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Anatomical Studies on the Petiole of Some Species of *Indigofera*

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Abstract: Anatomical studies on the petiole of eight *indigofera* species of the tribe galegeae common in eastern Nigeria were carried out with the aid of a light microscope. An analysis of the features of the internal arrangement of the petiole shows that these taxa possess vital characters that could be used in their description with a view of establishing taxonomic relationship among the species. The epidermal layer and collenchyma cells as well as crystal types are some of the characters assessed and discussed among the *indigofera* species investigated. The presence of uniseriate, tubular and closely parked epidermal cells in *I. paniculata*, *I. stenophylla* and *I. terminalis* is of taxonomic. The well-developed large distinct angular and lacuna collenchyma cells in *I. prieureana*, *I. senegalensis* and *I. tinctoria* are significant. Furthermore, the presences of variously shaped crystals and tannins in most of the species studied conforms the highly tanninferous nature of these taxa and hence the mechanical and storage roles of these crystal and tannins in plants.

Key words: Anatomy, petiole, *Indigofera*, leguminoseae-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[1]. Knuth[2] regarded the *Leguminales* as being made up of three families namely Caesalpinaceae, Mimosaceae and Papilionaceae, each consisting of different genera and different species. Lowe and Soladoye[3] reported the unification of these families in *Leguminales* into three families known as Leguminosae-Caesealpinoideae, Leguminosae-Mimosoideae and Leguminosae-Papilionoideae. These later treatment was also used by Burkill[4] in the recognition of different families of the Legumes.

Prior to this latest development in the taxonomy of the Legumes, Hutchinson and Dalziel[5] recognized the family Papilionaceae and split this into nine tribes. These tribes have between one genus in *Loteae* to thirty-one genera *Phaseoleae*. According to Willis[6] 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[7] identified 200 species while Mattson[8] recognized over 300 species. Hutshinson and Dalziel[9] further identified 78 species two of which *I. heterocarpa* Welw.ex Bak and *I. variabilis* Berhaut are imperfectly known species. Furthermore, the fact that no specific investigation has been conducted on the petiole anatomical features of the genus *indigofera* to the authors best knowledge despite the various contribution of anatomy in the proper understanding and delimitation of taxa necessitated this study. The purpose of this study therefore is to highlight the relevance of petiole anatomical features as it relates to the classification and systematics of the genus *indigofera*. The works of[10-12] are typical examples.

In West Africa, Burkill[4] reported 60 species while Hutchinson and Dalziel[5] reported 78 species of *Indigofera*. In Nigeria, Burkill[4] reported 45 species while Hutchinson and Dalziel[5] identified 55 species. 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 were selected for the present investigation for botanical variability. They are *I. hirsuta L. I. paniculata* Guill et. Perr, *I. prieureana* Guill et Perr. *I. pulchra* Wild. *I. senegalensis Lam. I. stenophylla* Guill and Perr. *I. terminalis* Baker and *I.*

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tinctoria L. The economic importance of *Indigofera* goes for 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[4,13]. Medicinally, the Chinese use *Indigofera tinctoria* to clear the liver, detoxify the blood, alleviate pains and reduce fever. 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 decorction is applied in most parts of Nigeria to counteract various poisons[4].

Indigofera species include plant from annual 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[1]. 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[8]. The use of anatomical features in the systematic characterization of different taxa is no more a recent event. The works of Okoli[11] in the family Curcurbitaceae, Edeoga and Osawe[14] in Caesalpinoideae-Leguminosae, Gibson in Anacardicaceae, Heo in Monimiaceae and Devades and Beck[15] in Rosaceae and Leguminosae are classical examples. Though this information on the anatomy of these plants is available, little or no specific investigation has been conducted specifically on the petiole anatomy of the *Indigofera* species. This study therefore described the anatomical features of the petiole 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 during the period of 2003-2004. Each root is 0.1 cm in diameter. Sections of 26mm thick prepared from the stems were fixed in FAA (1:1:18) glacial acetic acid. 40% formaldehyde 70% ethanol v Iv) for at least 72 h. 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 (3:1 1:1 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 24 h 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 forcepts 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 20-24$^{\mu}$ m. The ribbons were placed on clean slides smeared with a film of Haupt's albumen and allowed to dry and drops of water added prior to mounting. The slides were placed on a hot plate of 40°C for a few minutes to allow the ribbons to expand and were stored over night. The, slides were immersed in pure xylene for few minutes and in a solution of xylene and absolute alcohol with 1: 1 ratio (v Iv) 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 two minutes, then dehydrated in a series of alcohol 50, 70, 80, 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 Leitz Wetzlar Ortholus microscope fitted with a Vivitar-v-335 camera. The whole petiole anatomical process was carried out in the laboratory of Michael Okpara University of Agriculture Umudike, Umuahia, Abia State.

