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Studies on morphology and anatomy of *Strychnos spinosa* Lam. (Loganiaceae)

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***Strychnos spinosa* Lam. belongs to the family Loganiaceae. It is a small tree. The morphological and anatomical features of the leaves, stem, flower and fruit were studied. *S. spinosa* has a cylindrical stem with a pair of spines at irregular spaces. The leaves are opposite, decussate, petiolate with palmate venation. The petals are greenish in colour, tubular with five lobes. There are numerous white hair-like appendages at the neck of the corolla tube. The androecium is epipetalous and attached close to the bottom of the corolla tube. The flower is a cyme, occurring in threes, with the middle one being the oldest. The fruit is a berry with very hard pericarp. It turns from light green when unripe to bright yellow when ripe. The anatomy of the leaf revealed presence of rosette crystals and intraxylary phloem which is more pronounced in the mid rib and petiole. "Kranz" structure was observed in the transverse section of the leaf. Anisocytic stomata are numerous in the abaxial (lower) epidemis but absent in the adaxial epidemis. The anatomy of the stem showed the presence of crystals in the cortex. Both solitary and multiple vessels are found in the transverse section. In the tangential longitudinal section, most of the rays were four to seven cells wide, but a few were uniseriate, biseriate and triseriate. Most of the pollen grains were triangular in the polar view but a few were quadrangular. Those that were triangular exhibited tricolporate aperture while those that were quadrangular had tetracolporate aperture. The sculpturing was perforate and were not very compact.**

Key words: *Strychnos spinosa,* rosette crystals, intraxylary phloem, pollen dimorphism, heterogenous, rays.

INTRODUCTION

Strychnos spinosa Lam. belongs to the genus *Strychnos*, in the family Loganiaceae of the order Loganiales (Hutchinson, 1967). The delimitation of the family has been a matter of debate (Leeuwenberg, 1962, Backlund et al., 2000).

Strychnos spinosa is a small, medium-sized, spiny, deciduous tree. The leaves turn yellow in the dry season before they fall off. It also grows singly in well drained soil. It is found along river fringes and sandy forests from East Africa to South Africa (Watt and Breyer - Brandwijk, 1962). In Nigeria, it occurs in the savanna wood land (Keay, 1989).

The generic name is derived from the Greek word "strukhnos" that refers to the properties present in the

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poisonous night-shade. Many species of *Strychnos* produce alkaloids like strychnine and resinous substances used in preparing the arrow poison "curare" in South America. In other places like Malaysia and Central Africa, species of *Strychnos* have been used to prepare dart poison for hunting wildlife (Mwamba, 2006). The specific name *spinosa* refers to the spines characteristically found on some nodes (Watt and Breyer - Brandwijk, 1962; Mwamba, 2006).

The circumscription of Loganiaceae varies significantly among different taxonomists. Bremer and Struwe (1992) and Backlund et al. (2000) all reported that the debate has trailed the circumscription and the intra-familial relationship of this family. While Hutchinson (1967) placed Loganiaceae in the order Loganiales, Backlund et al. (2000) placed Loganiaceae in the order Gentianales. Metcalfe and Chalk (1989) however, rightly pointed out that opinions about the relative merit of the different systems notably differ. They remarked that there is not at present and probably there will never be, an all embracing system that will generally be accepted in any taxonomic work. They concluded that it is necessary to choose a sequence in which the families will be described. The system of Hutchinson (1967) for the family Loganiaceae has been adopted in this work. Mathé and Craker (1995), De Carvatha and Ferreira (2001) observed that despite the modern advances achieved in synthetic chemistry the most efficient drugs available have their origin directly or indirectly related to the plant kingdom. Leu and Amar (2000) reported that 74% of all plant-derived drugs in clinical use world wide have been discovered through follow-up investigation of the ethnobotanical uses of plants. They noted that from medicinal literature survey, the trend has been that plants represent most of all the traditional medicinal substances. They opined that since plants represent an important source of drug discovery and the search for new biologically active compounds usually starts in the field, there should be accurate, detailed and specific ethnobotanical information obtained from local practitioners. De Carvatho and Ferreira (2001) observed that indigenous communities have long used plant extracts to treat illnesses and many of such extracts have shown effective action, resulting in screening and extraction of new bioactive compounds every year. In line with the above, Ndubani and Höjer (1999) listed the root of *S. spinosa* as one of the plants used by traditional healers in conjunction with other plants to treat sexually transmitted diseases in rural Zambia. Kokwaro (1976) reported that juice from the root is dropped into the ear as remedy for ear ache. He also remarked that decoction of the root is used as a head wash for cold. The same decoction taken with milk is used as a cure for dropsy. Chewing of the roots or drinking the boiled root decoction effects healing from snake bite. The roots and leaves are used for treating veneral diseases and as a febrifuge in Zambia, while a decoction of the leaf or root is used as an analgesic in Central Africa (Mwamba, 2006). Oguakwa et al. (1980) isolated three alkaloids from the leaves of *S. spinosa*. They reported that the crude extracts from the leaves and stem bark have muscle relaxant effects.

