Comparative Study of the Leaf Morphology and Anatomy of Selected Strychnos Species: Strychnos Spinosa Lam., Strychnos Innocua Del. and Strychnos Usambarensis Gilg Found in Three Ecological Zones in Nigeria

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Abstract-- The morphological and anatomical study of three Nigerian species of Strychnos: S. spinosa, S. innocua and S. usambarensis was carried out. The leaves are dorsiventrally flattened, petiolate, with distinct palmate venation. The transverse section of leaf lamina revealed uniseriate epidermis with double-layered palisade mesophyll and loosely arranged spongy mesophyll. Rosette crystals were found in the mesophyll of S. spinosa and S. usambarensis. Stomata are hypostomatic and the measurement of the length of guard cells of S. innocua differed from those of S. spinosa and S. usambarensis. Petiole anatomy revealed seven variously sized and separate vascular bundles in S. innocua and S. spinosa but with a single arc shaped bundle in S. usambarensis.

Index Term-- Strychnos spinosa, S. innocua S. usambarensis, palmate venation, rosette crystals.

INTRODUCTION

Strychnos belongs to the family Loganiaceae and is made up of 200-400 species (Cruz, 2008; Rajesh et al. 2009). Strychnos is the largest genus of the family Loganiaceae (Fraiser, 2011). Mwamba, 2006; Rajesh et al.2009 and Orwa et al.2009 all reported that, it was first described by Linnaeus based on S. nux-vomica which is the type species.

The genus Strychnos is well known for its alkaloid production, particularly strychnine from S. nux-vomica which has been popularized for its potential nefarious uses. Angenot (1988) observed that although investigation into the genus Strychnos has been going on for some time, the African members suffered a long period of neglect. However, work on the uses of S. spinosa and S. innocua has been reported by Asesa et.al(2005); Mwamba (2006) Rajesh et al.(2009); Augustino et.al.(2011); Kokwaro (1976); F. A. O.(1983.); Mbuya et al (1994) and Orwa et al. (2009). Angenot (1988) and Cruz (2008) described the uses of S. usambarensis. Phytochemical studies on S. spinosa has been carried out by Bisset (1970); Adebiji and Sofowora(1978); Philippe et al. (2005); Morah (1982, 2011). Studies on the phytochemicals in S. innocua has been investigated by Corsaro et al. (1995); Philippe et al. (2005) and Bello et al.(2008). There is however still paucity of literature on the detailed description of the anatomy of the leaf lamina, midrib and stomatal types in the three Nigerian species of Strychnos. This study aims at examining the morphological and anatomical variables of the leaf lamina, midrib and stomatal types of the three Nigerian species of Strychnos.

MATERIALS AND METHODS

The species of Strychnos were collected randomly from three ecological zones in Nigeria where they grow. S. innocua was collected from Rigasa village near Kaduna (Sudan savanna), S. spinosa was collected from Kuje village near Abuja (Guinea savanna), while S. usambarensis was collected from Ohebe-Dim village near Nsukka (Derived savanna). Fresh leaves were collected from ten samples each. For the stomatal studies, freshly collected leaves of the three species were washed with distilled water to remove any dust or dirt clinging on the leaves. The leaves were put in three separate and well-labeled petri dishes. Fifteen to twenty ml of bleach (hydrogen peroxide) solution was poured into the petri dishes to completely submerge the leaves. The solution was left for 24-48 hours in order to bleach the leaves. The bleached leaves were removed carefully and rinsed thrice with distilled water to remove any trace of the bleach. The abaxial and adaxial surfaces of the leaves from each petri dish were scraped off. Viewing of the adaxial and abaxial surfaces of the scraped leaves was done using the Zeiss light microscope. Another set of leaves of freshly collected samples were cut into small pieces and stored in well labeled bottles containing F.A.A. (20 ml of 40% formaldehyde, 30 ml of distilled water, 30 ml of 100% ethanol and 20 ml of glacial acetic acid) in the Anatomy Laboratory of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. This mixture helped to preserve the specimens and prevent any form of microbial growth. Transverse sections of the midrib and petiole were made using a Reichert sliding sledge microtome. The sections were cut at 10μ thickness and stored in well-labeled petri dishes containing 70% ethyl alcohol.
RESULT
Leaf Morphology
The morphology of leaves of *S. spinosa*, *S. innocua* and *S. usambarensis* is presented in Table 1.

