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Leaf and stem anatomy of C*ymbopogon citratus* and *Cymbopogon schoenanthus* in Sudan

Ahlam Salih Eltahir*[1] and Bouran Ibrahim AbuEReish[2]

[1]*Department of Botany, Faculty of Science and Technology, Omdurman Islamic University*
[2]*Department of Botany, Faculty of Science, University of Khartoum*

ABSTRACT

In this study a comparison between the anatomical structures of the leaves and stems of the two medicinal plants Cymbopogon citratus and Cymbopogon schoenanthus was carried out to out line the diagnostic characters; thus helping to identify them, to classify them using the anatomical characters and to distinguish between them to avoid adulteration. This study showed that the differences between the two species are as follows: the spongy parenchyma of C. citratus is formed of 1-2 cells thick following the upper epidermis, in C. schoenanthus the upper epidermis is formed of small cells followed by patches of sclerenchyma cells only above the vein regions and the spongy parenchyma are formed of 3-5 layers of small cells. Kranz structure which is the vascular bundles are embedded in chlorenchyma cells, is found in the two species but it is well developed in C. citratus where the chlorenchyma are incircling the vascular bundles but in C. schoenanthus, the chlorenchyma are found only on lateral sides of the vascular bundles. In C. citratus, the lower epidermal cells just below the vascular bundles are projecting forming papillae, in C. schoenonthus the papillae are small. In the epidermal cells of the leaf of C. citratus there are tannin depositions they give positive results with $FeCl_3$ reagent they appear as large dark cells. Small quantities of oil was detected in the leaf epidermal cells and the mesophyll. The numbers of vascular bundles are more in the stem of C. citratus and the amount of sclerenchyma cells surrounding them are more. The number of xylem vessels in a vascular bundle is 3-4 xylem vessels.

Key words: C*ymbopogon citrates, schoenanthus,* microscopical, leaves, stems.

INTRODUCTION

This study is aimed to provide illustrated anatomical descriptions of the leaves and stems of *Cymbopogon citratus* Dc. Stapf and *Cymbopogon Schoenanthus* (L.)Sprengel, pl. Min.Cog. Pugil Prim 2:15 (1815), family Poaceae(Graminae) and to outline the differences between them. These plants were selected for their great importance in Sudanese folkloric medicine

Plant anatomy deals with the structure, contents and development of cells and tissues. It is of primary importance for all aspects of research in plant sciences such as morphogenesis, physiology, ecology, taxonomy, evolution, genetics, reproduction etc[1].

The systematic anatomy is mainly aimed towards relating structure particularly of vegetative organs, to taxonomic classification of the plants in which the characters are exemplified. Accurate microscopical and macroscopical descriptions of the medicinal plants must be carried out to maintain standards of safety and quality and to authenticate the crude drug materials properly [2]. Most of the drugs that are extracted from leaves, barks, roots and rhizomes may be difficult to identify from their macroscopical appearance only; they must be complemented by microscopical characterization.

The microscopical features of the medicinal plants were studied for different purposes They may be studied to outline the diagnostic features; thus helping to identify them, to classify them using the anatomical characters and to distinguish between similar species to avoid adulteration [3].

Family Poaceae(Graminae) plants are herbaceous or perennial herbs, rarely shrubs or trees; stems erect, ascending or prostrate and creeping, usually branched at the base. Leaves alternate, distichously, simple sometimes crowded at the bases of the stems, consisting of sheath, legule and lamina. Flowers usually hermaphrodite, sometimes unisexual, small and inconspicauos consisting of stamens and pistils.

EXPERIMENTAL SECTION

The plant materials for this study were collected from the Botanical garden of Khartoum and they were authenticated.

Experimental work:

Preparation of Permanent Slides:

The permanent slides were prepared from leaves and stems [4]. They were segmented and fixed for at least 72 hours using the standard fixative (FAA), formaldehyde- glacial acetic acid – 70% ethyl alcohol (5:5: 90 v/v). The fixed segments were washed with distilled water and then dehydrated using serial concentrations of ethyl alcohol 50%, 70%, 90% ,95% and 100% respectively. The segments were kept for one day in each concentration. For clearing the segments, 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 where the plant segments were transferred every 40 minutes from a mixture of wax and xylene, into

pure wax and finally into another container of pure wax. The melted wax, containing the plant segments were poured into a mold, cooled in water and trimmed.

