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Comparative pharmacognostical and histochemical studies on the three different species of *Tinospora* on stem and leaf

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Abstract
The present communication deals on the pharmacognostical and histochemical studies on the three different species of Tinospora – *T. cordifolia*, *T. sinensis* and *T. crispa* on stem and leaf. Studies revealed that presence of calcium oxalate crystals, vascular bundles 5-7 and are collateral, open, in *T. cordifolia* (stem) and leaf elongated glands are present in the lower side, stomata Anomocytic. In *T. sinensis* (stem), multicellular trichomes are present, vascular bundles are 5-8, collateral and open, secretory sacs and calcium oxalate crystals are absent, starch grains present in the cortical region. In leaf uni to biseriate trichomes are present, stomata Anomocytic type, starch and oil globules are absent in laminar region. In case of *T. crispa* (Stem) vascular bundles are 5-7, collateral and open, starch is present in cortical and pith region, in leaf rounded glandular trichomes are present, Anomocytic type of stomata in the lower side. Starch grains and oil globules are present in the midrib region only.

Keywords: comparative, pharamacognostical, histochemical studies, tinospora species, stem and leaf

Introduction
Plants are utilized extensively as raw drugs for many formulations in traditional systems of medicine. To check the genuineness of the raw drugs and to detect adulteration of these materials, an authentic pharmacognostical studies are needed for each raw drug. Usually, the drugs are collected by traditional practitioners who have inherited Ayurveda or other herbal practices [1]. Their identification is mostly based on morphological features or other traditionally known characteristics. In such cases, there is a chance of selecting incorrect raw drugs/adulterants. Therefore, an extensive macro and microscopical studies and phytochemical screening are needed for each raw drug used in the formulation to avoid any ambiguity and such a study will serve also as a reference for further studies [2]. Microscopical studies are helpful in describing a particular drug with a special emphasis on quantitative microscopies, such as sclereids, starch grains, crystals, stomata, and trichomes, and qualitative microscopy, such as xylem, phloem, and other tissues [3].
Family Menispermaceae consists of about 70 genera and 450 species that are found in tropical lowland regions. These are generally climbing or twining, rarely shrubs. Leaves are alternate or lobed, flowers small cymose, seeds usually hooked or reniform. This family is a rich source of alkaloid and terpenes. Tinospora is one of the most important genera of family Menispermaceae, consisting of about 15 species. Some medicinally important species include *T. cordifolia, T. malabarica, T. tementosa, T. crispa, T. uliginosa* etc [4].
Morphologically *T. sinensis* is distinguished from *T. cordifolia* by the tomentous leaves only. Macro and microscopical studies of this exceptionally giant Tinospora species is undertaken to distinguish its stem and leaves from that of *T. cordifolia* as they are often mixed as an adulterant or substitute to *T. cordifolia* in several Ayurvedic compound preparations used against debility, dyspepsia, fever and urinary disorders [5].
Comparative Pharmacognostical and Histochemical studies are the reliable sources to identify the genuine raw drug from their substitutes and adulterants.

Materials and methods
Macro and Microscopical Studies
The plant specimens used in the present study were collected from the Herbal garden of the Foundation of Revitalization of Local Health Traditions, Bangalore, Karnataka authentication, and voucher specimens were preserved. *Tinospora sinensis* (Lour.) Merr. *Tinospora cordifolia* (Willd.) Miers, *Tinospora crispa* (L.) Hook. f. & Thomson care was taken to ensure the

selection of healthy plants and their normal organs. Fresh materials are cut into small pieces and take freehand sectioning and dried raw drugs stem only used in powder microscopy stained with safranin and TBO mounted with glycerin. All the photomicrographs were taken with an Olympus CX33 microscope.

Histochemical studies

The useful parts of the mature stems of genuine plants were studied by taking sections using freehand sectioning stained with appropriate staining procedure lignin Potassium iodide-iodine-sulphuric acid method [6], Starch Iodine-Potassium-iodide reaction [7], and total proteins Fast green method [8], using histochemical techniques.

