

Original Research Article 1

A STUDY ON THE ANATOMY OF *ZANTHOXYLUM MACROPHYLLA* (RUTACEAE)

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 epidermal leaf study was carried out using impression technique. Results showed that the transverse section of various parts of the *Zanthoxylum macrophylla* had similar features including rays in the secondary stem and root. The epidermal leaf 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

INTRODUCTION

Z. macrophylla is a spreading shrub or tree growing to 6-12m tall. The trunk is generally rough with grey bark. The stems are glabrous, grey with solitary prickles [1, 2]. It is well known for its varied 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 bio-pesticide for stored food protection [3]. They are considered antiseptic, analgesic and diaphoretic. [4, 5, 6, 7, 8]. To classify plants, taxonomists make use of anatomy as one of the taxonomic lines of evidence to determine their similarities and differences in order to group them into various taxa. Anatomy is essentially of great importance in plant taxonomy. [9], defined anatomy as the “study of gross internal structure of plant organs by the technique of section cutting”. Anatomical characters of taxonomic importance include such characters as type of stomata, width of medullary rays, presence of bicollateral bundles, wood characters etc [9]. 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. 30 *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.

34 MATERIALS AND METHODS 35

Area of Study 36

The experiment was carried out at the Anatomy Laboratory, Department of Plant Science and 37 Biotechnology University of Nigeria, Nsukka. 38

Procurement and Identification of Plant species 39

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Comment [M2]: Recast as leaf epidermal study

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Comment [M11]: Look for publications on Anatomical and Micromorphological studies to make your introduction more robust. This is a little bit scanty.

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The species *Z. macrophylla* used in this work was collected between April-August 2016 from 40 Mbulu- owo, Nkanu East Local Government Area Enugu state. The species was authenticated at 41 Department of Botany, Nnamdi Azikiwe University, Awka where the voucher specimen was 42 deposited. 43

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Anatomical Studies 45

The following materials were used for the anatomical studies

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Photomicroscope, a staining jar, a wash bottle, a Reichert sledge microtome, a beaker, Carmel's 47 hair brush, light B.Bran microscope with the serial No. 4F8662206.

48 Transverse sections were made from middle part of fully grown leaves, midpoint of petiole,

49 centre of an internode of a young and mature stem and mature root.

50 The reagents and stains were 97% alcohol, absolute alcohol, 50/50 alcohol, /xylene, xylene, 51 safranin, fast green and Canada balsam as mountant.

52 **Procedure:** Sectioning of leaf, stem, petiole and root of specimens was done using a sledge

53 microtome. The sections were transferred into a staining jar and stained in safranin for 5 minutes.

54 The safranin was drained off and the sections washed three times with distilled water. The 55 sections were washed again with 97% alcohol and absolute alcohol for two minutes each and 56 counter stained in 1% fast green for 5minutes and then washed with absolute alcohol 4 times.

57 The sections were transferred into a staining jar containing 50/50 alcohol/xylene and washed

58 until they became very clear. Pure xylene was used to finally clear the sections in the staining

59 dish. Canada balsam was then used to mount the specimens on slides. Each slide was carefully

60 covered with a 22 mm x 22mm cover- slip. The mounted specimens were observed under a light

61 microscope and photomicrographs were made. This procedure is as outlined by [10]. 62

Leaf Epidermal Studies 63

Impression Technique 64

Foliar epidermis of adaxial (upper) and abaxial (lower) surfaces of the leaf of *Zanthoxylum 65 macrophylla* were prepared by impression technique method. The leaf samples were cleared by 66 washing with water and allowed to dry. Nail

varnish was applied using camel hair brush on a 67 small portion on both the abaxial and adaxial surface of the leaf

samples and left for about 10 – 68 15 minutes to dry. A second coating was applied and allowed to dry for the same length of time 69 after which the third coating was applied and allowed to stay for up to 25 minutes. The samples 70 were then

passed through an air current for 30 minutes to ensure maximum dryness. Then, the 71 epidermal strips of the leaves were peeled gently with the aid of a pair of forceps, and then 72 placed on a clean slide and covered with a cover slip. The slide was viewed under the light 73 microscope at different magnifications but the photomicrograph was taken at x400 74

