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QUALITY CONTROL OF MEDICINAL PLANTS II: ESTABLISHING SOME PHARMACOPOEIAL STANDARDS FOR THE LEAF OF *ARTOCARPUS CAMANSI* BLANCO MORACEAE

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ABSTRACT
As beneficial as medicinal plants in the health of populace, especially in emergent nations, their acceptance will be affected if quality cannot be assured. Quality eventually determines the safety and therapeutic value of these botanical drugs. This quality begins with proper identity of the plants. The leaves of *Artocarpus camansi* Blanco Moraceae are used worldwide in ethnomedicine in managing diverse diseases, so far, no pharmacopoeial standards have been established for it. This study is therefore aimed to provide some standards, pharmacognostically for *A. camansi* leaf in order to ensure its real identification. By employing standard methods, the macroscopy and microscopy of the leaf (fresh and dry powdered) were studied. Some physicochemical constants were also evaluated. The plant is composed of simple leaves, with entire margin, acuminate apex and a symmetrical base. The leaf is hypostomatous with anomocytic stomata, glandular trichomes with multicellular heads and unicellular stalks. The transverse section is dorsiventral with collateral vascular bundles. The established physicochemical constants such as moisture content, total ash, acid insoluble ash, water soluble ash, sulphated ash, water soluble and alcohol soluble extractives (11.0, 10.0, 2.0, 4.0, 7.3, 6.4 and 4.5w/w%, respectively) are within the ones for herbal medicine. This study is essential for establishing standards for the quality of *A. camansi* leaf.

KEYWORDS: Quality, *A. camansi*, physicochemical, standards, medicine, plants.

INTRODUCTION

Artocarpus camansi Blanco is endemic to New Guinea, and probably the Moluccus (Indonesia) and the Philippines.[1] It is a medium-sized, single-stemmed, evergreen tree growing up to at least 10 m tall and characterized by a spreading canopy.[2] Ethnomedicinally, all the various parts of *A. camansi* are useful. The fruit is used for laxative, furuncles.[1] Various preparations from the plant leaf are employed for rheumatism, hypertension, asthma, diabetes and thrush.[1,3,4] The latex from the tree and leaf is ethnomedicinally useful for treating hernia, broken bones, sprains, diarrhoea, stomach aches, dysentery and ear infections.[2-3] The bark of the tree is used for treating dysentery.[2-3] The flowers are insect repellent.[2-3] The fruits are a staple food in Nigeria.[5] Antioxidant[6-7], larvacidal[8], cytotoxic[9], antibacterial[6,7,10-11] antidiabetic[12-13], antiplasmodium[14] and alpha amylase inhibitory[15] pharmacological activities are among those reported for the various plant parts. Chemical constituents such as squalene, cycloartenol, cycloartenol acetate, beta- sitosterol, friedelino, lupeol acetate and beta-amyrin are some of the compounds that have been isolated from the plant.[16-18] This study is therefore aimed to provide some quality control standards for the leaf of *A. camansi.*

MATERIALS AND METHODS

Collection, Identification and Authentication of Plant

Collection of *Artocapus camansi* leaves was executed at Ologi-Ogbia, Bayelsa State (Figure 1), Nigeria, and its identification was done by Dr. A.T. Oladele of the Department of Forestry and Wild Life Sciences, University of Port-Harcourt, Rivers State, Nigeria. It was authenticated at the herbarium of the Department of Pharmacognosy and Herbal medicine, Faculty of Pharmacy, Niger Delta University, Bayelsa State where a voucher number NDUP0179 was assigned.

The leaves were oven dried at 35o C and pulverized into coarse powder which was eventually used for moisture content, total ash, sulphated ash, acid insoluble ash, water soluble ash, water and alcohol soluble extractives evaluation and powdered microscopy. Macroscopy and microscopy of the surface as well as the transverse section of the fresh leaf sample were also evaluated.

