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A Study on the Anatomy of *Zanthoxylum macrophylla* (L.) Sarg. (Rutaceae)

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Authors' contributions

This work was carried out in collaboration between both authors. Author CVI designed the study, performed the statistical analysis, wrote the protocol, and authors CVI and NAI wrote the first draft of the manuscript. Authors CVI and NAI managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Anatomical studies were carried out on various parts of *Zanthoxylum macrophylla* to determine its taxonomical data with regards to anatomical characters. Anatomical study of the transverse sections of the parts (leaf, stem, petiole and root) was carried out using microtomy while leaf epidermal study was carried out using impression technique. Results showed that the transverse sections of various parts (leaf, stem, petiole and root) of the *Zanthoxylum macrophylla* had similar features including rays in the secondary stem and root. The leaf epidermal study showed the presence of stomata on the abaxial surface only with paracytic stomatal type. The obtained data can be used to enhance proper taxonomic characterization and identification of the plant species.

Keywords: Zanthoxylum macrophylla; Rutaceae; anatomy; taxonomy.

1. INTRODUCTION

Zanthoxylum macrophylla is of the genus *Zanthoxylum* and family Rutaceae. Rutaceae commonly known as rue or citrus family which consist of plants whose fruits are berries, drupes, hesperidia, samarias, capsules and follicles [1]. The family Rutaceae is of great economic

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importance in warm temperate and sub-tropical climate for its numerous edible fruits of the citrus genus, such as the orange, lemon, calamansi, lime, kumquat, mandarin and grape fruit. *Zanthoxylum* comprises about 549 species distributed worldwide mainly in tropical and temperate regions [2]. This genus includes trees and shrubs, usually dioecious. They are economically important because of their alimentary, industrial and medicinal applications [3,4].

Z. macrophylla is a spreading shrub or more often, small tree growing up to 6-12 m tall. The trunk is generally rough with grey bark and grows to about 0.25 m (0.82 ft) in diameter. The stems are glabrous, grey with solitary prickles [5,6]. Leaves are alternate, glabrous, imparipinnately compound with 5–11 opposite or alternate leaflets with a length up to (12–20) cm; petiole 2–5 cm long. It is well known for its various uses in trado- medical practice; the root, root-bark and other parts of the plant are used in treating dental diseases, elephantiasis, sexual impotence, gonorrhoea, malaria, dysmenorrhoea, abdominal pain and as bio-pesticide for stored food protection [7]. They are considered antiseptic, analgesic and diaphoretic [8,9,10,11,12]. The leaf, stem and root are of high nutritional values when compared to some vegetables and fruits [13].

Anatomical characters are very useful in the determination of relationship in orders and genera and their features have played an increasingly important role in phylogenetic relationships. [14] discovered that trichome anatomy is of immerse significance in classification at levels from the circumscription of the family down to the separation of species and even varieties. In particular it has led to an improved tribal classification within the largest genus *Combretum*. In Costaceae differences in features of vegetative anatomy suggested a separate specific status for *Costus afer* and *Costus lucanusianus* as opposed to the conspecific treatment given to them by previous researchers [15]. Anatomical characters of taxonomic importance include such characters as type of stomata, width of medullary rays, and presence of bicollateral bundles, wood characters etc [16]. The characteristics of the wood (xylem) that possess greatest taxonomic value and diagnostic features are – the vessels: the distribution, pattern diameter and frequency as seen in transverse sections etc.

Z. macrophylla is a species of great medicinal importance. However, no research work has been done especially on its anatomy, hence the need for the present study. Accordingly, the problem and focus of the researcher is to ascertain the gross internal structure of plant organs as seen in a section.

2. MATERIALS AND METHODS

2.1 Area of Study

The experiment was carried out at the Anatomy Laboratory, Department of Plant Science and Biotechnology University of Nigeria, Nsukka.

2.2 Procurement and Identification of Plant Species

The species *Z. macrophylla* used in this work was collected between April-August 2016 from Mbulu- owo, Nkanu East Local Government Area Enugu state. The species was authenticated at Department of Botany, Nnamdi Azikiwe University, Awka where the voucher specimen was deposited.

2.3 Anatomical Studies

2.3.1 The following materials were used for the anatomical studies

Photomicroscope, a staining jar, a wash bottle, a Reichert sledge microtome, a beaker, Carmel's hair brush, light B.Bran microscope with the serial No. 4F8662206.

