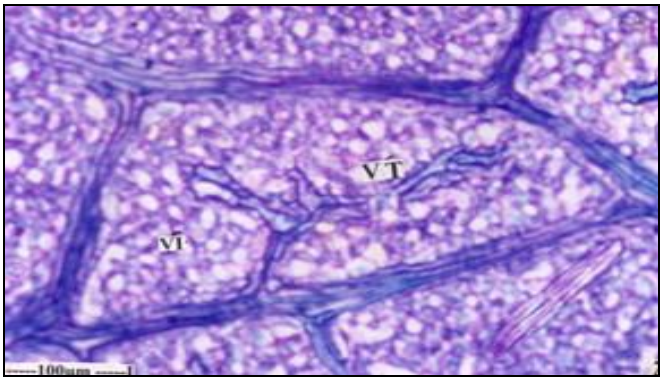

FIG. 18: VEIN – ISLETS AND VEIN – TERMINATION ENLARGED

Abs – Abaxial side; Ads – Adaxial side; GT – Ground Tissue; La – Lamina;
ML – Marginal loop; MR – Midrib; Ph – Phloem; VS – Vascular strand; X – xylem;
EP – Epidermis; AbE – Abaxial epidermis; AdE – Adaxial epidermis; LV – Lateral vein;
PM – Palisade mesophyll; Ra – Raphide; SM – spongy mesophyll; LW – Lateral wing;
GP – Ground parenchyma; Sc – Sclerenchyma; St – Stoma; GC – Guard cell;
EC – Epidermal cell; VI – Vein-islets; VT – Vein-termination

Lamina: The lamina is uniformly thick with smooth and even surfaces. It is 250 µm thick. The adaxial epidermis consists of thick, squarish cells with prominent cuticle. It is 40 µm thick. The abaxial epidermis is thin, and the cells are narrowly oblong with prominent cuticle. The mesophyll tissue is differentiated into the adaxial zone of palisade tissue which consists of two rows of thin cylindrical cells. The abaxial part consists of above ten layers of small, circular, loosely arranged spongy parenchyma cells **Fig. 7** the palisade zone is 70 µm in height.

The lateral veins occur in the median zone of mesophyll tissue. They are circular with a small group of xylem and phloem elements. The strand is surrounded by a single layer parenchymatous bundle sheath **Fig. 7**. Thick cylindrical raphide bundle is often seen in ventricle position in the palisade zone **Fig. 8** and the raphide may also be oblique or horizontal in orientation **Fig. 7** and **8**.

Anatomy of the Petiole: In cross-sectional view the petiole is semicircular with broadly convex adaxial side and semicircular abaxial side **Fig. 9**. There are two short, thick lateral wings on the adaxial side. The epidermal layer consists of thin rectangular or squarish, thick-walled cells. The ground tissue is homogeneous and parenchymatous. The cells are circular fairly thick-walled and less compact **Fig. 11** and **12**.

The vascular system consists of broad V-shaped strand with the free ends in curved **Fig. 10**. The vascular system is collateral with numerous, long, parallel lines of angular, thick-walled xylem elements. Discrete prominent masses of phloem strands are seen all along the outer part of the xylem strands **Fig. 12**. Apart from the wing strand, there are two strands located in the wing portion.

The wing strands are circular, collateral with phloem encircling the xylem elements **Fig. 11**. The petiole is 3.75 mm thick and 4 mm wide. Raphide bundles are fairly common in the phloem parenchyma of the petiole **Fig. 13**. The raphides occur inside normal parenchyma cells.

Epidermal Morphology: The adaxial epidermis is apostomatic (without stomata) consists of polygonal cells with thick and straight anticlinal walls **Fig. 14**. The abaxial epidermis is stomatiferous. The stomata are paracytic – type. There are two equal or unequal subsidiary cells which occur parallel to the guard cells on either side, and the stomatal pore is broad and elliptical in outline **Fig. 15** and **16**.

The epidermal cells are mostly rectangular with fairly thick and straight anticlinal walls. Midrib consists of broad adaxial side and semicircular wide abaxial side.

Venation: The lateral veins and veinlets are thick and prominent. They form distinct vein – islets which are small having mostly branched wavy, vein – terminations **Fig. 17** and **18**.