The segments were sectioned transversally using a rotatory microtome (Leitz 1512 west Germany), adjusted at 4-7 microns. Using a brush, the ribons of sections were collected on glass slides, which had been wetted with egg albumin to keep the sections attached to the slides. The slides were left overnight on a hot plate to give maximum expansions of the tissues. Dewaxing was done by immersing the slides with their sections in pure xylene for one minute. The sections were then dehydrated by transferring them into series of ethyl alcohol concentrations 100%, 95%, 90%, 70% and 50% respectively and stained by flooding them with safranin stain dissolved in 50% ethyl alcohol. They were then dehydrated back into 50% ,70%, 90%, 95% and 100% respectively and stained with fast green stain dissolved in absolute ethyl alcohol, washed in clove oil, covered with a drop of Canada balsam, and covered with a cover slip. The prepared slides were left to dry in an oven adjusted at 60˚ C for at least three days.

Microscopical Examinations:
The prepared permanent slides were examined using (Leitz Dialux 22 EB) microscope. The eye piece lens was (x10) whereas the objective lenses were (x4, x10 and x25). Measurements were carried out for all materials studied using the eye piece micrometer which was calibrated using the stage micrometer. Ten readings were carried out for each parameter and then the mean value was calculated. Drawings were made for the temporary slides using the drawing tube fitted in the microscope. The prepared slides were photographed using (Leitz Dialux 20) microscope fitted with (Wild PMPS II) camera, using Kodak coloured films 36 ExP. 24 x 36mm ISO 100/21o..

RESULTS AND DISCUSSION

Morphology
Cymbopogon citratus has short underground stems, leaves simple, alternate, linear, 5.0-7.0 cm. long. 0.5-1.5 cm. wide, sheathed, apex acute, parallel venation, central vein appear more in lower epidermis. *Cymbopogon schoenanthus* stems erect, tufted 9cm. long, culms slender, erect, glabrous 3-4 noded. Leaf simple, alternate, linear 5-7 cm. long., 1cm. wide, sheathed apex spiny entire.

Anatomy
The upper epidermis consists of a single layer of elongated cells, they are interrupted by large bulliform cells in *C. citratus*, these cells are larger than the typical epidermal cells, they are thin walled and have large vacuole they are called bulliform cells and are described as motor cells involved in involution and folding of leaves. These cells are often found in Gramineae and other monocotyledons [5]. The spongy parenchyma of *C. citratus* is formed of 1-2 cells thick following the upper epidermis (plate 1 a). In *C. schoenanthus* the upper epidermis is formed of small cells followed by patches of sclerenchyma cells only above the vein regions (plate 1 b). The spongy parenchyma are formed of 3-5 layers of small cells. The vascular bundles are embedded in chlorenchyma cells thus making a Kranz structure in the two species, but it is well developed in *C. citratus* where the chlorenchyma are incircling the vascular bundles but in *C. schoenanthus*, the chlorenchyma are found only on lateral sides of the vascular bundles. Kranz

structure is reported to occur in many monocotyledonous plants notably in certain grasses and sedges [6,7].

The vascular bundles are surrounded by sclerenchyma sheath, typical of monocotyledons, they are formed of xylem vessels separated by xylem parenchyma cells followed by the phloem tissue. The base of the vascular bundle is formed of about 3-4 layers of sclerenchyma. The lower epidermis is formed of one layer of small cells. In *C. citratus*, the lower epidermal cells just below the vascular bundles are projecting forming papillae but in *C. schoenonthus* the papillae are small. There are depositions of tannins in the cell walls of all abaxial epidermis of *C. citratus*, few are found on the upper epidermis. *C. citratus* and *C. schoenanthus* leaves when tested using Sudan III stain, large quantities of oil was detected. In the leaf epidermal cells and the mesophyll. This agrees with what was reported in the literature. [8] stated that in *C. citratus* leaf blade the essential oil droplets were observed in the epidermis and mesophyll cells and at a lower frequency in the bulliform cells and phloem. In the epidermal cells of the leaf of *C. citratus* there are tannin depositions they give positive results with $FeCl_3$ reagent they appear as large dark cells in the section.

