Open Access Library Journal

Comparative Anatomical Study of the Stems and Leaflets of *Tribulus longipetalous*, *T. pentandrus* and *T. terrestris* (Zygophyllaceae)

Hatil Hashim Elkamali, Ahlam Salih Eltahir, Ibrahim Saeed Yousif, Ahmed Mohamed Haroun Khalid, Elneel Adam Elneel

Botany Department, Faculty of Science and Technology, Omdurman Islamic University, Omdurman (Khartoum), Sudan
Email: ahlam.eltahir@yahoo.com

Received 8 June 2016; accepted 12 August 2016; published 15 August 2016

Abstract

In this study the anatomical characters of the stems and leaflets of *T. longipetalous* Viv., *T. pentandrus* Benth. and *T. terrestris* L. were compared to assist as a relevant source of information and contribute towards the standards to dispose the quality and identity of these plants to avoid adulterations. Transverse sections of the stems and leaf lets of the three species were done using wax method technique. The anatomical structures of the three studied species are very similar. The stems are formed of one layer epidermis, parenchymatous cortex, pericyclic fibers above the collateral vascular bundles, primary and secondary xylems and phloems, narrow medullary rays and wide parenchymatous pih. The densities of the epidermal hairs in the stems are found to be larger in *T. pentandrus* followed by *T. longipetalous* and lastly *T. terrestris*. There are sclrenchymatous fibres in the pith of *T. longipetalous* and *T. pentandrus* but not in *T. terrestris*. The leaflets are dorsiventral, showing kranz structure and different types of hairs. The densities of the epidermal hairs in the leaflets are found to be larger in *T. longipetalous* followed by *T. pentandrus* and lastly *T. terrestris*.

Keywords

Anatomy, Stem, Leaflets, *Tribulus longipetalous*, *T. pentandrus*, *T. terrestris*

Subject Areas: **Plant Science**

How to cite this paper: Elkamali, H.H., Eltahir, A.S., Yousif, I.S., Khalid, A.M.H. and Elneel, E.A. (2016) Comparative Anatomical Study of the Stems and Leaflets of *Tribulus longipetalous, T. pentandrus* and *T. terrestris* (Zygophyllaceae). *Open Access Library Journal*, **3**: e2810. http://dx.doi.org/10.4236/oalib.1102810

1. Introduction

The use of medicinal plants has become very popular in recent years. Standardization and quality control are essential analytical tools to assure the correct identification of drugs. Microscopy allows the identification of herbal drugs and the detection of individual components of a mixture. It is highly important to ensure quality and purity of herbal medicines in order to maximize the efficacy and minimize adverse side effects [1].

Zygophyllaceae is a family of trees, shrubs and herbs; they are mostly restricted to arid and semi-arid areas in the tropics and subtropics [2]. The genus *Tribulus* belongs to family zygophyllaceae Comprises approximately 25 species which grow as prostrate [3]. *Tribulus* species are perennial, but some grow as annuals in colder climates. The leaves are opposite and compound. The flowers are perfect (hermaphroditic) and insect-pollinated, with fivefold symmetry. The ovary is divided into locules that are in turn divided by "false septa" (the latter distinguish *Tribulus* from other members of its family). Some species are cultivated as ornamental plants in warm regions. Some, notably *T. cistoides*, *T. longipetalus*, *T. terrestris*, and *T. zeyheri*, are considered weeds.

T. terrestris had been subjected to different anatomical, taxonomical and chemical studies. Both morphological and microscopical characters of the fruits of *Tribulus terrestris* were outlined to help in reducing the problems of substitutions of the genuine drug [4]. Whereas the macroscopical and the microscopical characters of the flower parts were studied [5]. Hashim *et al.* (2014) [6] studied the medicinal properties, phytochemistry and pharmacology of *T. terrestris*. The morphology and anatomy of the xerophytes (dicot) of district Karak were reported within which was *T. terristris* [7].

This study aimed to describe and compare the anatomical characters of *T. longipetalous* Viv., *T. pentandrus* Benth .and *T. terrestris* L. to assist as a relevant source of information and contribute towards the standards to dispose the quality and identity of these plants in future exploration, The literature survey revealed that no anatomical studies were carried out on any morphological part of neither *T. longipetalous* nor *T. pentandrus*.

