



Pharmacognostic Specifications of *Calotropis procera* (Aiton) W. T. Aiton Leaf and *Tribulus terrestris* L. Fruit: Important Medicinal Plants Used in Veterinary Products

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Authors' contributions

ALL Authors have contributed equally to the preparation of manuscript and the experimental work done in the manuscript.

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ABSTRACT

Present article deals with the pharmacognostic specification of selected medicinal plants used in veterinary products for the purpose of their standardization, as evidential support concerning quality determination of plant material in veterinary medicine is scarce and there is poor documentation on primary studies of many species. The plants undertaken for the study were *Calotropis procera* (Apocynaceae) and *Tribulus terrestris* (Zygophyllaceae) on the basis of their usage relevance in veterinary products. Both plants are official in Ayurvedic Pharmacopoeia of India. Morphology as well as various pharmacognostic aspects of different parts of the plant were studied and have been described, which will help in authentication and quality control. The observed microscopic characters of *Calotropis procera* leaf were rubiaceous type apostomatic stomata, epidermis, collenchymas, parenchyma, vascular bundles, palisade cells, cortical fibres

and lamina, trichomes on the cuticle of lamina. Microscopic characteristics of *Tribulus terrestris* fruit were epicarp cells showing clusters of calcium oxalate crystals, xylem vessels, group of thin walled sclerenchymatous fibres, group of stone cells of mesocarp. Powder microscopy of the *Tribulus terrestris* fruit shows abundance of epidermal trichomes - simple, short, long, thick walled, multicellular covered trichomes, sclereids. The qualitative microscopic features would prove useful for laying down their pharmacopoeial standards. The present study also provides the information with respect to identification and authentication of crude drug and serves as a reference point for the proper identification of *Calotropis procera* leaf and *Tribulus terrestris* fruit, thereby contributing to the scientific world of research.

Keywords: *Calotropis procera*; *Tribulus terrestris*; pharmacognosy; microscopy; cortex fibres; vermiform trichomes.

1. INTRODUCTION

Medicinal plants diversity and biogeographic position of India is unique in the sense that all types of agro climatic and ecologic conditions are interdependent to each other. The rural people constitute 70 to 75% of the Indian populations and earn their livelihood through agricultural and livestock products. They take care of their health as well as of animals by self managed home-remedies [1]. Local health tradition and practice of indigenous traditional knowledge (ITK) for animal health care system have proven to be effective. The demand for plant based-drugs is increasing both in human and veterinary medicine as these are natural products, having negligible side-effects. These can be made available easily and at affordable prices. Medicinal plants sector has traditionally occupied an important position in the socio-cultural, spiritual and medicinal arena of rural and tribal lives of India. These herbal medicines hold considerable range of pharmacologically active chemical constituents beneficial in health care. Each herb has its own health benefit; properties of each plant when combined with other under one medicine can serve the purpose of prescribing herbal medicines in animal health [2].

Veterinary herbal medicines consist of plant-based medicines and have their application in prophylaxis, diagnosis and therapeutic action in animal health. These plants are known to treat diseases in animals like poisoning, constipation, dermatitis, bone fractures, snake bites, abdominal pain, foot and mouth diseases [3]. Herbal medicines for veterinary use can be given or prepared by mixing the herbs with food, infusions, decoctions, tinctures, powders and tablets for commercial purpose [4]. The advantage of herbal drugs is their easy

availability, economic and minimum side effects but there lies the trouble of adulteration with low grade or exhausted plant material. Since herbal global market generates huge revenue there is a need for standardization of crude plants used in industries to maintain the quality, reproducibility and consistency of veterinary herbal medicines [5]. Also, acceptability remains one of the major concerns in introducing modern technology for production of traditional medicines as practitioner(s) have sole faith in extemporaneous preparations. There is a need to motivate and educate people for its acceptability in the masses.