Macroscopic Evaluation

The macroscopic characters of the leaf were evaluated by using standard method.[19]

Microscopic Evaluation

The leaf microscopy was studied by employing standard procedures[20] so as to study the anatomical structures of the adaxial and abaxial surfaces as well as the transverse section across the mid ribs. The sectioned surfaces and the coarse powder were cleared with chloral hydrate, mounted with glycerol and examined under the microscope at x40, x100 and x400 magnifications. Pictures of the structures were taken using a digital camera.

Determination of Loss on Drying of *A. camansi* leaf

Exactly 5g of *A. camansi* powdered leaves was dried in the oven at 105^{o}C until a constant weight was attained. Loss on drying was expressed with reference to the air-dried powdered plant drug in percentage.[21] It was carried out in ten replicates and the average value obtained.

Determination of total ash of *A. camansi* leaf.

Powdered *A. camansi* leaf (2g) was transferred into a tarred crucible and incinerated at a temperature not exceeding 450°C until it was free of carbon. This was cooled in a desiccator and weighed until a constant weight was obtained. The total ash relatively to the dried powdered leaf was expressed in percentage.[21] The experiment was carried out in ten replicates and the average value obtained.

Determination of acid insoluble ash of *A. camansi* leaf

The obtained total ash was allowed to boil in 25 mL of 10% hydrochloric acid for 5 minutes, and filtered using an ashless filter paper. It was rinsed with hot distilled water. The ashless filter paper together with the insoluble mater was ignited. The acid in-soluble ash relatively to the dried drug was expressed in percentage.[21] It was carried out in five replicates and the average value obtained.

Determination of Water-Soluble Ash of *A. camansi* leaf

The obtained total ash was allowed to boil for 5 minutes with 25 mL of distilled water, and the insoluble mater was collected using an ashless filter paper. The ashless filter paper with the content was ignited at a temperature not exceeding 450^{0}C. The weight of the ash obtained was subtracted from the weight of the total ash, this weight difference represented the water-soluble ash. The water-soluble ash relatively to the air-dried leaf was expressed in percentage.[21] It was carried out in five replicates and the average value obtained.

Determination of sulphated ash of *A. camansi* leaf

Powdered *A. camansi* leaf (2g) was transferred into a tarred crucible, moistened with concentrated sulphuric acid. It was then ignited gently and again moistened with sulphuric acid and ignited at about 500^{o}C. The percentage sulphated ash relatively to the air-dried leaf was determined.[21] It was carried out in ten replicates and the average value obtained.

Determination of water and alcohol soluble extractives of *A. camansi* leaf.

Exactly 5g of A. camansi powdered leaf was macerated in Chloroform water BP (100 mL) for a day, agitating regularly in the first quarter of the day and left standing for the remaining 18 hours. It was filtered and 20 mL of the filtrate evaporated to dryness in a 100 ml beaker at 105oC to a constant weight. Then the percentage water soluble extractive was determined relatively to dried drug.[21] It was performed in ten replicates and the average value obtained. The same method was repeated for alcohol soluble extractive but with absolute ethanol.

RESULTS AND DISCUSSION

Macroscopically, the leaf is simple with acuminate apex, symmetrical base and an entire margin (Figure 1, Table 1).

Figure 1: *Artocarpus camansi* Blanco tree growing at the of Ologi village in Ogbia Local Government Area, Bayelsa State, Nigeria (with the leaf, fruits and seeds).

Table 1: Macroscopic characters of *A. camansi.*

Leaf size	length 31 – 105 cm, width 44 – 58 cm
Margin	Entire
Apex	Acuminate
Composition	Simple
Base	Symmetrical

Microscopically, the walls of the epidermal cells are straight and anticlinal and are polygonal shaped, the presence of glandular trichomes with multicellular head and unicellular stalks was visible in the upper layer. The upper layer is devoid of stomata (Figures 1- 3). Anomocytic stomata were found only on the abaxial leaf surface which shows its hypostomatous nature (Figures 4-5). This will benefit the plant because it is able to control water loss through transpiration. Plants having leaves which are accompanied by stomata on either side of the surfaces, known as amphistomatous, are infrequent when juxtaposed with those having them on both surfaces. Amphistomatous character can also be advantageous for the leaves that are thick by reducing the carbon dioxide transport pathway betwixt chloroplasts and the atmosphere leading to an enhancement of the capacity of the gas exchange between the leaf and the atmosphere but they can also cause an increase in the rate at which water is lost from the plant through transpiration.[22] The transverse section is dorsiventral in shape with collateral vascular bundles (Figure 6).

