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Microscopic Characters and Phytochemical screening of *Pandanus candelabrum* (P. Beauv., Pandanaceae) Leaves

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ABSTRACT

Microscopy and phytochemical screening of the leaves of Pandanus candelabrum were studied. Microscopical examination of the leaves showed paracytic stomata which are more numerous on the lower surface with prisms of calcium oxalate cystals. Preliminary phytochemical screening of the 80 % ethanol extract of the leaves revealed the presence of anthraquinones, saponins and traces of tannins and alkaloids.

Keywords : Microscopy, *Pandanus candelabrum*, Phytochemical screening.

INTRODUCTION

In developing countries, communities rely heavily on traditional herbal medicines in order to meet their primary health care needs. In many industrialized coutries herbal medicines are gaining popularity as alternative and complimentary therapies [1]. Due to some morphological similarities and lack of correct identification, crude drugs are often adulterated or substituted in commerce which obviously results in the loss of drug efficacy [1]. Correct identification of herbal drug is the foundation of the safe use of plant based natural health products. Consumers have a right to expect that these products can be used with confidence regarding safety and quality [2]. Superficial resemblance of plant species within the same family [3] and the problem of adulteration in medicinal plants arise due to the potential use of different species for similar ailments [4]. *Pandanus candelabrum* P. Beauv., family Pandanaceae is a mangrove shrub and commonly known as Screw pine [5]. It is known as 'Ebo' among the Edo people of Edo state of Nigeria. Folklorically, the fresh leaves of *P. candelabrum* are used to treat sore throat; they are

chewed and the liquid content swallowed thrice daily [6]. An infusion of the bark is used in curing diarrhoea, dysentary and enteritis [7], while the leaves are also used in making mats [8]. A related specie, *P. odoratissimus* L. is used as a support for bandage. Oil prepared from its young leaves is smeared on the burned portion in the case of burns [9] while the powder of the roots is used to prevent miscarriage among the tribe of Bhil of Bibdod [10] and also for treating leucorhoea, absess and oedema in Thailand [11]. Economically, the leaves of Pandanaceae family are used to make baskets, mats, hats, and roof thatch. Fibres are taken from the aerial roots to make cords and brushes. Other species are used for medicinal concoctions, and as spices and perfumes in India, Thailand and Malaysia. Some species such as *P. utilis* and *P. andamanensium*; *P. lendam* are used as starchy food sources Other species like *P. veitchii* plants which have glossy dark green leaves with a white border are used as ornamentals [12, 13]. Both macroscopic and microscopic characters can be a very useful tool for the detection of botanicals and non-botanical adulterants [14].

Collection and Identification of Plant

The leaves of *P. candelabrum* (Figure 1) were collected from Ogbia waterside in Ogbia town, Ogbia Local Government Area of Bayelsa State, Nigeria. It was identified and authenticated in the Department of Botany, Faculty of Sciences, Obafemi Awolowo University, Ile- Ife, Nigeria. An herbarium specimen with a voucher number (UHI 16175) was made and deposited in the same place. The leaves were cut into pieces and air dried for two weeks, then powdered and kept for extraction.

Figure 1
A –*Pandanus candelabrum* P. Beauv., Pandanaceae in its natural habitat; B- uprooted plant showing rhizome; C-leaves

Phytochemical screening

30 g of the dried ground leaf powder was extracted in the cold successively for 7 hours, with 200 ml of 80 % Ethanol. The extract was dried *in vacuo* at 30^{o}C. The ethanol extract of the leaves of *P. candelabrum* was subjected to preliminary phytochemical screening, to identify the chemical constituents. The methods of analysis employed were those described by [15].

Microscopy of dry powdered drug, transverse sections and surface preparations

A spatula full of the powdered drug was put into a 15 ml glass sample bottle and soaked with a dilute solution of Sodium Hypochlorite TS and allowed to stand for a few hours until discolouration had occurred. The sample was then washed several times with water. Chloral hydrate was then added to the sample and allowed to stand for 24 hours. Small samples were placed on a glass slide and washed several times with water, then mounted in dilute glycerin.