Adulteration of plant raw material is one of the major concerns as suppliers very often mix the low grade plant material or any foreign substances to meet the demand of the natural drug. The misuse of herbal products starts with wrong identification. Proper pharmacognostic (macroscopy and microscopy) studies of medicinal plants can curb the adulteration problem where authentication of commonly used plants is done through morphological, phytochemical and physicochemical analysis. Standardization parameters done on plants include organoleptic, macroscopic, microscopic, powder study, phytochemical analysis and physicochemical analysis [7]. Also there is scarce of pharmacognostic studies on veterinary herbals in literature. The selected plants for the present study were *Calotropis procera* (Apocynaceae) and *Tribulus terrestris* (Zygophyllaceae) which accounts for their role in autoimmune diseases, antimicrobial, adaptogenic, analgesic, anti-inflammatory properties and specially in GI diseases [8,9], in livestock and other animals. The important formulations of these plants are HIMROP Plus[®] by Himalaya, Bangalore; Exapar[®], Restobal Liquid[®], Stresomix[®], Ruchmax[®] by Ayurved, Baddi

Rumaxon[®] by Indian herbs, Saharanpur, INDIA. Taking into account above mentioned facts (adulteration menace, scarce of pharmacognostic study, important pharmacological attributes) of selected plants; the objective of the study is improvement of anatomical description including estimation of the value of microscopy for the proof of identity and purity of selected plants. Additionally, powder microscopy of *Calotropis procera* leaf is described for the first time.

2. MATERIALS AND METHODS

The parts of the selected plants used in marketed products were collected, authenticated and were subjected to pharmacognostical analysis including morphological, macroscopic and microscopic evaluation.

2.1 Collection and Authentication of Plant Material

The leaves of *Calotropis procera* and fruit of *Tribulus terrestris* were collected from roadsides of Baddi, Himachal Pradesh, India and were authenticated by Dr. A.S. Sandhu, S.A.S. Nagar, Mohali, which were further subjected to macroscopic and microscopic studies. Voucher specimen of *Calotropis procera* (CPB- 2021/01) and *Tribulus terrestris* (TTB-2021/01) were deposited in Department of Pharmaceutical and Nursing, Vivekanand Global University, Jaipur, Rajasthan, India.

2.2 Chemicals

Chloral hydrate-HIMEDIA, Safranin-(Sd.Fine Chem Limited, Mumbai) Phloroglucinol- Sigma-Aldrich, Japan) Conc. Hydrochloric acid and glycerine of analytical grade were purchased and used in the study.

2.3 Macroscopic and Microscopic Analysis

The macroscopic features of *Calotropis procera* leaf and *Tribulus terrestris* fruit were studied for shape, size, color, surface character, texture and odor. For microscopic studies plant parts were boiled in 20% solution of chloral hydrate to remove chlorophyll and fatty substances [10]. Studies were further carried out by making very thin cross sections as per the standard procedures. Hand sections were made by simply holding the moistened plant part to be sectioned between the thumb and forefinger of one hand

and other and for cutting the sections using blade. Wherever necessary, sections were stained with safranin, phloroglucinol, concentrated hydrochloric acid and mounted in glycerine medium [11]. Images were taken using Zeiss Digital Microscope (Primo star) resolution 4X, 10X, 40X with PROCAM camera.

3. RESULTS AND DISCUSSION

3.1 Macroscopic and Microscopic Analysis of *Calotropis procera* Leaf

Calotropis procera leaf (Hindi - aak) *Calotropis* is a large, bushy shrub with decussate, broadly obovate-oblong, glabrous leaves on both the sides with acute, sessile apices. The leaves are large, pale green, succulent, and covered with cottony surface [12,13]. For microscopic analysis of leaf, leaf specimens were cut into rectangular pieces that included the midrib and a portion of the lamina. Abaxial surface of the leaf shows the presence of rubiaceous type apostomatic and hypostomatic tetra-cyticstomatal complex surrounded by guard cells (Fig. 1). Open and close stomata can be seen clearly with the presence of simple trichomes on leaf cuticle (Fig. 2 A, B).

The transverse section of *Calotropis procera* leaf (midrib with lamina) stained with safranin and phloroglucinol showed the presence of epidermis, collenchymas, parenchyma, vascular bundles, palisade cells, cortical fibers and lamina. Upper and lower epidermal cells are small and polyhedral in shape. 3 to 5 layers of collenchymas cells on both upper and lower epidermis. Vascular bundles with the presence of xylem and phloem tissues. The lamina of the leaf shows the presence of mesophyll differentiated into palisade and spongy cells. The cuticle shows the presence of simple trichomes (Fig. 3). Presence of cortical fibers within the midrib extended towards the arms of the leaf can be clearly seen in (Fig. 4). Single layered epidermis with distinct cuticle and 2-3 layered spongy parenchymas are present below the upper epidermis in the wavy outline. Collenchymas cells can further be seen as upper and lower spongy tissues. Vascular bundles were in ovoid shape, between the upper epidermis and vascular bundles are 5-6 layers of irregular shaped collenchymas cells. Xylem can be seen on the upper side while phloem was seen to the lower side of the epidermis. Cambium was present in between the xylem and phloem cells (Fig. 5).