Studying the internal structures of plants having medicinal values is vital to their proper identification.[23-24] Internal structures like the epidermal type/shape, type of stomata, trichomes are diagnostic. Study aimed at determining the type of stomata has provided solutions to many taxonomic problems.[23-24]

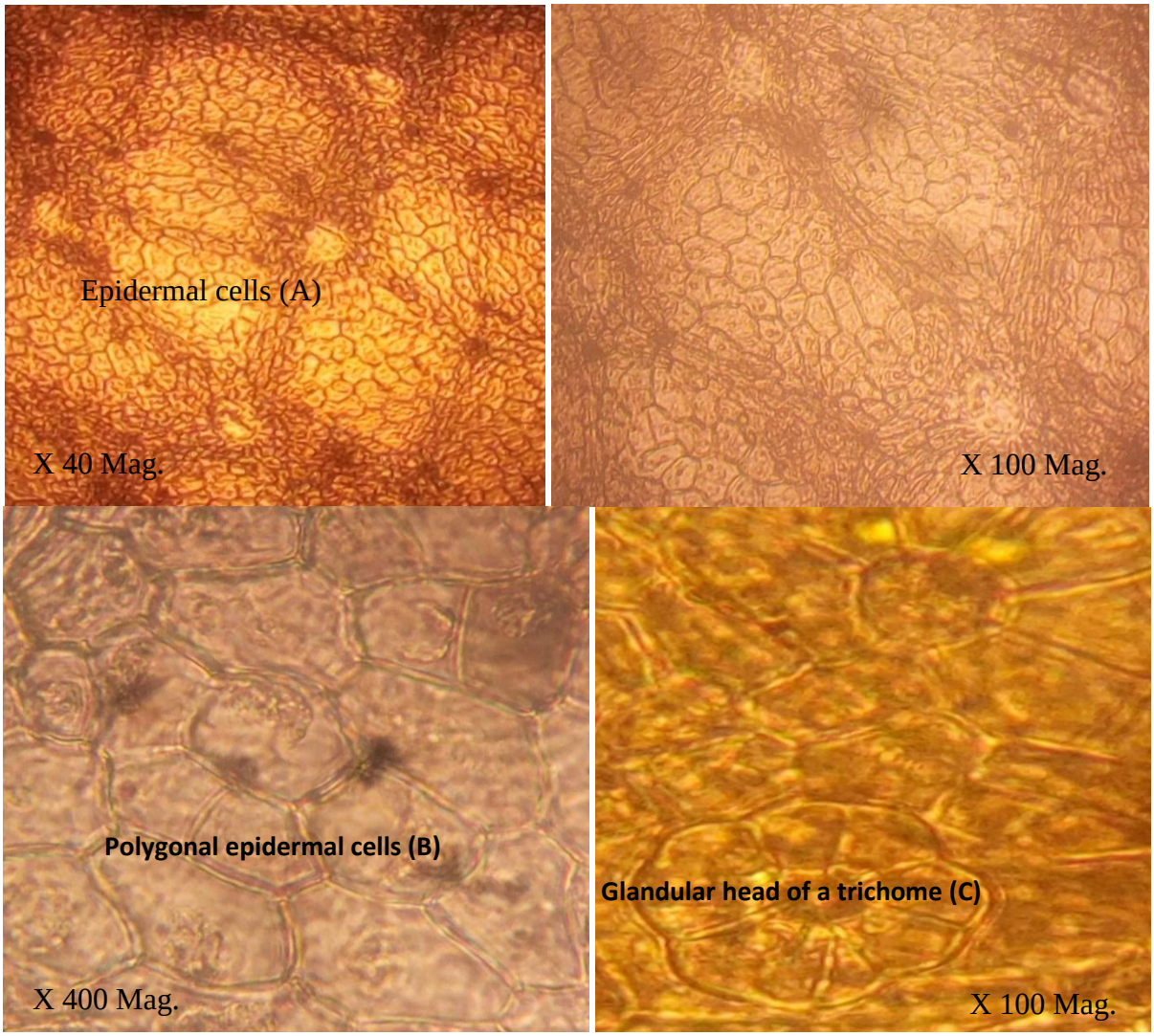


Figure 2: Microscopy of the upper (adaxial) surface of the leaf of *A. camansi* Blanco with straight anticlinal walls (A), polygonal in shape (B) and glandular multicellular head of a covering trichome (C).

