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Pharmacognostic Evaluation and Standardization of Capparis Incanescens DC. Leaves

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ABSTRACT: Pharmacognostic evaluation plays a vital role in the authentication, quality evaluation and standardization of medicinal plants. Capparis incanescens DC. is a medicinal plant of the family Capparaceae but there is limited pharmacognostic information of its leaves. The present work has been undertaken to develop the pharmacognostic profile of leaves of Capparis incanescens by macroscopic, microscopic, powder microscopic, quantitative microscopic and histochemical parameter.The leaves are simple, coriaceous, dark green on the upper surface and light green on the lower surface, with a characteristic odour and slightly bitter taste. Under the anatomical examination, the single layer epidermis was observed with thick cuticle, multicellular trichomes, parenchymatous cortex, collateral vascular bundles, dorsiventral mesophyll, palisade and spongy tissues and scattered sclereids. Powder microscopy revealed covering trichomes, epidermal fragments, palisade cells, spiral and palisade vessels, petiole epidermis, sclereids and cluster crystals. Leaf was hypostomatic and anomocytic stomatal type. Epidermal number, stomatal number, stomatal index, palisade ratio, vein-islet number and vein-termination number were recorded. Histochemical tests revealed the presence of cutin, alkaloids, phenolic compounds, mucilage and lignin in certain tissues. Macroscopic, anatomical, powder, quantitative, and histochemical features are valuable tools for the authentication and quality standardization of C. incanescens leaves, and can be used as reference standards in future pharmacognostic and phytochemical studies.

1. INTRODUCTION

Medicinal plants form an important component in traditional medicines and are important sources of drugs and drugs related chemicals (pharmacognostic materials). Traditional medicine is still a component of health care in many nations, such as India, where numerous plant species are utilized for therapeutic purposes. Identification and authentication of raw herbal materials is essential for the quality, safety, efficacy and reproducibility of herbal medicines. The pharmacognostic evaluation involves a systematic approach to establish the identity and quality of crude drugs by the following characteristics; macroscopic, microscopic, powder microscopic, quantitative microscopic and histochemical. These parameters are useful especially for the identification of adulteration, substitution and for the development of diagnostic standards for medicinal plant materials [1,2].

Capparis L. Capparaceae is a family of plants that includes many species, mostly found in tropical and subtropical regions of the Old World, particularly Africa [3]. Its medicinal use and the presence of phytochemicals in the genus has led to the investigation of several species. Phytochemical studies on *Capparis species* report the presence of glucosinolates, fatty acids, flavonoids, alkaloids, phenolic compounds and other secondary metabolites which confer the significant medicinal and pharmacological value to the genus [4]. But a proper quality control parameter and reliable identification of botanical origin are essential for safe and standard usage of *Capparis species* as medicinal resource. Morphological, anatomical and microscopic investigations are important tools for the authentication and to minimise the danger of adulteration or substitution of the herbal materials in this context [5,6].

Capparis incanescens DC. is a species of India from the area of Tamil Nadu. The species was first described by de Candolle (1824) and its taxonomy has been complicated by the use of earlier botanical literature under *Capparis sepiaria* L. [7,8]. According to the current taxonomic information *C. incanescens* DC. is an accepted species and its native range in India is recorded as the state of Tamil Nadu [8]. The species features a very branched shrubby growth, tomentose young branches, recurved stipular thorns and leaves with a

prominent midrib and lateral veins, which are pubescent. These characters are helpful in the field but might not be enough if the material is dry, broken and powdered for authentication purposes.

The earlier pharmacognostic studies on *Capparis sepiaria* L. have indicated the unique characteristics of its leaf such as macroscopic, microscopic, physicochemical and phytochemical parameters which are beneficial for standardisation [9]. The results suggest that there is a need for detailed pharmacognostic evaluation in the genus. The previous study was performed on C. sepiaria, but C. incanescens has been accepted as a different species [8,9]. Thus, it is necessary to document the leaves of C. incanescens from a chemospecific point of view to establish the diagnostic characters suitable to the taxonomic position accepted at the moment.

Besides the anatomical considerations, quantitative microscopy offers extra numerical characteristics. For authentication and standardization of leaf drugs, some parameters, like stomatal number, stomatal index, palisade ratio, vein-islet number and vein-termination number can be used as additional diagnostic criteria [10].

These are numerical parameters that are especially helpful because they give measurable parameters which can help in distinguishing between similar or morphologically similar plant materials. The histochemical study gives additional information, as it shows the distribution of cellular components like starch, lipids, phenolics, mucilage, lignified tissues, cutin, suberin, and alkaloids by specific chemical reactions [11]. Thus the combination of macroscopic, microscopic, powder microscopic, quantitative microscopic and histochemical observations gives a pharmacognostic profile for the authentication and quality assessment of medicinal plant materials.

Although *Capparis incanescens* DC. is taxonomically important and the genus has great pharmaceutical interest, a detailed pharmacognostic documentation has not been reported so far, which is specific for C. incanescens leaves. The present study is the first comprehensive pharmacognostic investigation which has been carried out specifically on *Capparis incanescens* DC. leaves keeping in view the macroscopic, microscopic, powder microscopic, quantitative microscopic and histochemical features. Hence, the present work was undertaken with the aim to develop a comprehensive diagnostic profile of *C. incanescens* leaves by following standard pharmacognostic techniques. The results can be used as reference standards for the authentication, quality assessment, adulteration and substitution prevention, and standardization of *C. incanescens* leaf material.

2. MATERIALS AND METHODS

2.1. Collection of Plant Material and Authentication

The plant material of *Capparis incanescens* DC. was collected from Tiruchirappalli (Trichy) District, Tamil Nadu, India and was submitted for botanical authentication. The authenticated specimen of *Capparis incanescens* DC. was obtained from Siddha Central Research Institute (SCRI), Central Council for Research in Siddha (CCRS), Ministry of AYUSH, Government of India, Chennai – 600106 on 21 March 2025.

