

LEAF ANATOMY OF LETESTUDOXA BELLA PELLEGR (ANNONACEAE)

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Abstract

A study of leaf anatomy (epidermis, architecture and midrib) of *Letestudoxa bella* was carried out using light microscope in order to provide useful anatomical features that will aid its identification. Hence, both qualitative and quantitative data of the leaf were assessed for their taxonomic significance. The shape of epidermal cells and its cell wall, type of wax depositions, type and number of stomata, areola formation, nerves ending, vein category, venation, type, shape and nature of vascular bundle, presence or absence of parenchyma and sclerenchyma are reliable criteria useful for identifying the plant. Outstandingly, the record of stephanocytic stomata in the genus is novel in the family Annonaceae. Other important findings in this study are the epidermal cell and stomatal number, length and width, and stomatal index which varied reasonably across the individuals of the species studied. The stomata number was recorded and its index calculated. A combination of these features proved can be reliably used to identify the species even when the leaf samples are fragmentary.

Keyword: Annonaceae, architecture, epidermis, stephanocytic stomata

Introduction

The Annonaceae Juss. is a pantropical family consisting of about 130 genera and 2300 species (Fries, 1959). Most of the species occur primarily in tropical lowland rainforest. The family is often represented by high numbers of individuals and species. The Annonaceae are clearly delimited (Fries 1959) and are regarded monophyletic in all studies including those based on molecular data (Qiu *et al.*, 2000). The genus *Letestudoxa* Pellegr. is a member of the family Annonaceae in the major group Angiosperms (Flowering plants). It is represented by three species worldwide, namely *L. glabrifolia* Chatrou & Repetur and *L. lanuginosa* Le Thomas (Chatrou, *et al.*, 2012) and is restricted to central Africa (Couvreur *et al.*, 2012).

They are most trees, sometimes lianas, with spiral phyllotaxis; indument of lepidote, stellate and/or simple hairs; bracts present; inflorescences rhipidiate, terminal; flowers bisexual; carpels free in flower, in fruit usually fused; placentation basal; stipe absent; aril rudimentary, rarely absent; ruminations lamelliform; pollen inaperturate, sometimes sulcate (Chatrou & Saunders, 2012). It occurs as one of the plant species in the flora of Cameroon.

Taxonomically, *Letestudoxa bella* is in the tribe *Duguetieae*, other genera include *Duguetia*, *Fusea*, and *Pseudartabotrys*. This tribe featured genera with both apocarpous (*Uvaria*, *Cleistopholis* Pierre ex Engl.) and syncarpous [*Letestudoxa*, *Pachypodanthium* Engl. & Diels (now *Duguetia*)] fruits. Parsimony analysis resulted in two strongly supported clades: a *Fusaea* clade, comprising *Fusaea*, *Duckeanthus*, *Letestudoxa*, and *Pseudartabotrys*, and a *Duguetia* clade, comprising all Neotropical and African species of *Duguetia*. Characters used to resolve relationships between and within the two clades are difficult to separate by outgroup comparison (Chatrou, et al., 2000).

Anatomical features are valuable to scientists who need to identify small fragments of plant materials and the taxonomic significance of epidermal morphology is well documented in botanical literature (Dehgan 1980). Though, a series of publications have contributed to a better understanding of the taxonomy of African *Annonaceae* (Couvreur, 2009; Botermans et al., 2011) but there has not been any information on the anatomical characters of genus *Letestudoxa*. Therefore, the present study is carried out in order to present and document leaf anatomical characters of *Letestudoxa bella* Pellagr. so as to complement the existing identification criteria upon which the recognition and delimitation of the plant could be based.

Materials and Methods

Leaf Epidermal Preparation

Dry materials of leaf were used for the study. Nomenclatural authentication was carried out in the National Herbarium Yaounde, Cameroon (YA). Herbarium abbreviation follows Holmgren et al., 1990. All existing data sources (e. g. Metcalfe and Chalk, 1950; Olowokudejo, 1993; Kadiri et al., 2009) were also consulted for further information.