Histochemical Localization:

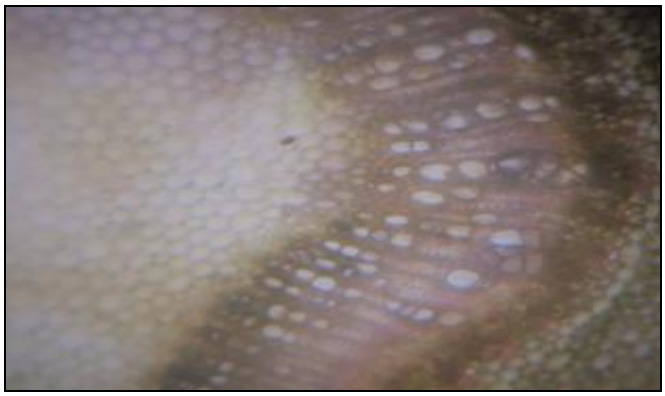

FIG. 19: CARBOHYDRATES

FIG. 20: PROTEINS

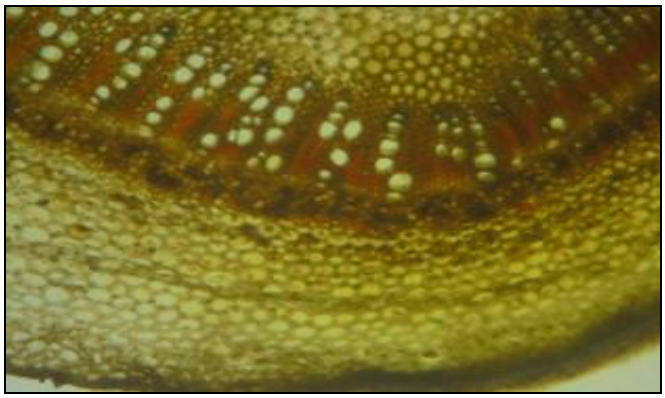

FIG. 21: ALKALOIDS

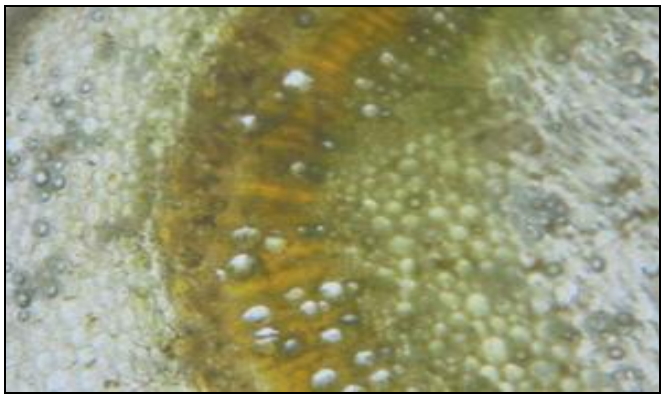

FIG. 22: FLAVONOIDS

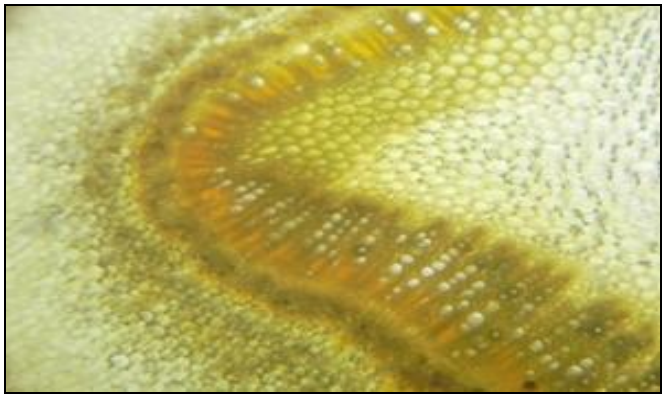

FIG. 23: PHENOLS

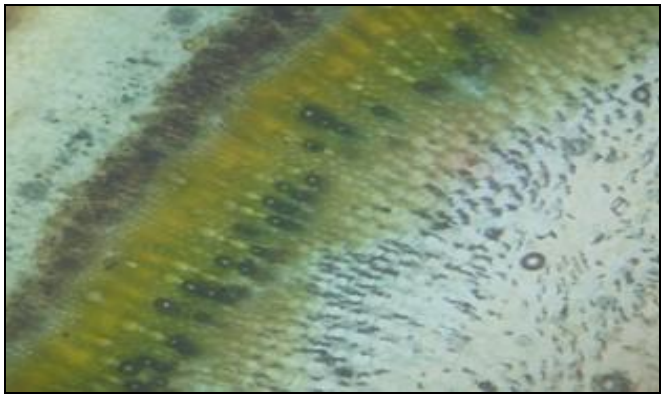

FIG. 24: TANNINS

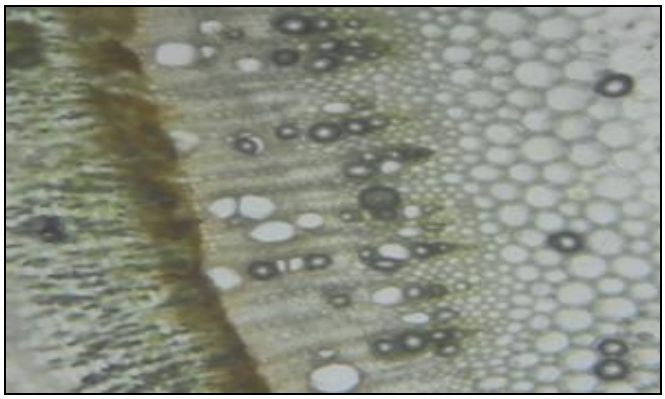

FIG. 25: SAPONINS

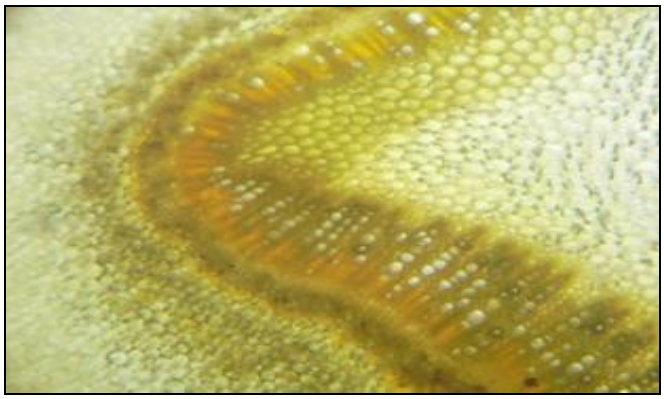

FIG. 26: LIPIDS

TABLE 1: HISTOCHEMICAL LOCALIZATION OF VARIOUS METABOLITES IN *MORINDA CITRIFOLIA* L.

Metabolite group	Reagents	Colour observed
Carbohydrates	Felhing's reagent	Pink
Proteins	Biurette reagent	Blue
Alkaloids	Dragendorff's reagent	Reddish orange
Flavonoids	Lead acetate	Yellow
Saponins	conc. Sulphuric acid	Blue to bluish black
Tannins	Ferric chloride and sodium carbonate	Dark blue
Lipids	Iodine water	Yellow to brown
Phenols	Alcoholic ferric chloride	Green

Histochemical techniques are fast and cheap methods that can be used to identify bioactive classes of phytocompounds in tissues and cell compartments precisely [35]. The main classes of primary and secondary metabolites localized from the stem of *Morinda citrifolia* after histochemical analysis are presented in **Table 1**. Carbohydrates were localized in the parenchymatous ground tissue of stem and pith regions of *Morinda citrifolia* appeared as pink patches in response to Fehling's reagent in stem **Fig. 19**. The presence of starch granules can be important for taxon identification [3]. Biuret test performed by immersing the sections in strong potassium hydroxide solution followed by addition of few drops of aqueous solution of 1% copper sulfate showed positive results for proteins and were localized as blue patches in vascular region **Fig. 20**.

The reactions with Dragendorff's reagent were positive and indicating the presence of alkaloids in the parenchymatous cells in the stem **Fig. 21** was explained by Cavalcanti *et al*., 2014. Lead acetate revealed the presence of flavonoids in stem which showed yellow color **Fig. 22**. The cortex region of stem reacted positively to lead acetate indicating the presence of flavonoids.