The authentication certificate is issued under No. PCOG002-ACF and has been numbered 1193.28022504, and was issued on the basis of a specimen code C280225041. The authenticated specimen upon which the identification was made was the twig and the authenticated part of the plant was *Capparis incanescens* DC. The authenticated leaves were then used in the present study for macroscopic, microscopic, powder microscopic and quantitative microscopic and histochemical studies.

Capparis incanescens Leaves

Scientific Classification

Kingdom: Plantae
Phylum :Streptophyta
Class :Equisetopsida
Subclass: Magnoliidae
Order :Brassicales
Family :Capparaceae
Genus :*Capparis*
Species :*incanescens* DC

2.2. Macroscopic evaluation

The external morphology of the test sample was recorded following SOP No. PCOG-004-SOP. Macroscopic characteristics of the leaves such as size, shape, texture, colour, margin, apex, base, surface, odour and taste were observed and recorded(Plate 1). Photographic documentation was conducted by taking photos with a Digital camera, Nikon D-5600.

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Plate 1. Macroscopical Studies of the Plant *Capparis incanescens* DC Twing(stout,zig-zag,pubescent or rarely,Puberulous on maturity); Thorn (numerous, recurved 4-6mm long);Leaves(blades lanceolate to elliptic lanceolate or oblanceolate 3-5cm*1-1.5 cm long); Inflorescence(Corymb to subumbels, 8-12mm long,10 to 15 flowers, terminal and on lateral branches)

2.3. Microscopic evaluation

The specimen was examined under the microscope as per SOP No. PCOG-005-SOP. This sample was preserved with FAA for over 48 h. The material was preserved and cut into thin slices with a sharp knife to make a cross section. They stained the sections with 0.8% safranin and 0.5% Astra blue.

The transverse sections of the petiole and the leaves were observed under bright field illumination with Axiolab 5 trinocular microscope equipped with Zeiss Axiocam 208 colour digital camera. Photomicrographs were taken and recorded. Scale bars were used to show the magnifications.

2.4. Powder microscopy

Powder microscopy was done as per SOP No. PCOG-006-SOP. The powdered sample of leaf was cleared by adding a drop of saturated solution of chloral hydrate and mounted in 50% glycerol on a microscopic slide. The powder was also soaked in the iodine solution for starch grains detection.The characteristics of powder were examined using a bright field illumination Nikon ECLIPSE E200 trinocular microscope with a Zeiss ERc5s digital camera. Photomicrographs were taken for documentation.

2.5. Quantitative microscopy

The quantitative microscopic evaluation was performed as per the SOP No. PCOG-007-SOP. About 5 × 5 mm pieces of the leaves were put into the test tube containing about 5 mL of saturated chloral hydrate solution, and heated in a water bath for 10-15 min. The cleared material was placed in glycerin and then looked at under a microscope. The microscope was fitted with a camera lucida and using 4× objective and 10× eyepiece. Qualitative parameters measured were epidermal number, stomatal number, stomatal index, palisade ratio, vein-islet number and vein termination number.

2.6. Histochemical studies

Histochemical examination was carried out based on SOP No. PCOG-008-SOP with standard procedures and specific histochemical reagent.

Crystals

This section was placed in water and acetic acid was added under the coverslip. Filter paper was used on the opposite side of the water to remove the water. The presence of air bubbles was taken as evidence of the presence of calcium carbonate crystals. If the

air bubbles were not used, the experiment was repeated using concentrated hydrochloric acid. The presence of crystals of calcium oxalate was thought to be indicated by the dissolution of crystals and the formation of calcium sulphate needles.

Fats, fatty oils, volatile oils and resins

A few minutes were left to react with one to two drops of Sudan IV added to the section. Orange red, pink or red globules were considered as fatty oil substances while red coloured irregular content were considered as resin.

Starch

A drop of 2% iodine solution was added to the section. The presence of starch resulted in the formation of a blue colour.

Phenolic compounds

A drop of alcoholic ferric chloride was added to the section. Bluish-black coloured contents showed the presence of phenolic compounds such as tannin-type compounds.

Mucilage

A drop of ruthenium red was added to the section. The mucilage was present when the tissues were pink to red in colour.

Lignified cell walls

The section was placed in a drop of phloroglucinol and left to stand for about 2 min or until it was nearly dry. Then 50 % HCl was added. Lignification was indicated by pink to cherry red staining on cell walls.

Suberized or cuticular cell walls

Several minutes allowed for Sudan red III to drop and react. When applicable, gentle warming was used. Staining of cell walls orange-red or red showed deposition of suberin or cutin.

Alkaloids

The section was treated with a drop of Wagner's reagent. Yellow to reddish brown coloured contents were taken as an indication of the presence of alkaloids.

3. RESULTS AND DISCUSSION

Macroscopic characteristics

The leaves of *C. incanescens* were simple, coriaceous and elliptic to oblong or ovate. The apex was acute to mucronate and the base cuneate. The margin was smooth. The upper surface had a dark green colour and was glabrous while the lower surface was light green. Petiole was short. The leaves had a unique scent and had a faint bitterness (Fig. 1).

Figure 1. Macroscopic Characteristic of *Capparis incanescens* leaf

The above-mentioned macroscopic features are preliminary diagnostic characteristics for the identification of the crude leaf material. Microscopic examination may be more suitable for providing diagnostic evidence of powdered and/or fragmented material, but leaf shape, texture, surface features, colour and organoleptic properties may be useful for routine authentication.