2. Materials and Methods

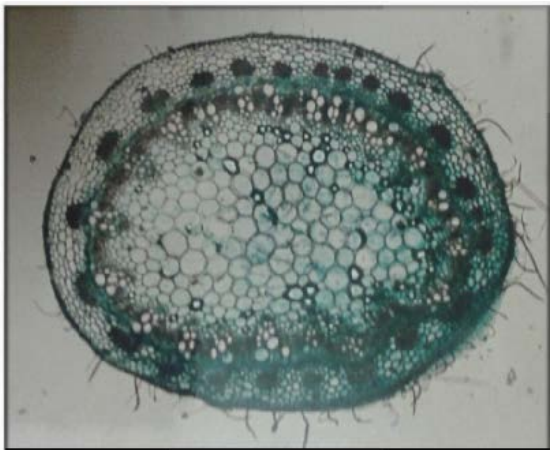
The studied plants *Tribulus longipetalous*, *T. pendulatus* and *T. terrestris* (Zygophyllaceae) were collected from Khartoum State (Central Sudan), taxonomically identified and herbarium specimens were deposited in Botany Department Faculty of Science and Technology, Omdurman Islamic University.

Stems and leaflets of the studied plants *T. longipetalous*, *T. pendulatus* and *T. terrestris* were sectioned and studied anatomically. Wax method technique [8] was used. The plants parts were fixed for at least 72 hours using FAA (formalin:acetic acid:alcohol 90:5:5) fixative, washed several times using distilled water, dehydrated using serial concentrations of alcohol 50%, 70%, 90%, 95% and 100%, respectively. For clearing, they were transferred every three hours from a mixture of 1:1 cedar wood oil: absolute alcohol, into pure cedar wood oil followed by a mixture of cedar wood oil and xylene and finally left overnight in pure xylene. Wax embedding was carried out in an oven adjusted at 60˚C, embedded in clear wax and sectioned using a rotatory microtome. Staining was done using safranine and fast green stains, sections were mounted in a drop of Canada balsam, covered and left to dry. Five prepared slides of each of the studied plant parts were repeatdly examined under the microscope; the eye piece lens was (×10) whereas the objective lenses were (×4 and ×20), the slides were photographed using a camera fitted with the microscope.

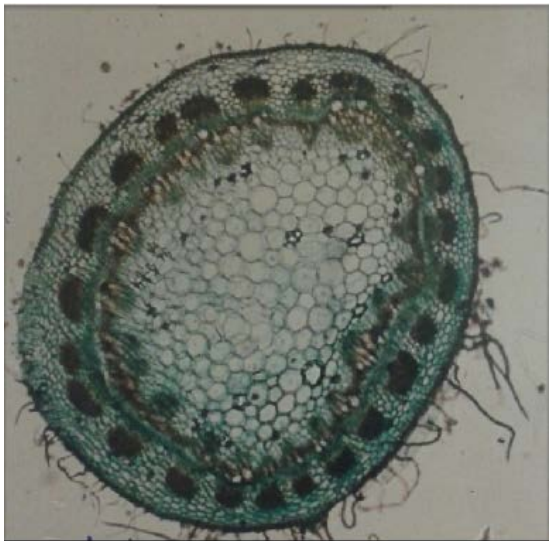
3. Results and Discussion

3.1. The Stems

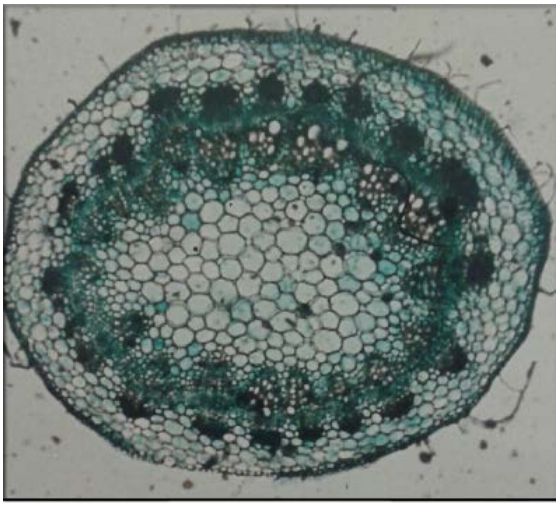
The transverse sections of the stems of the three species were found to be circular to oval in shape (**Figures 1(a)-(c)**). The outermost layer is the epidermis, it is formed of one layer of small polygonal cells covered by thick cuticles. Epidermal hairs are long, simple unicellular and they are much denser in *T. pentandrus* followed by *T. longipetalus*. The cortical region is preceded by the hypodermis which is formed of 1 - 2 cells thick of collenchyma, followed by 3 - 4 of parenchyma cells which are isodiametric with small intercellular spaces. Pericyclic fibers are formed of groups of sclerenchyma cells (6 - 8 cells thick) they are separated by parenchyma cells. Primary and secondary phloems are found below the pericyclic fibers. The fasicular cambium is connected with the interfasicular cambium forming a complete vascular cambium ring of 3 - 4 cells thick. The secondary xylem is formed of large vessels separated by xylem parenchyma cells, the primary xylem is found in the inner part it is formed of xylem vessels separated by primary xylem parenchyma.



