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Studies of the wood of some Nigerian alkaloid-rich *Strychnos* species

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The wood of three Nigerian species of *Strychnos* Linn. growing in different ecological zones was studied. The transverse, transverse longitudinal and radial longitudinal sections of the wood were made with sliding sledge microtome. The wood was macerated to measure its fiber and vessel dimensions. The wood was diffusing porous and intraxylary phloem occurred in the three species. Interxylary phloem in discontinuous ring occurred in the xylem cylinder of *Strychnos innocua* Del. and *Strychnos usambarensis* Gilg. Parenchyma was paratracheal and formed aliform to confluent patterns in *S. spinosa* Lam. but scanty in *S. innocua* and *S. usambarensis.* Vessel elements occurred singly and in radial and tangential multiples of two to four. The fiber lumen dimension of *S. spinosa* had the least value while its fiber cell wall thickness also had the greatest value. In *S. spinosa,* alkaloids and sterols were detected in the leaves and stem extracts. Alkaloids were found in the leaves, stem bark and fruits of *S. usambarensis*. The micro morphological features in each species were useful in making them adapt to the climatic conditions of the ecological zones where they grow.

Key words: Interxylary phloem, Intraxylary phloem, *Strychnos spinosa, Strychnos innocua, Strychnos usambarensis.*

INTRODUCTION

Strychnos Linn. belongs to the family Loganiaceae and has been variously circumscribed by different authors. It is the most specious genus of the family with about 200 species and is pantropically distributed (Krukoff and Munachino, 1942; Leeuwenberg and Leenhout, 1980). Frasier (2008) stated that the family was first suggested by Robert (1814) but validly published by Von Marius (1827). Some species in the genus produce alkaloids like strychnine and also have a history of being used in folk medicine to treat fever and malaria (Bisset, 1970) and other conditions. Interest in the genus has been ongoing for a long time because of its alkaloid rich content. Some studies have been done in the species from Africa (Adebowale et al., 2009, 2012; Oduoye and Ogundipe, 2013). According to Angenot (1988), indole alkaloids are the most active ingredients of *Strychnos* and more of the alkaloids are produced in the stem bark and roots than in the leaves (Quetin-Leclercq et al., 1990).

Although Metcalfe and Chalk (1989), Mennaga (1980) and Dayal et al. (1984) examined the wood of some Loganiaceae, Carlquist (1984) and Frasier (2008) opined that investigation into various aspects of the wood of the

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genus should be made. Carlquist (1984) reiterated that several phylogenists have shown interest in delineating natural relationships and plausible groupings among dicotyledons. He also stated that wood anatomy appears to offer some clues about affinity among sympetalous families of dicotyledons. According to Stace (1991), a good taxonomic classification relies on a wide range of characters obtained from morphology, systematic anatomy, chemical taxonomy, cytology and phylogenetics. None of these characters (like anatomical character) can on its own form the basis of classification but when used in synergy, a good taxonomic classification can be achieved. There is no literature that compared the wood of these three species of *Strychnos*. This prompted the present authors to examine these woods of Nigerian species of *Strychnos* from sections Brevitubae, Densiflorae and Spinosae.

MATERIALS AND METHODS

Fresh samples of the wood of *Strychnos innocua* were collected from Rigasa village at the outskirts of Kaduna city, *S. spinosa* was collected from Kuje village at the outskirts of Abuja city and *Strychnos usambarensis* from Ohebe-Dim at the outskirts of Nsukka. Ten stands of each species were randomly collected from their natural regions of provenance in Nigeria. The stem and root of freshly collected samples were cut into small pieces about 2 cm cubes and stored in labeled bottles containing FAA in the Anatomy Laboratory of the Department of Plant Science and Biotechnology, University of Nigeria Nsukka. Three dimensional structures of the stem (transverse section, transverse longitudinal section and radial longitudinal sections) measuring 5-10 um thick were made using a Reichert sledge microtome and stored in well labeled petri dishes containing 70% ethyl alcohol.

Wood of the species of *Strychnos* were split into chips measuring 2 mm in thickness and put into well labeled long test tubes. The chips were macerated with Jane’s method (Oladele, 1991).The macerated chips were transferred into well-labeled specimen bottles and stained with crystal blue and safranine before mounting on slides with Canada balsam and covering with cover slips. The fiber dimensions, the vessel element length and the diameter taken at the widest point were measured and recorded. The cut sections of the stem and root stained with safranine and counter stained with fast green according to Sass’s method (Oladele, 1991) were mounted with Canada balsam and left on the laboratory bench to dry for 3-4 days. They were then examined under a Zeiss light microscope.