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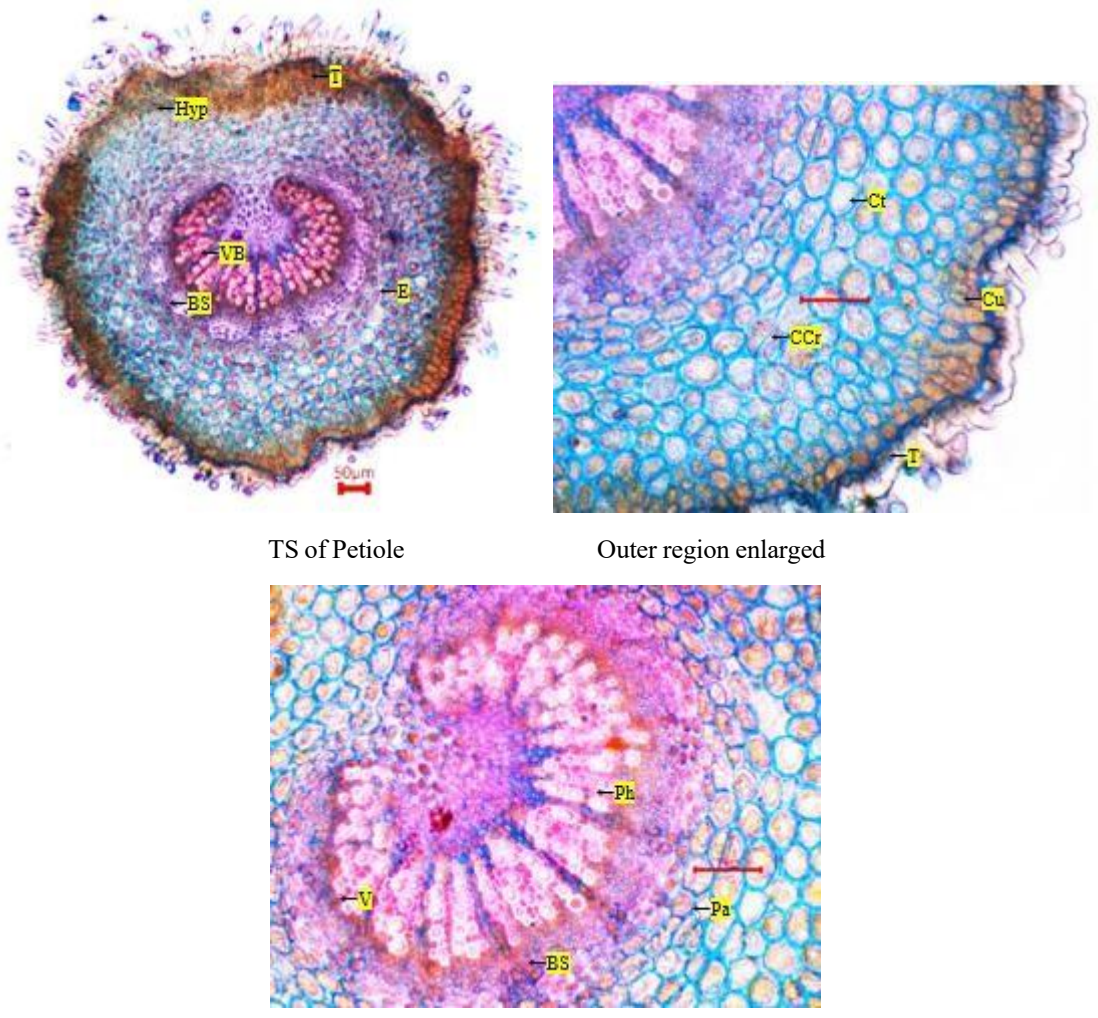
Microscopic characteristics

Petiole

The TS is circular to slightly ridged in outline with a wavy margin; outermost epidermis is made up of single layer of compactly arranged cells covered with thick cuticle; multicellular trichomes are seen arising from the epidermal cells particularly on the ridges; region under epidermis is composed of single layer of thick walled and angular collenchyma which are evident under the ridges and reduced in the furrows; cortex is parenchymatous, comprising several layers of thin-walled, rounded to polygonal cells loosely arranged and contains starch and mucilage; vascular bundles are placed in a ring; bundles are collateral and open, with xylem on the inner side and phloem on the outer side; bundle sheath are seen surrounding the vascular bundles (Fig.2).



TS of Petiole

Outer region enlarged

Inner region enlarged

Figure 2. TS of *Capparis incanescens* petiole

Leaf

The leaf is dorsiventral, the epidermii are distinct, the midrib area is prominent and the lamina areas are narrower on either side.

Midrib

TS has outer single layered epidermis, ground tissue is parenchymatous, loosely packed with intercellular spaces, vascular bundles are collateral, xylem on upper side and phloem on lower side of the bundle, vascular bundles are surrounded by parenchymatous bundle sheath (Fig.3).

Lamina

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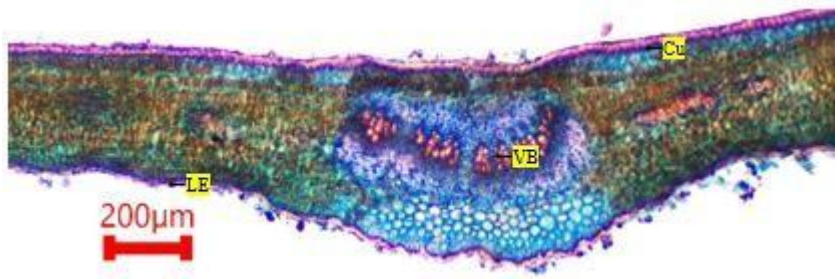
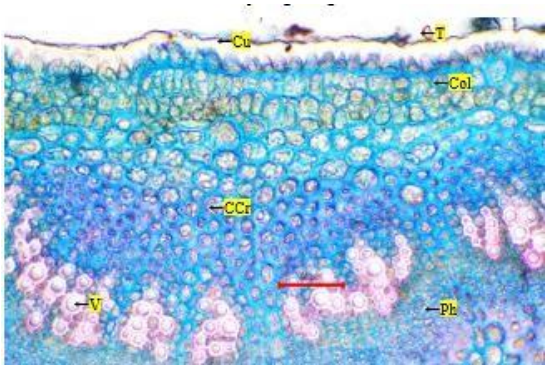
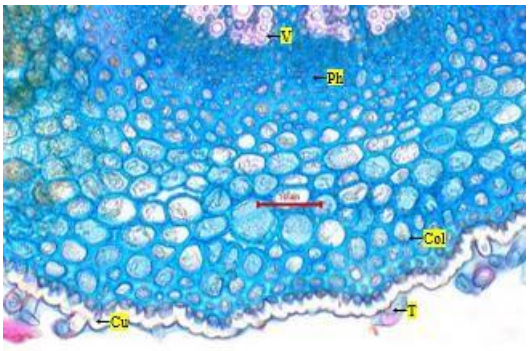
The upper epidermis is composed of a single layer of compact, rectangular cells with thick cuticle, while the lower epidermis is also a single layer but has more trichomes. Trichomes are observed in both epidermidi with more in lower epidermis, mesophyll is Divided into upper 1-2 layers of elongated columnar cells of palisade parenchyma followed by 6-8 layers spongy parenchyma made up of loosely arranged rounded cells with large intercellular spaces, few groups of sclereids are seen in the mesophyll region, 3-4 developing vascular bundles are observed at the lamina region (Fig.3).