This study was undertaken in cognizance of the great medicinal potentials of this plant and the scanty literature available on the accurate and detailed description of its morphology and anatomy. The aim of this study is to carefully examine the morphology and anatomy of *S. spinosa*.

MATERIALS AND METHODS

Ten stands of *S. strychnos spinosa* were randomly collected from Kuje village near Abuja and Igbeji along Lagos-Ilorin road (Guinea savanna). The species was authenticated by Prof. Dr. M.O Nwosu of the Department of Plant Science and Biotechnology UNN, Mr. A. Ozioko of Bioresource Centre Nsukka in conjunction with the type specimen accessed from the website of Royal Botanical Garden Kew. Voucher specimens were deposited at the Herbarium of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Detailed examination of the morphological features of twenty five leaves, petioles, flowers and seeds was done with the help of hand lens and dissecting microscope. Measurement of the dimensions of leaves, petiole, stem, flowers, fruit and seeds was done with a meter rule.

Anatomical studies

Freshly collected leaves were washed with distilled water to remove dirt and dust and allowed to dry under a fan. They were further soaked in domestic bleach for 24 h to digest the mesophyll layer according to the method of Olowokudejo (1993). The leaves were washed in water twice and the abaxial and adaxial epidermises teased off carefully with fine forceps and dissecting needles. The membranes were stained with safranin, mounted in glycerine and viewed under the microscope.

The transverse sections of the median part of lamina, midrib and petiole were cut with the aid of sliding sledge microtome mounted on slides for microscopic examination. Transverse section (TS), transverse longitudinal section (TLS) and radial longitudinal section (RLS) of the root and stems were also made using a sliding sledge microtome. The sections were stained with safranin and counter stained with Fast green. The materials were washed in a series of alcohol and with 50/50 alcohol and xylene. They were picked with brush and mounted on slides with Canada balsam as mountant. Photomicrographs of the good slides were taken with a Sony digital camera fitted on a Zeiss microscope.

Pollen study

Anthers were teased off from mature buds of flowers of *S. spinosa* and squashed with a glass rod to release pollen grains. This was sieved with fine mesh of wire gauze, washed twice with distilled water and centrifuged at 2000 revolutions per minute for 5 min. The resulting precipitate was acetolysed using a modified version of Erdtman (1971) acetolysis method. Pollen grains were mounted on slides and examined with Zeiss microscope fitted with Sony digital camera at X 400 and X1000 magnifications. Terminologies for pollen descriptions are adapted from Eidtman (1971) and Punt et al.

Plate 1a. Inflorescence of *S. spinosa***.**

Table 1. Reproductive characteristics of *Strychnos spinosa.*

Sepal	Petal	Anther	Filament	Ovary
5 Green in colour Persistent. Almost linear in shape. Acuminate tip.	5 Tubular with five lobes White hair - like projections at neck of corolla tube Light green in colour Petals fall off easily from flower	Has two conspicuous lobes with two chambers each Anther lobes covered with hair-like projections Bright orange in colour Dorsifixed and intorse	Five Epipetalous Inserted at lower part of corolla tube Filament and anther fall off with corolla.	Small Round Has numerous ovules
Style	**Stigma**	**Fruit**	**Seed**	**Embryo**
Short	Single Orange-yellow in colour	Round berry Apple green in colour when unripe Bright yellow when ripe Very hard pericarp Light brown pulp inside Plate 1b	Light brown in colour Measure 22-24mm in length and 6-18mm in width Thin testa Fleshy endosperm	Small Embedded in endosperm Thin cotyle- dons with distinct veins like those of leaves.

(2007).

The primitive and advance features of the species were deduced from the morphological and anatomical features examined.

