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Stem anatomical studies of some species of *Indigofera* L. (Leguminosae-Papilionoideae)

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Stem anatomical studies of eight species of the genus *Indigofera* species common in Eastern Nigeria was carried out with the aid of a light microscope. This is with a view of establishing taxonomic relationship among the species. The type of epidermal and hypodermal layers, nature and size of cortical vessels and vascular bundle arrangement are some of the characters assessed and discussed among the *Indigofera* species studied. The 3-5 lignified hypodermal layers found in *I. paniculata*, *I. pulchra* and *I. tinctoria* distinguish these species from the other species of *Indigofera* studied. Similarly the conjoint collateral and open vascular bundle type present in *I. paniculata*, *I. senegalensis* and *I. stenophylla* are remarkable and diagnostic for taxonomic characterization of these species. The cortex is distinct with the variations in size and number of vessels with bigger vessels occurring at the hypodermal region and gradually reducing in size towards the center as observed in *I. paniculata* and *I. pulchra* and possess cells that are closely packed with little or no intercellular spaces. The nature of the endodermal layer shows that it is sclerenchymatous in *I. paniculata*, *I. priureana* and *I. pulchra*. The presence of tannins and other ergastic substance in the stem of these species further confirms the highly tanniferous nature of these plants and hence their economic values to man. The biosystematic implications of these findings have been discussed in the light of current literature.

Key words: Anatomy, *Indigofera*, stem, Leguminosae-Papilionoideae, taxonomy.

INTRODUCTION

The genus *Indigofera* L. is a member of the family Leguminosae-Papilionoideae among the dicotyledons. A very large family of herbs, shrubs and trees with a great variety of habit, including hydrophytes, xerophytes and climbers (Dallwitz and Watson, 2000). Knuth (1930) regarded the Leguminales as being made up of three families namely Caesalpinaceae, Mimosaceae and Papilionaceae, each consisting of different genera and different species. Hutchinson and Dalziel (1958) also recognized the three families namely Caesalpinaceae with 58 genera Mimosaceae, with 24 genera and Papilionaceae with 9 tribes. Lowe and Soladoye (1990) reported the unification of these families in Leguminales into three families known as Leguminosae-Caesalpinoideae,

Leguminosae-Mimosoideae and Leguminosae-Papilionoideae. These later treatment was also used by Burkill (1995) in the recognition of different families of the Legumes.

Prior to this latest development in the taxonomy of the Legumes, Hutchinson and Dalziel (1958) recognized the family Papilionaceae and split this into nine tribes namely Sophoreae, Dalbergia, Galegea, Genisteae, Loteae, Trifolieae, Phaseoleae, Viciae and Hydysareae. These tribes have between one genus in Loteae to thirty-one genera in Phaseoleae. According to Willis (1985) the genus *Indigofera* which belongs to the tribe Galegeae is made up of about 700 species which are found in warm tropical and subtropical regions of the world. Worthy of note is the inconsistency in the number of species identified by various authors. To this end Daniel (1960) identified 200 species while Mattson (1983) recognized over 300 species. Hutchinson and Dalziel (1968) further identified 78 species two of which *I. heterocarpa* Welw.

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Ex Bak. and *I. variabilis* Berhaut are imperfectly known species.