magnification. 75

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RESULTS AND DISCUSSION 79

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

80 The transverse section of leaf of *Z. macrophylla* (Fig 2) showed single-layered epidermis which
81 remains covered with thick cuticle. Beneath the epidermis are 2 – 3 layers of collenchyma cells, t
82 followed by 3 – 4 layers of parenchyma cells. The central midrib consists of the vascular bundle;
83 xylem (vessel) and phloem. Xylem is internal while phloem is external; the vessels are arranged
84 in radial multiple. The transverse section of the petiole (Fig 3) showed a single-layered
85 epidermis. Beneath the epidermis are 2-3 layers of collenchyma cells, followed by 3 – 4 layers of
86 parenchyma cells. The vascular bundle consists of internal xylem and external phloem. The
87 vessels are arranged in radial multiple. In the transverse section of secondary stem (Fig. 4) the
88 vessels are diffuse- porous and not occluded by tyloses. The pores are round in shape.
89 Multiseriate and uniseriate rays are present. The radial longitudinal section of stem (Fig. 5)
90 showed vessels that are not occluded by tyloses. There are procumbent ray cells. Tangential
91 longitudinal section of stem (Fig. 6) showed multiseriate, heterocellular and procumbent rays,
92 vessels not occluded by tyloses. In the transverse section of root (Fig. 7) the vessels are diffuse-
93 porous and not occluded by tyloses. The pores are round in shape. Multiseriate and uniseriate
94 rays are present. The radial longitudinal section of root (Fig. 8) showed vessels that are not
95 occluded by tyloses. There are procumbent ray cells. Tangential longitudinal section of root
96 (Fig.9) showed multiseriate, heterocellular and procumbent rays, vessels not occluded by tyloses. 97 The leaf
epidermal peel (Figs 10 and 11) showed no stomata on the adaxial surface but had
98 stomata confined to the abaxial epidermis only (hypostomatic). The plant has paracytic stomata.
99 Result of the study showed that the transverse sections, the radial and tangential sections of
100 various parts (petiole, stem and root) of *Z. macrophylla* had similar features respectively. The
101 epidermal leaf study showed the presence of stomata on the abaxial surface only with paracytic
102 stomatal type. No previous work has been done on the anatomy of *Z. macrophylla*, but the result
103 is in line with anatomical observations of most dicotyledonous plants such as the family
104 Rutaceae and also in line with the anatomical observations of [11] who reported that the
105 transverse sections of various parts of *Morinda lucida* had similar features, stomata paracytic and
106 exist only on the abaxial epidermis only. 107