RESULTS

Phytochemical constituents of *Strychnos*

In *S. spinosa,* Philippe et al. (2005) isolated two alkaloids namely saringo sterol and 2,4-hydroperoxy-24-vinyl cholestorel and these showed anti-trypanosomal activity while Rajesh et al. (2009) detected four sterols from the leaf extract of *S. spinosa*. According to Morah (2011) and Oguakwa et al. (1980), the stem and leaf extract of *S. spinosa* collected from different locations in Nigeria yielded some alkaloids. However, Kingsley and Lofgreen (1942), working with stem and leaf extracts grown in Florida, found no alkaloids from the samples they analysed.

Corsaro et al. (1995) isolated polysaccharides from the seeds of *S. innocua* while Bello et al. (2008) reported the presence of trypsin inhibitor that caused diminished growth in rats, chicken and other experimental animals when eaten raw from the fruit juice and seed of *S. innocua.* Corsaro et al. (1995) also reported the presence of sterols, and fatty acids in the oil of the seed.

Coprasse and Angenot (1982) isolated iso strychnopentamine and dihydro usambarensine which showed strong activity against *Plasmodium falcipariun in vitro*. Federick et al. (2002) also isolated strychnopentamine from the leaves of S. usambarensis and it exhibited antiplasmodial activity *in vitro* against *P. falciparum* that is sensitive and resistant to chloroquine. Cruz (2008) reported that combination of curare alkaloids from the leaves of *S. usambarensis* with anaesthetic material, allowed for the use of less of the latter thereby, reducing the risk inherent in the use of the normal dose of anaesthesia.

Philippe et al. (2005) isolated tertiary and quaternary alkaloids as well as anydronium bases from the root bark of *S. usambarensis*. Federick et al. (1998) also isolated alkaloids from the stem bark of *S. usambarensis*. The fruits of *S. usambarensis* which look like cherries and have an attractive yellow colour when ripe have caused poisoning in children in Africa. This alkaloid has never been isolated from any member of Loganiaceae but has been earlier identified in *Ochrosia* (Apocynacae) by Angenot (1988).

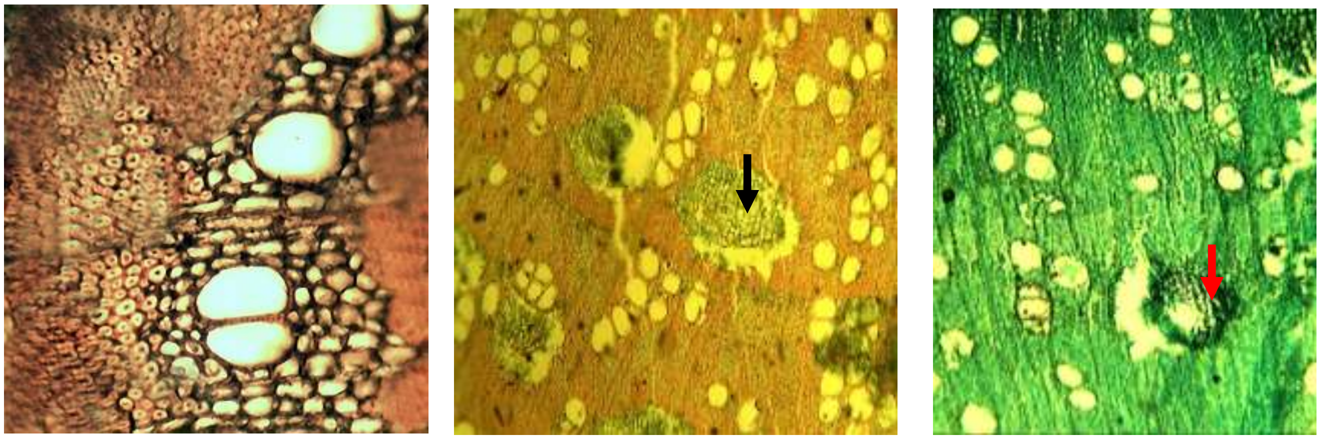
Transverse section of stem

S. spinosa has more than 70% of solitary vessels, less than 29% are in twos or more*. S. innocua* has about 50% vessels occurring singly while the other 50% occur in radial or tangential patterns of 2-3(4). *S. usambarensis* has almost 60% of vessels in singles while the vessels in multiples are in radial patterns.