(a)



(b)



(c)

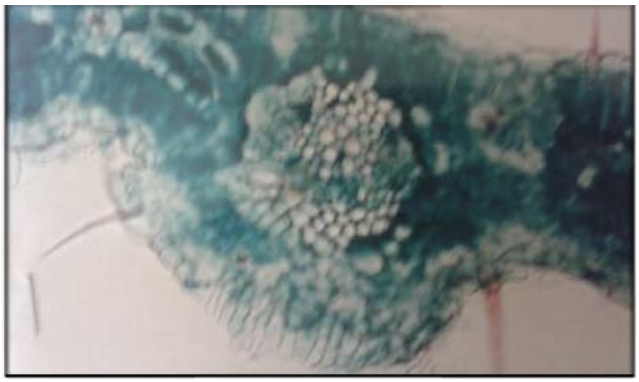
Figure 1. Transverse sections (×40) of the stems of (a) *T. longipetalus* (9 mm ≡ 50 µ); (b) *T. pentandrus* stem (7 mm ≡ 50 µ); (c) *T. terrestris* (3 mm ≡ 50 µ).

The vascular bundles are located very close to each other and they are separated by narrow primary and secondary medullary rays. The pith is wide and it is formed of compact parenchyma cells, some sclrenchyma fibers are found in between the parenchyma cells, they are of larger numbers in *T. longipetal* followed by *T. pentandrus* and they are not found on *T. terrestris.*

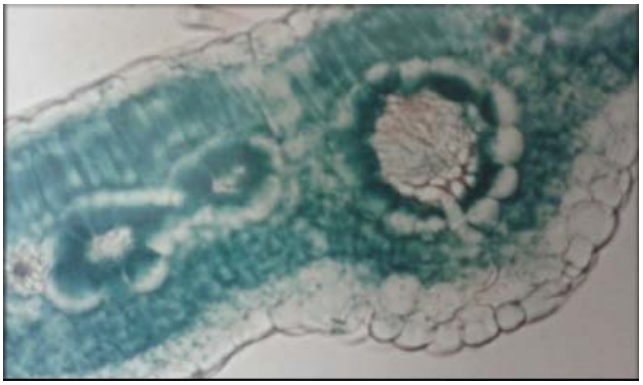
3.2. The Leaflets

The transverse sections of the leaflets of the three species are formed of one layer of polygonal epidermal cells of different sizes, they are interrupted by small stomata (**Figures 2(a)-(c)**). Unicellular, simple glandular and non-glandular hairs are found, the epidermal hairs densities are larger in *T. longipetalus* and *T. pentandrus* than in *T. terrestris.* The epidermal cells are covered by thick cuticles.

(a)



(b)



(c)

Figure 2. Transverse section of *T. terrestris* leaflet (a) *T. longipetalus*; (b) *T. pentandrus*; (c) *T. terrestris* (×200).

The leaflets are dorsiventral; the upper epidermisis followed by one layer of palisade cells which are long compact chlorenchyma cells. The spongy parenchyma layers are found below the palisade layer and they are formed of 3 - 4 layers of chlorenchyma cells, druses of calcium oxalates are scattered within the spongy parenchyma. The vascular bundles are found on the region between the palisade and spongy parenchyma layers. The vascular bundles are surrounded by sheaths of chlorenchyma cells (kranz structures). In the midrib region the vascular bundles are preceded by a palisade layer and on the lower side followed by chlorenchyma and collenchymas cells. The vascular bundles are collateral formed of xylem to the upper epidermis and phloem to the lower epidermis. The lower epidermis is formed of cells of smaller sizes than the cells of the upper epidermis but the epidermal hairs are denser and the stomatal numbers are larger.