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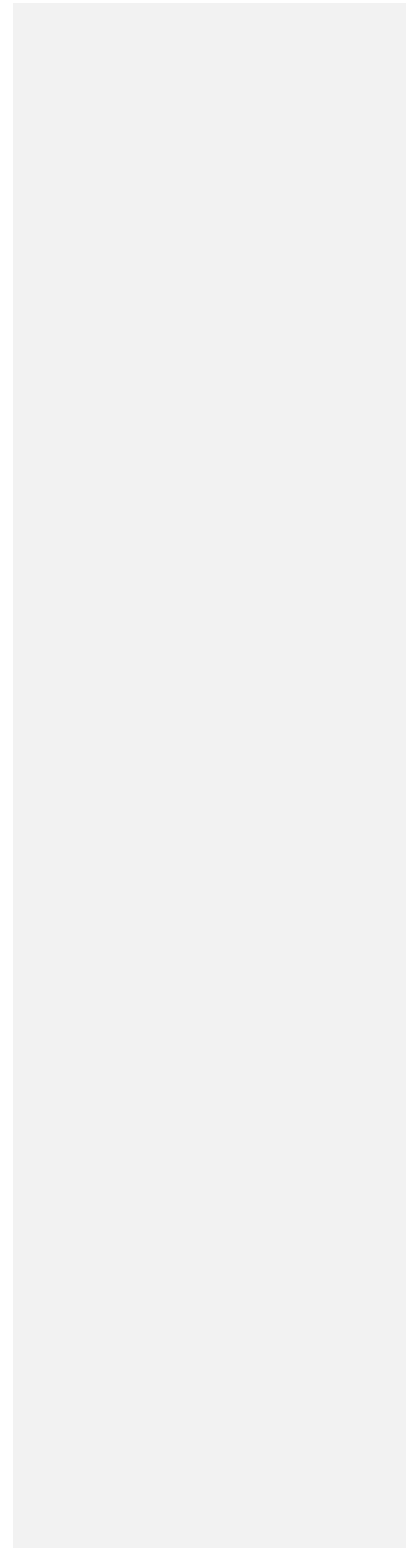
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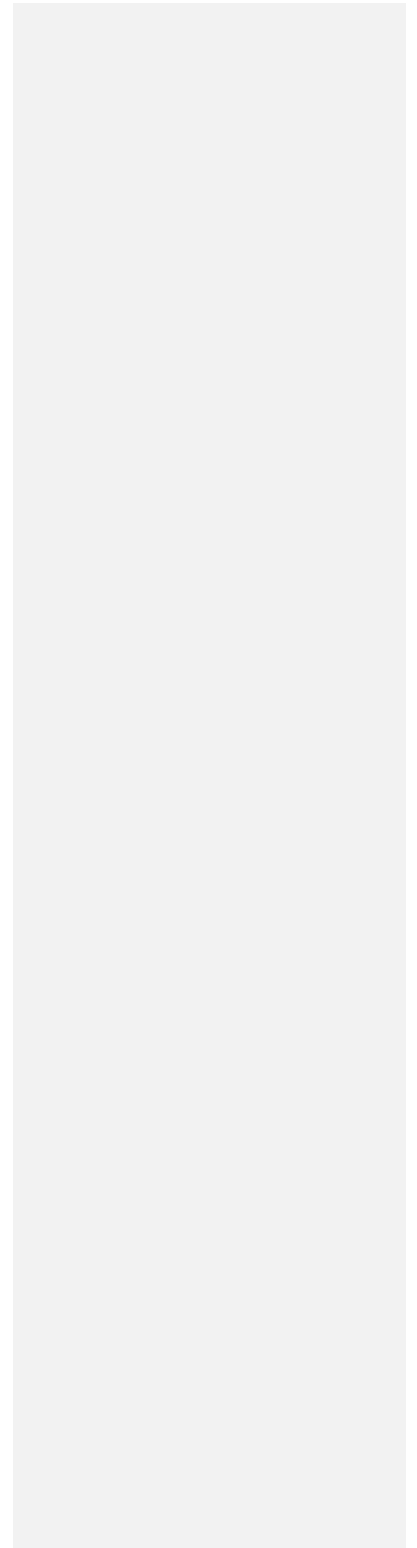
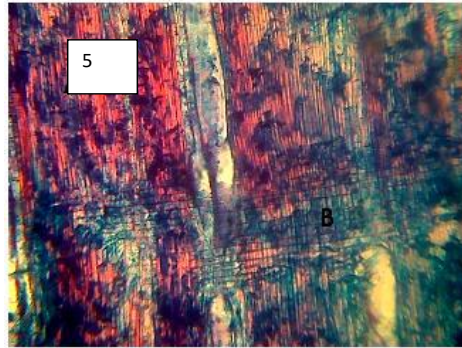
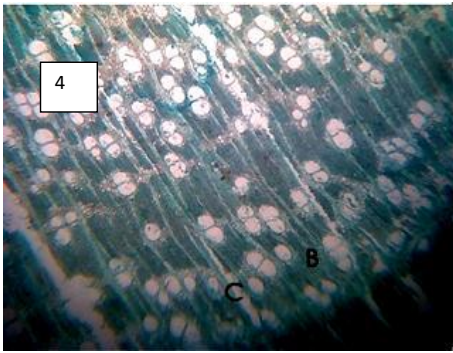
