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MORPHOLOGICAL, ANATOMICAL AND POLLEN STUDIES OF *STRYCHNOS INNOCUA* DEL. FROM KADUNA STATE, NIGERIA

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ABSTRACT

The anatomical description of *Strychnos innocua* Linn, a Nigerian species growing in the Sudan savannah, was described. Pollen grains, epidermal peel from adaxial and abaxial leaf surfaces, transverse section of the leaf lamina close to the midrib at median part of the leaf, median part of petiole, stem and root were cut and examined. Some of the striking features possessed by *S. innocua* include the possession of hypostomatic leaves, bicollateral vascular bundle, sclerenchyma sheath around the vascular bundle of midrib, interxylary phloem in TS of stem and presence of tricolporate and triangular pollen grains.

Keywords: *Strychnos innocua*, bicollateral vascular bundle, interxylary phloem

INTRODUCTION

Strychnos Linn. belongs to Loganiaceae and in the family are trees, shrubs and lianas (Rajesh *et al*., 2009). The genus was first described by Linnaeus based on *S. nux-vomica* which is the type species (Mwamba, 2006; Rajesh *et al*., 2009; Orwa *et al*., 2009). Many species of *Strychnos* produce alkaloids like strychnine used in preparing arrow poison curare in South Africa and hunting wildlife in Central Africa (Mwamba, 2006). Mwamba (2006) noted that the name *Strychnos* was coined from a Greek word 'Strukhnos' which refers to toxic properties found in the poisonous night shade.

The specific epithet *innocua* was derived from the fact that it lacked spines and was considered to lack poisonous properties and so harmless (Orwa *et al*., 2009). *Strychnos* was reported by Rajesh *et al*. (2009) and Fraiser (2011) to be distributed in three geographically separated zones with the number of species in each zone as follows: Africa 75, America 73, Asia and Australia 44. The species of *Strychnos* are generally continent-specific, that is, the ones that are found in Africa are not normally found in Asia and *vice versa*, with the exception of *S. potatorum* found in Asia and Africa. In Nigeria, about twenty-one species of *Strychnos* are found and deposited in the Forestry Herbarium Ibadan. Of these twenty-one species, the following occur in the savannah: *S. floribundu, S. aculeata, S. nigritana, S. innocua* and *S. spinosa* (Forestry Herbarium Ibadan). Most of other species grow in the rain forest or the derived savannah. Its morphology has been described by Mwamba (2006) and Orwa *et al*. (2009). There are a few conflicting reports about the morphology of the leaf, flower and seeds, thus, the author thought it necessary to include the description of the morphology of the species collected from Kaduna, Nigeria. It is possible that the discrepancies may be due to ecological or other factors in the places of collection. Mbuya *et al*. (1994) described its uses, while the phytochemical components were described by Phillipe *et al*.

(2005) and Bello *et al*. (2008). However, there is very scanty information in literature on detailed description of the stomatal type, anatomy of the transverse section of the leaf, stem and root, type and size of fiber in the wood, pollen grain morphology, among others. This paper presents detailed description of the anatomy of the leaf, stem, root, stomatal type, size of fiber and pollen grain present in *Strychnos innocua*.

MATERIALS AND METHODS

Fresh leaves, flowers, stem and root of *S. innocua* were collected at Rigasa village near Kaduna in northern Nigeria (Sudan savannah). Identification was done by comparing the specimen with herbarium material at Forestry Herbarium at Ibadan, Nigeria and with that of the Royal Botanic Garden Kew, United Kingdom.

The leaves were soaked in 10 ml of hydrogen peroxide (commercial bleach) in a Petri dish to macerate the mesophyll for 24 hours. The adaxial and abaxial epidermises were teased off with Carmel's hair brush and a pair of forceps and examined under the microscope. The length and width of the widest part of the guard cells of ten stomata were measured using a calibrated ocular micrometer fitted into the eyepiece of the Zeiss microscope. Stomatal index was calculated using the formula of Evans (2002).

$$SI = \frac{S}{S+E} \times 100$$

Where, S = the number of stomata in each unit area, E = the number of epidermal cells in the same unit area.