Result and Discussion

Macroscopically characters

Morphologically the plants show differences in stem and leaf size, shape and thickness etc. *T. cordifolia* is a deciduous woody climber, up to 10m high, entirely glabrous; stems seriate when young, often with scattered lenticels. Some time with aerial roots; bark corky, flaking off with age. Leaves broadly ovate-cordate, sinuate at base, abruptly cuspidate-acuminate at apex 4-15x 4.5 – 13cm, glabrous; basal nerves 5-7, palmate; petioles pulvinate 2-7cm long. Inflorescence pseudo racemose, axillary or on leafless branches; usually solitary 5-15cm long, slender. Fruit drupes globose. *T. sinensis* large fleshy climber; stems puberulous when young, striate with scattered lenticels; leaf-scales distinct. Leaves ovate to suborbicular, abruptly truncate or cuneate or suborbicular at the base, acutely acuminate at apex 7-15 x 4.5 -12cm, membranous sparingly pubescent above, pilose beneath with glandular patches in basal nerve axils; basal nerves 5-7; lateral nerves 1or2 on either side of midrib; petioles 4-10cm long, puberous. Inflorescence pseudo racemose, 3-12cm long slender. Fruit drupes globose. *T. crispa* shrubs climbing, up to 15cm, glabrous; stem prominently striate, tuberculate, often bearing aerial roots, with bitter milky sap. Leaves broadly ovate to oblong-orbicular, shortly or deeply cordate at base, acuminate at apex, palmately 5-7 nerved at the base, thinly papyraceous; domatia absent; petioles 5-15cm long. Inflorescence on old leafless stems, 9-25cm long; flowers yellowish green; bracts subulate, 2-3mm long fleshy. Fruit drupes ellipsoid ca 2cm long, orange colored, condyle deeply intrusive into seed cavity.

Microscopically characters

Microscopically and histochemical these three species show variations in many characters. In stem show variations in nature of epidermis, cortex and cell contents in different regions.

Leaves

Leaf microscopical anatomical characters are also shown differences in many characters like nature of the vascular region, mesophyll, palisade cells and stomatal type. *T. sinensis* TS of leaf through midrib region in plan convex, shows upper and lower epidermis with trichomes on both sides. Followed by epidermis upper and lower layer of tissue and in the center well developed collateral vascular bundles are present. T.S through laminar region shows typical dorsiventral leaf structure with mesophyll tissue and anomocytic type of stomata. Trichomes are on both adaxial and abaxial side (Plate 1 A-C). *T. cordifolia* TS of leaf through midrib region slight convex at the adaxial side, broad hump at abaxial side. T.S through laminar region shows typical dorsiventral leaf structure with mesophyll tissues and anomocytic type of stomata. Elongated glandular trichomes on abaxial side (Plate 1 D-F). *T. crispa* TS of leaf through midrib region shows convex nature, however, the glandular hairs are present in the abaxial surface. T.S through laminar region shows dorsiventral leaf structure with mesophyll tissues and anomocytic type of stomata (Plate 1 G-I). This species presents in different types of the stomata, trichomes, and glands show the photos (Plate 2). Details of anatomical characters of leaf given in Table 1.

Stem

T. sinensis: TS of the stem is circular in outline and most abundant in uniseriate trichomes are made up of two or more cells. Vascular bundle of this species is wedge-shaped and more in number (Plate 3 A-D). *T. cordifolia* TS of the stem is cylindrical, slender, wedge-shaped and hairs are absent (Plate 3 E-G). *T. crispa* TS of the stem is circular in outline (Plate 3 H-J). Histochemical studies of starch and lignin deposited, starch grains are present only in some cortical cells and pith cells, lignin deposited in the thick wall cells of *T. sinensis* (Plate 4 A-C). In *T. cordifolia* starch grains are present in all the cells of cortical, vascular and pith region, thick wall cells are lignin deposited (Plate 4 D-F). In *T. crispa* starch grains are present in all the cells of cortical and pith region (Plate 4 G-I). Details of anatomical characters of stem given in Table 2.