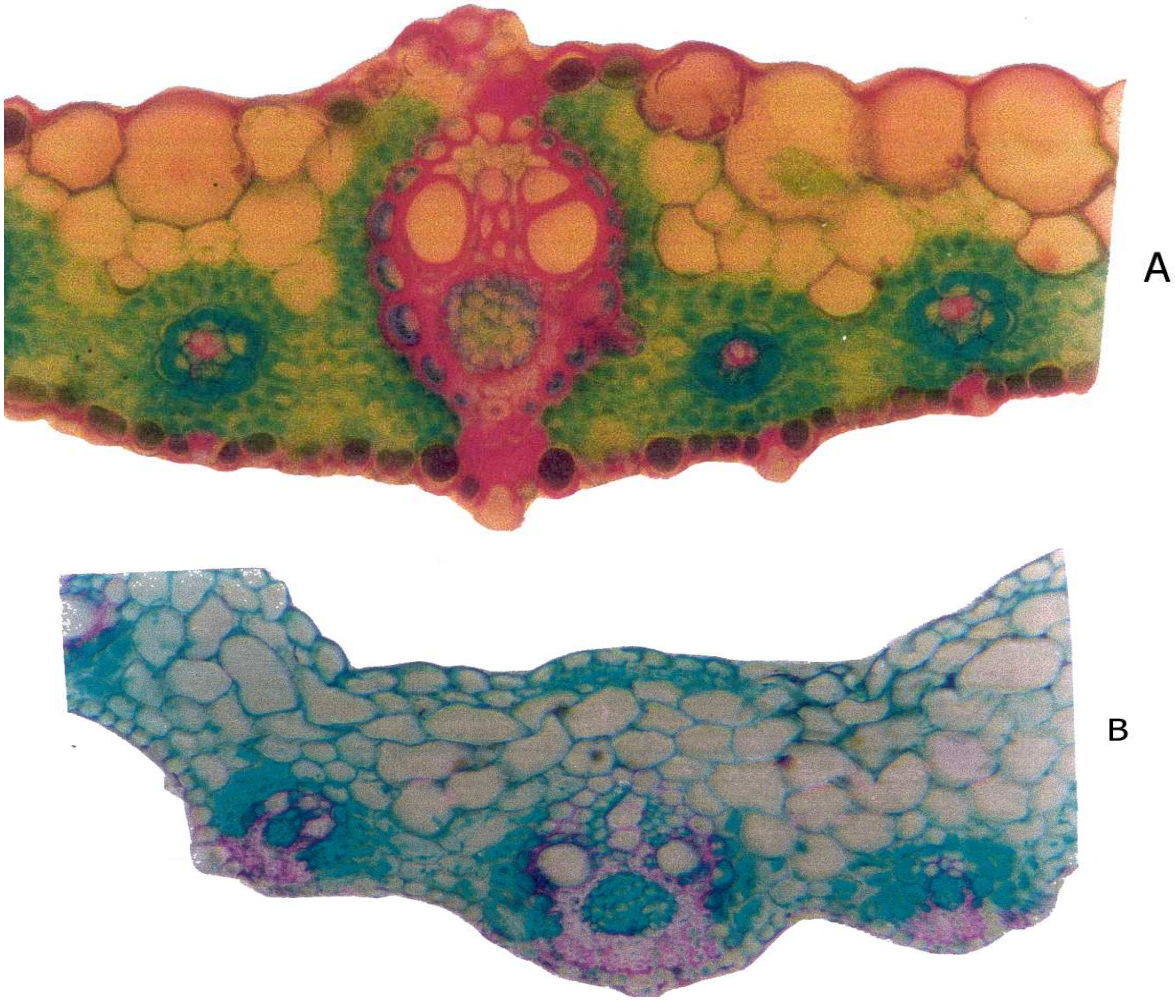
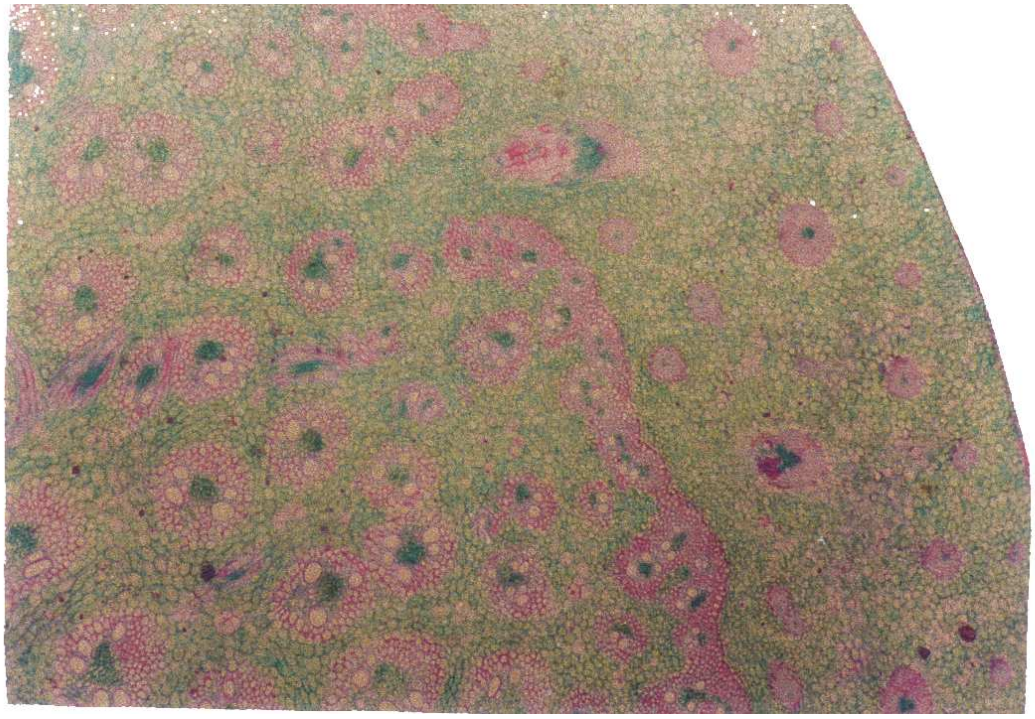