Transverse section of leaf lamina, midrib and median part of petiole and that of the stem and root were made using Reichart sliding sledge microtome. Tangential longitudinal sections (TLS) and radial longitudinal sections (RLS) of the stem and root were also made. Maceration of the wood of stem was made using the method of Jane (1970). The fibres from maceration were stained with crystal blue and safranin O and mounted on slides for microscopic examination. Measurements of the following dimensions were made namely, fibre length (L), fibre diameter (D), fibre lumen diameter (FL) and fibre cell wall thickness (FCWT). Measurement of length and width of guard cells were made using micrometer gauge. Acetolysis of mature polleniferous buds was used to avoid contamination from other pollen grain in the environment using the method of Moore and Webb (1978). Pollen parameters assessed were sculpturing type, aperture type and shape in polar and equatorial views. Pollen grains were mounted on slides and examined under Zeiss microscope at x400 and x1000 magnifications.

RESULT

S. innocua is a shrub that grows mainly in Sudan savannah part of Nigeria. The leaf margin is entire, base is acute and the apex is obtuse to round. Venation is palmate with a prominent midrib at abaxial surface. Petiole measures 0.3-0.7 cm long having a groove adaxially but in a semi-circle abaxially. Leaves are opposite and decussate, stem is terete (cylindrical), colour is cream or milky and there are no appendages.

Inflorescence is cymose and flowers are bisexual and hypogynous. Sepals are green, gamosepalous and persistent in the fruit. Petals are four, orange-yellow, tubular at the lower part with lobes at the upper part. Filaments are four, basifixed and are attached near the numerous white hair-like projections (corona). Anthers are yellowish, introrse with two lobes. Ovary is superior, globular in shape, bicarpellate and syncarpous. Style is single, terminal with a simple stigma. Fruit is a berry, green when unripe, turning to yellow when ripe. Shape is

round; epicarp is dry, measuring between 3.00-4.00 mm in thickness. Ten to twelve seeds are embedded in a scanty yellowish pulp. Seeds are triangular and light brown in colour, measuring 1.8- 1.9 cm in length and 1.5- 1.6 cm in width.

Epidermal strip

Epidermal strip examination revealed that the leaves were hypostomatic with stomata only on the abaxial surface. Length and width of guard cells, number of stomata/field of view, cell wall thickness and stomatal index were measured and calculated (Table 1, Plate1a & b). There were distinct cutcular markings at the adaxial surface, the epidermal cells were mainly pentagonal with a few being hexagonal. Anticlinal walls were straight. Stomata were found only on the abaxial surface (hypostomatic). Stomata were of animocytic type (Plate 1b). Length and width of guard cells were 0.187 and 0.029 mm, respectively, number of stomata (12.2), number of subsidiary cells per field of view (92) and Stomatal Index (11.70) were all calculated and recorded as shown in Table 1.

Transverse section of leaf lamina

Transverse section of the lamina revealed uniseriate epidermis having rectangular-shaped cells (Plate 1c). The palisade mesophyll had two layers of rectangular- shaped cells. There were seven to eight large and loosely arranged cells in the spongy mesophyll. Rosette crystals, bundle sheaths and air-spaces were also present. The abaxial epidermis was not even because of the presence of numerous anisocytic stomata.

Midrib

The midrib had a slightly concave adaxial surface and a semi-circular abaxial surface (Plate 1d). Vascular bundle was semi-circular, bicollateral and embedded in the ground tissue. Xylem elements were 7-8 at the median part but 2-3 at the
extreme left and right- hand corners. Phloem occurred at the adaxial and abaxial surfaces sandwiching the xylem (Plate 1d.). Sclerenchyma sheath, 3-6 celled thick, surrounded the vascular bundle (Plate 1d).

Petiole

The adaxial epidermis of petiole was uniseriate with slight undulating surface. There were seven bicollateral vascular bundles with the biggest one at the centre. The abaxial surface was semi-circular with uniseriate epidermis (Plate1d).

Transverse section (TS), Transverse Longitudinal Section (TLS) and Radial Longitudinal Section (RLS) of the stem

In the transverse section of the stem were pockets of interxylary phloem occurring in discontinuous ring around the xylem cylinder. Vessels were predominantly solitary while a few occurred in radial multiples. The wood was diffuse porous (Plate 2a). Transverse longitudinal section revealed heterogeneous rays with uni, bi and triseriate rays. In the radial longitudinal section were heterocellular cells with both upright and procumbent cells. Vessel walls had simple pitting (Plate 2b). The fibre length, fibre lumen diameter and fibre cell wall thickness obtained after maceration of stem were measured and presented in Table 2 (Plate 2c).

Transverse section (TS), Transverse Longitudinal Section (TLS) and Radial Longitudinal Section (RLS) of Root

Most of the vessels of the root were solitary and circular/spherical in shape (Plate 3a). More interxylary phloem and many more vessels per field of view occurred in the roots than what was recorded in the stem. Vessel wall had reticulate pitting (Plate 3b) and numerous fibres. Procumbent and upright rays wee present (heterocelluar) (Plate 3c). Square and upright rays were more in number.