Table 1: Comparative Leaves Anatomical Characters

S.No	Characters	*T. sinensis*	*T. cordifolia*	*T. crispa*
1	Cuticle	Thin layer	Thin layer	Lower midrib thin layer
2	Epidermis Upper Lower	Rounded and rectangular Rounded	Both side angular.	Both side polygonal.
3	Palisade tissue	Single layered, columnar cells	Single thin walled, columnar cells	Single layered, columnar cells.
4	Trichomes & glands	Uniseritate of more than 2 cells.	Elongated glands.	Rounded glands.
5	Stomata type	Anomocytic	Anomocytic	Anomocytic Abaxial side only.
6	Midrib vascular bundles	Single median open vascular bundles. Cup shaped xylem phloem. Cap like structure of sclerenchyma cells.	Single median well developed collateral vascular bundles. Absent of sclerenchyma cells.	Well-developed collateral vascular bundles. Absent of sclerenchyma cells.
7	Starch grains Oil globules	Absent lamina region.	Absent.	Midrib region present. Absent.

Table 2: Comparative Stem Anatomical Characters

S. No	Character	*T. sinensis*	*T. cordifolia*	*T. crispa*
1	Shape in TS	Circular	Wedge	Circular
2	Epidermis	Epidermis is two layers, multi cellular trichome is present.	Epidermis is single layer.	Epidermis single layer
3	Cortex	Cortex is collenchyma and parenchyma cells. Pericycle is sclerenchymatous 9-10 layers, thick walled cells and cap-like structure.	Cortex is parenchyma cells are round elongated cells. Pericycle is sclerenchymatous 5-6 layers, thick walled, cap-like structure.	Cortex is parenchyma cells are rows like elongated cells. Pericycle is sclerenchymatous 5-6 layers, thick walled cells and cap-like structure.
4	Vascular bundles Xylem	Vascular bundles are 5-8, collateral and open. P. xylem initiation is observed in pith. Secretory sacs and calcium oxalate crystals absent.	Vascular bundles are 5-7, collateral and open. P. xylem initiation is not observed. Present.	Vascular bundles are 5-7, collateral and open. P. xylem initiation is observed in pith. Absent.
5	Starch grains	Present in cortical region.	Present in cortical, vascular and pith region.	Present in cortical and pith region.
6	Pith	Thin walled, parenchymatous, compactly arranged.	Thin walled, large parenchymatous, compactly arranged. Crystals present.	Thin walled, parenchymatous, compactly arranged.