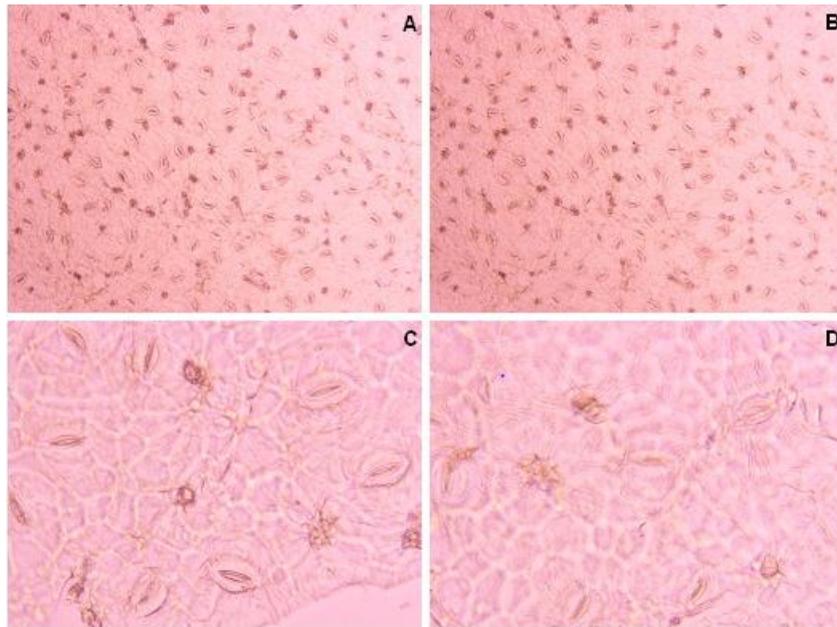


Fig. 1. Apostomatic and hypostomatic tetra-cytic stomatal complex (rubiaceous type), (A, B; 4X; C, D: 40X), epidermal cells, subsidiary cells; with rosette type crystals

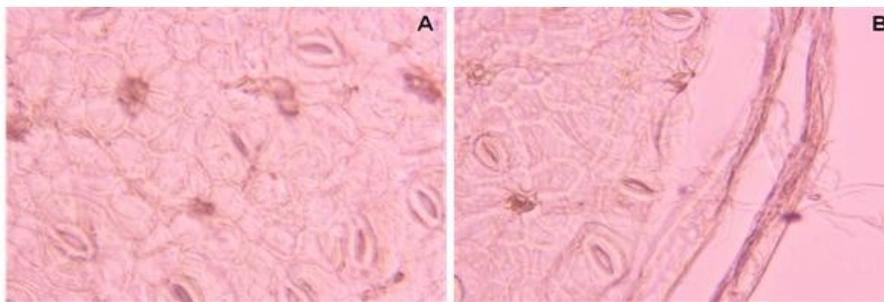


Fig. 2. A, B) Rubiaceous Stomata with trichomes (40X)

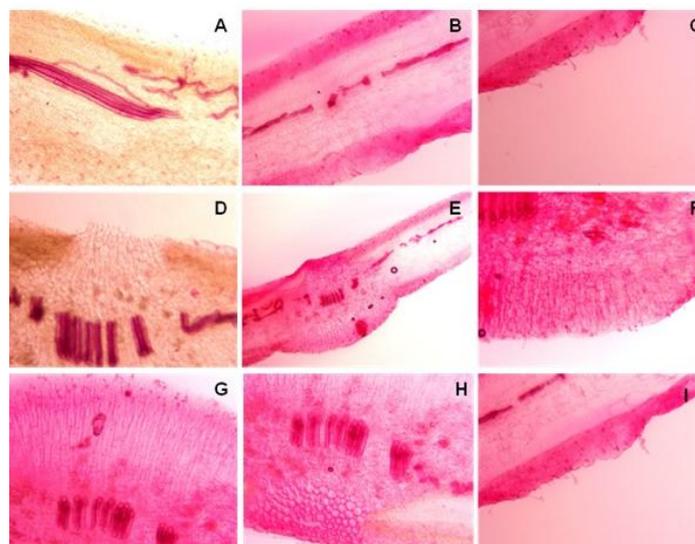


Fig. 3. Transverse section (TS) of leaf; A, D): Stained with phloroglucinol, stained with saffranin (B, C, E, F, G-I) showing spongy parenchyma, upper epidermis, lower epidermis (I) chlorenchyma (D, H) palisade cells (G) , vascular bundles (D, E), trichomes (C,I)