Cross sections of the samples were obtained with a razor blade by free-hand method. For foliar epidermis, leaf samples were examined using the standard method established for tropical plant taxa including *Annonaceae*, e.g. Ogundipe and Kadiri (2012) on *Amaranthaceae*, Olowokudejo (1993) on *Jatropha*, Kadiri et al. (2009) on *Acalypha*, Yang and Lin (2005) on *Schisandraceae*. However, modifications were introduced where necessary for optimal result. The method involved cutting 1- 5cm portions from the standard median portion of the leaf lamina i.e. near the mid-rib

and then boiled in water for 20-30 minutes. The leaf pieces were later soaked in concentrated Trioxonitrate (v) acid (HNO_3) in capped specimen bottles for about 8-24 hours to macerate the mesophyll. Tissue disintegration was indicated by bubbles, and the epidermises were transferred into Petri dishes containing water for cleansing and then, epidermises were separated with forceps and mounting needles. Tissue debris was cleared off the epidermises with fine hair brush and washed in several changes of water.

For examination of the petiole and midrib, this was done using hand free sectioning to make semi-permanent slides for microscopic studies. Drops of different grades of ethanol- 50%, 75% up to 100% were added in turn to harden the cells. Preparations were later stained with Safranin O in 50% alcohol before mounting in glycerine on a glass slide. The epidermises were mounted on a glass slide with upper surfaces facing up and then covered with cover-slips and ringed with nail varnish to prevent dehydration. Scraped epidermis of the seed was obtained and stained with Safranin O and then examined under the microscope. Slides were examined with light microscopes at x100 and x400.

For quantitative analysis of epidermal characters, 20 epidermal cells and 10 stomata were randomly selected for measurement per microscope field. Stomata index was calculated using the formula reported by Stace (1965).

$$\text{StomataIndex} = 100 \left(\frac{S}{S + E} \right)$$

Where S= No of stomata present per unit area

E= No of Epidermal cell per unit area

Leaf Architecture

The areolation patterns and higher order venation in the leaf lamina were studied. Dried leaf samples were boiled in water for about five minutes and later transferred into commercial bleach solution for diaphanization (approximately 5% sodium hypochlorite), for 30 to 120 minutes. Thereafter, the samples were transferred into Petri dishes containing water for cleansing and then, drops of different grades of ethanol were added in turn to harden the cells after mounting on the slides. The adopted method follows Foster (1952), Hickey (1973) and Dilcher (1974), with some modifications. Slides were examined with light microscope at x100 only while photomicrographs were taken using Toupview 3.2 Image Camera attached to a computer.

Midrib Anatomy

Dried specimens were used through free hand sectioning. The sections were obtained transversely and boiled for about five minutes then drop of Sodium hypochlorite was added; washed in several changes of water and then stained with few drops aqueous Safranin. Samples were mounted in glycerin, covered with cover-slips and ringed with nail polish to prevent dehydration. Specimens were observed at x40 magnification and hand drawings were taken.

Results

Table 1: Leaf anatomical characteristics of *Letestudoxa bella*

S/N	CHARACTERS	UPPER SURFACE	LOWER SURFACE
1	Epidermal cell shape	Polygonal	Polygonal
2	Epidermal cell crystal	Absent	Absent
3	Anticlinal wall pattern	Straight	Straight
4	Stomata type	-	Stephanocytics
5	Trichome type	Absent	Present
6	Epidermal cell number	251(261.9±1.42)272	168(174.1±0.55)177
7	Epidermal cell length	5(6.4±0.16)7	4(5.55±0.32)7
8	Epidermal width	4(4.7±0.23)7	2(2.75±0.17)4
9	Epidermal cell thickness	0.5(0.5±0)0.5	0.5(0.5±0)0.5
10	Stomata number/mm	-	15(15.9±0.12)17
11	Stomata length	-	3(3.35±0.10)4
12	Stomata width	-	1.5(1.5±0)1.5
13	Stomata Index	-	8.4%
	Leaf Architecture		
14	Angle of Divergence	82-100	
15	Nerves Ending	1-2	
16	Areola Formation	Well developed	
17	Areola shape	4	
18	Vein Category	Alternate	
19	Vein course	Straight	
20	Venation	Eucamptodromous	
21	Leaf Shape	Elliptic	
22	Leaf base	Round	
23	Leaf Margin	Entire	
24	Leaf Apex	Acute	
25	Leaf Type	Simple	
26	Leaf Arrangement	Alternate	
27	Leaf Surface	Glabrous	
	Midrib		
28	Collenchyma layers	2-3	
29	Pith shape	Oval	
30	Parenchyma layers	3-4	
31	Parenchyma shape	Polygonal	