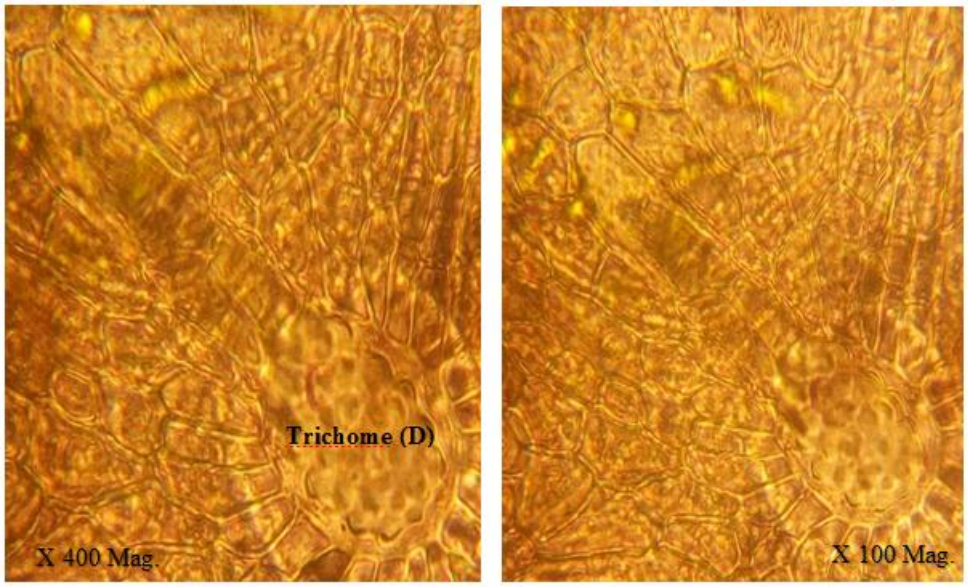


Figure 3: Microscopy of the upper (adaxial) surface of the leaf of *A. camansi* Blanco showing a covering trichome with a multicellular head and a unicellular stalk (D).