RESULTS

The leaf is obovate with the broadest part near the apex. The margin is entire, the base is rounded and apex has a little projection that is acute. The colour of the leaf is light green above and lighter green beneath. The venation is palmate with 2 pairs of veins on both sides of the midrib, all emanating from the petiole at the base and terminating at the apex. The two veins closest to the midrib on the right hand and on the left hand, run parallel to the midrib for a distance of 0.4 to 0.6 cm before curving and terminating at the apex. The outermost veinlet to the margin is joined by a number of arches. Leaf has no odour and is smooth (glabrous). Leaf arrangements are opposite and decussate.

The petiole is rounded at the abaxial (lower) side and grooved at the adaxial (upper) side with some hairs in the groove. It is greenish in colour and measured 0.5 to 0.9 cm in length.

The stem is fissured, the degree of fissuring increases with the age of the plant. The nodes on the young stem are swollen. Axillary spines occur but not in any particular sequence. The spine measures 0.4 to 0.7 cm in length. A black line that circles the stem exist just above the point of attachment of the leaves to the node.

The inflorescence is a cyme and terminal and appears soon after the new leaves form between March and June. The main axis ends in a flower and produces two lateral branches. These two lateral branches also behave in the same manner. The terminal flower is older than the lateral flowers (Plate 1a and b). The details of the floral parts, fruits, seeds and embryo is summarised in Table 1.

Anatomical study

Leaf

The transverse section of the leaf revealed a single layer

of upper epidermis that consisted of compactly arranged rectangular cells with the upper part that is concave. A thin layer of cuticle covered the epidermis (Plate 1e).

Palisade mesophyll consists of compactly arranged rectangular shaped cells with numerous chloroplasts. It is single-layered in some places and double at other places. The upper most palisade is longer than wide. The lower palisade consists of cells that are nearly square in shape.

The spongy mesophyll contains loosely arranged parenchyma cells thus creating numerous air spaces. Rosette crystals are found in the spongy mesophyll. Bundle sheaths consisting of a single layer of compactly arranged parenchyma cells in a circle with few vascular bundles that are enclosed in the circle occur in the spongy layer. The lower epidermis is less compactly arranged having numerous stomata for gaseous exchange.

In the midrib, the upper epidermal cells are composed of compactly arranged rectangular cells that are barrel shaped. The ground tissue immediately below the epidermis is made up of 5 to 7 layers of thin walled parenchyma cells that lack chlorophyll. The vascular bundle clearly shows the bicollateral arrangement where internal and external phloems sandwich the xylem (intraxylary phloem). Rosette crystals are present in the ground tissue. Three to four layers of ground tissue occur at the lower portion of the vascular bundle (Plate 1e). The latter resemble the cells at the upper side of the vascular bundle. The lower epidermis is single layered (Plate 1f). Examination of epidermal strip, revealed that stomata is absent on the adaxial surface. On the abaxial epidemis, the stomata are numerous and are of the anisocytic type. The epidermal cells have walls that are curved (Plate 1c and d)

The upper epidermis of the petiole is made up of compactly arranged cells with an undulating surface. There are two small projections at the ends of the upper surface (Plate 1g). The vascular bundle is arranged in form of an arc and this affects the number of cells in the ground tissue. At the adaxial side, the cells range in number from nine - eleven at the distal ends to about twenty-three at the median part. The cells range in number from nine at the distal ends to sixteen at the median part on the abaxial side. The cells in the ground tissue are parenchymatous, round or hexagonal in shape and compactly arranged. The lower epidermis consists of closely packed cells with an outer convex surface.
There are seven separate vascular bundles. In the middle is the biggest, flanked on either side by three bundles in decreasing order of sizes. The bicollateral arrangement is very pronounced with the xylem in the middle and the phloem on both the abaxial and adaxial sides.

Transverse section (T.S.) of stem: The periderm contains eighteen - twenty-six layers of rectangular shaped cells with their long axis oriented horizontally and compactly arranged. The cortex contains twenty seven – thirty one layers of closely packed, thin walled parenchyma cells. The endodermis consisting of thick walled sclerenchyma cells form a continuous ring that is

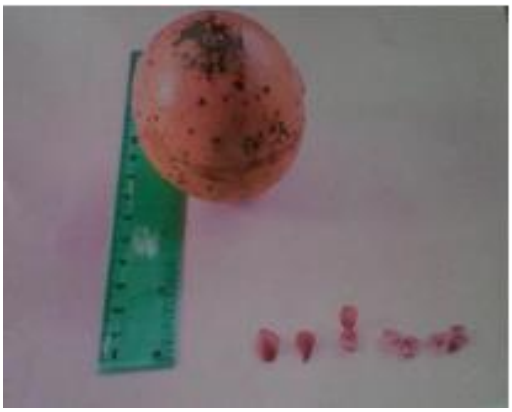

Plate 1b. Fruit of *S. spinosa.*

Plate 1c. Adaxial leaf surface of S. *spinosa* with no stomata present x400.