Anatomical Evaluation
There were no visible stomata on the adaxial surface of the three *Strychnos* species. Numerous stomata were found on the abaxial surface and range in number from 25 to 40 per field of view. Comparison of the dimensions of the guard cells is shown in the Table 1.

Transverse Section of the leaf
Epidermis: The abaxial and adaxial epidermis in the three species all have uniseriate layers. The adaxial epidermis is rectangular in shape and has thin walls. The outer surface is convex and is covered with a thin cuticle.

Mesophyll layer: The mesophyll in the three species is differentiated into palisade and spongy. The palisade mesophyll is double layered, rectangular shaped and longer than wide in the three species. There are seven to eight layers of cells that are large and loosely arranged in the spongy mesophyll in the three species. Air spaces, rosette crystals and bundle sheaths are also present. The abaxial epidermis is not even because of the presence of stomata of anisocytic type. Plate 3a-c.

Midrib: The midrib is plano convex in the three species with semicircular abaxial surface. Plates4a-c.

Adaxial surface: There is uniseriate layer of epidermal cells in the three species. The cells are rectangular with convex shape at the outer surface. There is a thin layer of cuticle over the adaxial epidermis.

Ground tissue: The ground tissue is parenchymatous, thin walled and the number of layers of cells in each of the species is in Table 2.

The palisade mesophyll does not extend across the adaxial part of midrib and the vascular bundle is embedded in the ground tissue.

Vascular tissue: The vascular strand is single, semicircular and bicolateral in *S. spinosa*, *S. innocua* and *S. usambarensis*. The xylem elements consist of radially oriented lines of xylem vessels and narrow bands of adaxial and abaxial phloem. The xylem elements in *S. spinosa* and *S. innocua* are predominantly polygonal in shape but a few are circular. (See Table 3).

Sclerenchyma sheath surrounds the vascular bundle in *S. usambarensis* and *S. innocua*. In the former, the sclerenchyma layer is one to two celled thick at the adaxial surface. In the latter, the sclerenchyma layer is three to six celled thick right round.

Abaxial epidermis: The abaxial epidermis is semicircular and single layered in the three species.

Petiole:
Adaxial epidermis: The adaxial epidermis in the three species is uniseriate. *S. spinosa* has two projections that are bluntly conical with compact thin walled parenchyma cells.

*S. innocua* lacks these lateral projections at the adaxial surface. The adaxial surface of *S. spinosa* and *S. innocua* are slightly undulating, while that of *S. usambarensis* is concave. Plates4 a-c

Ground tissue: The ground tissue is homogenous, parenchymatous, thin walled, circular in shape and compact. The number of layers of parenchyma cells in the ground tissue of petiole is shown in Table 4.

Vascular tissue: *S. spinosa* and *S. innocua* each has seven distinct and separate vascular bundles of varying sizes, with the middle one as the biggest *S. usambarensis* has one big vascular bundle embedded in the ground tissue (Plates5a-c). In the three species, the xylem cylinder has closely arranged radial files of vessel elements with phloem on the adaxial and abaxial surfaces. In *S. spinosa* and *S. usambarensis*, the xylem elements are predominantly spherical to circular in shape, while in *S. innocua*, the xylem are angular. The vessel elements range from eleven in the middle to six at the right and left hand lateral corners in *S. spinosa* while in *S. usambarensis*, the rows of xylem elements are about twenty-eight in the single vascular bundle. In addition, there is a pronounced region of protoxylem at the abaxial layer, which is not visible in *S. spinosa* and *S. innocua*. In *S. innocua* there are about eleven rows of xylem elements in the median bundle and one at the extreme left and right hand corners. In each of the seven bundles in the petiole of *S. spinosa* and *S. innocua*, there are sieve cells and phloem cells at the adaxial and abaxial surfaces. In *S. usambarensis*, the phloem tissue occurs at both adaxial and abaxial surfaces. The abaxial epidermis is semicircular in shape and uniseriate in the three species.

DISCUSSION
Metcalfe and Chalk (1979) and Stace (1980) recognized the use of leaf architectural design as an important tool in field taxonomy. Thus the differences in leaf margin and apex in the three species is of taxonomic interest in delimiting them.

Significant difference was recorded in the dimensions of the length of guard cells. *S. innocua* growing in Sudan savanna with drier climatic conditions possessed guard cells with the longest length. *S. usambarensis* growing in Derived savanna had the least length of guard cells. This is probably a feature to enable each adapt to it’s particular vegetation zone and a response to the climatic conditions in that zone.