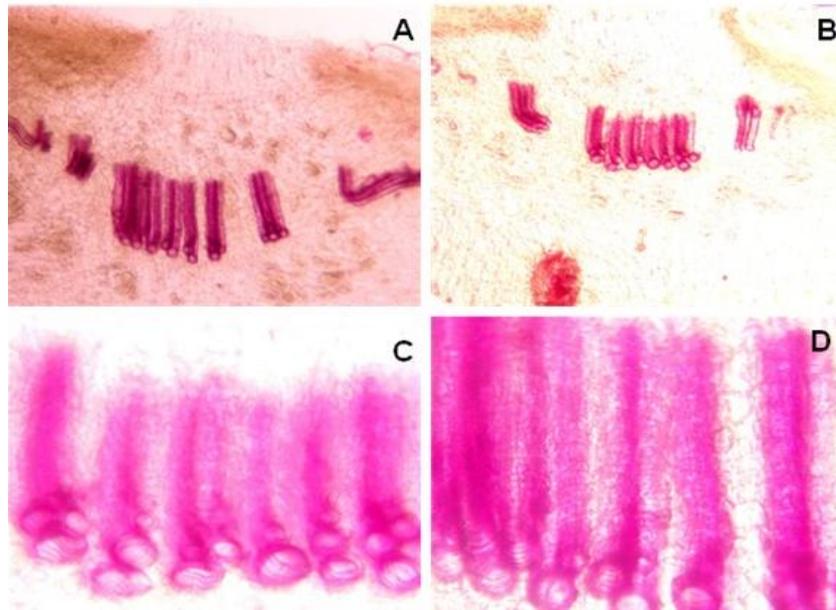


Fig. 4. TS of leaf of *Calotropis procera* leaf showing cortical fibres; A: 4X; B: 10X; C, D: 40X

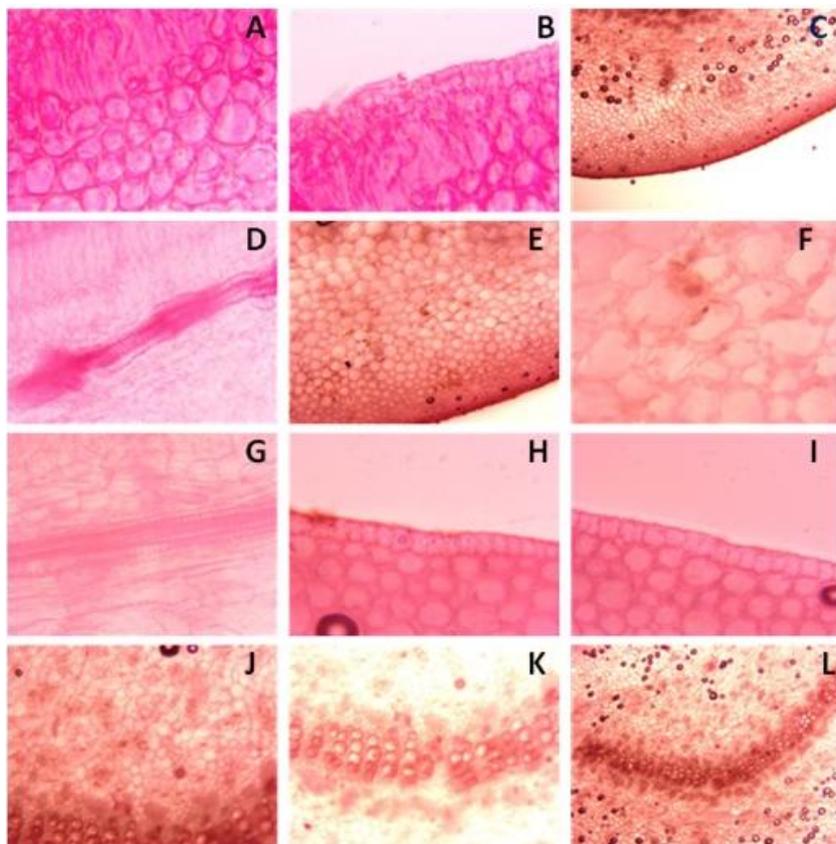


Fig. 5. Transverse section (TS) of leaf; Stained with safranin - pink slides (A,B D,F,G,H,I); stained with phloroglucinol-light brown(C, E, F, J, K, L); showing collenchyma (A, F); upper epidermis (B), cuticle lower epidermis, collenchyma lower epidermis (H, I) secondary xylem (K 40X, L 10X) outer phloem (J)