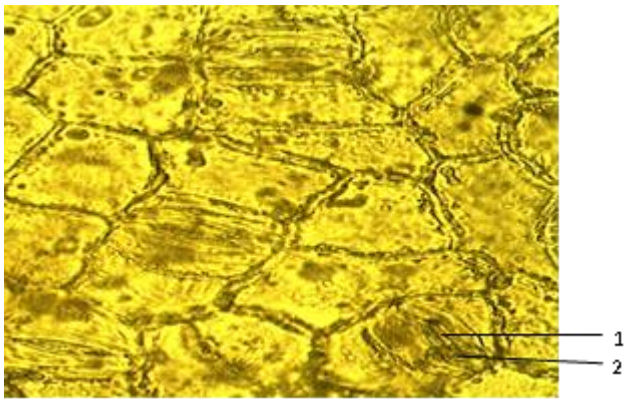

Plate 1d. Abaxial view of leaf of *S. spinosa* x400; 1, stomatal pore; 2, guard cell.

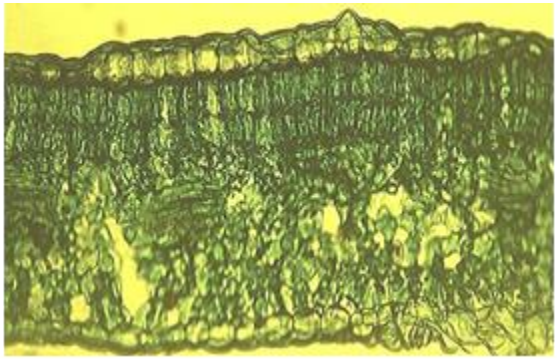

Plate 1e. TS leaf lamina *S. spinosa* x 200. **1**, Adaxial epidermis; **2**, Palisade mesophyll; **3**, Spongy mesophyll.

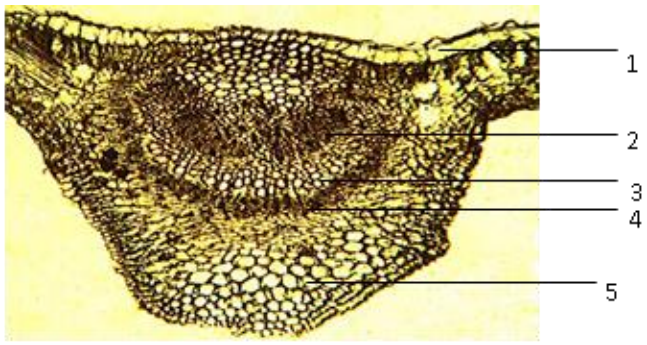

Plate 1f. Midrib of leaf*S.spinosa*x40; 1, adaxial epidermis; 2, internal phloem; 3, xylem elements; 4, external phloem; 5, ground tissue.

Plate 1g. petiole *of S. spinosa* x 40; 1, Adaxial epidermis; 2, vascular bundle; 3, ground tissue.

one or two layers, about eight cells away from the phloem. The pericycle is made up of a discontinuous

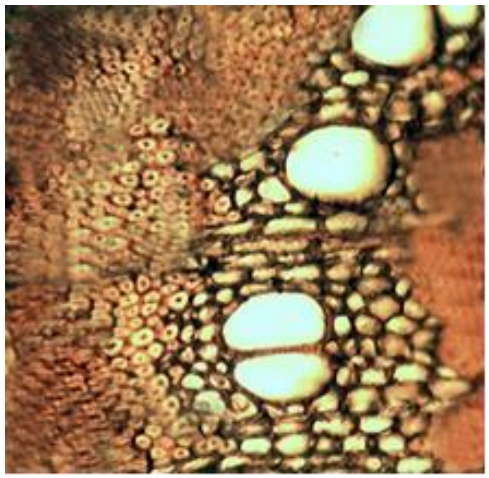

Plate 2a. TS stem of *S. spinosa* x100.

