Nigerian Journal of Science Volume 34 No. 1 (2000) 9-13

FOLIAR EPIDERMAL CHARACTERISTICS OF THE GENUS *PLUMBAGO* LINN (PLUMBAGINACEAE) IN NIGERIA

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Abstract

The epidermal morphology of adaxial and abaxial leaf surfaces of the two *Plumbago* species represented in Nigeria were studied using light microscopy. The epidermal cells are polygonal with straight, thin anticlinal walls. Both taxa are amphistomatic with anisocytic stomata type on both surfaces. *P. zeylanica* contains larger cells and stomata. The occurrence of crystal sands and chalk glands on the surfaces of the leaves is of generic importance in the family *Plumbaginaceae*. Epidermal cell size and number, stomatal size, number and index are characters of taxonomic importance sufficient in separating the two taxa even in sterile specimens. A key is presented for the identification of the species.

Introduction

Plumbago Linn. is a genus of herbs or shrubs which is represented in Nigeria and West Africa by two species - *P. zeylanica* Linn. and *P. capenis* Thunb., the latter usually cultivated in gardens (Hutchinson and Dalziel, 1963). *P. zeylanica* is of considerable economic importance in West Africa because of its numerous medicinal uses in the traditional African medicine. The root of the plant is known to be vesicant and counter-irritant (Irvine, 1961). The dried pulverished roots according to Irivine (1961) with certain spices are added to hot pap and drunk for a parasitic skin disease. The roots are ground and mixed with the juice of *Citrus aurantifolia* (Christm.) Swingle for the treatment of skin diseases. In Ghana, the root is administered in enema for piles (Dalziel, 1948). In South Africa, the roots with *Eleusine coracana* (Linn.) Gaertn. are used locally for leprosy (Irvine, 1961). The roots when scraped and boiled for a long time in sea water are used as a lotion for ringworm (Irvine, 1961). In southern Nigeria, the leaves are put in soup as a remedy for worms and fever (Dalziel, 1948) while they are used for other various skin troubles (Irvine, 1961).

The leaves can also be added to the stem barks of *Khaya grandifoliola* C.D.C. and *Securidaca longepedunculata* Fres. and the fruits of *Lagenaria breviflora* (Benth.) G. Roberty and *Tetrapleura tetraptera* (Schum. & Thonn.) Taub. for the treatment of tuberculosis (Ashidi *et al.*, 1994). The anti-cancer, anti-biotic, anti-bacterial and anti-implantation properties of *P. zeylanica* are known to be due to the presence of pulmbagin (Adesina, 1990). This substance, i.e. plumbagin, which induces the production of blisters, is said to have been characterized as a yellow naphthaquinone containing the properties of vitamin K and having anti-biotic properties active on different germs which are pathogenic to man (Irvine 1961, Oliver 1959, Chropra & Chopra 1955).

P. capensis on the other hand is not known to be medicinally important. Its value does not transcend the ornamental use. This then constitutes a danger of substitution and adulteration of *P.zeylanica* with *P. capensis* particularly when in sterile or fragmentary state, considering the close resemblance of the two species. The works of Stace (1965), Dilcher (1974), Olowokudejo & Pereira-Sheteolu (1988), Pereira-Sheteolu (1992), Sheteolu & Ayodele (1997)

and many others have shown that characters derived from the leaf epidemis could assist in the taxonomic identification and classification because of their high structural diversity. The leaf epidemal characters of the two *Plumbago* species occurring in Nigeria are thus examined through light microscopy to discover the extent to which these features could enhance the accurate determination of each of the two species

Materials and Methods

Specimens of both taxa were studied at the Forestry Research Herbarium (FHI) Ibadan, Nigeria and the University of Ibadan Herbarium (UIH) Ibadan, Nigeria. Fresh leaf materials were collected from plants growing at the botanical nursery and beside the Department of Botany, University of Ibadan, Ibadan. The leaf material was preserved in Formal - Acetic - Alcohol, F.A.A. (5ml of 40% formaldehyde solution: 5ml of acetic acid: 90ml of 70% alcohol). Voucher specimens are deposited at the University of Ibadan herbarium. Three specimens were used from each species. An area of about $1cm^2$ was taken from the standard median portion of the leaf. Each sample was macerated in dilute trioxononitrate V acid in glass petri dishes for about 2-5 hours. The appearance of air bubbles on the surface of the leaf sample indicated its suitability for separation. Each sample was consequently transferred into water in a petri dish with a pair of forceps. Both epidermal layers were carefully separated by teasing up the corner of the leaf fragment and pulling back the upper epidermis on itself. The epidermises were cleaned with camel hair brush. These were rinsed in distilled water and transferred into 50% alcohol for about five minutes to harden. They were then stained in aqueous safranin for about five minutes. Each membrane was dehydrated by passing serially through 50%, 70%, 90% and absolute ethanol and mounted in glycerine on a slide. Thirty epidermal cells and stomata were chosen randomly from each taxon and measured using a micrometer eye-piece. The range, mean, standard deviation and standard error were determined for both taxa. Phytomicrographs were made using Reichert Microstar IV microscope to which a camera is fitted. Descriptive terminology of the stomata and cells follows that of Dilcher (1974).