Pollen grains

Pollen grains were tricolporate, triangular with pronounced convex shape and reticulate sculpture. The equatorial view was subspheroidal to spheroidal (Plate 4a, 4b).

Table 1: Stomatal dimensions of abaxial surface of *S. innocua*

Name of Plant	Length of guard cell (mm)	Width of guard cell (mm)	No of stomata/ Field of View	No of subsidiary Cells/Field of View	Stomatal Index (%)
S. innocua	0.187	0.029	12.2	0.92	11.70

Table 2: Fibre dimensions of stem of *S. innocua*

Plant of name	Fibre Length x100 (μm)	Fibre Diameter x400 (μm)	Fibre Lumen Diameter x400 (μm)	Fibre Cell Wall Thickness x400 (μm)
S.innocua	0.66	0.022	0.059	00.0035

DISCUSSION

Many features in *S. innocua* are diagnostic for the genus; example is the possession of palmate venation which was reported in other species by Keay (1989) and Orwa *et al.* (2009). Occurrence of cymose inflorescence is another feature also observed by Albert and Struwe (2002) and Orwa *et al.* (2009) in other *Strychnos* species. The presence of tubular petal with lobes, epipetalous filaments and white hair-like projections (corona) were equally found in *S. spinosa* and *S. usambarensis* (Asuzu and Nwosu, 2019). Hypostomatic nature of *S. innocua* was reported by Asuzu and Nwosu (2019) for *S. spinosa* and other species of *Strychnos.*

The features of leaf epidermal strip like dimensions of length and width of guard cells, Stomatal Index and number of epidermal cells per field of view can be used for the correct identification by future researchers who would embark on studies on Nigerian *Strychnos* species. The work of Asuzu and Nwosu (2019) revealed that

epidermal features exhibit variations in different species that can be used for their delineation. According to Taia (2005), the study of leaf epidermal features has been used to resolve conflicts encountered in taxonomy.

The presence of bicollateral vascular bundles in the midrib, petiole and stem is of taxonomic importance. Metcalfe and Chalk (1989) noted that it has restricted occurrence but was found in members of family Loganiaceae and other closely related families like Asclepiadaceae, Rubiaceae and Apocynaceae. Sclerenchyma sheath that occurred right round the vascular bundle was equally reported in *S. usambarensis* by Asuzu and Nwosu (2019). Evert (2006) stated that the presence of this feature enhances strengthening of the leaf.

Vessels were predominantly solitary though a few vessels occurred in multiples. Dayal *et al*. (1984) noted the presence of vessels that were predominantly single with a few that occurred in radial multiples in *S. nux-blanda*, *S. nux-vomica* and *S. potatorum* from India. Evert (2006) stated that the possession of vessel multiples had the advantage of helping plants to overcome embolism whenever it occurred. This was achieved by allowing neighbouring vessels to carry on with the transport of water and mineral salts, thereby ensuring a continuous flow.

The tricolporate pollen grains observed in this study corroborates the finding of Dutta and Ghosh (1982) who reported the presence of tricolporate grains in *S. nux-vomica* from Asia. Perween and Quiser (2007) noted that pollen grains do not differ much in a family and can, therefore, be used to establish affinity.

CONCLUSION

S. innocua is an important medicinal plant in northern Nigeria where it grows in the wild and the features obtained in this study will be found helpful in identifying the plant and to prevent adulteration as it is marketed by local herbal men in northern Nigeria.