In West Africa, Burkill (1995) reported 60 species while Hutchinson and Dalziel (1958) reported 78 species of *Indigofera*. In Nigeria, Burkill (1995) reported 45 species while Hutchinson and Dalziel (1958) identified 55 species. Similarly Hutchinson and Dalziel (1968) further subdivided the genus *indigofera* into five subgenera on a global scale. These are *Acanthonotus*, *Amecarpus*, *Indigofera*, *Indigastrum* and *Microcharis*. further subdivided The reason for the confusion and discrepancies in estimation of the number of taxa in this group of plants could be due to the perceived similarities in structural and reproductive biology of these Legumes. Eight *Indigofera* species commonly found in Eastern Nigeria are *I. hirsuta* L. *I. paniculata* Guill et. Perr, *I. priureana* Guill et Perr. *I. pulchra* Wild. *I. senegalensis* Lam. *I. stenophylla* Guill and Perr. *I. terminalis* Baker and *I. tinctoria* L. The economic importance of *Indigofera* goes far back into the past. *Indigofera* is one of the oldest colouring agents known to man and is among the most widely used natural dye in the world (Horward, 1988, Burkill, 1995). Medicinally, the Chinese use *Indigofera tinctoria* to clear the liver, detoxify the blood, alleviate pains and reduce fever (Simon et al, 1984) Sap from the whole plant of *I. hirsuta* is used in the case of injury to the eyeball and inflammation of the eyelids. A root decoction is applied in most parts of Nigeria to counteract various poisons (Burkill, 1995).

(Stern 2000) *Indigofera* species include plants from herb to trees and can reach a height of 2.5-45cm. The stem may be slender, erect and conspicuously branched with bright green or tinged with colour (Dallwitz and Watson, 2000). The leaves are mostly, dark green, alternate, compound, obovate to oblanceolate in shape and leaflets occurring in pairs of 4-7. The fruit is a pod usually smooth, reddish brown and cylindrical with 2-15 seeds in most species (Mattson, 1983, Simon et al., 1984). The use of anatomical features in the systematic characterization of different taxa is no more a recent event and cannot be overemphasized Akinnubi et al (2013), Adedeji (2004), Adedeji and Iloh (2014), Palmer and Tucker (1981) stressed that the taxonomic importance of anatomical features along with other characters are useful for identification and classification of plants. Similarly anatomical features are widely used in systematics for identification, placing anomalous groups in a satisfactory positions in classifications and for indicating patterns of relationship that may have been observed by superficial convergence in morphological features (Kemka, 2002). In the recent past, plant species have been removed from families or genera and classified under another taxon based on anatomical evidences, Metealf and Chalk, (1979). The study of Okoli (1987) in the family Curcubitaceae, Gibson(1981) Edeoga and Osawe (1996) in Caesalpinoideae-Leguminosae and Devades and Beck (1971) in Rosaceae and Leguminosae, Kumar et al (2010).

in leaf, stem and node of *Withaniasomnifera*, Nwachukwu and Edeoga (2006) in Leguminosae-Papilionoideae are classical examples. Though information on the anatomy of these plants are available, little or no specific investigation has been conducted specifically on the anatomy of the *Indigofera* species. This paper therefore described the anatomical features of the stem of eight *Indigofera* species that are common in Eastern Nigeria and discusses the possibility of utilizing this in the characterization of these taxa.

MATERIALS AND METHODS

Mature and fresh stems of the eight *Indigofera* species were obtained from living samples, collected from different parts of eastern Nigeria. Each root of the plant is 0.1cm in diameter. Sections of 26 mm thick prepared from the stems were fixed in FAA (1:1:18 glacial acetic acid. 40% formaldehyde 70% ethanol v/v) for at least 72hrs. These were then rinsed in several changes of distilled water and passed through alcohol series (30, 50, 70, 95 and 100%). The dehydrated materials were infiltrated with wax by passing through different proportions of alcohol and chloroform (3:11:1 1:3 v/v). As the chloroform and wax gradually replaced the alcohol, pure chloroform and wax were put in the bottles to gradually infiltrate the tissue with wax which would be hard enough for microtomy.