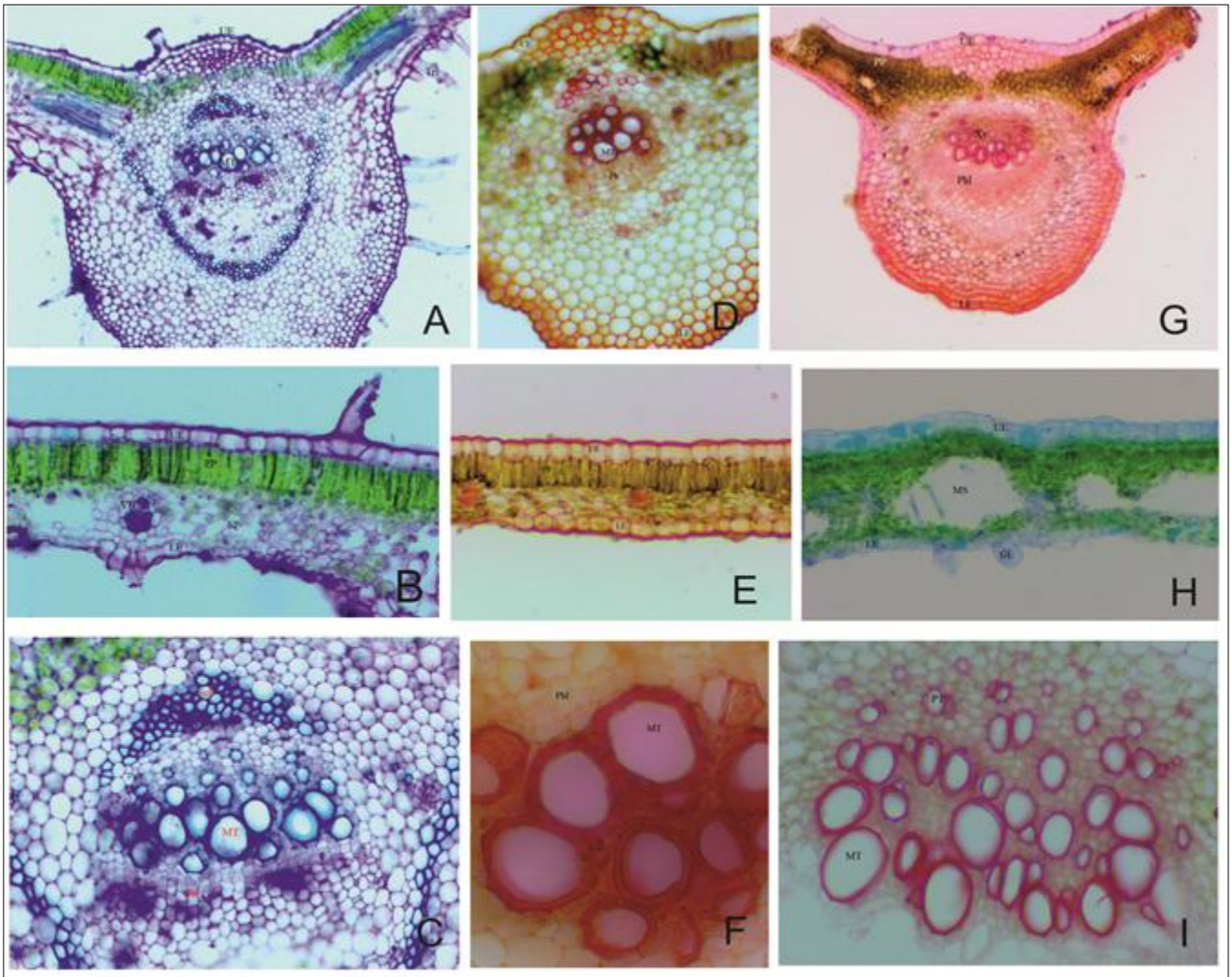

Plate - 1 (A-C) TS of T.sinensis Leaf A. Midrib enlarged x 40: B. Leaf lamina showing Mesophyll x40: C. Midrib enlarged showing Xylem x400. (D-F) TS of T.cordifolia leaf D. Midrib enlarged x 40: E. Leaf lamina showing Mesophyll x40. F. Midrib enlarged showing Xylem x400. (G-I) TS of T.crispa leaf G. Midrib enlarged x 40: H. Leaf lamina showing Mesophyll x40. I. Midrib enlarged showing Xylem x400. UE – Upper epidermis; LE – lower epidermis; PP – Polysade parenchyma; SP – Spongy parenchyma; Sch – Schlerenchyma; Tri – Trichome; MT – Metaxylem; PT – Protoxylem; Gl – Gland: MS – Mucilage sheath.