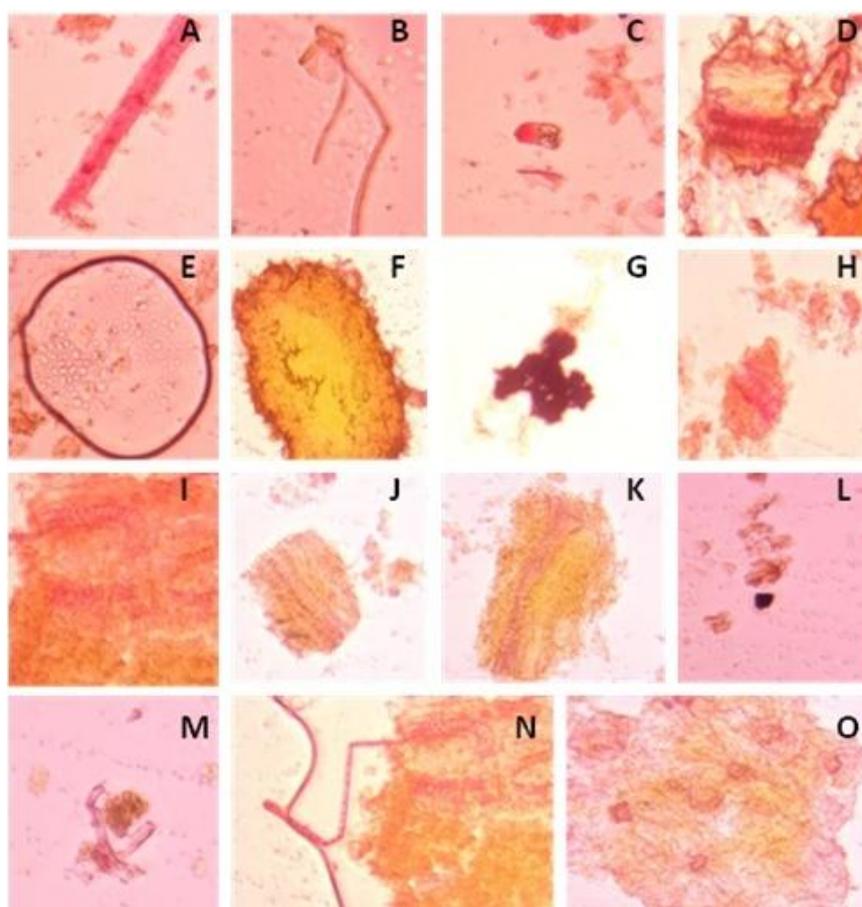


Fig. 6. Powder microscopy of *Calotropis procera* leaf showing; phloem vessel (A), fibre (B, M), xylem and phloem vessels in parenchyma (D, H, I, J, K), vascular bundle within the bubble (E), trichome (F), pit (G, L), xylem vessels embedded with parenchymatous tissues, extended phloem fibres (N), stomata (O)

Powder microscopy of the *Calotropis procera* leaf shows the presence of phloem vessel, fibre, xylem and phloem vessels in parenchyma, vascular bundle within the bubble, trichome, pit, xylem vessels embedded with parenchymatous tissues, extended phloem fibres and stomata, while no calcium oxalate crystals were found which is accordance with the previous findings [14].

Calotropis procera leaf surface view shows the presence of rubiaceous type apostomatic stomata along with epidermis (Figs. 1-2). Other characteristic features of leaf like epidermis, collenchymas, parenchyma, vascular bundles, palisade cells, cortical fibres, lamina and trichomes on the cuticle of lamina were also seen in the TS studies (Figs. 3-4). These observations are in accordance with the previous studies on *Calotropis procera* leaf [11,12]. Powder microscopy shows the occurrence of stomata, xylem and phloem vessels embedded in

parenchymatous tissues, and extended phloem fibers. There is no previous report of powder microscopy of *Calotropis procera* leaf.