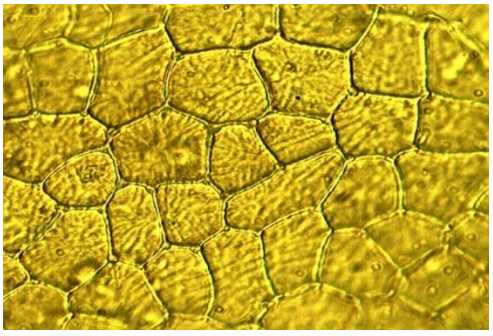


Plate 1a: Adaxial leaf surface of *S. innocua* with no visible stomata

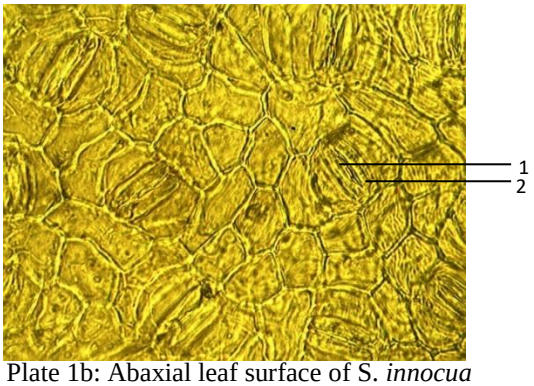


Plate 1b: Abaxial leaf surface of S. *innocua* x400

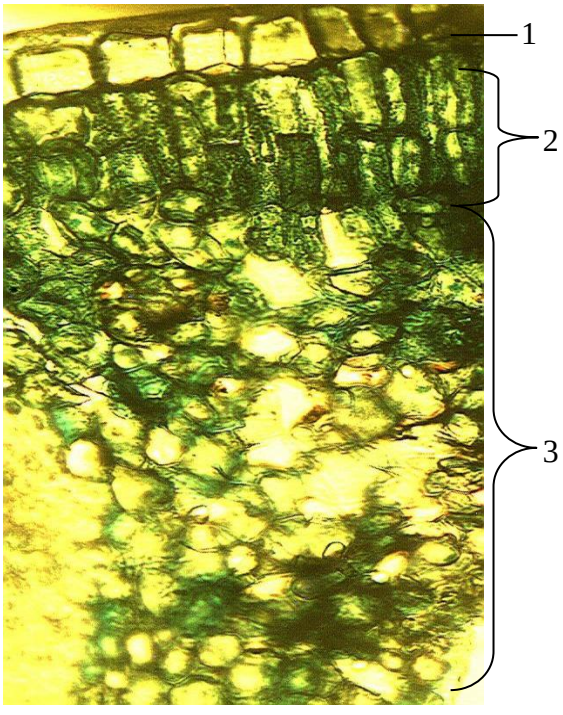


Plate 1c TS leaf *S. innocua* x 200
1 – Adaxial epidermis, **2** – Palisade

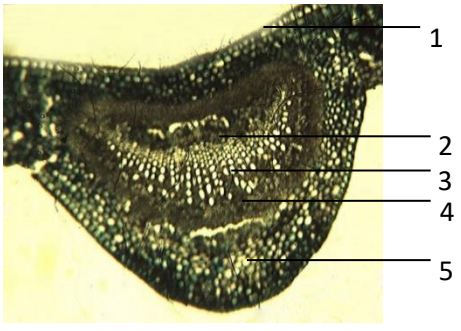
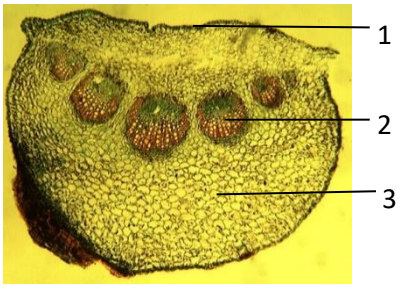
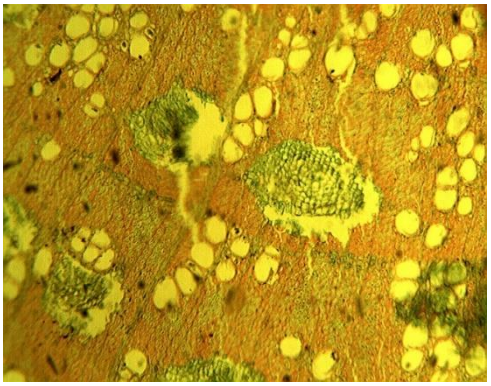


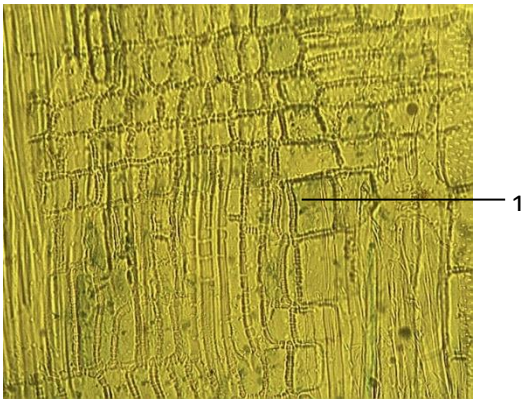
Plate 1c: Midrib of leaf *S.innocua* x40
1 – adaxial epidermis, 2 – internal phloem,