Transverse sections were made from middle part of fully grown leaves, midpoint of petiole, centre of an internode of a young and mature stem and mature root.

The reagents and stains were 97% alcohol, absolute alcohol, 50/50 alcohol, /xylene, xylene, safranin, fast green and Canada balsam as mountant.

2.3.2 Procedure

Sectioning of leaf, stem, petiole and root of specimens was done using a sledge microtome. The sections were transferred into a staining jar and stained in safranin for 5 minutes. The safranin was drained off and the sections washed three times with distilled water. The sections were washed again with 97% alcohol

and absolute alcohol for two minutes each and counter stained in 1% fast green for 5minutes and then washed with absolute alcohol 4 times. The sections were transferred into a staining jar containing 50/50 alcohol/xylene and washed until they became very clear. Pure xylene was used to finally clear the sections in the staining dish. Canada balsam was then used to mount the specimens on slides. Each slide was carefully covered with a 22 mm x 22 mm cover- slip. The mounted specimens were observed under a light microscope and photomicrographs were made. This procedure is as outlined by [17].

2.4 Leaf Epidermal Impression

2.4.1 Impression technique

Foliar epidermis of adaxial (upper) and abaxial (lower) surfaces of *Zanthoxylum macrophylla* were prepared by impression technique method. The leaf samples were cleared by washing with water and allowed to dry. Nail varnish was applied using camel hair brush on a small portion on both the abaxial and adaxial surfaces of the leaf samples and left for about 10 – 15 minutes to dry. A second coating was applied and allowed to dry for the same length of time after which the third coating was applied and allowed to stay for up to 25 minutes. The samples were then passed through an air current for 30 minutes to ensure maximum dryness. Then, the epidermal strips of the leaves were peeled gently with the aid of a pair of forceps, and then placed on a clean slide and covered with a cover slip. The slide was viewed under the light microscope at different magnifications but the photomicrograph was taken at x400 magnification.

3. RESULTS AND DISCUSSION

The results of the study are presented in Figs. 1-11.

Z. macrophylla is a spreading shrub or more often, small tree growing up to 6-12 m tall. The trunk is generally rough with grey bark and grows to about 0.25 m (0.82 ft) in diameter (Fig. 1). The transverse section of leaf of *Z. macrophylla* (Fig. 2) showed single-layered epidermis which remains covered with thick cuticle. Beneath the epidermis are 2 – 3 layers of collenchyma cells, t followed by 3 – 4 layers of parenchyma cells. The central midrib consists of the vascular bundle; xylem (vessel) and phloem. Xylem is internal while phloem is external; the vessels are arranged in radial multiple. The transverse section of the petiole (Fig. 3) showed a single-layered epidermis. Beneath the epidermis are 2-3 layers of collenchyma cells, followed by 3 – 4 layers of parenchyma cells. The vascular bundle consists of internal xylem and external phloem. The vessels are arranged in radial multiple. In the transverse section of secondary stem (Fig. 4) the vessels are diffuse- porous and not occluded by tyloses. The pores are round in shape. Multiseriate and uniseriate rays are present. The radial longitudinal section of stem (Fig. 5) showed vessels that are not occluded by tyloses. There are procumbent ray cells. Tangential longitudinal section of stem (Fig. 6) showed multiseriate, heterocellular and procumbent rays, vessels not occluded by tyloses. In the transverse section of root (Fig. 7) the vessels are diffuse- porous and not occluded by tyloses. The pores are round in shape. Multiseriate and uniseriate rays are present. The radial longitudinal section of root (Fig. 8) showed vessels that are not occluded by tyloses. There are procumbent ray cells. Tangential longitudinal section of root (Fig. 9) showed multiseriate, heterocellular and procumbent rays, vessels not occluded by tyloses. The leaf epidermal peel (Figs. 10 and 11) showed no stomata on the adaxial surface but had stomata confined to the abaxial epidermis only (hypostomatic). The plant has paracytic stomata. Result of the study showed that the transverse sections, the radial and tangential sections of various parts (petiole, stem and root) of *Z. macrophylla* had similar features respectively. The leaf epidermal study showed the presence of stomata on the abaxial surface only with paracytic stomatal type. No previous work has been done on the anatomy of *Z. macrophylla*, but the result is in line with anatomical observations of most dicotyledonous plants such as the family Rutaceae and also in line with the anatomical observations of [18] who reported that the transverse sections of various parts of *Morinda lucida* had similar features, stomata paracytic and exist only on the abaxial epidermis only. Also in agreement with [19] who observed that stomata are present on both surfaces of leaf but are usually more on the lower epidermis in species of *Amaranthus* and *Vigna*, respectively. The stomatal result of this study is similar to the report of [20] who worked on South African species of *Combretum* and reported that majority of *Combretum* species have their stomata confined to the abaxial surface but minimal species have stomata on the adaxial surface. The stomatal types are important diagnostic character in taxonomy. This is