3.2 Macroscopic and Microscopic Analysis of *Tribulus terrestris* Fruit (Zygophyllaceae)

Tribulus terrestris L. commonly known as *Gokshru* (Hindi) belongs to family *Zygophyllaceae*. *Gokshru* is an important component of many herbal formulations used in the treatment of various diseases [15]. Decoction of *Gokshuru* has been found useful in lower back pain, sciatica, inflammation of the pelvic and sacral region, dry cough and respiratory disorders. It can be used as tonic to strengthen the system in *Vata Prakopa* (ayurvedic *dosha*). The fruits have been used traditionally for the treatment of eye trouble, edema, abdominal distention, emission [16].

Macroscopy: The most diagnostic morphological characters of *Tribulus terrestris* fruits are globose or with five cocci, ribbed, pubescent, woody, warty consisting two spreading spines at each cocci. It was attached to pedicel with greenish yellow color. Fruit is slightly aromatic, bitter and astringent in taste. It has simple, straight, unicellular with bulbous base are short narrow, usually bent at the base forming “U” curvature and sickle shaped and some are with huge convex bulging base [15,17].

The transverse section showed the presence of trichomes-wooly and glandular, epicarp cells showing clusters of calcium oxalate crystals, xylem vessels, group of thin walled sclerenchymatous fibres, group of stone cells of mesocarp (Fig. 7). Presence of trichomes and calcium oxalate crystals can be seen in the epidermal cells of parenchyma and sclerenchyma in different views (Fig. 8). Slide stained with safranin shows the layers of

epidermal cells in between separated by the fibre sclerids, are long narrow fibre like in shape, their ends are tapering (Fig. 9).

Powder microscopy of the *Tribulus terrestris* shows abundance of epidermal trichomes – simple, short, long, thick walled, woody material, multicellular covered trichomes. Wide elliptical thick walled parenchyma cells are seen in clusters in powder. Calcium oxalate crystals cubical in shape were spread in the powder and presence of stone cells was also observed. Fiber sclereids in the bundle were seen (Fig.-10a, 10b).

Previously authors have shown the presence of rows of cylindrical palisade cell, vascular bundles, xylem, phloem, and palisade mesophyll, spongy mesophyll [16, 17] in microscopic studies of fruits of *Tribulus terrestris*. They have also reported the presence of trichomes in abundance.

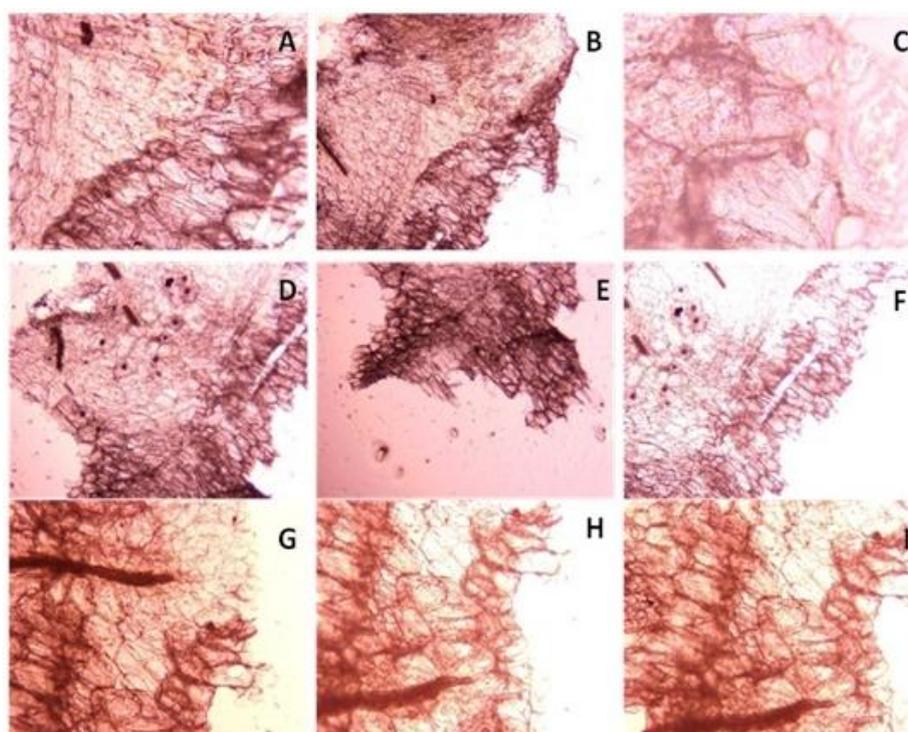


Fig. 7. Transverse section (TS) of *Tribulus terrestris* fruit; Unstained slides (A-F); Stained with phloroglucinol – light brown slides (G,H,I); showing parenchyma and sclerenchyma in the epidermal cells (A 10X; F 40X); stone cells of mesocarp (D,F), Parenchyma and sclerenchyma cells and fibre sclereids (thick line) stained with phloroglucinol (G, H, I 40X)