32	Outer surface	Slightly uneven
33	Trichome	Present
34	AB Shape	Slightly crescentiform
35	AD shape	Slightly crescentiform
36	Vascular Bundle type	Collateral
37	Vascular Bundle Shape	Oval
38	Vascular Bundle Nature	Continuous
39	Sclerenchyma	Present

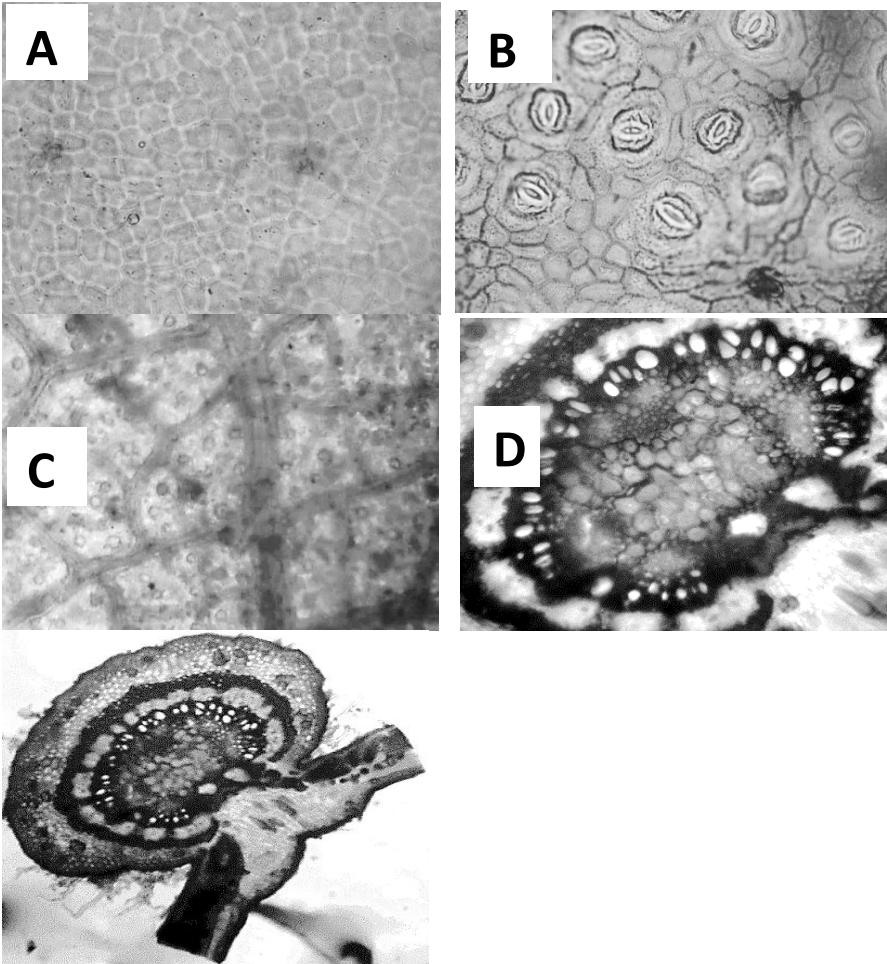


Figure 1: Character of leaf anatomy under light Microscopy: A- Adaxial surface, B- Abaxial surface, C- lamina architecture, D&E- Midrib of *Letestudoxa bella* Scale bar- 25 μ m.