James and Bell (1995) working on six clones of *Eucaliptus camaldalensis* from five Australian locations found that there was a lack of correlation between leaf characteristics and climatic data. They suggested that availability of ground water, root structure and internal transport of water may have greater influence on leaf structure than atmospheric demand. In the same vein Zu and Zhou (2008) opined that different effects of abiotic factors on stomatal size might depend on plant species varieties. Martinez et al. (2007) however reported that under water
deficit, a decrease in cell size occurred indicating that an adaptation to drought is probable. The shapes of epidermal and subsidiary cells at the adaxial and abaxial surfaces of the leaves of the three species are markedly different such that if there is need to identify the species when they are not flowering, they could be successfully separated without any mix up.

Oladele (2002) pointed out the importance of plant stomata in humidification of the atmosphere. He opined that the efficiency of the stomatal complex in carrying out this feature depends on factors like stomatal size, stomatal density and the number of subsidiary cells per stoma. Olafinobin and Oladele (1970) and Obiremi and Oladele (2001) stated that the higher the number of subsidiary cells per stoma, the greater the capacity of such stomatal complex in humidifying the atmosphere. According to Oladele (2002), the presence of high number of subsidiary cells per stoma enhances the presence of stomatal opening and consequently making humidification of the atmosphere to be more rapid. The three species of *Strychnos* possessed a minimum of four subsidiary cells around the stoma. From the fore mentioned, it follows that there will be enhanced stomatal opening and rapid humidification of the atmosphere by these species of *Strychnos*.

Bicollateral vascular bundles were clearly shown in the midrib and petiole of leaf and stem of the three species. This feature is taxonomically important at the generic level since bicollateral vascular bundles were regarded as anomalous structure because of their restricted occurrence. It was reported by Metcalfe and Chalk (1979) to occur in the family Loganiaceae and other related families like Asclepiadaceae, Rubiaceae and Apocynaceae. *S. usambarensis* has a uniseriate layer of sclerenchyma at the adaxial surface of the midrib but two to three at the abaxial surface, while *S. innocua* has three to six layers of sclerenchyma sheath right round the vascular bundle. Evert (2006) stated that the presence of sclerenchyma around vascular bundles performs the function of strengthening the leaf. The variation in thickness of the sclerenchyma is of taxonomic interest in delimiting the species.

Rosette crystals that are of taxonomic importance at the genus level as cited by Metcalfe and Chalk (1979) were observed in the petiole, leaf, mesophyll and midrib of *S. spinosa* and *S. usambarensis*. Franceschi and Niakata (2005) reported that the presence of small crystals in higher plants is related to physical protection, storage of calcium and regulation of light during photosynthesis for plants growing in the shade. In the species studied, the presence of rosette crystals confirms that they possess the characteristic feature of the family Loganiaceae as stated by Metcalfe and Chalk (1979). Metcalfe and Chalk (1950) considered the petiole to be of taxonomic importance, as it is not influenced much by environmental changes. The organization of the vascular system in the petiole consisted of seven separate and biconvex bundles in *S. spinosa* and *S. innocua* but one big bundle in *S. usambarensis*. The petiole outline in *S. spinosa* and *S. innocua* are plano convex with *S. spinosa* having lateral projections at the adaxial surface. *S. usambarensis* has petiole that the adaxial surface is concave. Thus the petiole outline can furnish information for the delimitation of the three species. *S. nux-vomica* is reported by Metcalfe and Chalk (1979) to have three separate vascular bundles. It is thus suggested that the organization of the vascular system in form of separate and variously sized bundles is not diagnostic for the genus.

In conclusion, this study revealed that the three species studied are hypostomatic with presence of stomata only at the abaxial surface. The varying thickness of sclerenchyma around the vascular bundle of the midrib suggests more strengthening. The presence of rosette crystals in the leaf mesophyll, midrib and petiole suggests additional physical protection and storage of calcium.

