Research Article

MACROSCOPIC AND MICROSCOPIC CHARACTERIZATION OF THE AERIAL PARTS OF *TRIBULUS ALATUS* (ZYGOPHYLLACEAE) GROWING IN EGYPT

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ABSTRACT

Tribulus alatus is a promising medicinal plant. Macroscopic and microscopic studies were carried out on leaflet, rachis, stem and fruit as well as powdered aerial parts. Powder microscopy revealed the presence of anomocytic stomata, numerous non glandular unicellular trichomes with different sizes, large cluster crystals of calcium oxalate, pericyclic fibers and lignified xylem vessels of leaflet, rachis and stem, in addition to sclerides of the fruit. The results of this study will provide a valuable tool for identification and quality control of the plant.

Keywords: *Tribulus alatus*, Zygophyllaceae, aerial parts, microscopy.

INTRODUCTION

The genus *Tribulus* of the Zygophyllaceae comprises about 25 species which grow as shrubs or herbs in subtropical areas around the world^{1,2}. Among these species is *Tribulus alatus* found in dry sandy soil in warm-temperate regions. Its fruits are used in Pakistan for the treatment of urinary disorders and cough^{3,4}. In a previous study the isolation and characterization of eight steroidal saponins, together with six flavonol glycosides have been reported from *Tribulus alatus* growing in Egypt⁵. Several biological studies have been carried out on the extracts of *Tribulus alatus* aerial parts and fruits. It was reported to have antioxidant⁶, diuretic⁷ and testosterone-increasing activities⁸. Till date no work was reported in the current literature describing the botanical features of *Tribulus alatus*. In this study the authors reported the macroscopic and microscopic diagnosis of leaflet, rachis, stem and fruit of this promising plant.

MATERIALS AND METHODS

Plant material

The aerial parts of *Tribulus alatus* Del. (Zygophyllaceae) were collected from Nasr-City, Cairo, Egypt, and identified by late Professor Nabil El Hadidi, Faculty of Sciences, Cairo University, Egypt. A voucher specimen was deposited at Pharmacognosy department, Faculty of Pharmacy, Al Azhar University and at Herbarium Horti Botanici Pisani (Flora Aegyptiaca), Pisa, Italy (number 3978/1).

Chemicals

Formalin, acetic acid, ethanol, xylol and safranin were purchased from Sigma-Aldrich Co.

Method used for anatomical study

Anatomical sections of the leaflet, rachis, stem, fruit and powdered samples were prepared for the microscopic studies. The staining was done using standard laboratory methods.^{9,10} Fresh material was fixed in (formalin-acetic acid –70 % ethanol, 5:5:90, V/V). The leaflet, rachis, stem, and fruit were

sectioned using rotary microtome to the thickness of 10 to 30 µm. All sections were stained in safranin (1 % solution in 50 % ethanol) for 15 minutes, then washed in ethanol (50, 70, 100 %) respectively for 5 minutes, then immersed in xylol for 5 minutes. The slides were dried at 50°C for 72 hours and examined by Carl Zeiss transmitted light photomicroscope II using different magnifications.

RESULTS AND DISCUSSION

Macroscopic Diagnosis

The plant (Figure 1) is an annual herb attaining about 60 cm long. It produces alternate, paripinnate compound leaves with 4-6 pairs of leaflets. The leaflets are green on the upper surface, and dark green on the lower one. The lamina is oblong to elliptical, having acute apex, entire margin, asymmetric base and pinnate reticulate venation. The mid rib is more prominent on the lower surface. It measures 0.4 - 0.9 cm in length and 0.1 - 0.4 cm in width. The odor is characteristic and the taste is slightly bitter. The leaf rachis is cylindrical, pale green, hairy, and measures 2 - 3.5 cm in length and 0.2 - 0.3 cm in diameter. The stem is nearly cylindrical in shape, about 20 to 60 cm in length, green to grayish green in color with pubescent surface. The fruit is small globular in shape, pubescent, schizocarp, separating into five indehiscent one-seeded fruitlets. Each fruitlet has two broad wings and few spinescent teeth.

Microscopic Diagnosis

The leaf

The transverse section of the leaflet (Figure 2) showed isobilateral structure. The epidermal cells are rectangular to square in shape, covered with smooth cuticle and bearing numerous non-glandular trichomes. The mesophyll consists of a single layer of palisade beneath each epidermis enclosing layers of spongy tissue. The upper palisade layer is continuous over the mid rib as well as in the leaflet margin regions (Figure 3). The palisade cells are short cylindrical with straight anticlinal walls.

Figure 1 Macroscopic characteristic of *Tribulus alatus*

Figure 2 Transverse section of *T. alatus* leaflet. a: T.S of the whole leaflet; (X100) b: vascular bundle; c: mesophyll and lower epidermis in the midrib region (X400).