Results

Tables 1 and 2 are summaries of the observed epidermal characteristics of both taxa. Photomicrographs of the micromorphological featues are shown in Figure 1. The epidermal cells are polygonal with straight anticlinal walls on both surfaces of the two taxa. The cells are generally larger in *P. zeylanica* (Table 1). The adaxial epidermal cells are more than those on the abaxial surface in both taxa, however, *P. capensis* recorded a higher number of epidermal cells on both surfaces (Table 1). The cells are generally thin-walled and those on the abaxial surface are wider than cells on the adaxial surface. Leaves of both taxa are amphistomatic with stomata occurring on both surfaces (Fig. 1, Table 2). Anisocytic stomata with three cells unequal in size enclosing the guard cells were recorded for both taxa (Fig. 1).

P. zeylanica has more stomata on the adaxial surface with an index of 3.56% while on the abaxial surface, *P. capensis* has more stomata with an index of 16.06% (Table 2). Stomata are generally larger in *P. zeylanica* (Table 2). Both taxa are entirely glabrous on both surfaces. The presence of large deposits of crystal sands on the epidermal cell and guard cells of both taxa (Fig. 1), is noteworthy. Chalk glands were also recorded on both surfaces of the two taxa. These comprise four secretory cells encircled by four subsidiary cells (Fig. 1).

Table 1: Epidermal cell characters of Plumbago species in Nigeria

Taxa	Leaf surface	Epidermal cell shape	Anticlinal cell wall pattern	Epidermal cell width	No. of epiderma cell/mm^2	Epidermal cell wall thickness m
P. capensis	Adaxial	Polygonal	Straight	25.00-50.00	314	2.00-2.50
				35.0± 2.2		2.35± 0.06
	Abaxial	Polygonal	Straight	30.00-75.00	115	1.75-2.50
				54.4± 3.4		2.23± 0.09
P. zeylanica	Adaxial	Polygonal	Straight	35.00-60.00	217	2.00-2.50
				46.8± 2.1		2.38± 0.05
	Abaxial	Polygonal	Straight	40.00-90.00	99	1.75-2.50
				64.0± 3.3		2.29± 0.07

All measurements in microns (Range, Mean ± Standard Error)

Table 2: Epidermal features of Plumbago species in Nigeria

Taxa	Leaf surface	Stomatal frequency	Stomatal length	Stomatal width.	Stomatal Index (%)	Stomata type
	Adaxial	3	15.00-22.50	15.00-20.00	0.95	Anisocytic
			19.17± 0.56	17.92± 0.42		
P. capensis	Abaxial	22	22.50-25.00	17.50-22.50	16.06	Anisocytic
			23.75± 0.38	19.38± 0.45		
	Adaxial	8	25.00-32.50	22.50-30.00	3.56	Anisocytic
			28.96± 0.78	24.79± 0.78		
P. zeylanica	Abaxial	15	27.50-32.50	22.50-25.00	13.16	Anisocytic
			29.79± 0.48	24.17± 0.36		

All measurements in microns (Range, Mean ± Standard Error)

Discussion

The two species exhibit considerable resemblance in their gross morphology but characters such as the epidermal cell size, number of epidermal cells, stomatal size, number and index are sufficiently distinct to facilitate easy recognition of each taxon. The anisocytic stomata recorded for both taxa is in conformity with Metcalfe and Chalk (1950) who recorded the same type for *P.zeylanica*. The larger sizes of epidermal cells and stomata observed in *P. zeylanica* are reflected in the external morphology of the leaves. Mueller (1966) has shown that the leaf surface patterns are of great taxonomic significance and are noted to be under strong genetic control. Although in the view of some authors (Yapp, 1912, Salisbury, 1927, and Watson, 1942) variations in the characters of the leaf may be under different environmental pressures in different habitats, it is believed that the environment plays very little if any part in the determination of the appearance of the leaf (Cutler, 1972, Cutler and Brandham, 1977).

Crystals have been of considerable taxonomic importance (Franceschi and Horner, 1980). Crystal sands are found in the two taxa. These and the Chalk glands are characterisitic of the family Plumbaginaceae. Most importantly, the chalk secreting glands which occur on the leaf surfaces of *Plumbago* species according to Cutler (1978) are characteristic of the genus.