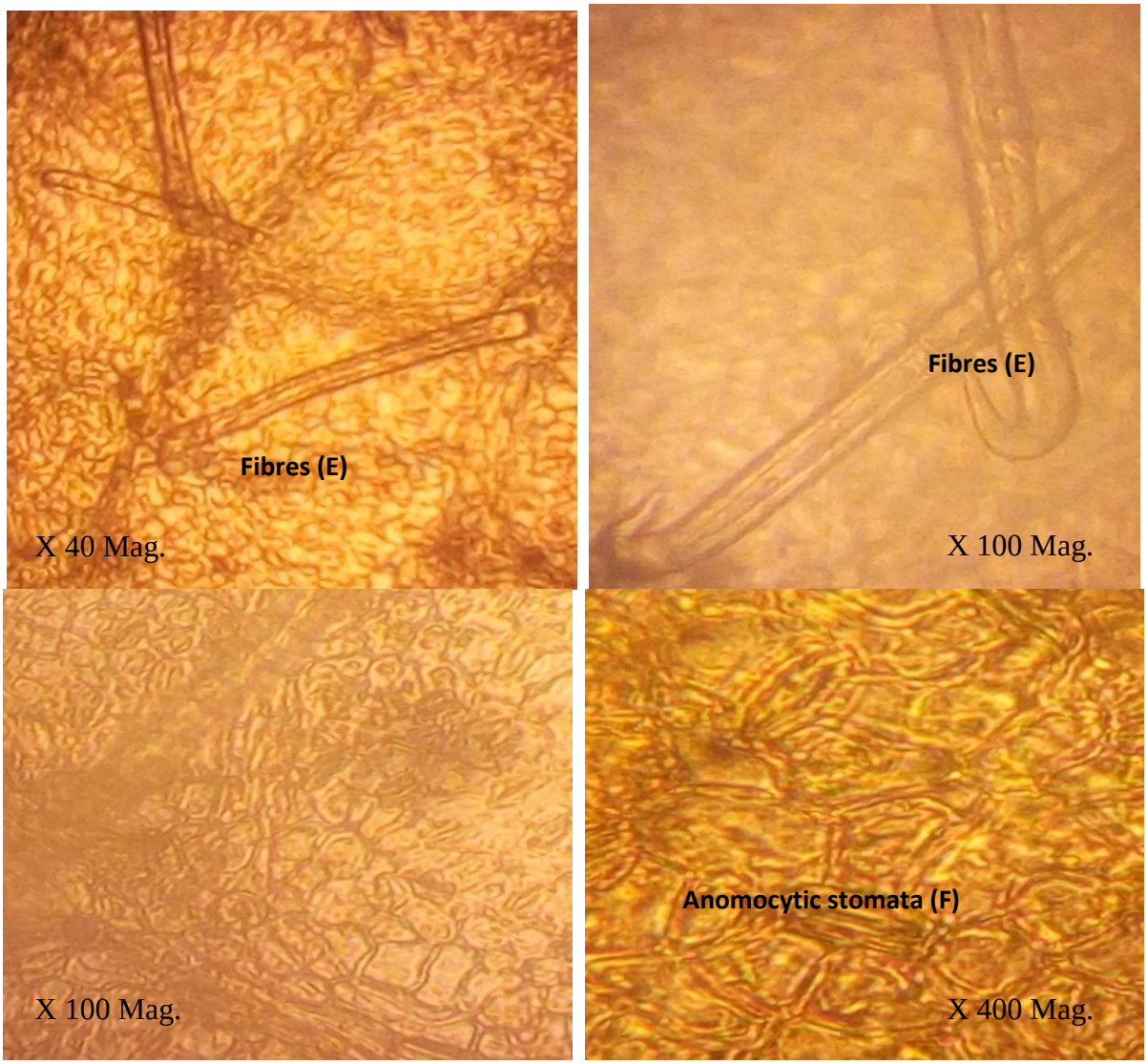


Figure 4: Microscopy of the lower (abaxial) surface of the leaf of *A. camansi* Blanco showing fibres (E) and anomocytic stomata (F).