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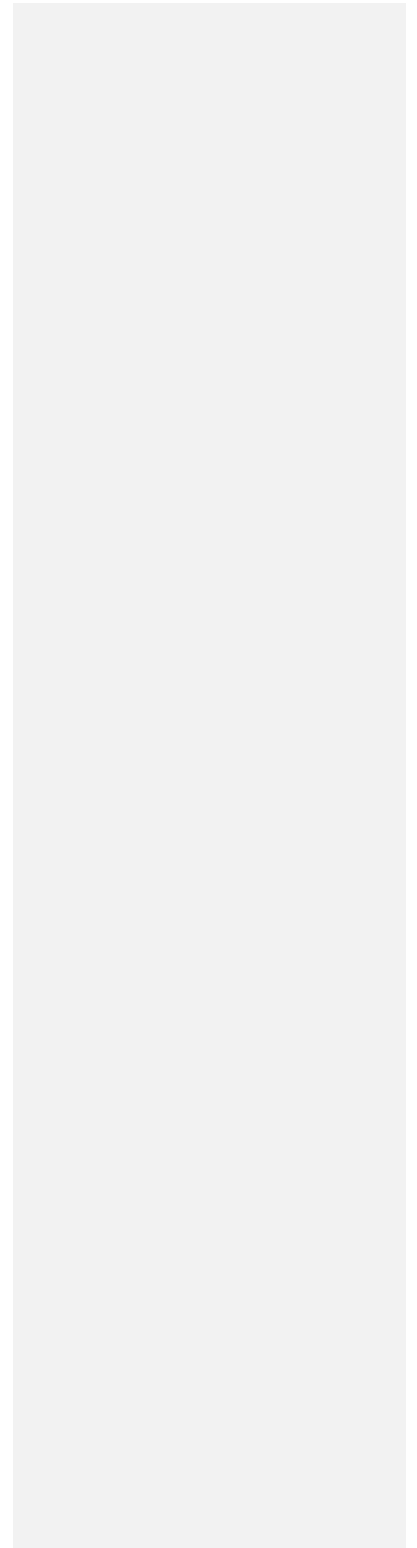
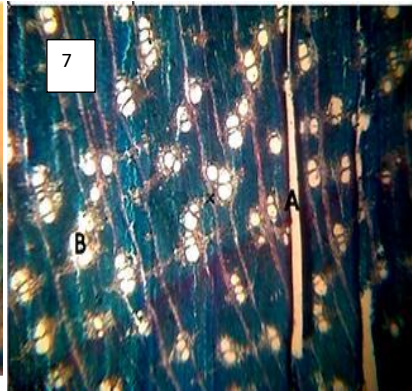
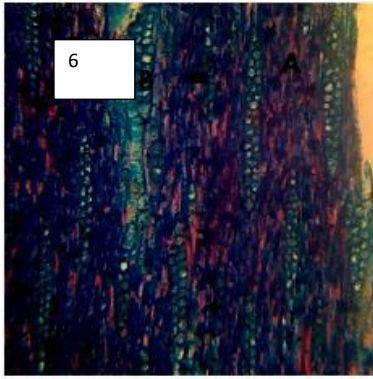
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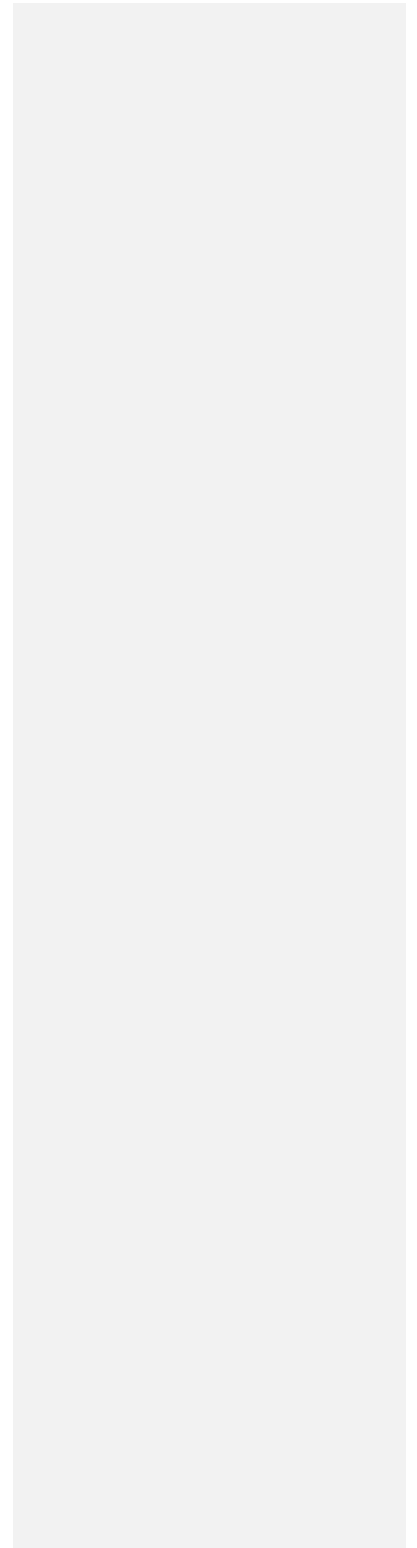