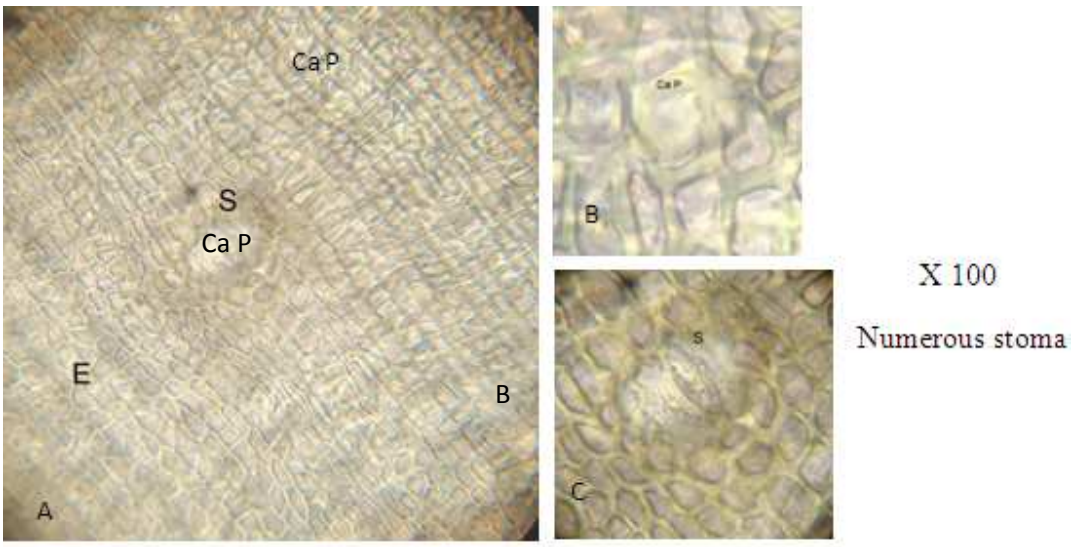
The transverse section, the upper and lower surfaces of the leaves were prepared and cleared using chloralhydrate. The cleared sample specimen was placed on a glass slide and washed carefully with water after which the specimen was mounted in dilute glycerin. The slides were then viewed at magnification of X 400 using a microscope to which a 8.0 mega pixel camera was attached. After obtaining a sharp image of the features, the pictures of the microscopic characters were taken.

RESULTS AND DISCUSSION

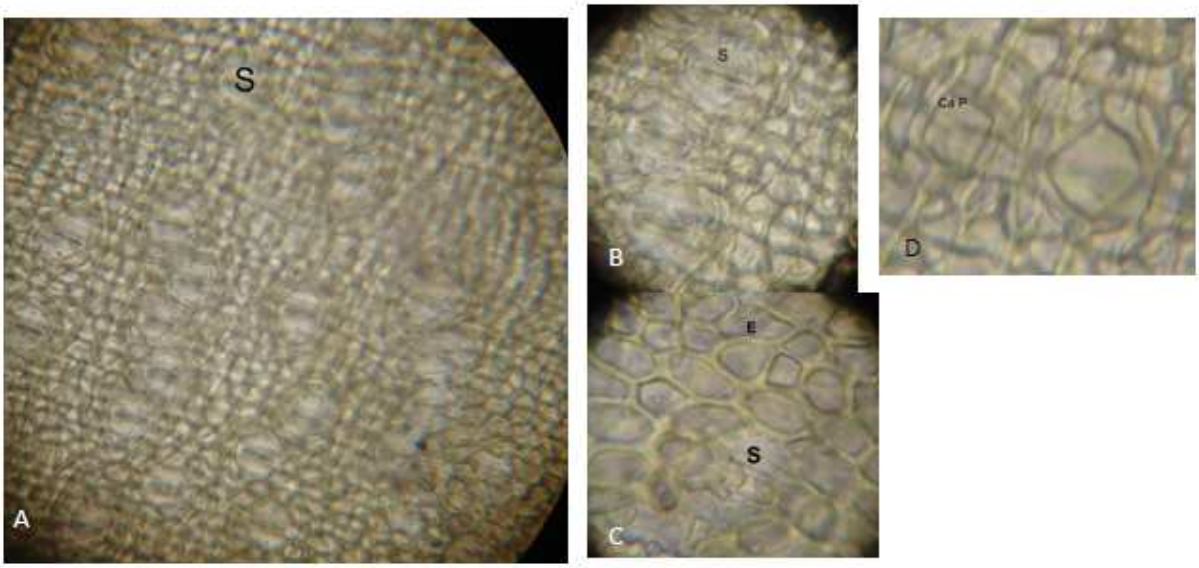
P. candelabrum is shown in its salt water habitat (Figure 1). The epidermal surfaces of the leaf of *P. candelabrum* show polygonal cells with thick walls. The stomata which are paracytic show the unique characteristic of being sunken and the epidermal cells partially cover the stoma itself (Figure 2). There are numerous calcium oxalate prisms which are characteristically embedded in cells. The transverse section of *Pandanus candelabrum* showed the presence of a series of cell structures repeated throughout. This consists vascular system interspaced by large parenchyma cells which also make up a schizolysigenous gland (Figure 3and 4) the vascular system is surrounded by fibers which are thick walled and numerous.