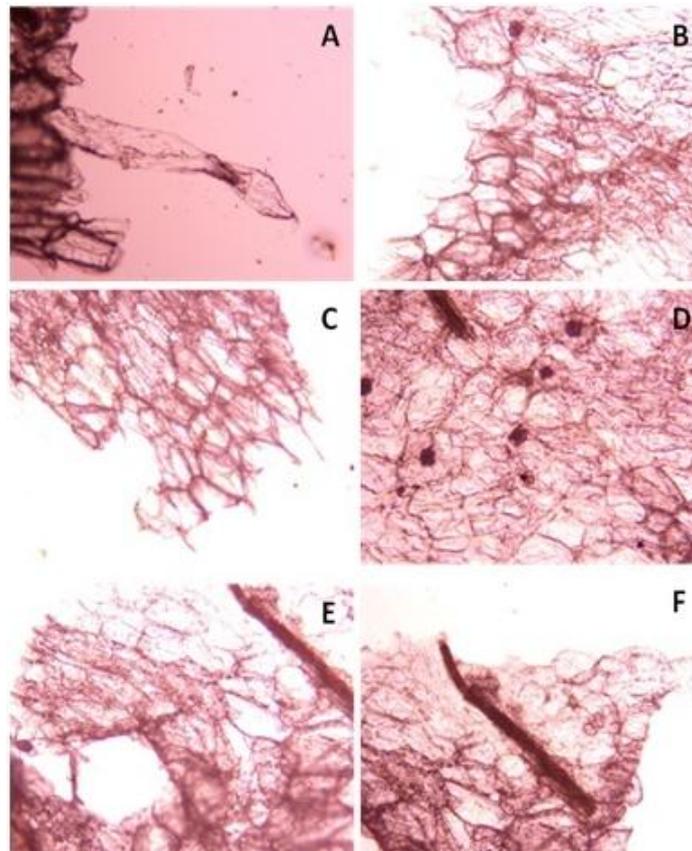


Fig. 8. Transverse section (TS) of *Tribulus terrestris* fruit; Unstained slides (A-F); showing presence of trichome (A 40X); epidermal cells showing the presence of parenchyma and sclerenchyma in different views

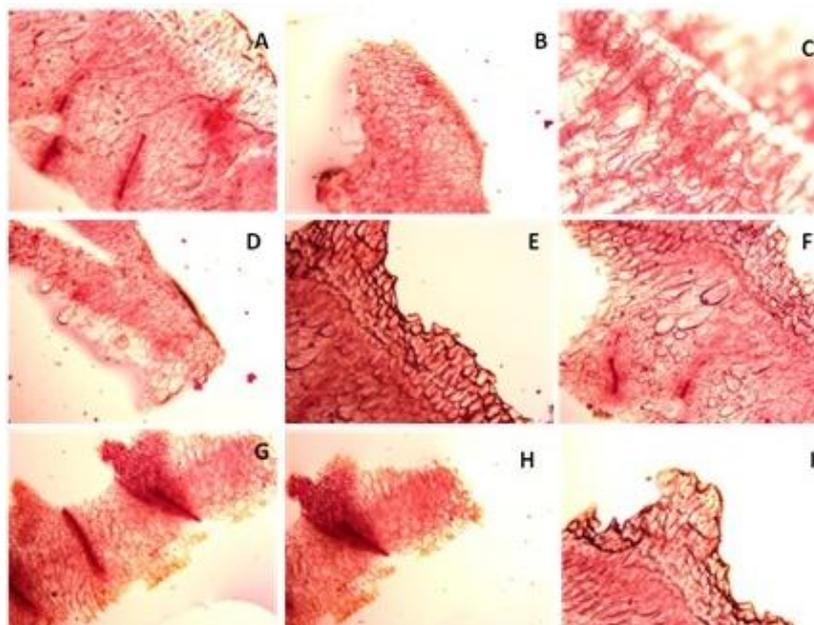


Fig. 9. Transverse section (TS) of *Tribulus terrestris* fruit; Stained slides (A-I); Stained with safranin-pink slides; showing parenchyma and sclerenchyma in the epidermal cells (A, B, D, E, F, G, H, I 10X; C 40X); thin walled sclerenchyma cells (G, H)