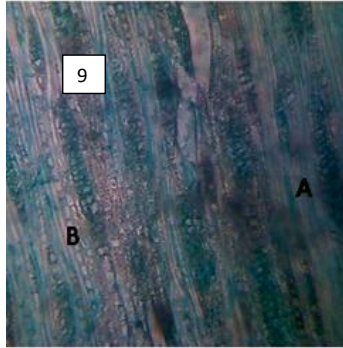
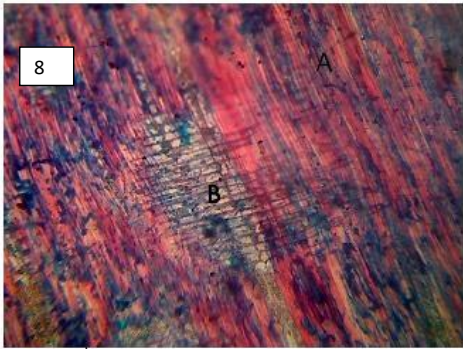
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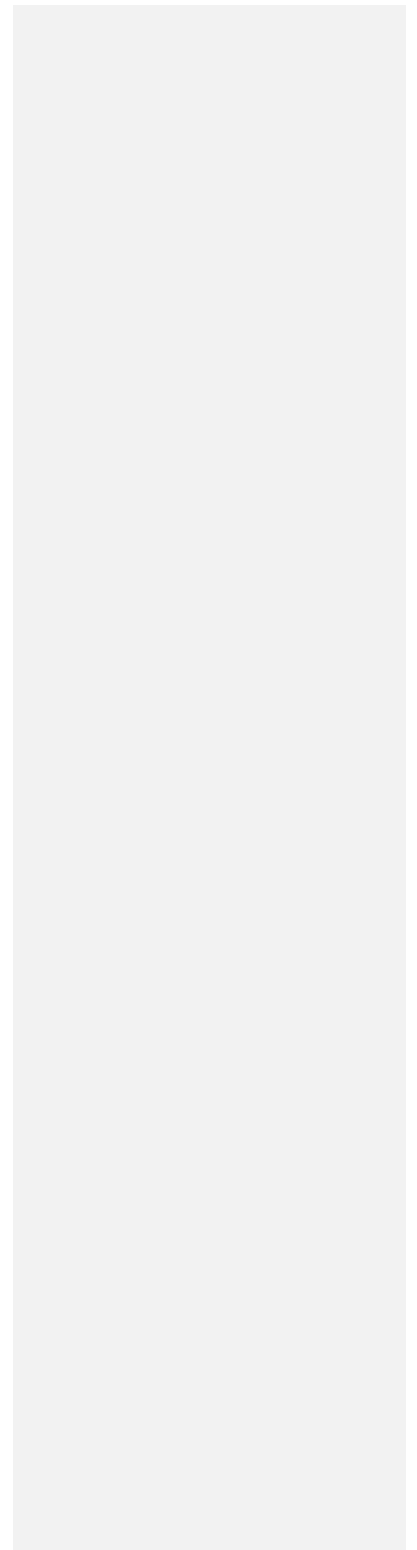
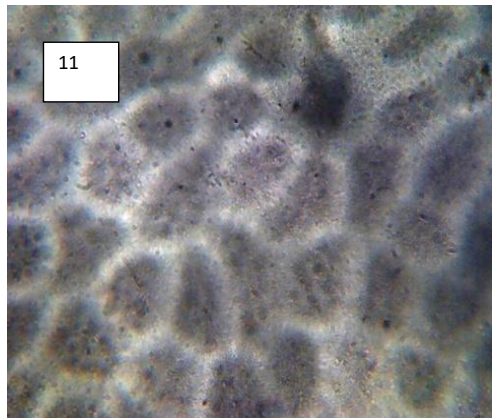
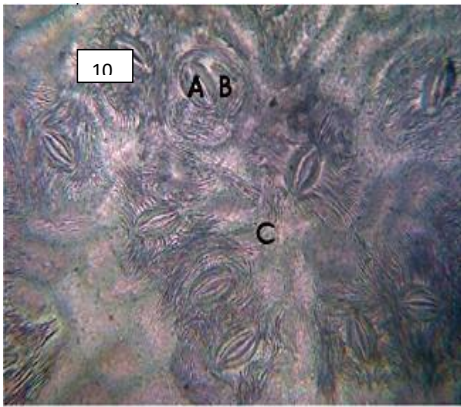