Plate 1: Transverse sections of the leaves of
a. *Cymbopogon citratus* **b. *Cymbopogon Schoenanthus***

The stem

The general structures of the transverse sections of the stems appeared as complete circles (plate 2 a and b). The stem of *C. citratus* is larger than *C. schoenanthus* stem. The epidermis is the outer most layer it is formed of rectangular cells followed by two cells thick of sclerenchyma cells in *C. schoenanthus* only. The ground mass cells are parenchymatous filling the rest of the sections except in the regions occupied by the vascular bundles. The vascular bundles (typical monocotyledonous types of vessels) are found scattered within the section they are formed of two larger metaxylem vessels to the outside and small protoxylem vessel to the inside forming V-shaped xylen. A narrow phloem region (greenish) found outside the metaxylen vessel. The numbers of vascular bundles are more in the stem of *C. citratus* and the amount of sclerenchyma bundles sheath surrounding them are more. The number of xylem vessels in a vascular bundle is 3-4 xylem vessels. An outer secondary thickening region is clear outside the primary part which could be a leaf sheath.



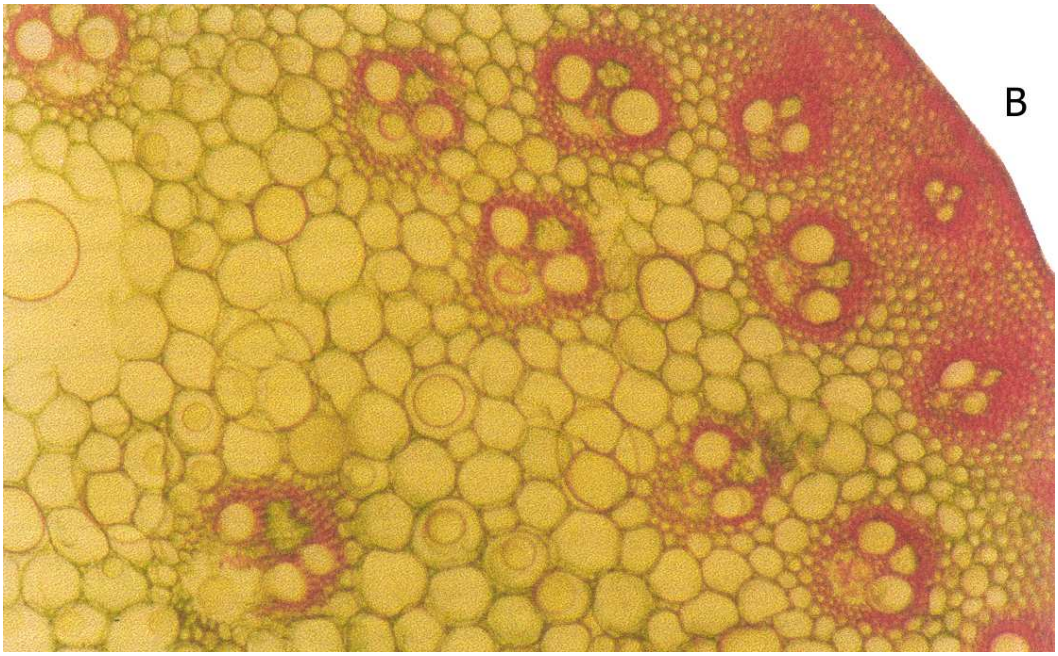


Plate 2 : Transverse sections of th stems of
a. *Cymbopogon citratus* **b. *Cymbopogon Schoenanthus***

The vascular bundles of the leaves and stems are surrounded by a bundle sheath. [9] declared that the bundle sheaths of grasses are characteristic structures used in grasses systematic. In C. schoenanthus leaf; there are batches of sclerenchyma cells following the epidermis and the cell walls are sinuous whereas those of C. citratus are straight.

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