The bottles were left on a hot plate (37-40°C) for 24hrs before transforming to the oven (58-60°C). This step was designed to evaporate the chloroform. The wax having reached its melting point completely infiltrated the tissues in it. After a period of 2-3 days with constant addition of paraffin melted wax. This was accomplished by a quick orientation of the specimens in the mould with a hot mounted needle and forceps and quick cooling on ice block. The metal mould, were later removed and the specimens within the wax cube were trimmed and section on Reichert rotary microtome at 20-24µm. The ribbons were placed on clean slides smeared with a film of Haupt's albumen and allowed to dry and drops of water added on a hot plate of 40°C for a few minutes to allow the ribbons to expand and were stored overnight. The slides were immersed in pure xylene for few minutes and in a solution of xylene and absolute alcohol with 1:1 ratio (v/v) for 5 min. The slides were then transferred to another solution of xylene and alcohol in the ratio of 1:3 (v/v) for 5 min to 95, 90, 70 and 50% alcohol.

Drops of alcian blue were put on the specimen for five minutes, washed off with water and counter stained with safranin for 2 minutes, then dehydrated in a series of alcohol 50, 70, 80 and 90% xylene/absolute alcohol solution (i.e. 1:3 and 1:1 v/v) and pure xylene at intervals of a few seconds and mounted in Canada balsam. Photomicrographs were taken from the slides using a LeitzWetzlar Ortholus microscope fitted with a Vivitar-v-335camera.

This study was carried out in October between the year

Table 1. Stem anatomical features of *Indigofera* investigated.

Attributes	<i>I. hirsuta</i>	<i>I. paniculata</i>	<i>I. prioureana</i>	<i>I. pulchra</i>	<i>I. senegalensis</i>	<i>I. stenophylla</i>	<i>I. terminalis</i>	<i>I. tinctoria</i>
Epidermal Layer type	Multiseriate and thick walled	Multiseriate	Multiseriate	Multiseriate	Multiseriate	- Multiseriate	Multiseriate	Multiseriate
Hypodermal layer	Lignified	3-5 lignified	Lignified	3-5 lignified	Lignified	Lignified	Lignified	3-5 Lignified
Nature and size of cortical cells (vessels)	Varied in size	Varied in size and parenchymatous	Varied in size and parenchymatous	Closely parked parenchymatous	Varied in size	Varied in size	Varied in size	Varied in size
Nature of Endodermal Layer		sclerenchymatous	sclerenchymatous	sclerenchymatous	-	-	-	-
Vascular bundle	Cojoint Collateral and open	Cojoint Collateral and open	Poorly differentiated	Poorly Differentiated	Collateral and open	Conjoint Collateral and open	Poorly Differentiated	Poorly differentiated
Nature of crystal	Usually Developed	Tannins bag and crystals	Scattered tannins	Weakly Developed	Tannin bags and crystals	Tannins bags and crystals	Tannins	Scattered tannins
Pith	Large and Parenchymatous	Present	Present	Present	Present	Present	Present	Present

2008 to the year 2009 in the laboratory of Michael Okpara University of Agriculture Umudike, Umuahia, Abia State, Nigeria.

RESULTS

The anatomical features of the stem of the eight species of *Indigofera* are given in Table 1 and showed in (Figure 1 and 2). The epidermal layer of *I. hirsuta* is multiseriate, thick-walled and tubular. Below the epidermis is a lignified hypodermal layer which separates the epidermis from the cortex. The cortex is made of variation in vessel size and number. The vascular bundles are collateral and lies between the pericycle and the pit. The pit is thin-walled parenchyma cells which occupies a greater part of the stem. In *I. paniculata* the epidermis is multiseriate separated from the cortex by a 3-5 lignified layer of the hypodermis. The cortex is distinct with variations in the size and number of vessels.

Tanniferous bags were found within the cortex (Figure 1b) In *I. prioureana*, the hypodermal wall is lignified. There are also variations in size of cortical cells and presence of scattered tannins.