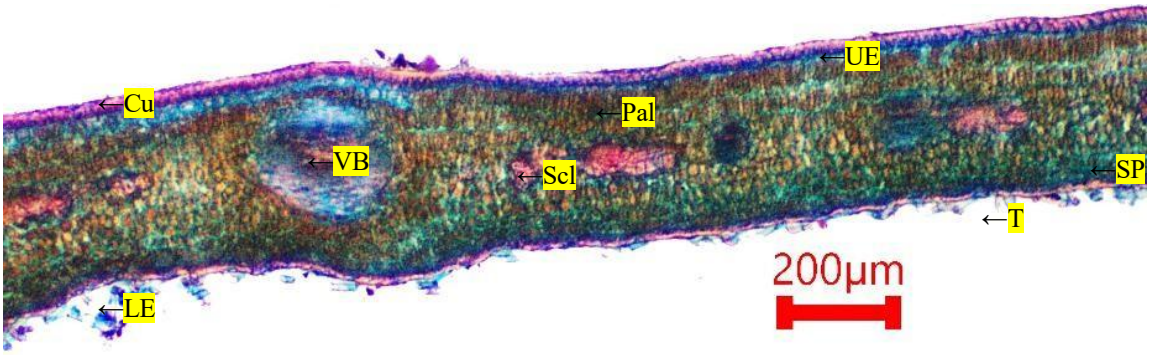
TS of lamina passing through midrib



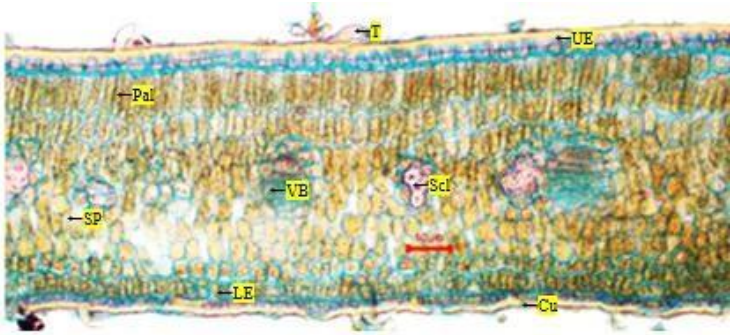
Upper portion enlarged



Lower portion enlarged



TS of lamina



Lamina region enlarged

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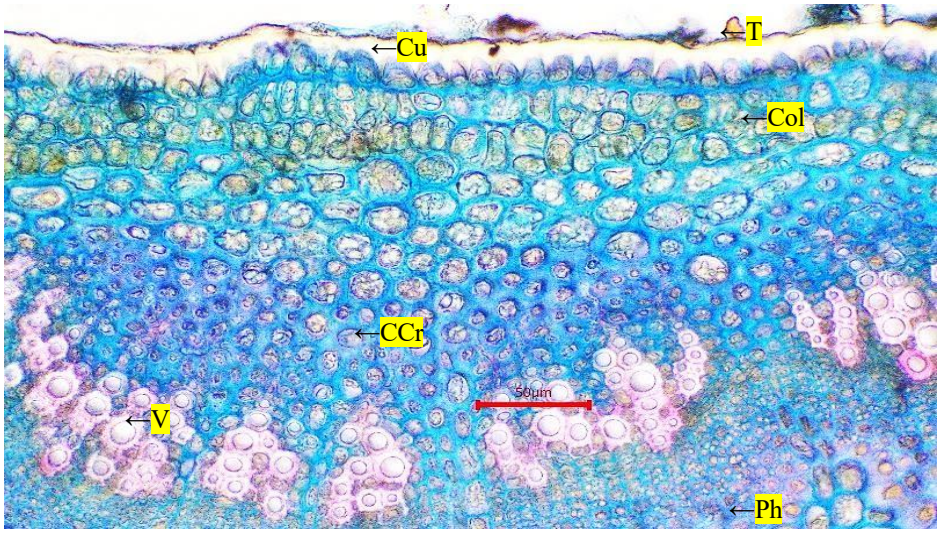
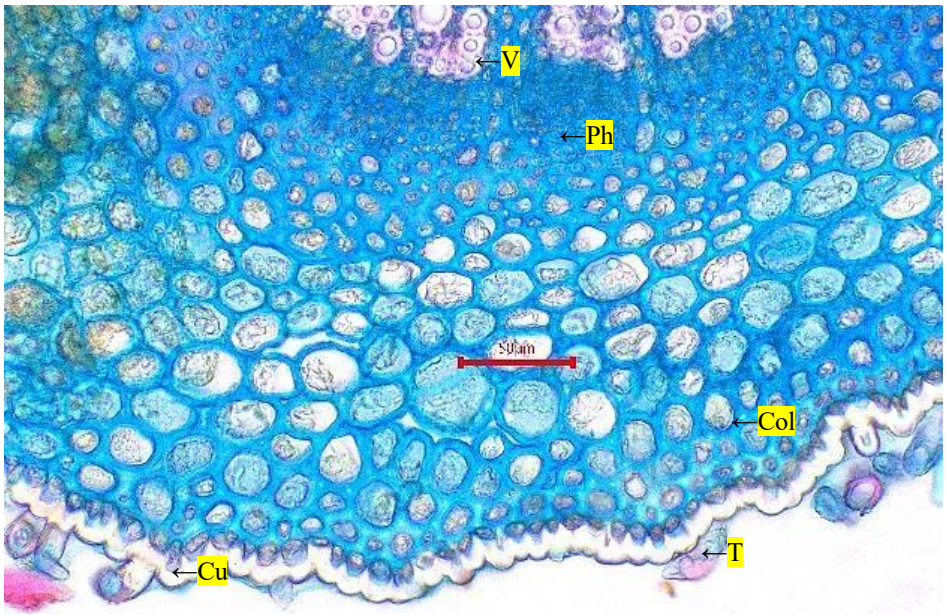
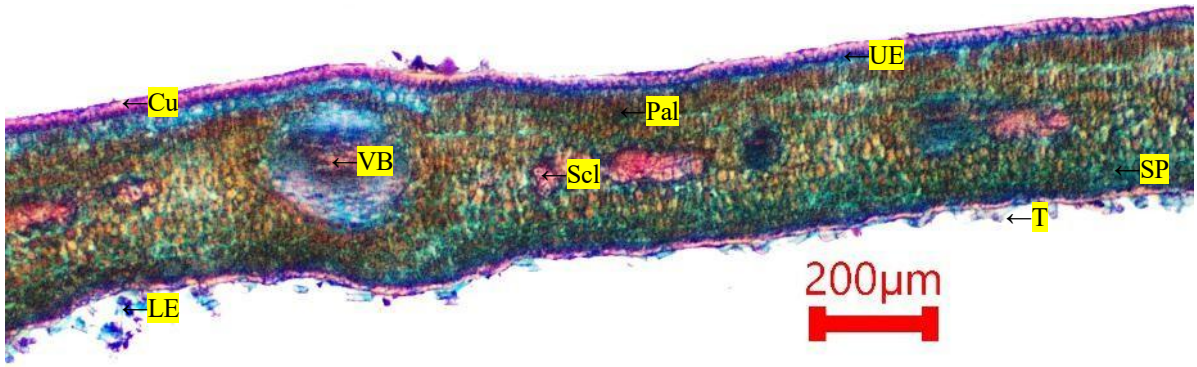