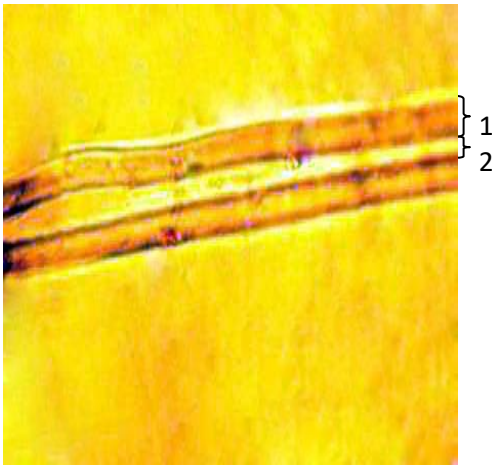
1d: Petiole of *S. innocua* x40
1 – Adaxial epidermis, 2 –



2a: TS older stem of *S.innocua* showing interxylary phloem x40



2b: RLS stem of *S.innocua* x200
1 - upright ray



2c: Fibre of *S. innocua* x400
1 – fibre cell wall , 2 – fibre lumen

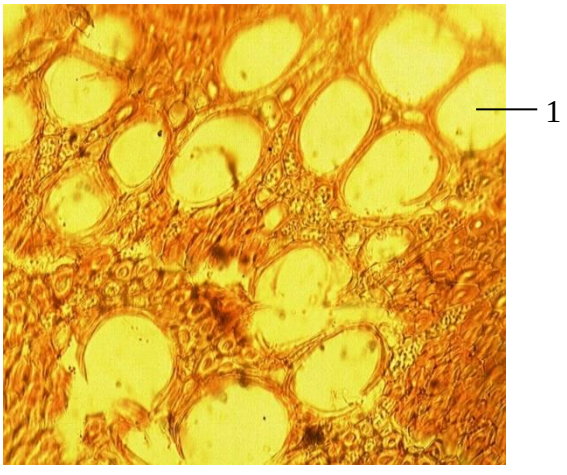


Plate 3a: TS root of *S. innocua* x200
1 – xylem

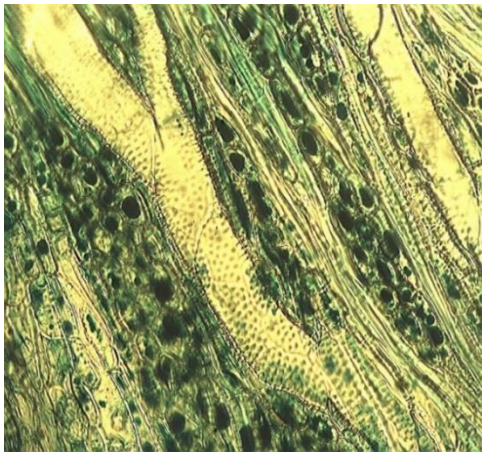


Plate 3b: TLS root of *S. innocua* x40

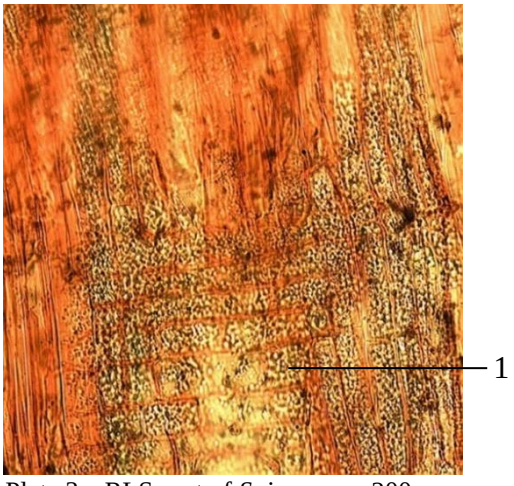
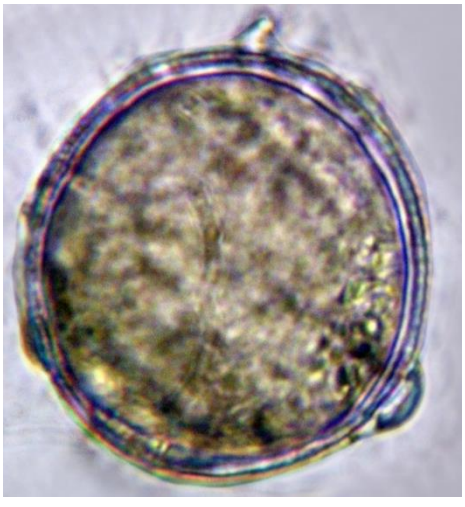


Plate 3c: RLS root of *S. innocua* x200
1 – procumbent ray cell

4b: Polar view *S. innocua* x1000 (triangular)



4a: Pollen of *S. innocua* at equatorial view

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