RESULTS

The anatomical features of the petiole of eight species of *indigofera* studied are summarized in tTable 1 and illustrated in (Fig\s. 1 and 2). The presence of uniseriate, tubular and closely parked epidermals cells in *I. paniculata*, *I. stenophylla* and *I.terminalis* separates these taxa from the other taxa. This is of taxanomic importance. The petiole in the eight *Indigofera* species studied is characterized by the presence of crystal sand and tannins in the cortex region of *I. hirsuta* and *I. prieureana*.

I. pulchra has star shaped aggregated crystals and tannins scattered within the vessels. This observation is distinctive and taxonomically important. The presence of a large angular collenchyma cell noticed between the cortex and the epidermis in *I. prieureana* and *I. senegalensis* documented for the first time is a new discovery while in *I. tinctoria* the presence the lacunar collenchyma was noticeable.

DISCUSSION

The conspicuous uniseriate, tubular, closely parked and thick layer epidermis in *I. terminalis*, *I. paniculata* and *I. stenophylla* c ould reflect an intraspecific affinity

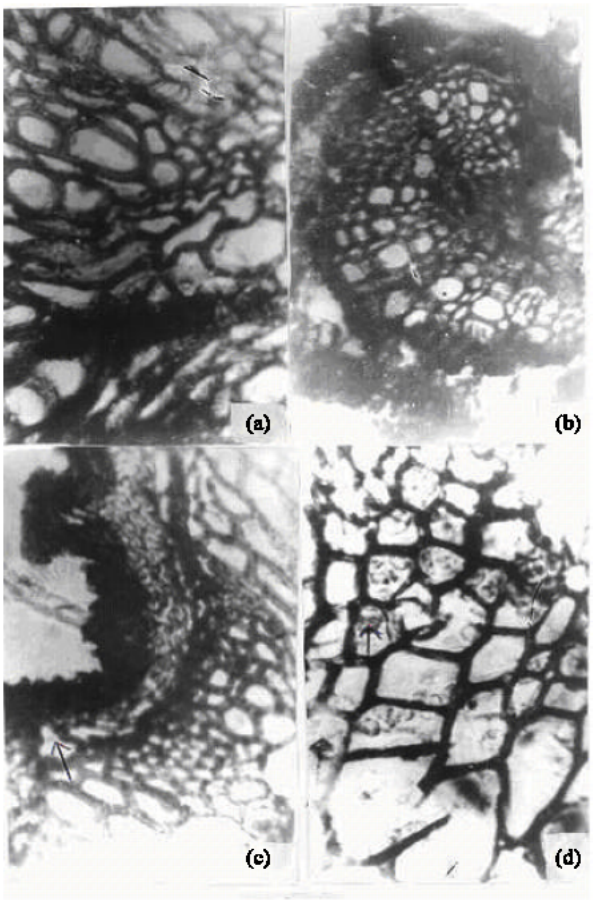

Fig. 1: (a-d).Transverse section of petiole of *indigofera.*

(a) *I. hirsuta* showing presence of crystal sand and irregular cell size x 100

(b) *I. paniculata*. Note closely parked cortex cells shown x 100

(c) *I. prieureana*. Presence of angular collenchyma and crystal sand shown arrowed x 100