Plate 2b. TLS stem of *S. spinosa* x 200.

band of sclerenchyma that goes round. Vessels are of various shapes, while most are spherical, some have egg shape and a few have hexagonal shapes. The sizes of vessel also vary with both big and small ones. Vessel diameter measures 0.03 and 0.8 mm (at x 100). Both multiseriate and uniseriate rays occur and bands of fibers form a pattern in the section (Plate 2a).

Transverse longitudinal section (TLS): There are numerous rays of varying sizes and heights. Uniseriate rays are numerous, while multiseriate rays are few and vary from seventy - ninety cells high. Biseriate and triseriate rays are twelve - forty-five cells high (Plate 2b).

Vessels are of varying lengths ranging from 90 to 160 µ and vessel members measure between 12 to 15 µ, each. Vessel walls have simple pitting. Numerous fibers that are variously branched ramify the section.

Radial longitudinal section (RLS): The rays are hetero-

cellular, containing both upright and procumbent cells (Plate 2c). The procumbent cells have shapes that vary from square to rectangular, a few are somewhat round in shape. The height of the procumbent cells range from thirteen - twenty. The upright rays consist of cells that are all rectangular with their long axis oriented vertically and their height range from six - fifteen cells. Thus the procumbent cells outnumber the upright ones. Vessels are of varying lengths. Some vessels have one - four members and others have ten - fifteen members. There are numerous fibers that ramify the section (Plate 2c).

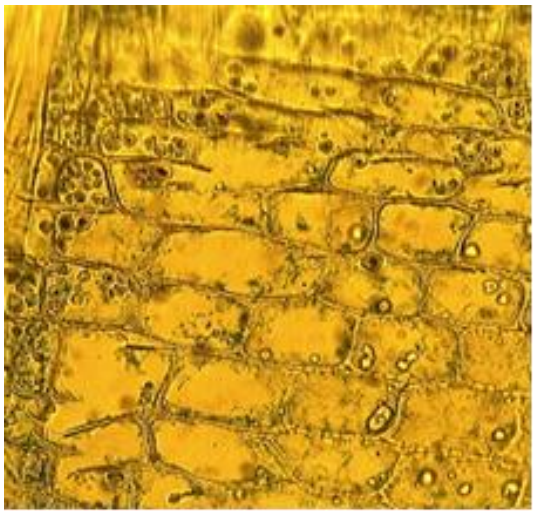

Plate 2c. RLS stem of *S. spinosa* x 200.

Root

Transverse section (TS): The cork cells contain eleven - fifteen layers of thin-walled rectangular shaped cells that are compactly arranged. The cortex contains ten - eighteen layers of thin-walled parenchymatous cells of various shapes. The endodermis consisting of a continuous ring of two to four layers of thick-walled sclerenchymatous cells is conspicuous and occurs close to the vascular tissue. Other patches of sclerenchyma are scattered in the cortex. There are numerous vessels and most of them are single though vessel multiples of twos and threes also occur (Plate 3a). Thick fibers forming bands abound and numerous rays transverse the section.

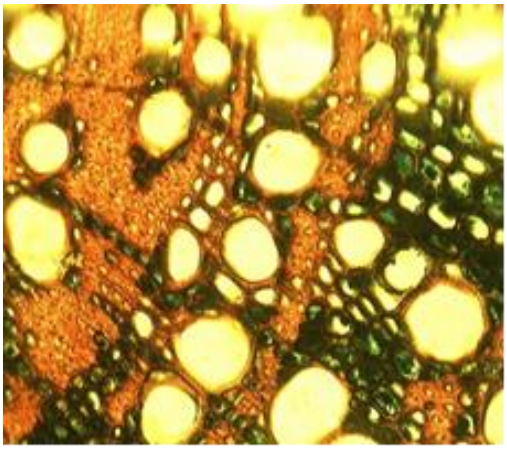

Plate 3a. TS root of *S. spinosa* x 200.

Tangential longitudinal section (TLS): There are very few uniseriate rays. Most rays are multiseriate varying in width from four - eight cells and in height from eleven - fifty four cells (Plate 3b). There are numerous fibers and vessel walls have simple pitting.

Pollen study: Pollen grains exhibit dimorphism in shape in *S. spinosa* with occurrence of 70% of pollen with triangular shape while 30% has quadrangular shape (Plate 4a and b).