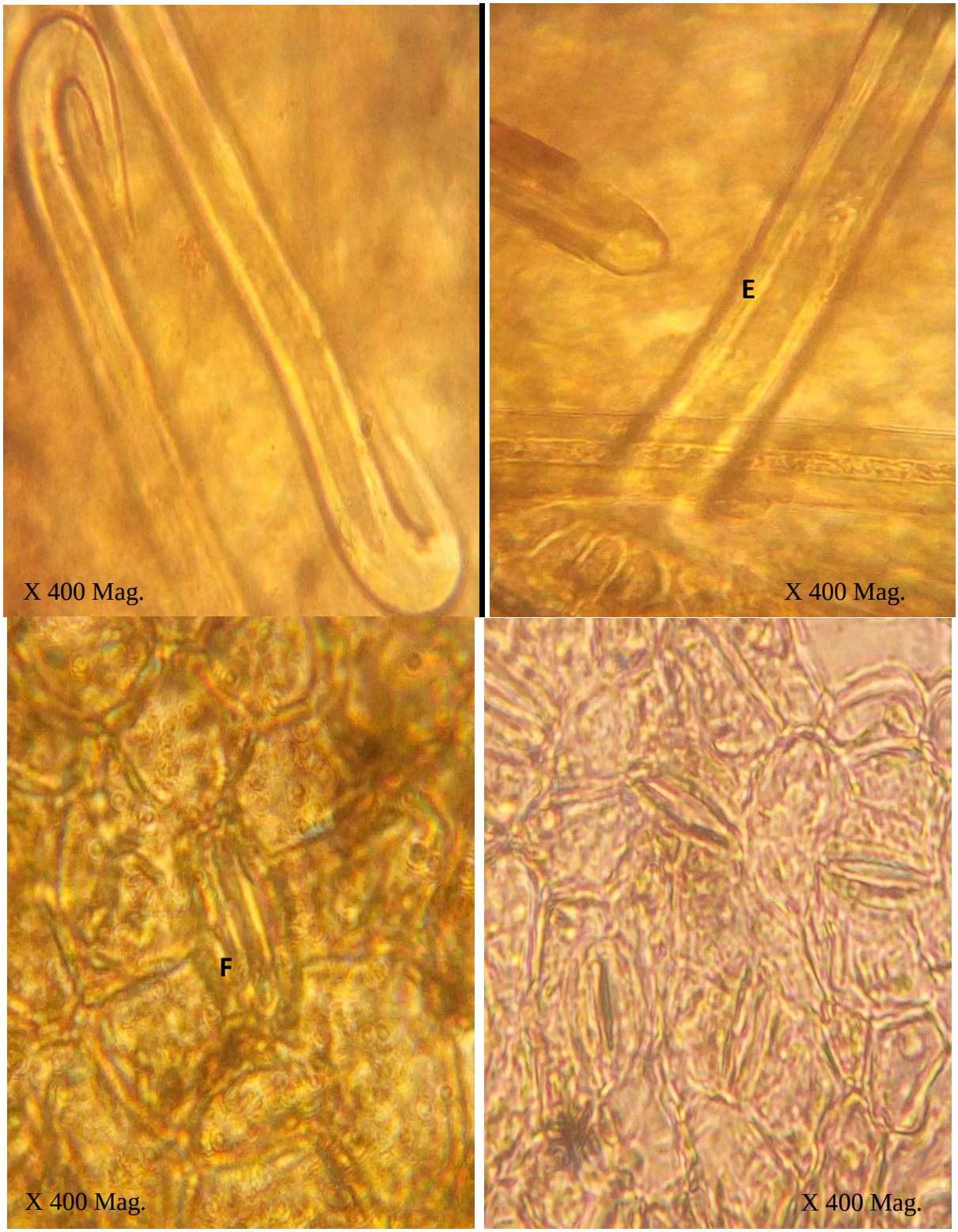


Figure 5: Microscopy of the lower (abaxial) surface of the leaf of *A. camansi* Blanco showing fibres (E) and anomocytic stomata (F).