I. pulchra possess a 3-5 lignified hypodermal layer. The cells of the cortex are closely parked with little or no intercellular spaces and variable size and number of vessels (Figure 1b). *I. senegalensis* has uniform vessels with intercellular spaces within the cortex. Tanniferous bags and crystal sand were equally noticed (Figure 1c). In *I. stenophylla* the cortex shows a distinct variation in size of vessels. Tannins and crystal sands were observed within the cortex (Figure 2a). The vascular bundles are distinct. The epidermis of *I. terminalis* is multiseriate, thick-walled and separated from the cortex by a lignified hypodermal layer (Figure 2b). *I. tinctoria* has a thick-walled multiseriate epidermal wall separated from the cortex by a 3-5 lignified hypodermal wall. Variations in sizes of vessels were observed (Figure 2c).

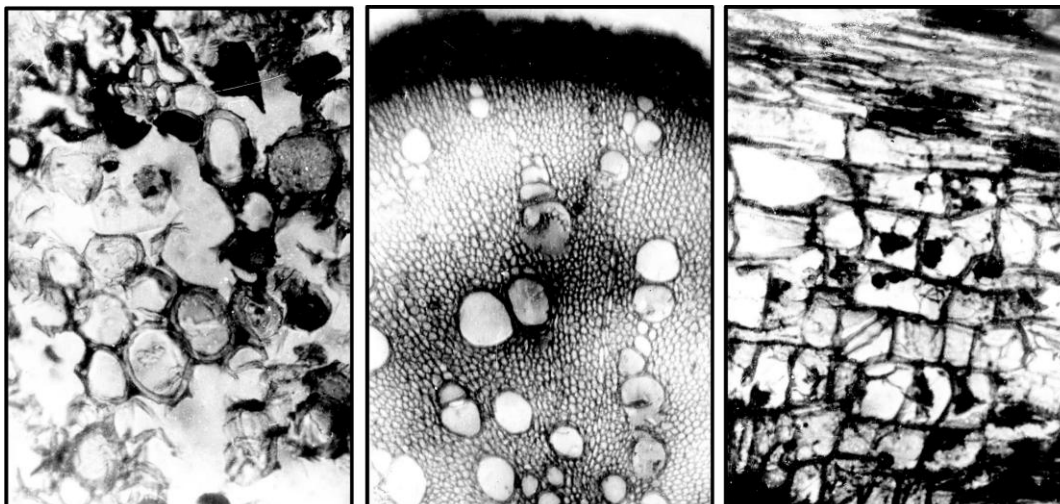


Figure 1(a-c). Transverse section of stems of *I. paniculata*, *I. pulchra* and *I. senegalensis*. (a) *I. paniculata*. Note the variation in size of tannin bags in the cortex x100 (b) *I. pulchra* showing variation in corticular cells with little or no inter cellular spaces x100. (c) *I. senegalensis*. Presence of scattered tanniferous bags x100.