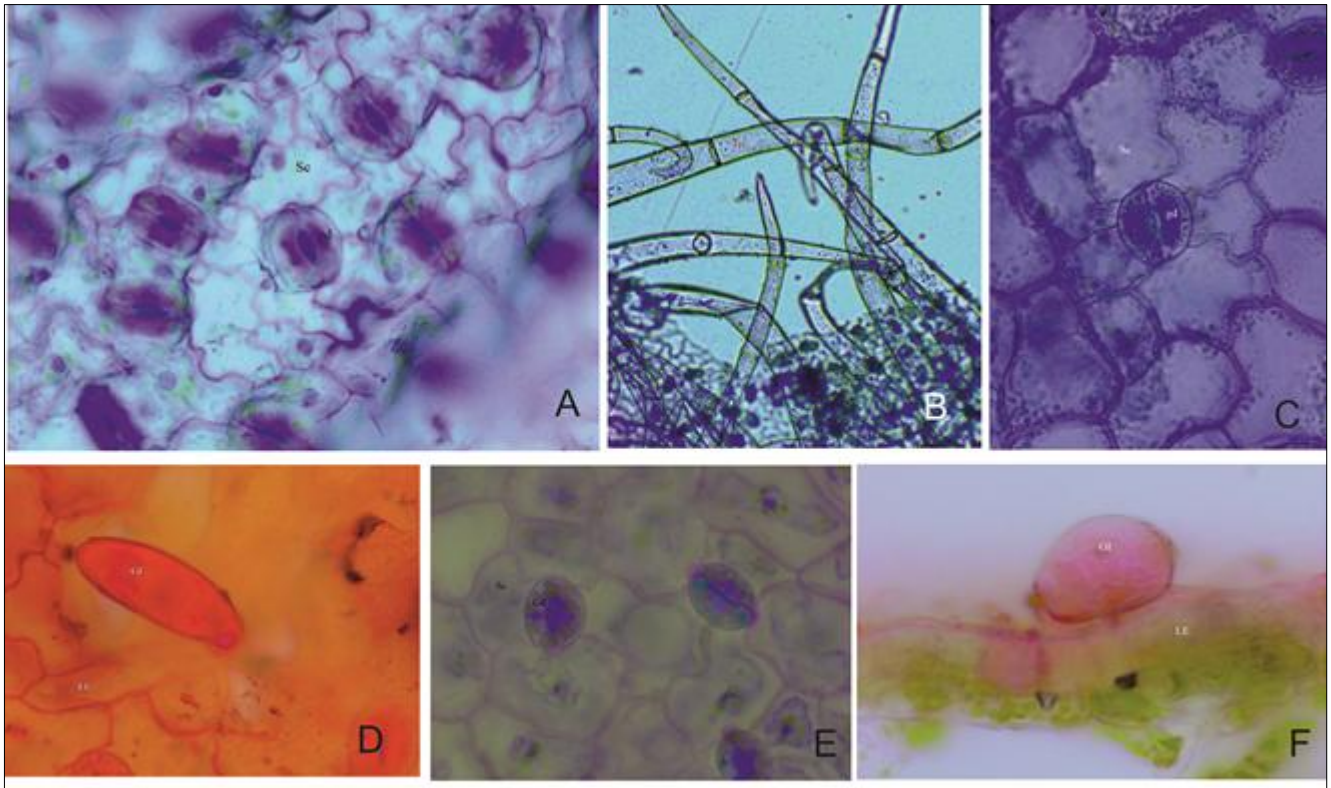

Plate 2: A- T.sinensis leaf stomata, B- Uniseriate trichomes, C- T.cordifolia leaf stomata, D- Elongated glands, E- T.crispa leaf stomata, F- Rounded glands, Gd – Guard cells, Tri – Trichome, Gl – Gland, Sc – subsidiary cells.