Figure 3: T.S of leaf margin region of *T. alatus*
a: the whole margin (X200); b: the upper part (X400)

Figure 4: T.S. of the leaf - rachis of *T. alatus*.
a: leaf rachis (X100); b: cortical region; c: vascular bundles (X400)

Figure 5: T. S. of the stem a: T.S. of young stem; b: T.S. of old stem (X100); c: cortical region; d: vascular bundles of the young stem; e: vascular bundles of the old stem; f: pith parenchyma of the young stem (X400).

Figure 6: T. S. of the fruit of *T. alatus*. a: T.S. of the whole fruit (X25); b: T.S. of fruitlet (X50); c: epicarp and mesocarp (X400); d: non glandular unicellular hairs (X200).

Figure 7: Microscopic characterization of powdered aerial parts of *T. alatus*. 1: epidermis with anomocytic stomata; 2: non glandular trichomes; 3: pericyclic fibers; 4: spiral and annular xylem vessels; 5: sclerides of the endocarp and mesocarp; 6 : cluster crystals of calcium oxalate.

The spongy tissue consists of nearly rounded parenchyma cells. Numerous large cluster crystals of calcium oxalate are present in the mesophyll. The cortical tissue of the midrib region is formed of thin parenchyma cells, the inner most layers consist of large barrel-shaped cells forming a distinct endodermis. The vascular bundle consists of radiating lignified xylem tissue situated above a narrow zone of phloem. The pericycle is arranged in two bands of lignified fibers above and below the vascular bundle. The transverse section of the leaf - rachis (Figure 4a) is nearly rounded in shape with two lateral projections or ridges. The cortical tissue is formed of parenchyma cells with minute intercellular spaces. Large clusters crystals of calcium oxalate are scattered especially near the vascular bundles (Figure 4b). Several vascular bundles are present and arranged in a circle. Additional smaller vascular bundles are found under each ridge and a group of pericyclic sclerenchyma is present abutting each vascular bundle. The vascular bundles are formed of groups of thin phloem elements separated from radiating lignified xylem tissue by two layers of thin cambium cells (Figure 4c).

The Stem

The transverse section of the stem is circular in shape in both young and old stems (Figure 5a and b respectively). The epidermal cells are slightly axially elongated, covered with smooth cuticle and carrying non-glandular trichomes with different lengths. The cortex is relatively narrow consisting of several layers of thin-walled parenchyma containing scattered cluster crystals of calcium oxalate (Figure 5c). The pericycle consists of patches of lignified fibers interrupted by parenchyma cells forming a complete circle around the stele (Figure 5d). The stele consists of a ring of vascular bundles separated by parenchyma in the young stem (Figure 5a and d), but forming a complete ring in the old one (Figure 5b and e). The vascular bundles are formed of groups of thin phloem elements separated from radiating xylem tissue by two layers of thin cambium cells. The xylem is composed of lignified vessels, parenchyma and xylem fibers separated by uni- or biseriate medullary rays. The pith in young stem is formed of a wide zone of parenchyma cells containing scattered cluster crystals of calcium oxalate (Figure 5f), while there is a

central cavity and remains of parenchyma cells in the old one (Figure 5b).

The Fruit

The transverse section in the fruit (Figure 6a) showed 5 indehiscent fruitlets which are conical shaped, having two broad wings and each fruitlet shows one locule containing one seed (Figure 6b). The cells of the epicarp are polygonal tabular cells with straight anticlinal walls, covered with smooth cuticle and showing non glandular unicellular hairs (Figure 6c), some of them have characteristic large size, thick striated walls and with enlarged bases (Figure 6d). The mesocarp consists of polygonal thin cellulosic parenchyma and stone cells, vascular strands extended through the mesocarp from the endocarp in the wing regions. The endocarp is formed of sclerenchymatous cells which are highly thickened and lignified.

Powdered aerial parts

The powder of the aerial parts is greyish green in color having characteristic odor, slightly bitter taste and characterized microscopically (Figure 7) by the presence of:

- Fragments of upper and lower epidermis of leaflets showing anomocytic stomata and numerous non glandular unicellular trichomes. The epidermal cells are polygonal nearly isodiametric in top view with straight anticlinal walls covered with smooth cuticle.
- Numerous non glandular trichomes of different shapes and sizes (112 μ m to 1.12 mm in length) which are unicellular with enlarged bases, tapering ends and covered with either smooth or striated cuticle.
- Fragments of pericyclic fibers which are large fusiform with tapering ends and moderately thick lignified walls (825 μ m to 1.5 mm in length, 25 μ m to 37 μ m in width).
- Fragments of lignified spiral and annular xylem vessels.
- Sclerides of the fruit endocarp and mesocarp with highly thick lignified sinuous walls
- Large cluster crystals of calcium oxalate.

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