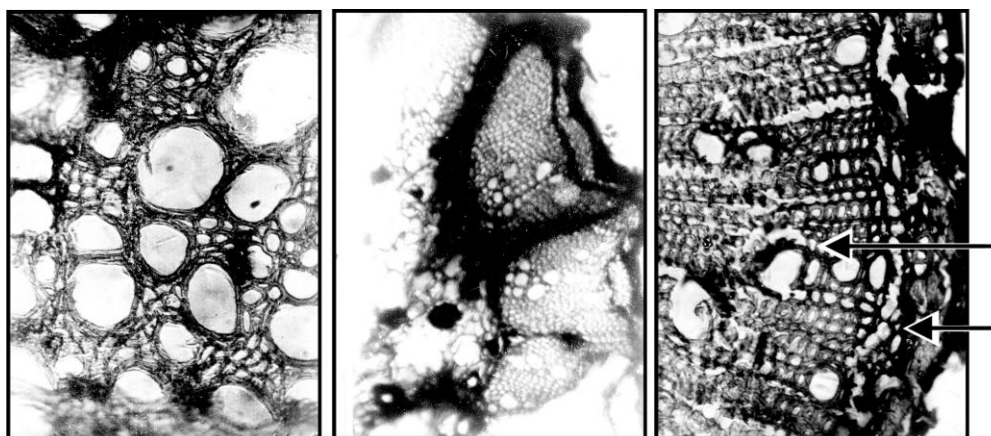


Figure 2 (a-e). Transverse section of stem of *I. stenophylla*, *I. terminalis* *I. tinctoria*. (a) *I. Priureana* and *I. hirsuta* (a) *I. stenophylla*. Presence of intercellular spaces between cells x100. (b) *I. terminalis*. Showing multiseriate epidermal layer x 100. (c) *I. tinctoria*. Note of presence of 3-5 lignified hypodermal layer x 100 (d) *I. stenophylla* *I. tinctoria* and *I. priureana*. Scattered tannins found within the cortex x 100 (e) *I. hirsuta*. Presence of conjoint, collateral and open vascular bundle x 100.

DISCUSSION

The result of stem anatomical studies in the eight species of *Indigofera* shows that variation in the number of lignified layer, nature of endodermal wall, and vascular bundle type are taxonomically significant. The internal structure of the stem of the eight *Indigofera* species in Figure 1 and 2 is characterized by an outer, multiseriate, tubular epidermal layer with closely packed cells and thick walled as observed in most of the species studied. Below the epidermis are a few layers of much thickened

hypodermis made up of 3-5 lignified sclerenchyma cells with little or no intercellular spaces in *I. paniculata*, *I. pulchra* and *I. tinctoria* (Figure 1a, b and 2c). This feature support the works of Nwachukwu and Mbagwu (2006), Mbagwu and Edeoga (2008) who used anatomical studies on the roots and stems of some species of *Indigofera* L to and Savi respectively to distinguish them hence reflecting close affinity between these three taxa and could be used diagnostically in taxonomic classification.

The parenchymatous cells of the cortex which are varied in size with bigger vessels at the hypodermal

region and gradually reducing in size towards the centre observed in *I. paniculata*, and *I. pulchra*, (Figure 1a and b) is been reported for the first time and could be of taxonomic importance. Scattered tannins found within the cortex of *I. paniculata*, (Figure 1a), *I. senegalensis*, (Figure 2c), *I. stenophylla*, (Figure 2a), *I. tinctoria*, (Figure 2c) and *I. prieureana* (Figure 2d) confirms the highly tanniferous nature of these species and could suggest that the crystals might have a storage function which support evidence from the works of Francheschi and Horner (1980) and Mathew and Shah (1984). Hence could be of great taxonomic importance in the characterization of these species. The vascular bundles are co-joint, collateral and opened. They are well arranged and lie between the pericycle and the pith as clearly observed in *I. paniculata* (Figure 1a), *I. stenophylla* (Figure 2a) and *I. hirsuta* (Figure 2e). This could suggest a close affinity among these three taxa and could be used taxonomically in the characterization of these taxa. The central pith consists of thin walled; much larger parenchymatous cells which appear rounded as

seen in *I. hirsuta* Figure 2e. The presence of tanniferous bags and crystal and scattered within the cortex of *I. paniculata*, *I. senegalensis*, *I. stenophylla* (Figure 1a, c and 2a) are distinctive and taxonomically important.

The systematic value of anatomical characters in different groups of plant could be seen in the contribution of Mbagwu and Edeoga (2008) on some species of *Vigna savi* Leguminosae-Papilionoideae, Edeoga et al (2007) in the stem and roots of some *Mimosa* species Leguminosae-Mimosoideae, Edeoga and Ogbemor (1991) in the Vegetative anatomy of some Nigerian species of *Aneilema* Commelinaceae, Nwachukwu and Edeoga (2006) in the roots of some species of *Indigofera*. Modern taxonomy rely sufficiently on synergy in multiple characters. Hence the usefulness of utilizing these stem anatomical features alongside data from other disciplines which can aid in the identification, description and characterization of taxa cannot be over emphasized.

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