Microscopy of Pandanus candelabrum

Figure 2: Surface preparations of *Pandanus candelabrum*

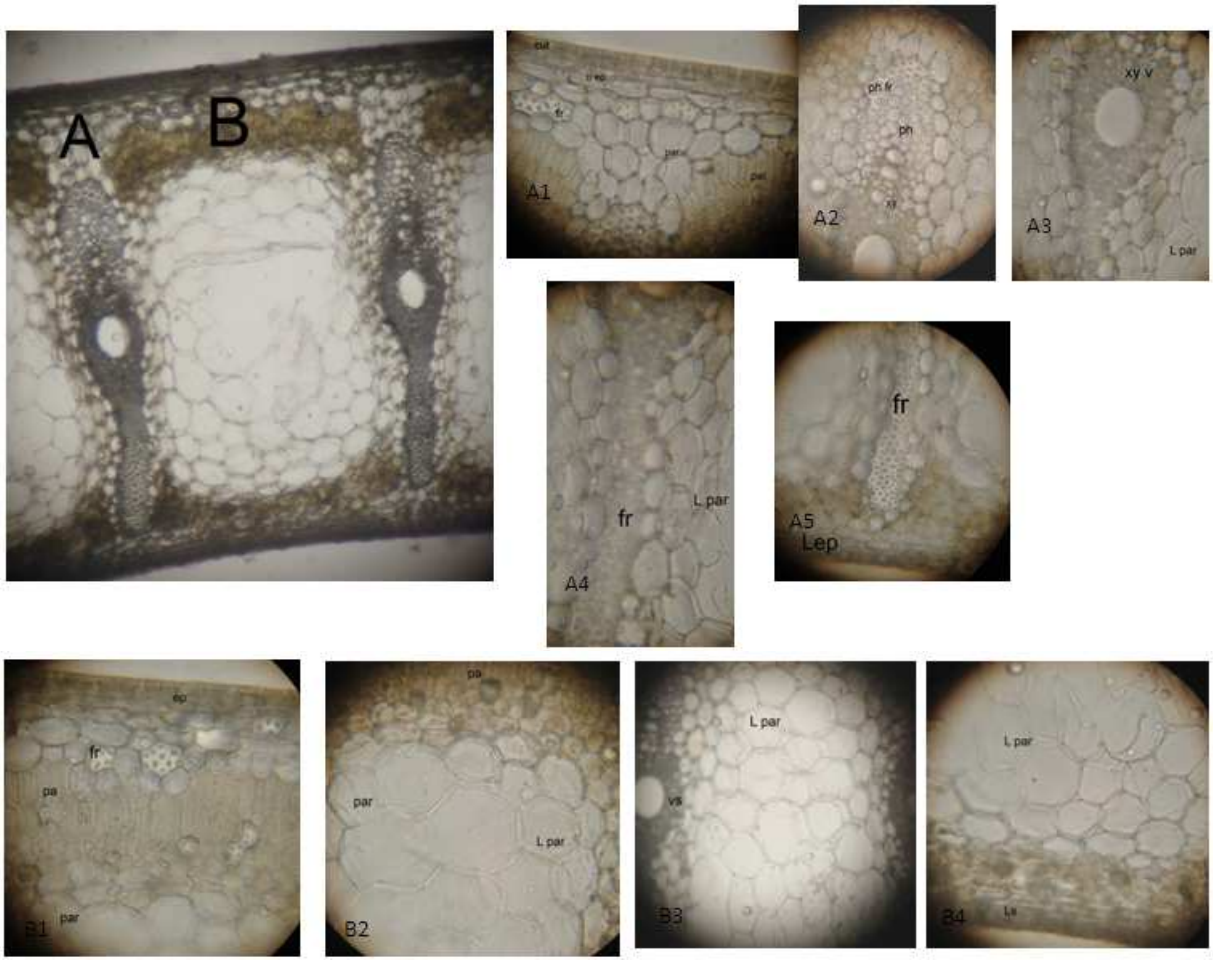


A-Upper epidermal surface of P. candelabrum leaf(x100) B- epidermal surface showing calcium oxalate prism C- epidermal surface showing parayctic stomata. E-epidermal cell, S- stomata, Ca P- calcium oxalate prism (X400)