3.3. Discussion

The three studied species show anatomical characters typical to that of the family Zygophyllaceae in that they are formed of compact stems, secretary cavities are absent, internal phloem absent, primary medullary rays are narrow. The structure of the leaflets showed distinguished xeromorphic adaptations as stated [9], the reduced stomatal density and the presence of trichomes are adaptations characteristics for plants to live in water limited areas. Leaflets veins are collateral composed of adaxial positional xylem and abaxialy situated phloem. The leaf lamina is dorsiventral, Anna and Andon (2011) [10] stated that the leaf lamina in *T. terrestris* is isobilateral, but they agreed with this study in that the mesophyll with reduced intercellular spaces and the stomata are small.

Kranz structure (which is the large green sheath cells, thick walled specialized chlorenchymas surrounding the veins) is very clear. The palisade cells extend radially from the bundle sheath cells where as in most of dicotyledons leaves there are no palisade cells in the mid rib region instead there are collenchymas cells. Spherical star-shaped druses occur throughout the middle part of the lamina, druse crystals are distribution in the mesophyll tissue of the leaf and cortex tissue of the stem [11]. Well developed cuticle on both surfaces reduces water loss and play role in controlling surface temperature by reflecting and alternating the incoming radiations. The larger densities of the epidermal hairs in *T. pentandrus* and *T. longipetalous* enable them to be found in drier regions than *T. terrestris*. Further studies using scanning electron microscopy technique are very important to reveal details of the plant surfaces specially epidermal features such as hairs, trichomes and cell types besides the gross morphological characters which are very useful in systematic plant anatomy.

References

[1] Serrano, R., da Silva, G. and Silva, O. (2010) Application of Light and Scanning Electron Microscopy in the Identification of Herbal Medicines. *Microscopy*: *Science, Technology, Applications and Education*, 182-190.

[2] Beier, B.A., Chase, M.W. and Thulin, M. (2003) Phylogenetic Relationships and Taxonomy of Subfamily Zygophylloideae (Zygophyllaceae) Based on Molecular and Morphological Data. *Plant Systematic and Evolution*, **240**, 11-39. http://dx.doi.org/10.1007/s00606-003-0007-0

[3] Boulous, L. (2000) Flora of Egypt. Vol. 2. Al Hadara Publishing, Cairo, 26-31.

[4] Kevalia, J. and Patel, B. (2011) Identification of Fruits of *Tribulus terrestris* Linn. and *Pedalium murex* Linn A Pharmacognostical Approach. *Ayu*., **32**, 550-553.

[5] Samy, N.M., Bishr, M.M., Ahmed, A.A., Sayed, M.H. and Kamel, S.M. (2013) Pharmacognostical Studies on Flower of *Tribulus terrestris* L. *Journal of Pharmacognosy and Phytochemistry*, **1**, 18-22.

[6] Hashim, S., Bakh, T., Marwat, K.B. and Asad, J. (2014) Medicinal Properties, Phytochemistry and Pharmacology of *Tribulus teresstris* L. (Zygophyllaceae). *Pakistan Journal of Botany*, **46**, 399-404.

[7] Bibi, H., Afzal, M., Kamal, M., Ullah, I., Sohail, Mulk, K.S., Sher, A.A., Ullah, I., Ziaulhaq, Ali, A., Ali Khan, S. and Ur Rahman, I. (2015) Morphological and Anatomical Characteristics of Selected Dicot Xerophytes of District Karak, Khyber Pakhtunkhwa, Pakistan. *Middle-East Journal of Scientific Research*, **23**, 545-557.

[8] Johansen, D.A. (1940) Plant Microtechnique. McGraw-Hill Book Co., New York.

[9] Semerdjieva, I. (2011) Studies on Leaf Anatomy of *Tribulus terrestris* L. (Zygophyllaceae) in Populations from the Thracian Floristic Region. *Biotechnology and Biotechnological Equipment*, **25**, 2373-2378. http://dx.doi.org/10.5504/BBEQ.2011.0038

[10] Nikolova, A. and Vassiler, A. (2011) A Study on *Tribulus terrestris* L. Anatomy and Ecological Adaptation. *Biotechnology and Biotechnological Equipment*, **25**, 2369-2372. http://dx.doi.org/10.5504/BBEQ.2011.0032

[11] Konyar, S.T., Öztürk, N. and Dane, F. (2014) Occurrence, Types and Distribution of Calcium Oxalate Crystals in Leaves and Stems of Some Species of Poisonous Plants. *Botanical Studies*, **55**, 32.