Upper portion enlarged



Lower portion enlarged



TS of lamina

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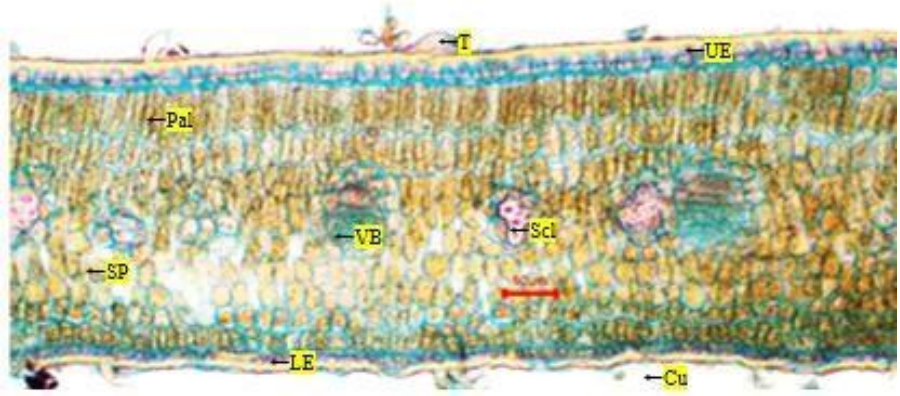


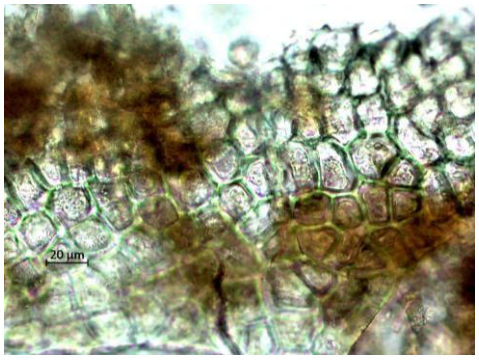
Lamina region enlarged

CCr, cluster crystal; Col, collenchyma; Cu, cuticle; LE, lower epidermis; Pa, parenchyma; Pal, palisade cells; Ph, phloem; Scl, sclereid; SP, spongy parenchyma; T, trichome; UE, upper epidermis; V, vessel; VB, vascular bundle.

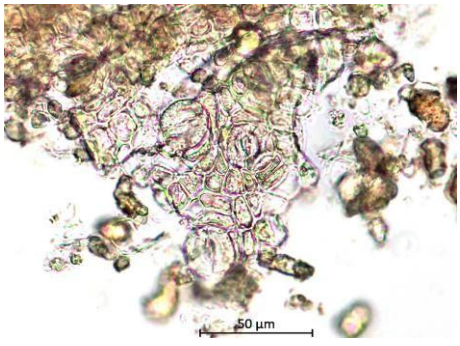
Powder microscopy

Powdered leaf was greyish-green in color and had a distinct aroma and taste. Many diagnostic features such as covering trichomes, upper and lower epidermal cells, palisade cells, epidermis of petiole and sclereids, spiral vessels, pitted vessels and cluster crystals were observed by powder microscopy (Fig. 4). These diagnostic fragments will help to authenticate powdered leaf material if their gross morphology is no longer available. The characteristic microscopic profile of the crude drug consists of trichomes, epidermal fragments, vascular elements, sclereids and cluster crystals in particular.