Parenchyma is paratracheal and forms aliform to confluent pattern in *S. spinosa*. In *S. innocua* and *S. usambarensis* parenchyma is apotracheal to scanty. Interxylary phloem occurs in discontinuous ring right round the xylem cylinder of *S. innocua* and *S. usambarensis* but was not observed in *S. spinosa*. Intraxylary phloem was present in the three species (Figure 1a to c). Fibers in *S. spinosa* have very thick walls.

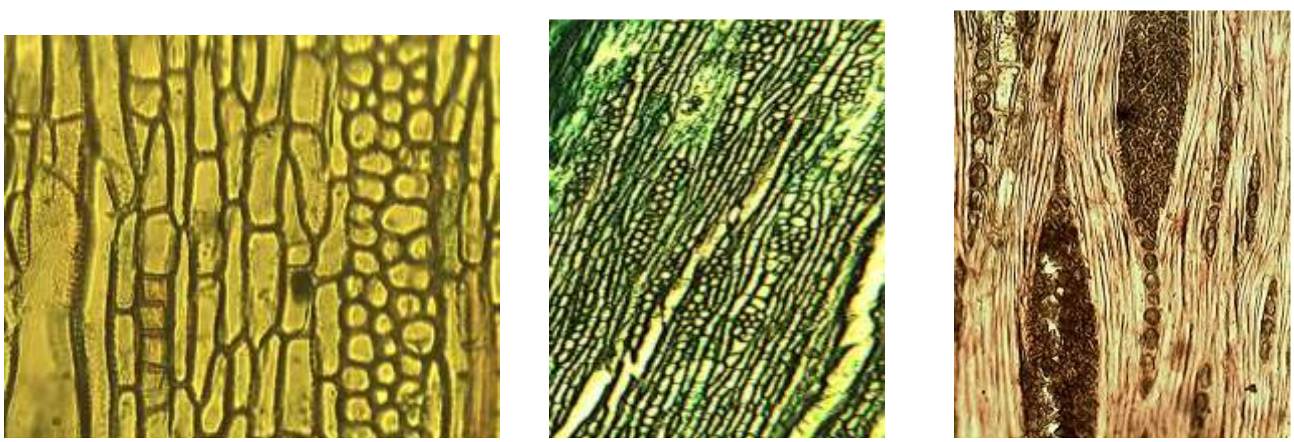
Transverse longitudinal section

Rays are heterogeneous, having uni and multiseriate



(a) (b) (c)

Figure 1. (a) TS stem of *S. spinosa* x100, (b) TS stem of *S. innocua* showing interxylary phloemx40, (c) TS stem of *S. usambarensis* showing interxylary phloemx40.



(a) (b) (c)

Figure 2. (a) TLS stem of S. spinosa x200, (b) TLS stem of S. innocua x40, (c) TLS stem of *S. usambarensis* x100.

types. Multiseriate rays are more abundant in *S. usambarensis* with some rays being more than four cells wide while in *S. innocua*, the rays are mostly 1-3 seriate and are short (Figure 2a to c). Rays are irregularly storied in *S. spinosa* and *S. innocua.*

Radial longitudinal section

The rays are heterocellular having both upright and procumbent types in the three species (Figure 3a to c). The fiber lumen diameter (FLD) of *S. spinosa* had the least value (Table 1). On the other hand, the fiber cell wall thickness (FCWT) of *S. spinosa* had the greatest value while the dimensions of that of *S. usambarensis* had the least value (Figure 4a to c). The vessel walls of the three species have simple reticulate pitting (Table 1, Figure 5a to c).