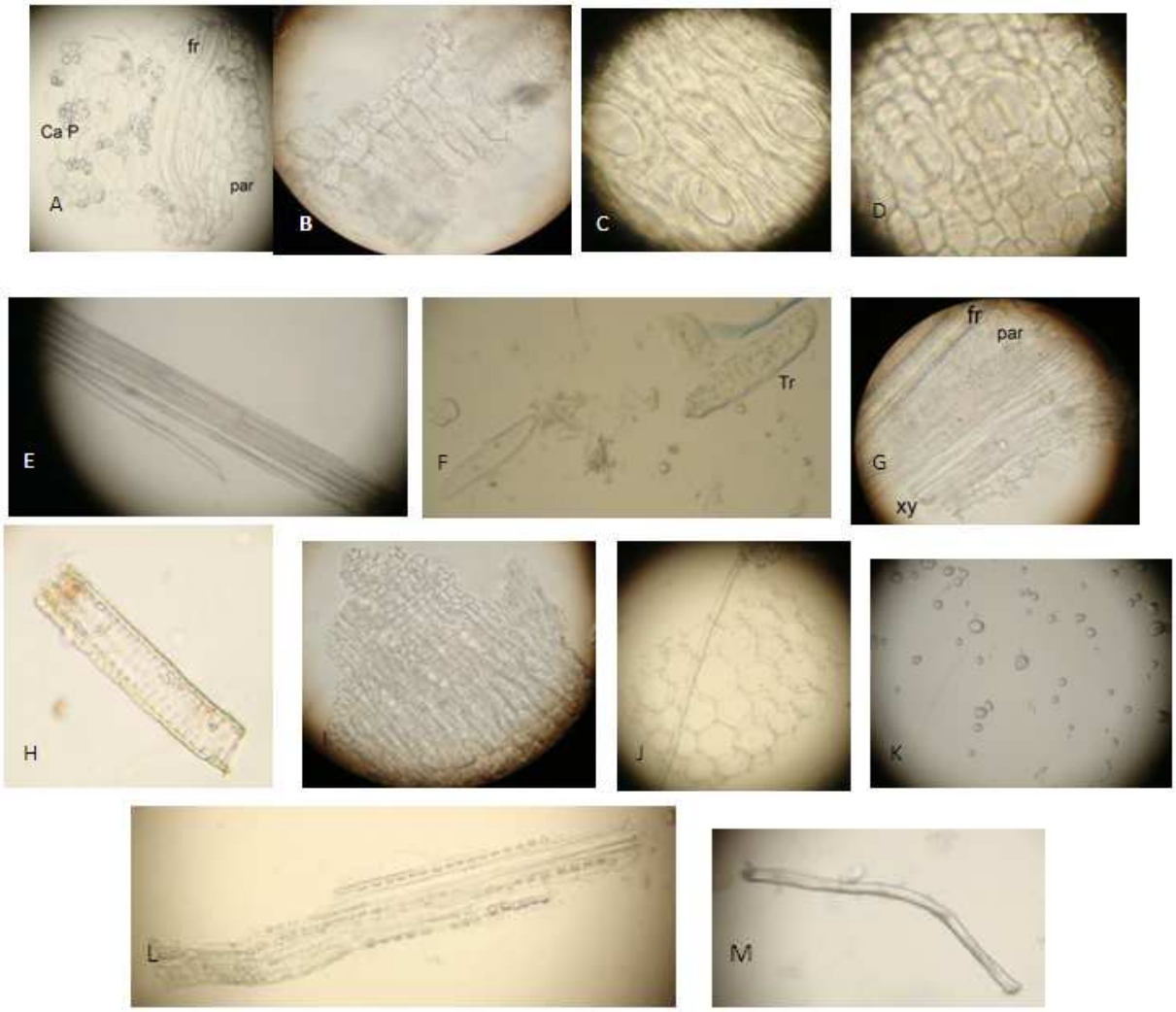
A-Lower epidermal surface of P. candelabrum leaf showing numerous paracytic stomata(x100); B- C- epidermal surface showing stomata; D-epidermal cells showing calcium oxalate prism. E-epidermal cell, S- stomata, Ca P-calcium oxalate prism(x400)