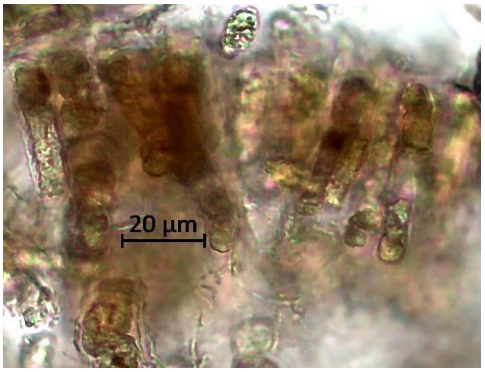
Covering trichome



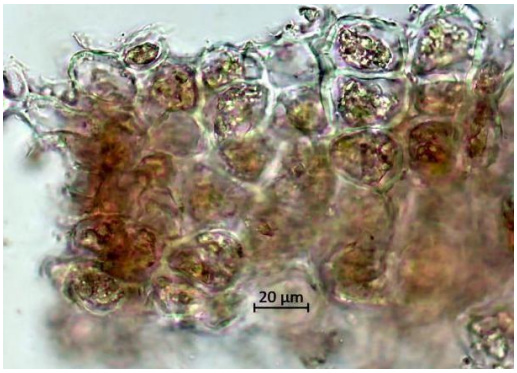
Surface view of the upper epidermis



Surface view of the lower epidermis

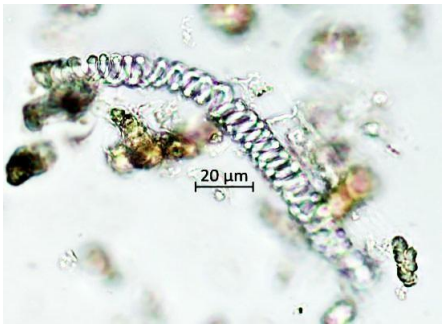


Palisade cells in sectional view

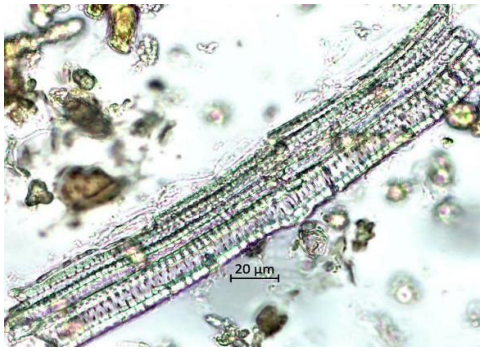


Petiole epidermis in surface view

Sclereid



Spiral vessel



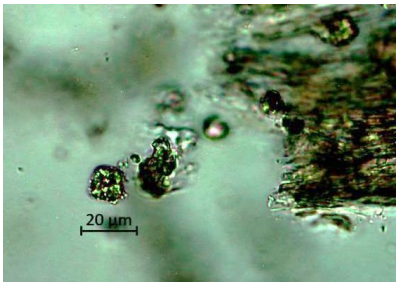


Figure 4. Powder microscopic characteristics of *Capparis incanescens* DC. leaves.

Labels: **covering trichome; upper epidermis in surface view; lower epidermis in surface view; palisade cells in sectional view; petiole epidermis in surface view; sclereid; spiral vessel; pitted vessel; cluster crystal.**

Quantitative microscopy

Table 1 shows the quantitative parameters of the leaf measured under a microscope. The leaf was hypostomatic, that is, stomata were only present in the lower epidermis. The stomata were of the anomocytic type (Fig. 5).

The upper and lower epidermis had epidermal numbers of 3300-3500 cells per mm² and 4600-4750 cells per mm² respectively. The stomatal number on the lower epidermis was 350–400/mm² and stomatal index 7–8%. The palisade ratio was 4–6. Veins was 13–15 and vein terminations was 7–9.The quantitative microscopic characters are numberable features that can be added to the qualitative anatomical features. Thus, the hypostomatic condition, anomocytic stomata, stomatal index, palisade ratio and venation parameters can be helpful as auxiliary criteria for authentication and quality assessment.

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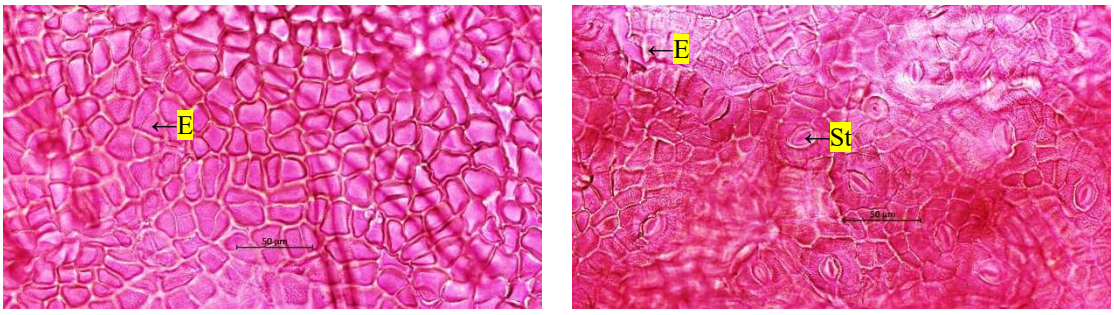
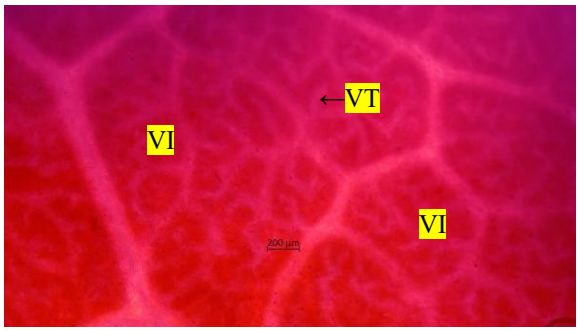
Table 1. Quantitative microscopy of *Capparis incanescens* leaves

Parameters	Upper epidermis (/mm²)	Lower epidermis (/mm²)
Epidermal number	3300 - 3500	4600 - 4750
Stomatal number	-	350 - 400
Stomatal index	-	07- 08
Palisade ratio	04 - 06	
Vein islet	13 - 15	
Vein termination	07 - 09	



Upper epidermis Lower epidermis



Vein islet and termination

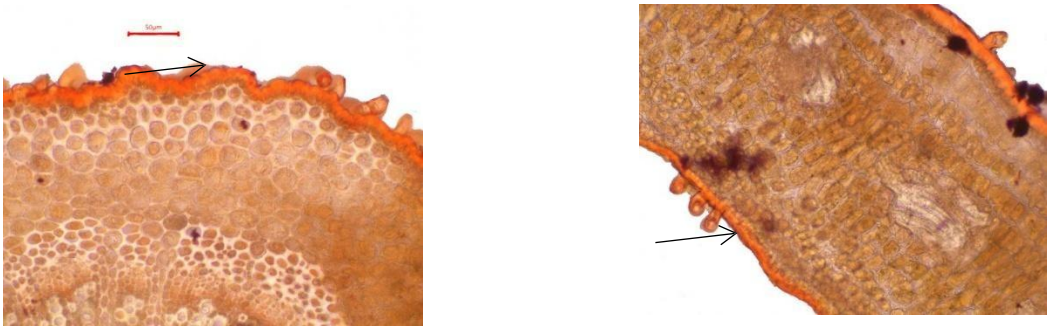
E - Epidermis; **St** - stomata; **VI** - vein islet; **VT** - vein termination