probably an adaptation to water loss. It might be an adaptive component of the plant to the environmental conditions of growth and/or a factor coded by the plant. Hypostomatous leaves might not only result in a higher resistance to water deficit but also in a reduced susceptibility of the plant to fungal infections, since most often fungi infect plants through stomata.

Fig. 1. *Zanthoxylum macrophylla* tree in its natural habitat

Fig. 2. T/S of *Z. macrophylla* leaf

Fig. 3. T/S of *Z. macrophylla* petiole

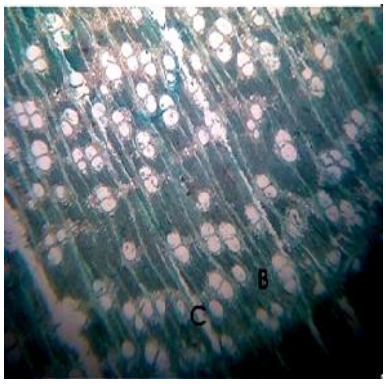

Fig. 4. T/S of *Z. macrophylla* secondary stem

Fig. 5. Radial longitudinal section of *Z. macrophylla* stem

Fig. 6. Tangential longitudinal section of *Z. macrophylla* stem

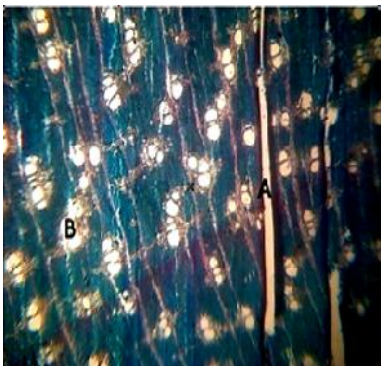

Fig. 7. T/S of *Zanthoxylum macrophylla* root

Fig. 8. Radial longitudinal section of *Z. macrophylla* root

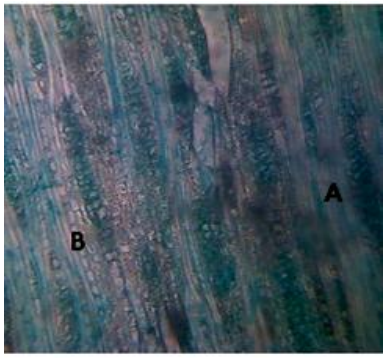

Fig. 9. Tangential longitudinal section of *Z. macrophylla* root

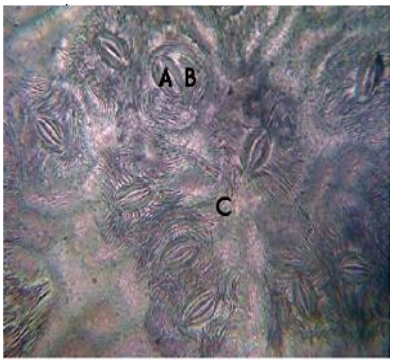

Fig. 10. *Z. macrophylla* abaxial leaf epidermal surface

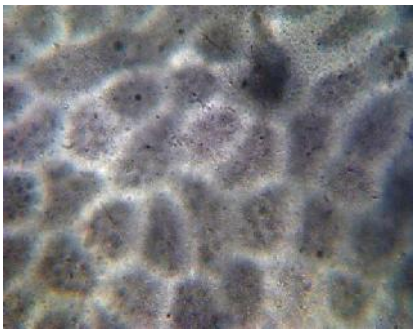

Fig. 11. *Z. macrophylla* adaxial leaf epidermal surfaces

4. CONCLUSION

The obtained data can be used to enhance proper taxonomic characterization and identification of the plant species.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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