Alcoholic ferric chloride produced a stable green color with phenolic compounds which were localized in ground tissue of stem **Fig. 23**. Tannins were localized in xylem fibers of collenchyma stem which appeared dark blue in reaction with ferric chloride and sodium carbonate **Fig. 24**. The reactions with concentrated sulphuric acid revealed positive results for saponins which produced blue to bluish-black color in ground tissues **Fig. 25**. Whereas the reactions with iodine water were positive indicating the lipophilic substances in the parenchymatous ground tissues of leaf and vascular and pith region of stem **Fig. 26**. Localization of primary and secondary metabolites with various histochemical indicators in stem of *Morinda citrifolia* indicates that these reagents can selectively identify specific substances within intact cells and tissues. Hence these techniques can be used in herbal industries for providing quality control measures.

CONCLUSION: According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken. Quality control of herbal drugs has traditionally been based on appearance and microscopic evaluation, which is indispensable in the initial identification of herbs, as well as in identifying small fragments of crude or powdered herbs, detection of foreign matter, substitutes and adulterants. The pharmacognostic studies provide useful information for identifying and authenticating the medicinal plants. Histochemical standardization of *Morinda citrifolia* could be useful to identify and determine the authenticity of this drug in the herbal industry. Noni is responsible for the treatment of skin infections, colds, fevers, and other bacterial-caused health problems.

Therefore, it may be of benefit to cancer patients by enabling them to use lower doses of anticancer drugs to achieve the same or even better results. Because of its benefits, pharmaceutical companies will pay attention to this research and explore the Noni plant as a potential source of drugs. The present pharmacognostic details of *Morinda citrifolia* would be helpful for further scientific studies in this plant.

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CONFLICTS OF INTEREST: We declare no conflict of interest for this research.

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