Figure 5. Quantitative microscopy of *Capparis incanescens* leaves

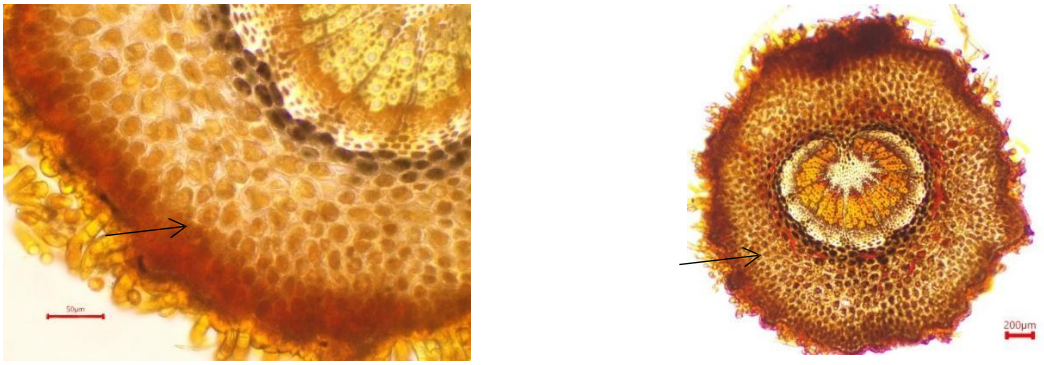
Histochemical characteristics

The histochemical studies revealed the distribution of various cellular constituents in the petiole and lamina (Fig. 6). Cutin was found in the epidermal cells of petiole and lamina. Contents were found to be positive for alkaloids in the outer cortical sections of the petiole. The bundle sheath was found to contain a number of phenolic compounds in abundance in the outer cortical layers and in the inner layers, and also in the lamina.

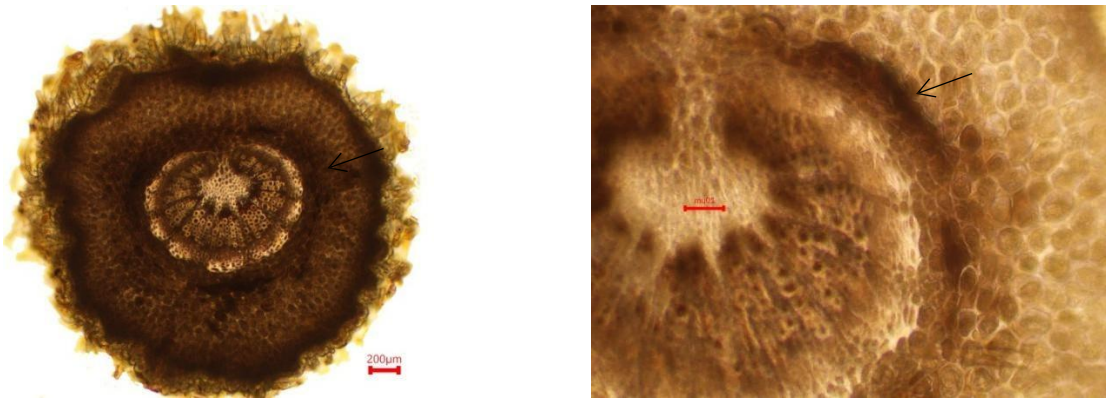
Mucilage was found in the petiole and lamina. After the phloroglucinol - hydrochloric acid reaction, the presence of lignified tissues was demonstrated in the petiole and lamina. The histochemical reactions complement the information on the localisation of leaf constituents. The results of these observations are qualitative and can only be considered as diagnostic histochemical features and not as quantitative determination of the phytochemical content of the tissues.