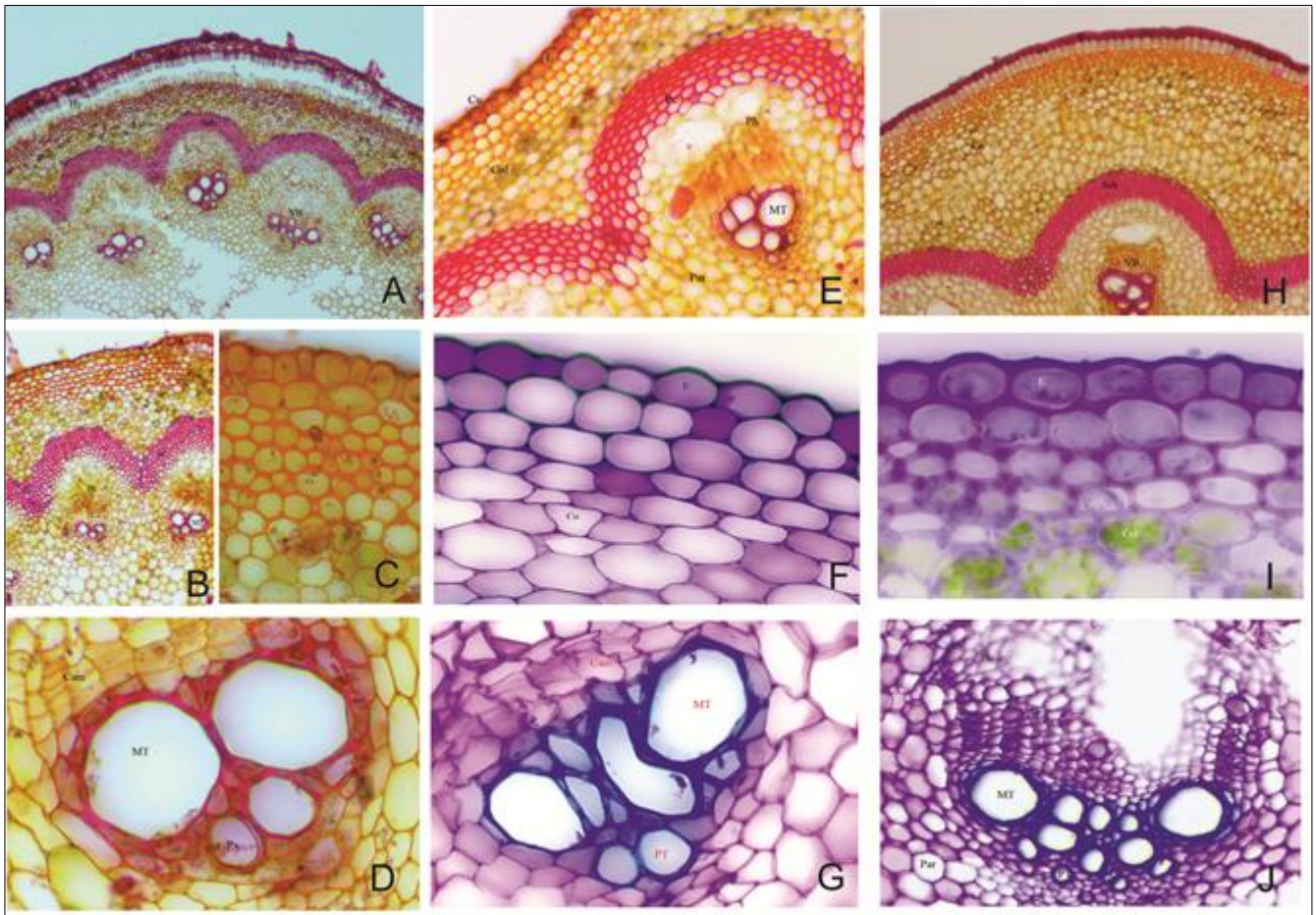

Plate 3: (A-D) TS of T.sinensis stem A. One portion enlarged showing x40, B. outer portion showing epidermis, cortex and vascular bundle x100, C. outer portion enlarged showing epidermis x400, D. Inner portion enlarged showing xylem x400. (E-G) TS of T.cordifolia stem E. One portion enlarged showing x100, F. outer portion showing epidermis x400, G. Inner portion enlarged showing xylem x400. (H-J) TS of T.crispa stem H. One portion enlarged x100, I. outer portion showing epidermis x400, J. Inner portion enlarged showing xylem x400. Cu – cuticle, Co – cortex, E – epidermis, Phl – phloem, Cam – cambium, col – collenchyma, MT – metaxylem, sch – schlerenchyma.