Figure 4: Transverse section of *P candelabrum* leaf showing details of cellular structure



A1-A5 Transverse section of P. candelabrum leaf showing cell structure of A portion; B1-B4 Transverse section of P candelabrum leaf showing cell structure of B portion ;cut-cuticle; ep-epidermal cell; fr-fibers; par-normal parenchyma cells; L par- large parenchyma cells; pa-palisade cells; ph- phloem cells; ph fr- phloem fibers; u ep-upper surface epidermal cells; xy-xylem vessels; Ls –lower surface epidermal cells (x400)

Figure 5: Microscopical characters of the powdered leaf of *P.candelabrum*



A-Fragments of tissue showing parenchyma cells, calcium oxalate prisms and fibres; B-fragments of palisade cells; C,D- fragments of epidermis showing stomata; E- groups of fibers; F- trachieds;G-fragment of xylem vessels, parenchyma and fibers ; H-Trachied; I-Fragment of epidermis showing polygonal thick walled cells; J- fragment of mesophyl and a fibre; K- starch granules; L- group of fibres lined with Calcium oxalate prisms; M-fibre;

This confirms and supports its use as a source of fibers for making mats etc. The tissues shown in the transverse section are in the following order cuticle, upper epidermis, parenchymatous cells, interspersed with groups of fibers, palisade cells of about one to two rows secretory glands consisting of large parenchymatous cells, vascular bundle consisting of phloem fibers, phoem cells and xylem vessels; fibers, parenchyma and the lower epidermis. The Vascular system is in series with the large parenchyma cells. Some of the large parenchyma contained starch granules. The arrangement of the cellular structure is a useful diagnostic tool for identification of the plant. The powered specimen showed many diagnostic structures figure 5.

Fragments of epidermis were easily identifiable. The inner structures which were not identifiable in the surface preparations and the transverse section could be seen clearly. Calcium oxalate prisms are numerous and seen attached to the cells of the veins and fibers. Broken large parenchyma cells, fragments of annular xylem vessels, tracheids, fibres, starch granules characterized the powder. A combination of all these characteristics would make for easy identification of this medicinal plant especially in powder form.

Table 1: Phytochemical constituents of the crude ethanol extract of *Pandanus candelabrum* leaf ethanolic extract

Plant metabolites	Inference
Alkaloids a. Preliminary screening	
i. Draggendorff	±
ii.Mayer	-
iii. Marquis	-
iv. Saturated picric acid	-
v. Wagner	-
b. Confirmatory test (TLC)	±
Anthraquinones a. Free	+
b. Combined	+
Cardiac glycosides	-
Flavonoids i. Lead acetate	-
ii. Sodium hydroxide	-
Saponins i. Frothing	+
ii. Blood haemolysis	+
Tannins a. True: ferric chloride	±
b. phlobatannins:	±

Keys: +: Positive, ±: Trace, - : Negative

The need for quality control of medicines has attracted attention in recent times, but it seems the fact that without standards in plant sources of drugs, we cannot evaluate standard in medicines has been forgotten. This long standing problem of accurate determination of drug sources is yet to receive adequate attention [1, 16]. Plants are sourced by professionals who may not be taxonomists or botanists. Similarly identity of crude drugs purchased from the market which is based on trade or vernacular name is taken for granted, without subjecting the plant material to stringent methods of botanical identification [1, 17]. It is clear that many of our medicinal plants now in use should have microscopically documented characteristics to enable easy and correct identification of the right drug. Phytochemical analysis of the ethanolic extract revealed the presence of anthraquinones, saponins and traces of tannins and alkaloids (Table 1). Further investigation of the biological activities of the plant is being carried out.

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