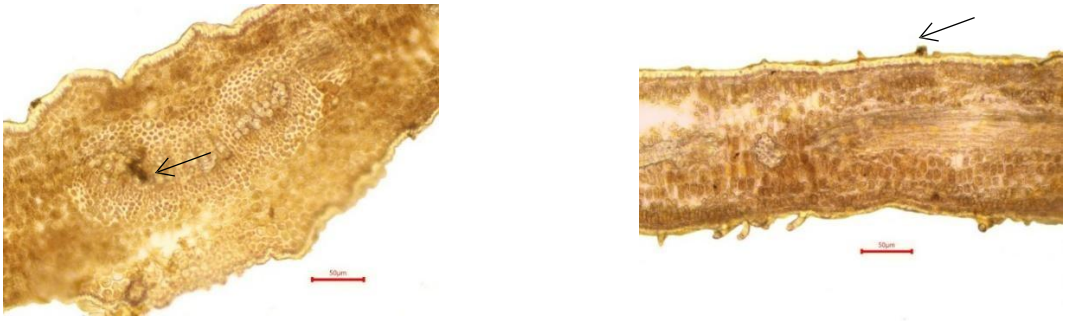
Presence of cutin in petiole and lamina



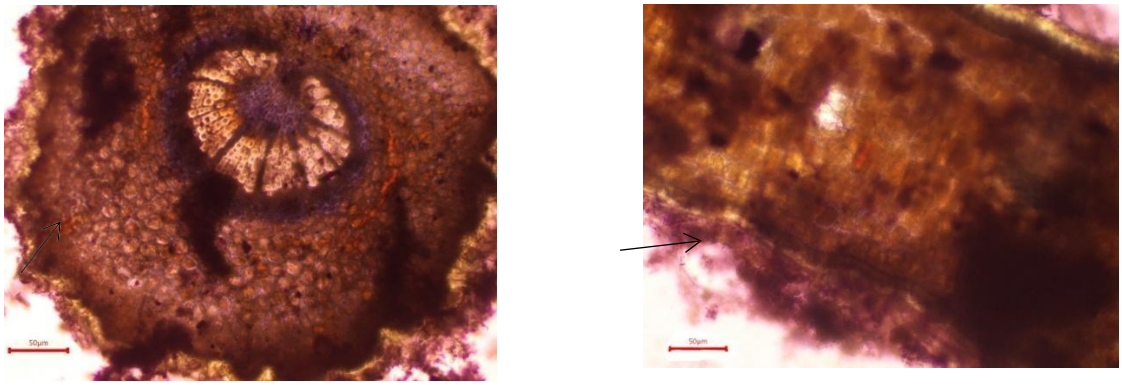
Alkaloid content in petiole



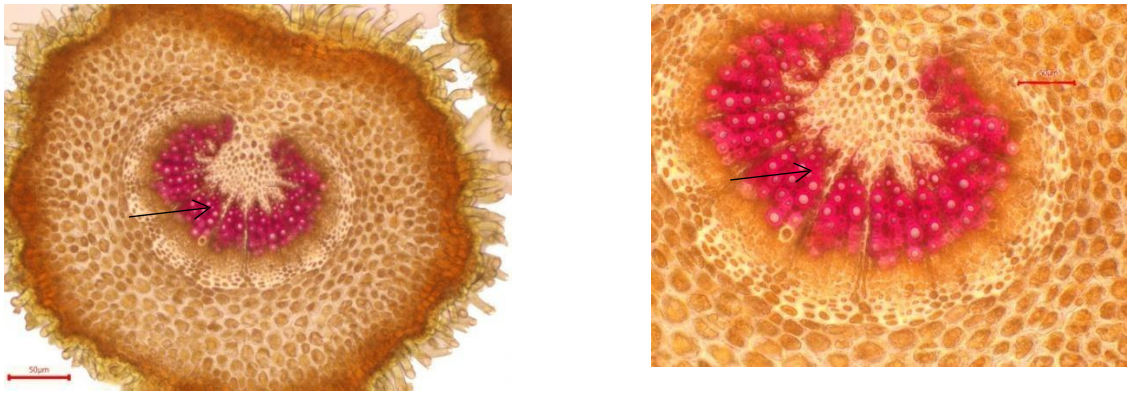
Phenol content in petiole



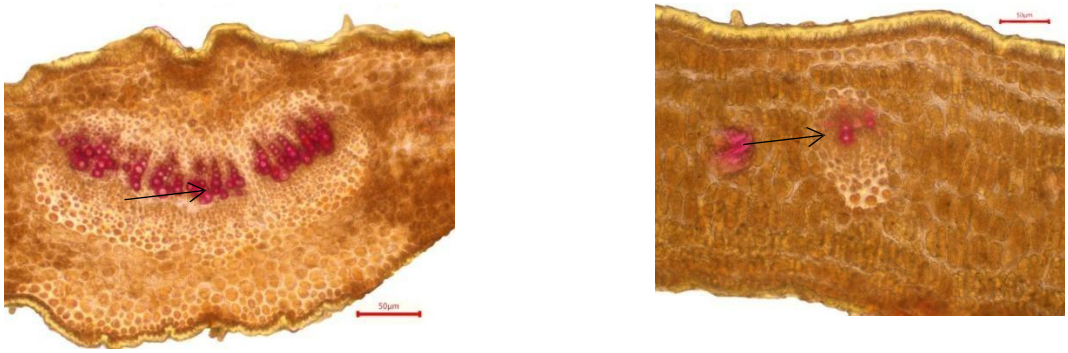
Phenol content in lamina



Mucilage in petiole and lamina



Lignin in petiole



Lignin in lamina

Figure 6. Histochemistry of *Capparis incanescens* leaves

Pharmacognostic significance

This study is the first one that gives an extensive diagnostic characterization of the leaves of C. incanescens, using both macroscopic and microscopic features, anatomical features, powder microscopic features, quantitative features, and histochemical features. The macroscopic characteristics give a preliminary diagnosis, and more specific diagnostic markers are given by the anatomical characteristics of the petiole and lamina. When the drug is given in broken or powdered, the powder microscopic characters are of great help for the identification. The quantitative parameters such as epidermal number, stomatal number, stomatal index, palisade ratio, vein-islet number and the number of vein terminations give numerical values which can supplement the qualitative anatomical observations. Likewise, the histochemical features of the localization of cutin, alkaloids, phenolic compounds, mucilage and lignin give further features for quality evaluation. The observations are in line with the significance of anatomical and pharmacognostic parameters of other *Capparis* species [8,9]. The documented characters, however, can serve as a good reference profile for the material of the *C. incanescens* leaves examined.

4. CONCLUSION

In the present study, *Capparis incanescens* DC. leaves were subjected to the macroscopic, microscopic, powder microscopic, quantitative microscopic and histochemical approaches to establish a pharmacognostic profile of the leaf. The leaf morphology, thick cuticle, multicellular trichomes, dorsiventral mesophyll, palisade and spongy tissues, sclereids, collateral vascular bundles, diagnostic powder elements and hypostomatic anomocytic stomata are useful authentication parameters. Other quantitative features, such as epidermal number, stomatal number, stomatal index, palisade ratio, vein-islet number and vein-termination number give additional numerical criteria. The diagnostic profile is complemented by the histochemical localization of cutin, alkaloids, phenolic compounds, mucilage and lignin.All the above results serve as reference pharmacognostic data for authentication and quality control of C. incanescens leaves and can be used as a reference for future phytochemical, pharmacological and standardization studies. The histochemical results are qualitative and should not be interpreted as quantitative determination of the phytochemical components.

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DECLARATION

AUTHOR CONTRIBUTION

S. Revathy took part in conceptualization, methodology, investigation, Pharmacognostic evaluation, data collection, microscopic and histochemical analysis, interpretation of results and preparation of the original manuscript. S. Akilandeswari provided supervision, conceptual guidance, methodology review, interpretation of findings, critical revision of the manuscript and final approval. Both the author(s) read and approved the final manuscript and take responsibility for the entire content.

CONFLICT OF INTEREST

The authors state that there is no conflict of interest related to the present study.

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