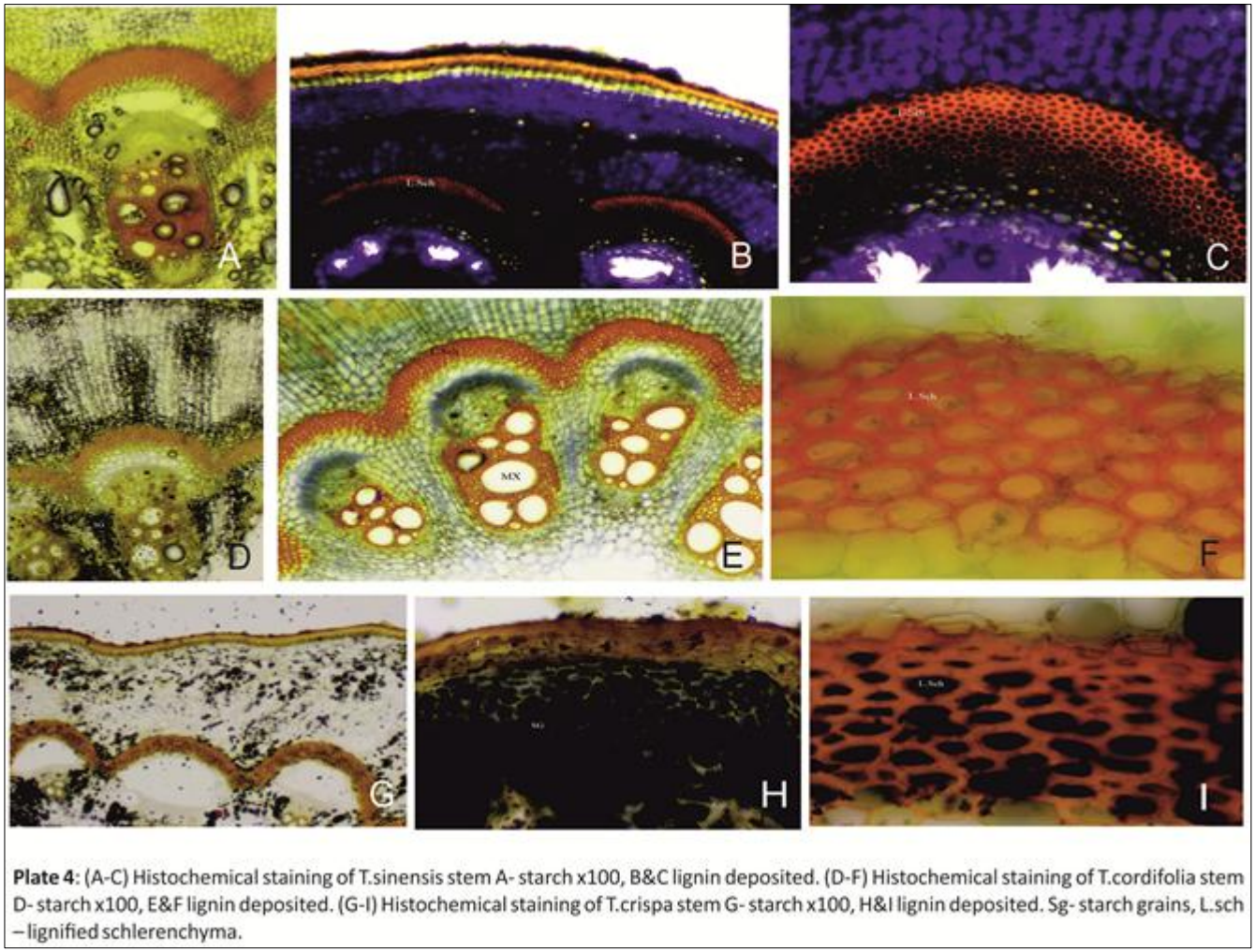

Plate 4: (A-C) Histochemical staining of T.sinensis stem A- starch x100, B&C lignin deposited. (D-F) Histochemical staining of T.cordifolia stem D- starch x100, E&F lignin deposited. (G-I) Histochemical staining of T.crispa stem G- starch x100, H&I lignin deposited. Sg- starch grains, L.sch – lignified schlerenchyma.

Conclusion

This comparative study helps in distinguishing the different characters of all the three species of *Tinospora*, and also helps in the Ayurveda preparations by making use up genuine drug of *Tinospora* species. Microscopic analysis is one of the cheapest methods to correctly identify the particular drug and the surely of raw materials. Morphological and microscopical studies of stem and leaf will be helpful in the identification of these parts of *T. sinensis*, *T. cordifolia*, and *T. crispa*. The histochemical analysis is highly essential that will aid the botanist to locate chemical substances and its properties in terms of tissues, cell, and cell parts. Thus, the histochemical study of the stem indicates the reports of support for the presence of the histochemical compounds in this three *Tinospora* species contained maximum plant metabolites such as lignin, tannin, and starch. This type of studies will help the Ayurvedic physicians, research scholars and students in order to differentiate the three different species of *Tinospora*.

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