DISCUSSION

The possession of vessels in radial and tangential directions occurring together with solitary vessels is a feature common to members of Loganiaceae (Krukoff and Munachino, 1942; Mennega, 1980; Dayal et al., 1984; Metcalfe and Chalk, 1989; Moya et al., 2017). Also the possession of vessels that are round in transaction is a feature of Loganiaceae (Carlquist, 1984; Metcalfe and Chalk, 1989). The presence of up to 50% of vessels in

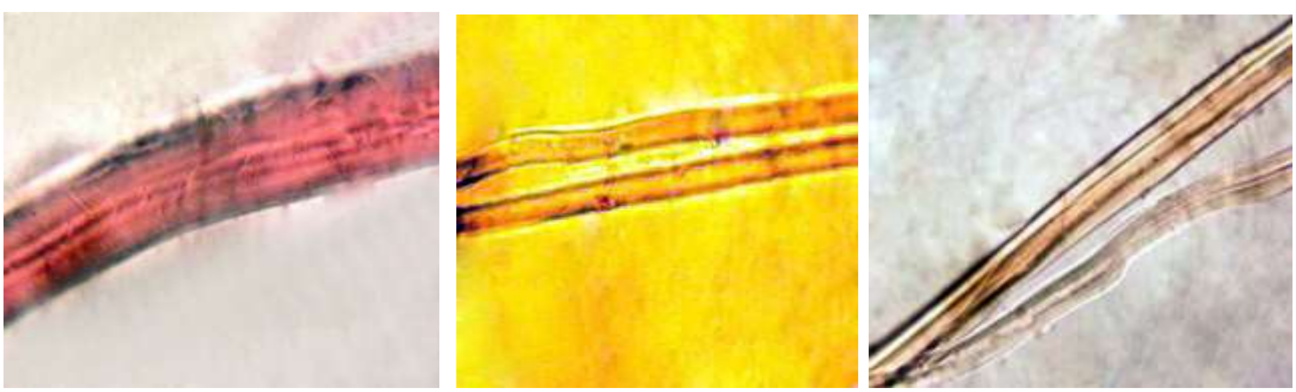
(a) (b) (c)

Figure 3. (a) RLS stem of *S. spinosa* x200, (b) RLS stem of *S. innocua* x100, © RLS stem of *S. usambarensis* x100.

Table 1. Vessel and fiber dimensions of stem of *S. spinosa, S. innocua and S. usambarensis.*

Name of Plant	VL (mm)	VD (mm)	FL (mm)	FCWT (mm)	FLD (mm)
S. spinosa	0.456±0.22[b]	0.133±0.04[b]	2.45±0.31[b]	0.014±0.00[b]	0.028±0.01[b]
S. innocua	0.396±0.16[a]	0.107±0.01[a]	1.773±0.11[a]	0.0133±0.00[b]	0.0017±0.0001[a]
S. usambarensis	0.453±0.18[b]	0.145±0.03[c]	1.88±0.001[a]	0.002±0.00[a]	0.002±0.00[a]

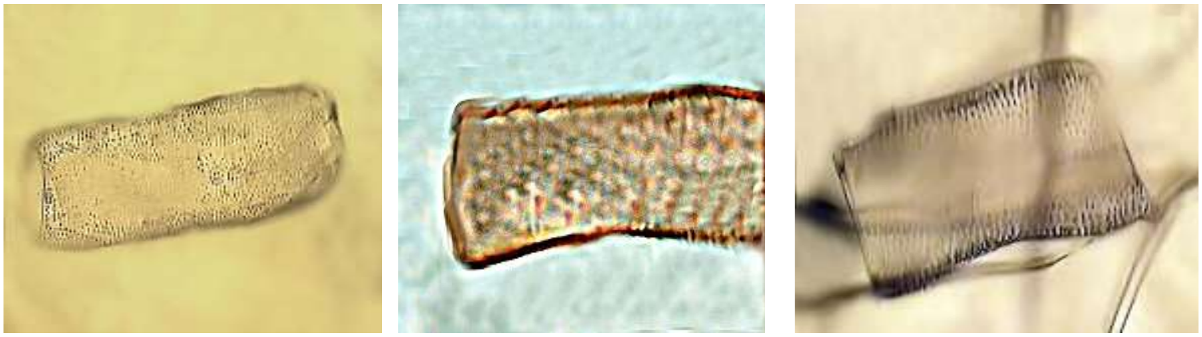
VL- Vessel length, VD- Vessel diameter, FL- Fiber length, FCWT- Fiber cell wall thickness and FLD Fiber lumen diameter. Means followed by different alphabets vertically are significantly different at $p<0.05$. Means ± standard deviation of three replicates.