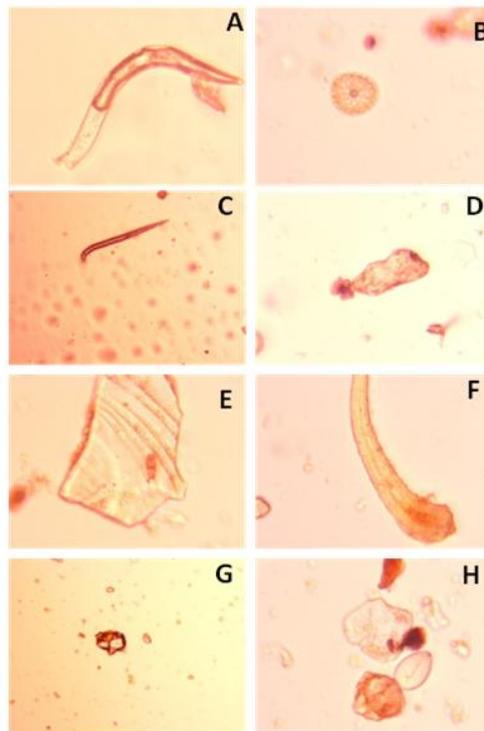


Fig. 10a. Powder microscopy of *Tribulus terrestris* fruit; Unstained slides (A-F); showing multicellular covered trichomes (A,C,F) ; sclereid (B), parenchyma cells (D) stone cells and calcium oxalate crystals (D, E, G, H)

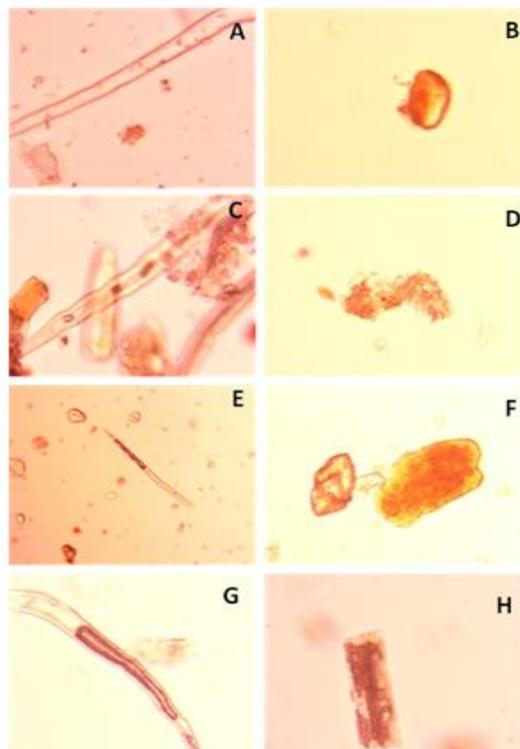


Fig. 10b. Powder microscopy of *Tribulus terrestris* fruit showing; epidermal trichomes - simple, short, long, thick walled, multicellular covered trichomes (A, C, E, G) ; sclereid (B), parenchyma cells (D) Parenchyma cells (D); stone cells (B, F) ; woody material (H)

4. CONCLUSION

Approaches like microscopical analysis offers a scientific basis for the use of right species of the plants to be used in medicines. The sections made for microscopy when treated with chemical reagents, different tissues exhibited different colours which can be used as standardization parameters. The anatomical characters can be used to differentiate between the different species of the same genus. Presence of rubiaceous stomata, epidermis, collenchymas, parenchyma, vascular bundles, palisade cells, cortical fibres and lamina, trichomes on the cuticle of lamina in *Calotropis procera* leaf and epicarp cells showing clusters of calcium oxalate crystals, xylem vessels, group of thin walled sclerenchymatous fibres stone cells, trichomes sclereids in *Tribulus terrestris* fruit can be used as identifying characters to check piracy and hence make available true botanicals to the manufacturers of drugs and consumers.

RESEARCH SIGNIFICANCE

The study highlights the efficacy of "Herbal medicine" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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