The summary of the result in Table 1 and Figs. 1 & 2

Leaf Epidermal

The leaf is hypostomatic, with stephanocytics stomata at the abaxial surface. The stomata are sparse. Epidermal cell shape and anticlinal wall pattern are polygonal and straight on both surfaces. Crystal can be found only on the abaxial. Quantitatively, the mean epidermal cells number is about 261.9 ± 1.42 on the adaxial and 174.1 ± 0.55 on the abaxial (Table 1). The range of epidermal cell size is $6.4 \pm 0.16 \mu\text{m} \times 4.7 \pm 0.23 \mu\text{m}$ on the upper surface and $5.55 \pm 0.32 \mu\text{m} \times 2.75 \pm 0.17 \mu\text{m}$ on the lower surface (Table 1). Mean epidermal cell number per square millimeter is 15.7 ± 0.09 and stomatal size is $3.35 \pm 0.10 \times 1.5 \pm 0$ on the abaxial.

Lamina Architecture

Leaves alternate, simple leaf with petiole. Leaf shape elliptical, leaf apex and base is acute and round or acute. Leaf margin entire, venation is eucamptodromous. Areoles well developed polygonal. Angle of divergence is between 82-100 degrees, Nerves ending mostly between 1 to 2, well developed areola, four shaped areola more like rectangular, vein category possesses alternate, the vein course straight.

Midrib

The midrib is slightly uneven on the surface with the presence of trichomes on both surfaces. Parenchyma cells are polygonal and most abundant with about 3-4 layers while collenchyma cell is about 2-3 layers. The upper and lower surfaces are slightly crescentiform. The vascular bundle is oval-shaped and its type is collateral. There is continuous ring of the vascular bundle. There are some of crystals in the vascular bundle. Pith shows deposit of sclerenchyma cell (Table 1).

Discussion

The Foliar epidermis is one of the most noteworthy taxonomic characters from the biosystematic point of view and the taxonomic studies of a number of families are made on the basis of leaf epidermis (Stace, 1984; Baranova, 1972). In contrast to the paracytic stomata of the family Annonaceae reported by different authors (Metcalf & Chalk, 1950; Olowokudejo 1990; Pereira-sheteolu 1994 and Michael & James, 2012), this investigation reveals stephanocytics stomata at the abaxial surface of *Letestudoxa bella*. This type is a diagnostic feature for the identification of the genus *Letestudoxa*. Metcalfe & Chalk, (1950) recorded the presence of clustered or solitary crystals in Annonaceae but were not observed in the epidermis.

Davis and Heywood (1963) and Radford *et al.* (1974) have enunciated the taxonomic significance of anatomical characters as an aid to establishing the interrelationships of taxa at and above the specific level. The midrib anatomy revealed useful taxonomic information for the recognition of the species in this genus. Van sulen *et al.*, 1994 reported simple hair in some of the species of

Duguetia, which is a sister genus to *Letestudoxa* in the tribe *Duguetieae* (Chatrou *et al.*, 2012). These simple hairs are also present in abaxial surface of *Letestudoxa bella*. Furthermore, Metcalfe and Chalk (1950) also reported sclereid elements of various kinds in the parenchymatous tissue of the leaf and axis. These are also present in the vascular bundles and crystals in the pith of midrib. Stace, (1965); Inamdhar & Gangadhara (1977) noted that trichome distribution can be under the influence of environmental factors. Presence of trichomes was reported by Metcalfe & Chalk (1950, 1979) in the family Annonaceae. Likewise in this study, trichomes are restricted to the lower surface of the epidermis and found on both surfaces of the midrib.

Vein architecture has been confirmed to be a taxonomic option to solving some problems of identification. The principal characteristics of the leaf venation pattern of a species are genetically fixed. This provides the basis for using the leaf venation as a taxonomic tool (Hickey, 1973). Doyle & Le Thomas (1996) asserted that Annonaceae vary more than the families in spacing, angle and course. Angle of divergence on the secondary vein is highest in between the angles of 82-100. Nerve ending is between 1 and 2. He further revealed that many members of Annonaceae have higher secondary angles, recorded brochidodromous to eucamptodromous in their works. This study revealed eucamptodromous venation in the studied species which falls within the conclusion of the previous (Hickey, 1973; Radford *et al.*, 1974). With all the characters described in this study, it will be easy to identify the quest to document leaf anatomy of *Letestudoxa bella* with its leaf samples even when they are in fragments.

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