(a) (b) (c)

Figure 4. (a) Fiber stem of *S. spinosa* x400, (b) Fiber stem of *S. innocua* x400, (c) Fiber stem of *S. usambarensis* x400.

radial chains suggests a feature that can help to enhance better conductivity of fluid. Evert (2006) stated that multiple vessels assist the plant in circumventing embolism if it occurs and ensures efficient flow of fluid through nearby vessels. *S. innocua* grows in a drier ecological region and will be faced with the challenge of conserving water and will probably be at an advantage with multiple vessels. Moya et al. (2017) in their study of *S. bredemeyeri* from Costa Rico also observed the occurrence of solitary vessels and those in radial and



(a) (b) (c)

Figure 5. (a)Vessel of stem of *S. spinosa* x100, (b) Vessel of stem of *S. innocua* x100, (c) Vessel of stem of *S. usambarensis* x100.

diagonal multiples.

The length of vessel elements in *S. innocua* that grows predominantly in drier parts (Sudan savanna) is shorter than that of *S. spinosa* and *S. usambarensis* that grow in Guinea savanna and Derived savanna respectively. According to Ekwutoziem (2015), shorter vessels are associated with drier habitats and longer vessels with wetter ones. The diameter of vessels of *S. innocua* growing in the drier ecological zone of Sudan savanna is smaller than those of *S. spinosa* in Guinea savanna and *S. usambarensis* in derived savanna. According to Ekwutoziem (2015) plants in the arid regions tend to have smaller vessel diameter and are also less vulnerable to cavitations. Sperry (2003) observed that wider conduits are more vulnerable to cavitation than smaller ones and are thus more exposed to embolism of vessels.

Rays of varying width can help to delimit the different species*. S. innocua* has rays with 1-3 seriate, while *S. usambarensis* has rays wider than 4- seriate. Moya et al. (2017) observed rays that were 2- to many celled wide being mixed up with few uniseriate rays in *S. bredemeyeri* that they studied. According to Evert (2006), the possession of wide rays is equally of importance in strengthening a plant.

The possession of intraxylary phloem in the three species is a diagnostic feature for family Loganiaceae and other families in the order Gentianales (Metcalfe and Chalk, 1989; Frasier, 2008). Carlquist (2013) also stated that interxylary phloem occurs in only a relatively small number of families and consisted of strands of sieve tubes, and companion cells embedded within the secondary xylem*. S. spinosa* lacks interxylary phloem while *S. innocua* and *S. usambarensis* possess it. Moya et al. (2017) noted that the possession of interxylary phloem is restricted to small number of dicotyledonous taxa and mentioned that it has been reported in many species of *Strychnos*. This is the first time the possession of interxylary phloem is being reported in *S. innocua* and *S. usambarensis*. Moya et al. (2017) observed that the development of interxylary phloem occurred at a later age in *S. bredemeyeri*. It was not found in the juvenile wood of that species. They suggested that further work should be done at different developmental ages of those species currently reported in literature that lack this feature. Mennega (1980) however noted that within a genus, some species may have this feature and others lack it with no clear difference in habit or size of plant.

Axial parenchyma is scanty in *S. innocua* and *S. usambarensis*. This confirms the earlier submission of Carlquist (1984); Metcalfe and Chalk (1989) on the possession of scanty axial parenchyma in Loganiaceae. Fibers of *S. spinosa* have very thick walls and this feature is important in performing, mechanical functions. Moya et al. (2017) reported that thick walled fiber is a characteristic of genus *Strychnos*. Herendeen and Miller (2000) reported that the thickness of fiber cell wall is closely related to density, stating that the thicker the fiber cell wall, the higher the density. The possession of thick walled fiber could be used to support the use of traditional folks of the wood of *S. spinosa* in construction of poles, agricultural and other tool handles (ICRAF, 2000). The possession of thick walled fibers of *S. spinosa* is been reported for the first time. Some of the features possessed by the species are of great importance in making the plants adapt to the climatic conditions of the ecological zones where they grow. This study is a part of the ongoing studies on the African *Strychnos*. DNA studies in all the members of the genus to better separate them into sections and also elucidate their phylogeny are desirable.

In conclusion, the *Strycnos* species studied possess alkaloids and other phytochemicals. The presence of interxylary phloem which is suggested to have physiological function is reported for the first time in *S. innocua* and *S. usambarensis*. The fiber cell wall thickness of *S. spinosa* which is greater than that of *S. innocua* and *S. usambarensis* is also reported for the first time. Fiber cell wall thickness is associated with mechanical function of the plant and explains the use of the wood by farmers in making farm implements.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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