Shape: The triangular ones are between oblate and subspheroidal. The quadrangular ones are oblate.

Aperture: The pollen that are triangular exhibit tricolpate aperture; while those that are quadrangular exhibit tetracolpate aperture Exine is tectate, sculpture is perforate. The perforation is irregularly arranged and not very compact.

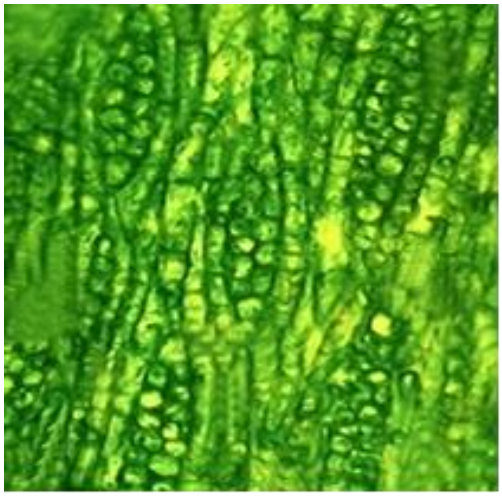

Plate 3b. TLS root of *S. spinosa* x 100.

Primitive and advanced features of *S. spinosa*

Summary of primitive and advanced features of *S. spinosa* are presented in Table 2.

DISCUSSION

The description of leaf morphology in the present study

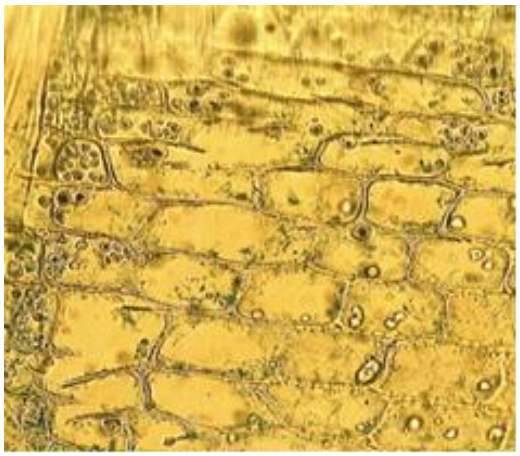

Plate 3c. RLS root of *S. spinosa* x 200.

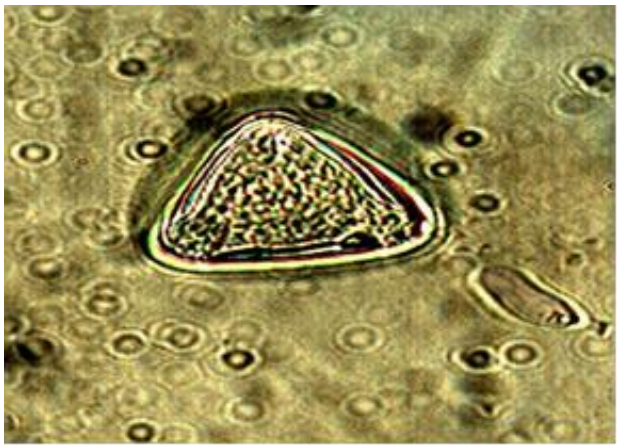

Plate 4a. Tricolporate pollen of *S. spinosa*. Triangular pollen in equational view showing perforate sculpturing x 400.

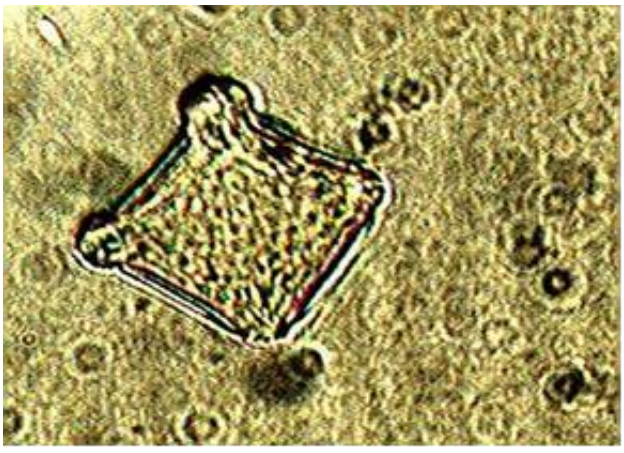

Plate 4b. Quadrangular pollen of *S. spinosa*. Quadrangular pollen in equational view showing perforate sculpturing x 400.

was found to be similar to the description of the family Loganiaceae made by Keay (1989).