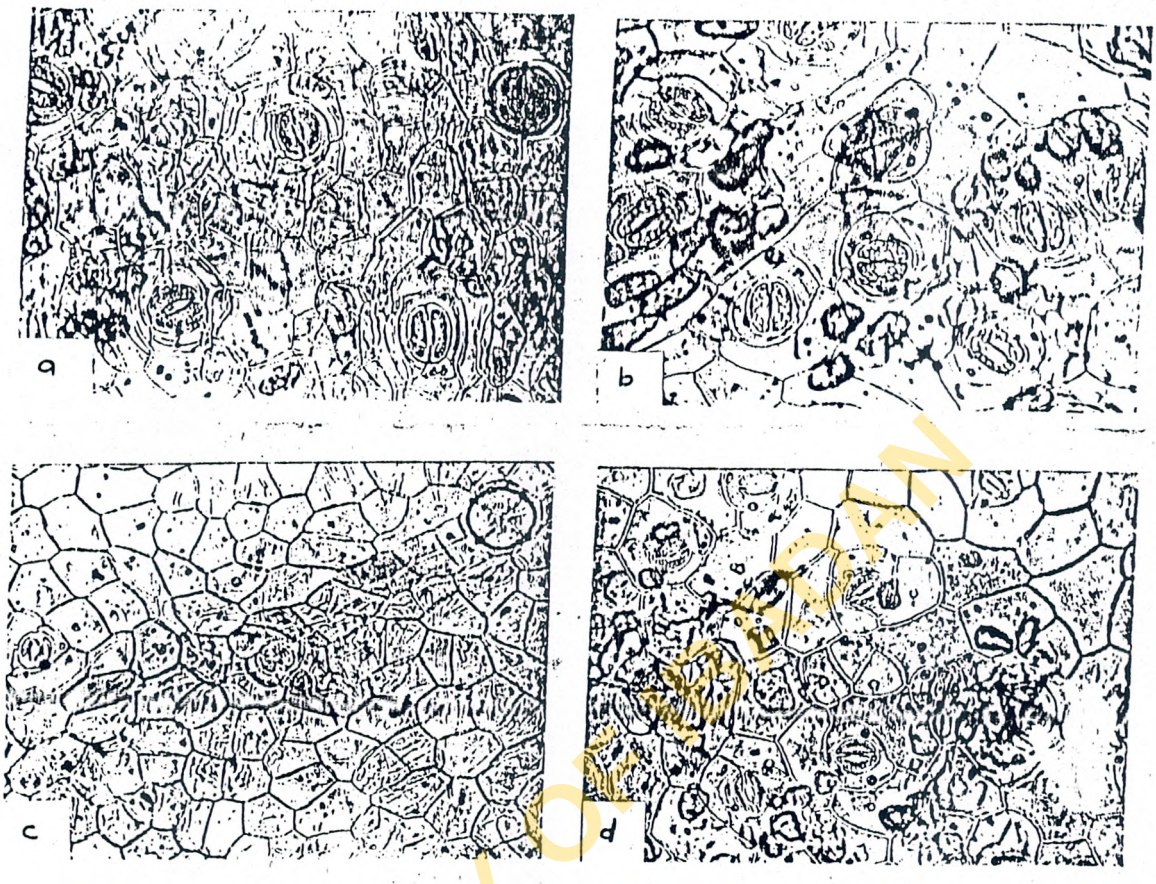

Figure 1: Photomicrographs of the adaxial and abaxial epidermis of *Plumbago* species.

a): Adaxial epidermis of *P. zeylanica* UIH 6893, Ayodele 021, 12-2-97

(b) Abaxial epidermis of *P. zeylanica* UIH 6893, Ayodele 021, 12-2-97

(c): Adaxial epidermis of *P. capensis* UIH 6892, Ayodele 022, 12-2-97

(d) Abaxial epidermis of *P. capensis* UIH 6892, Ayodele 022, 12-2-97

Key to Plumbago species in Nigeria

1. Stomata smaller, 19.17 x 17.92rm on the adaxial surface, 23.75 x 19.38rm on the abaxial surface, stomatal index less than 1% on adaxial surface, and more than 15% on the abaxial surface, epidermal cells smaller, 35rm and 54.38rm on adaxial and abaxial surface respectively. *P. capensis*.

2. Stomata larger, 28.96x24.79rm on the adaxial surface, 29.79 x 24.17rm on the abaxial surface, stomatal index more than 1% on the adaxial surface, epidermal cells larger, 46.87rm and 63.96rm on adaxial and abaxial surface respectively. *P. zeylanica*.

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