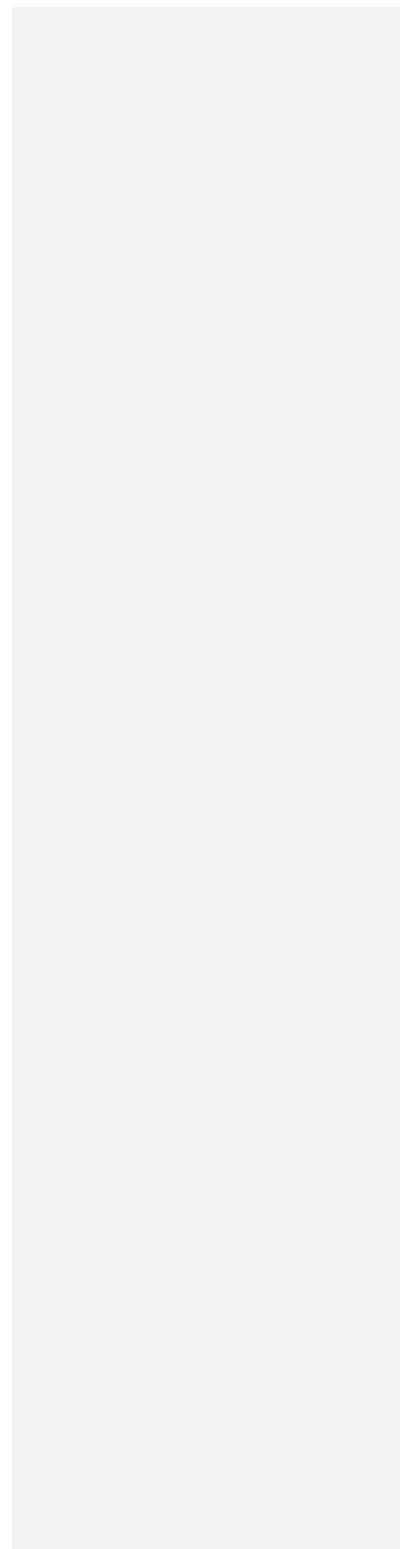


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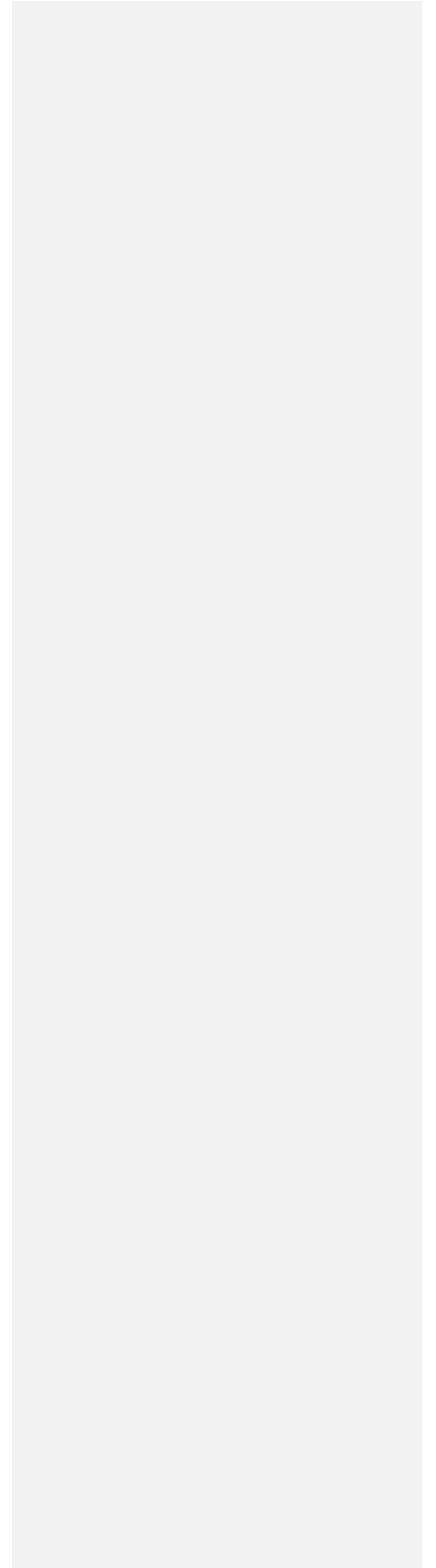
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LEGEND 124

Fig 1: *Zanthoxylum macrophylla* tree in its natural habitat **Fig 2:** T/S of *Z. macrophylla* leaf 125 **Fig 3:** T/S of *Z. macrophylla* petiole **Fig 4:** T/S of *Z. macrophylla* secondary stem **Fig 5:** Radial 126 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 128 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 130 surfaces 131

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CONCLUSION 133

The obtained data can be used to enhance proper taxonomic characterization and identification of 134 the plant species. 135

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Competing interests 137

Authors have declared that no competing interests exist. 138

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