The venation is diagnostic having all the veins emanating from the base or a little distance from the base (that is, palmate) and terminating at the apex. It can be used to spot the species in the field. Oduoye et al. (2013) reported the occurrence of similar venation in some Nigerian *Strychnos* spaces, they studied.

The occurrence of spines on the stem is another diagnostic feature that can be used to spot the tree in the field. Watt and Breyer Brandwijk (1962) noted that the specific name 'spinosa' is derived from the presence of spines and are often in pairs thus confirming the observation of Keay (1989).

The fragile nature of the petals was also observed in *Spigelia anthelmia,* another member of the family Loganiaceae (Asuzu and Nwosu, 2009). The transverse section of the leaf revealed a single layered, compactly arranged epidermis. This, together with the tough cuticle offers mechanical protection and reduction of water loss by transpiration (Evert, 2006).

The occurrence of "kranz" structure observed in *S. spinosa* was reported by Metcalfe and Chalk (1989) as a feature with restricted taxonomic distribution among the dicotyledon. It was suggested by Metcalfe and Chalk (1989) that plants with kranz structure probably arose in tropical surroundings. The occurrence of kranz structure in family Loganiaceae was observed by Asuzu and Nwosu (2009) in *Anthocleista djalonensis.*

Crystals were found in the mesophyll cells of the leaf and ground tissue of the petiole confirming the listing of the family Loganiaceae as one of the families where crystals occur (Metcalfe and Chalk, 1989).

In the midrib and petiole, intravascular phloem was observed. Metcalfe and Chalk (1989) reported that intravascular phloem is an anomalous structure and has restricted occurrence in plants. Evert (2006) remarked that intravascular phloem is found in families like Apocynaceae, Asclepiadaceae, Convolvulaceae, Cucurbitaceae, Mrytaceae, Solanaceae and Asteraceae. Bremer and Struwe (1992) observed that the families, Apocynaceae, Asclepiadaceae and Gentianaceae have close affinity and share some features in common with the Loganiaceae. The transverse section of the stem and root revealed vessels of varying sizes and numbers. Evert (2006) noted that wide vessels are more efficient in water conduction than narrow vessels. He postulated that the hydraulic conductivity of a vessel is roughly proportional to the fourth power of its radius or diameter. If the diameter of vessel is 2 for example, the relative volume of water flowing through it under normal conditions would be 2^4 (16). Though increased vessel diameter increases efficiency of water conduction, it also decreases safety. Vessels with larger size tend to be more vulnerable to water stress-induced cavitations. Cavitation is the formation of cavities filled with air within the conduits resulting in breakage of water columns

Table 2. Primitive and advance features of *S. spinosa*.

Primitive features	Advance features
Shrub	Opposite leaf arrangement
Perennial	Flowers arranged in an inflorescence
Simple leaves	Connate petals
Bisexual flower	Few carpels
Flowers with many parts	Fruit a berry
Petaliferous	Estipulate leaves
Hypogyny	Spine present
Endospermous seed with small embryo	
Separate stamens	
Taproot present	
Glabrous condition	
Seed present	

(Evert, 2006).

Both solitary vessels and vessel multiples were found in the sections. Evert (2006) reported that vessel multiples are safer than solitary vessels as they provide alternative paths for the xylem sap to bypass embolism or blockage of the conduit with air. When this kind of blockage occurs in a single vessel element, the wide vessel may soon be filled with water vapour and air and consequently lose the ability to conduct water.

There are many rays of varied sizes and heights. Rays in angiosperms occupy about 17% of the volume of wood and contribute substantially to the radial strength of the wood (Evert, 2006). On the issue of phylogeny, the specie *S. spinosa* has both primitive and advanced features. Jones (1979) opined that the rate of evolution is not the same for all structures of the plant. He noted that some parts of a plant may become more specialized than others leading to a situation where both advanced and primitive features occur in a taxon. The primitive features of this species outnumber the advanced features.

In conclusion, the study revealed that *S. spinosa* has the following features namely: stomata of the anisocytic type only in the abaxial section and the absence of stomata in the adaxial section; the presence of crystals in the anatomical section of leaf and stem; the presence of both solitary vessels and vessel multiples and pollen that are predominantly tricolporate with a few that are quadrangular.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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