(d) *I. pulchra* showing star shaped and aggregate crystals arrowed x 1000.93

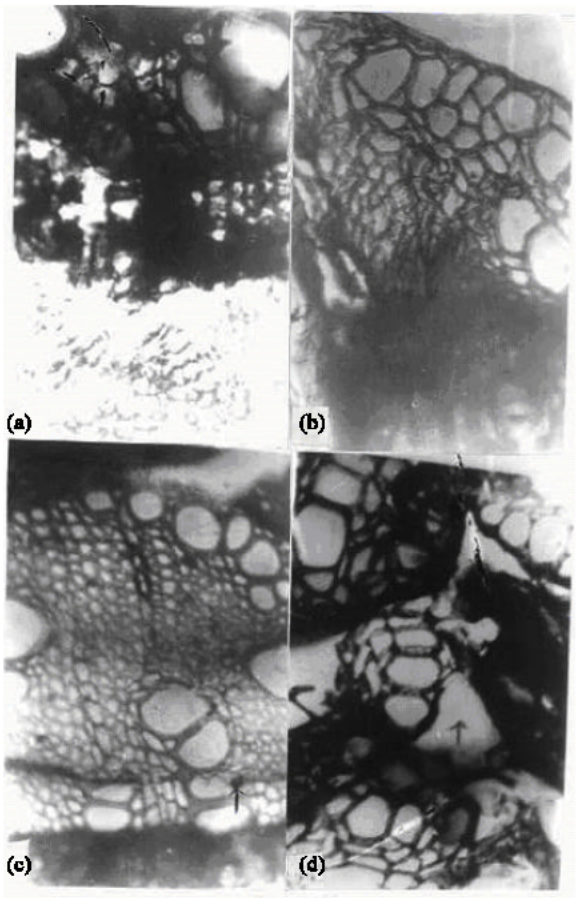

Fig. 2: (a-d).Transverse section of petiole of *indigofera.*

(a) *I. senegalensis*. Angular collenchyma and raphide crystals present x 100

(b) *I. stenophylla*. Note presence of crystal sand and tannins arrowed x 100

(c) *I. prieureana*. Uniseriate tubular epidermal layer present x 200

(d) *I. tinctoria*. Presence of lacunar collenchyma and intercellular spaces shown x 100

among these taxa. This observation is in line with earlier works of Okoli[11] who used the leaf anatomy in establishing relationship among the taxa in the family curribitaceae. The cells of the cortex in most of the *Indigofera* species did not show any variation though the cells are of irregular size. The presence of variation in collenchyma cells (large and distinct angular found in *I. prieureana* and lacunar found in *I. tinctoria)* could be used taxonomically in the delimitation of the *Indigofera* species investigated since no previous work on the petiole anatomy of these *indigofera* taxa has been reported. Similarly, the localization of crystals and tannins in the cortex region of *I. prieureana* and *I. hirsuta* confirms the highly tanninferous nature of these species and further support the notion of mechanical and storage roles of crystals in plants Edeoga and Ogbebor,[16]. Enright and Cumbie,[10]; Taylor *et al* and Fahn,[17]. Although previous works did not pay much attention to the anatomy of the petiole in describing the anatomy of the genus *indigofera*[8,12] evidence of the crucial role of these structures on the considerations of the systematics of these taxa still remains clear. Thus the necessity of including the results from the petiole anatomy with data derived from other botanical disciplines remains vital when formulating conclusions on the systematics of the *indigofera* species.

Table1:Petiole Anatomical Features of Eight Species of *Indigofera* Studied

Type of Epidermal layer	*I. hirsuta*	*I. paniculata*	*I. prieureana*	*I. pulchra*	*I. senegalensis*	*I. stenophylla*	*I. terminalis*	*I. t. tinctoria*
	-	thick uniseriate and differentiated	poorly uniseriate and tabular	- uniseriate and tabular	-	thick tabular	thick	-
types of closely		closely	-	-		closely	closely	-
epidermal cell		parked	parked	parked		parked		
Collenchyma type		-	-	angular	-	angular	--lacunar	
Collenchyma								
collenchyma collenchyma								
Nature of		crystal sand -	-	crystal sand -crystal & and tannins		aggregate and tannins	- crystals	

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