REFERENCES

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Plate 1a: S. spinosa adaxial no stomata present x400

Plate 2a: S. spinosa abaxial x400

Plate 1b: Adaxial leaf surface of S. innocua, no stomata present x400

Plate 2b: Abaxial leaf surface of S. innocua x400
Plate 3a: Leaf lamina *S. spiniosa* x 200
1 – Adaxial epidermis, 2 – Palisade mesophyll, 3 – Spongy mesophyll

Plate 3b: Leaf lamina *S. innocua* x 200
1 – Adaxial epidermis, 2 – Palisade mesophyll, 3 – Spongy mesophyll
Plate 3c: T.S leaf of *S. usambarensis* x200
1 – Adaxial epidermis, 2 – Palisade mesophyll, 3 – Spongy mesophyll, 4 – crystal
4 – Crystal

Plate 4a: Midrib of leaf *S. spinosa* x40
1 – adaxial epidermis, 2 – internal phloem,
3 – xylem elements, 4 – external phloem, 5 – ground tissue
Plate 4b: Midrib of leaf S. innocua x40
1 – adaxial epidermis, 2 – internal phloem, 3 – xylem elements, 4 – external phloem, 5 – ground tissue

Plate 5a: Petiole of S. spinosa x40

Plate 5b: Petiole of S. innocua x40

Plate 5c: Bicollateral vascular bundle of petiole of S. innocua x200

Plate 5d: Bicollateral vascular bundle of petiole of S. spinosa x200

Plate 5e: Bicollateral vascular bundle of petiole of S. innocua x200
Table I

Stomatal dimensions of *S. spinosa*, *S. innocua* and *S. usambarensis*

<table>
<thead>
<tr>
<th>Name of plant</th>
<th>Length of guard cell (mm)</th>
<th>Width guard cell (mm)</th>
<th>No of stomatal/field of view</th>
<th>No of subsidiary cell/field of view</th>
<th>Cell wall thickness of epidermal cells (mm)</th>
<th>Stomatal index</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. spinosa</em></td>
<td>0.176</td>
<td>0.027</td>
<td>10.9</td>
<td>66.2</td>
<td>0.016</td>
<td>14.13</td>
</tr>
<tr>
<td><em>S. innocua</em></td>
<td>0.187</td>
<td>0.029</td>
<td>12.2</td>
<td>92</td>
<td>0.037</td>
<td>11.70</td>
</tr>
<tr>
<td><em>S. usambarensis</em></td>
<td>0.158</td>
<td>0.040</td>
<td>11.6</td>
<td>97.8</td>
<td>0.014</td>
<td>10.60</td>
</tr>
</tbody>
</table>

Table II

Number of layers of ground tissue in midrib of the three species of *Strychnos*

<table>
<thead>
<tr>
<th></th>
<th><em>S. spinosa</em></th>
<th><em>S. innocua</em></th>
<th><em>S. usambarensis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Layers of cells at adaxial surface</td>
<td>11.7±0.82</td>
<td>6.5±0.52</td>
<td>4.3±0.48</td>
</tr>
<tr>
<td>Layers of cells at abaxial surface</td>
<td>7.5±0.51</td>
<td>8.5±0.53</td>
<td>8.5±0.53</td>
</tr>
</tbody>
</table>

Table III

Number of xylem elements in the vascular bundle of midrib

<table>
<thead>
<tr>
<th></th>
<th><em>S. spinosa</em></th>
<th><em>S. innocua</em></th>
<th><em>S. usambarensis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of xylem elements at median part</td>
<td>4.34±0.61</td>
<td>7.5±0.50</td>
<td>2.3±0.64</td>
</tr>
<tr>
<td>Number of xylem elements at left and right hand corners</td>
<td>1.5±0.52</td>
<td>2.3±0.45</td>
<td>4.5±0.50</td>
</tr>
</tbody>
</table>

Table IV

Number of layers of parenchyma cells in the ground tissue of petiole

<table>
<thead>
<tr>
<th>Name of plant</th>
<th>Median Adaxial</th>
<th>Extreme Left &amp; Right adaxial</th>
<th>Median Abaxial</th>
<th>Extreme Left and Right Abaxial</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. spinosa</em></td>
<td>21.5±1.56</td>
<td>9.50±2.00</td>
<td>18.5±3.55</td>
<td>8.5±1.33</td>
</tr>
<tr>
<td><em>S. innocua</em></td>
<td>13.9±2.34</td>
<td>9.87±0.98</td>
<td>11.45±0.87</td>
<td>6.50±0.55</td>
</tr>
<tr>
<td><em>S. usambarensis</em></td>
<td>13.55±0.65</td>
<td>16.50±1.87</td>
<td>10.55±1.76</td>
<td>9.50±0.50</td>
</tr>
</tbody>
</table>

Plate 5c: Petiole of *S. usabarensis* x40

Plate 5f: Bicollateral vascular bundle of petiole of *S. usabarensis* x200