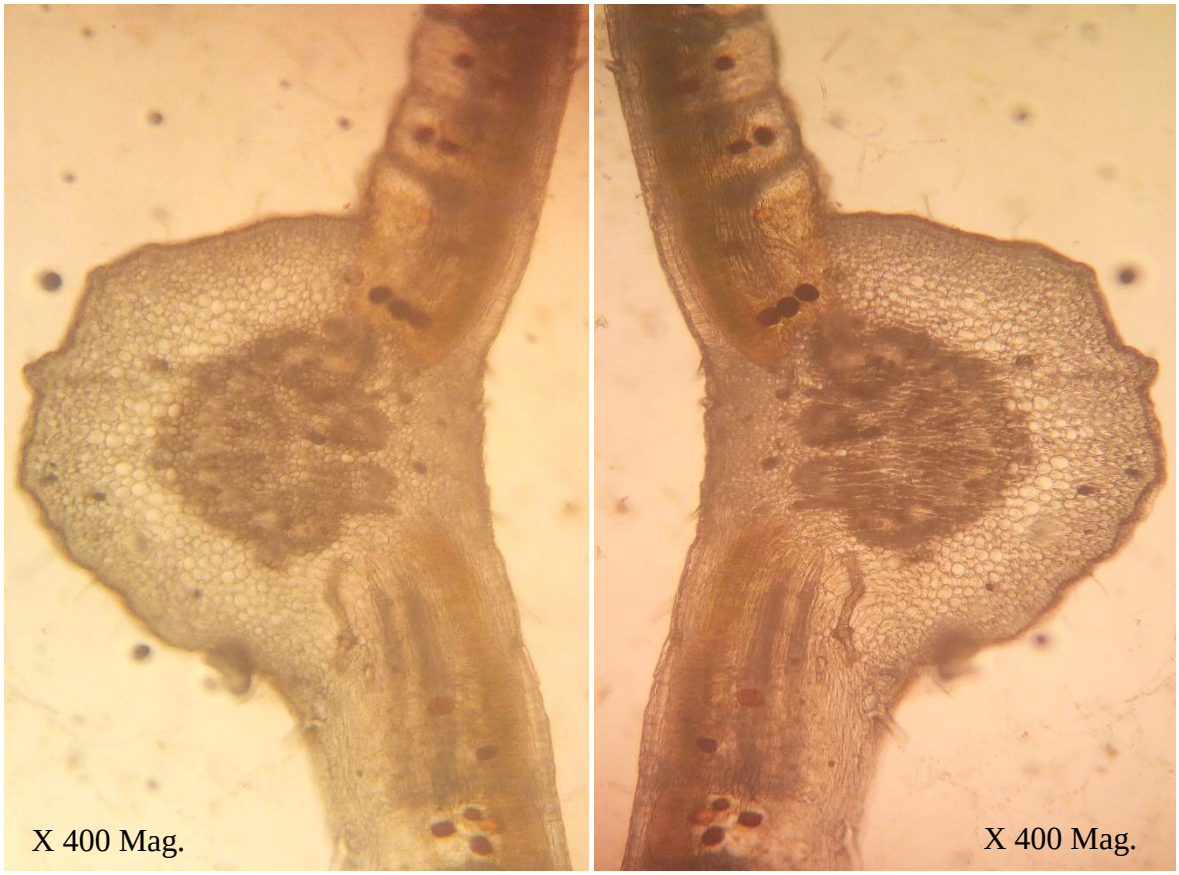
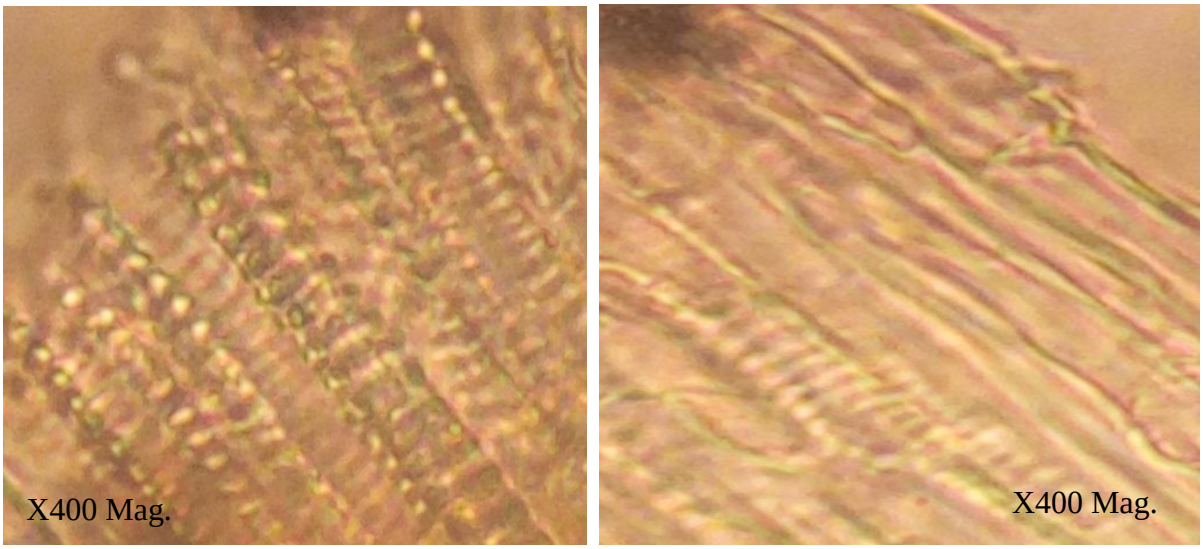


Figure 6: Microscopy of the transverse section (TS) through the midrib of the leaf of *A. camansi* Blanco showing a dorsiventral TS with collateral vascular bundles.



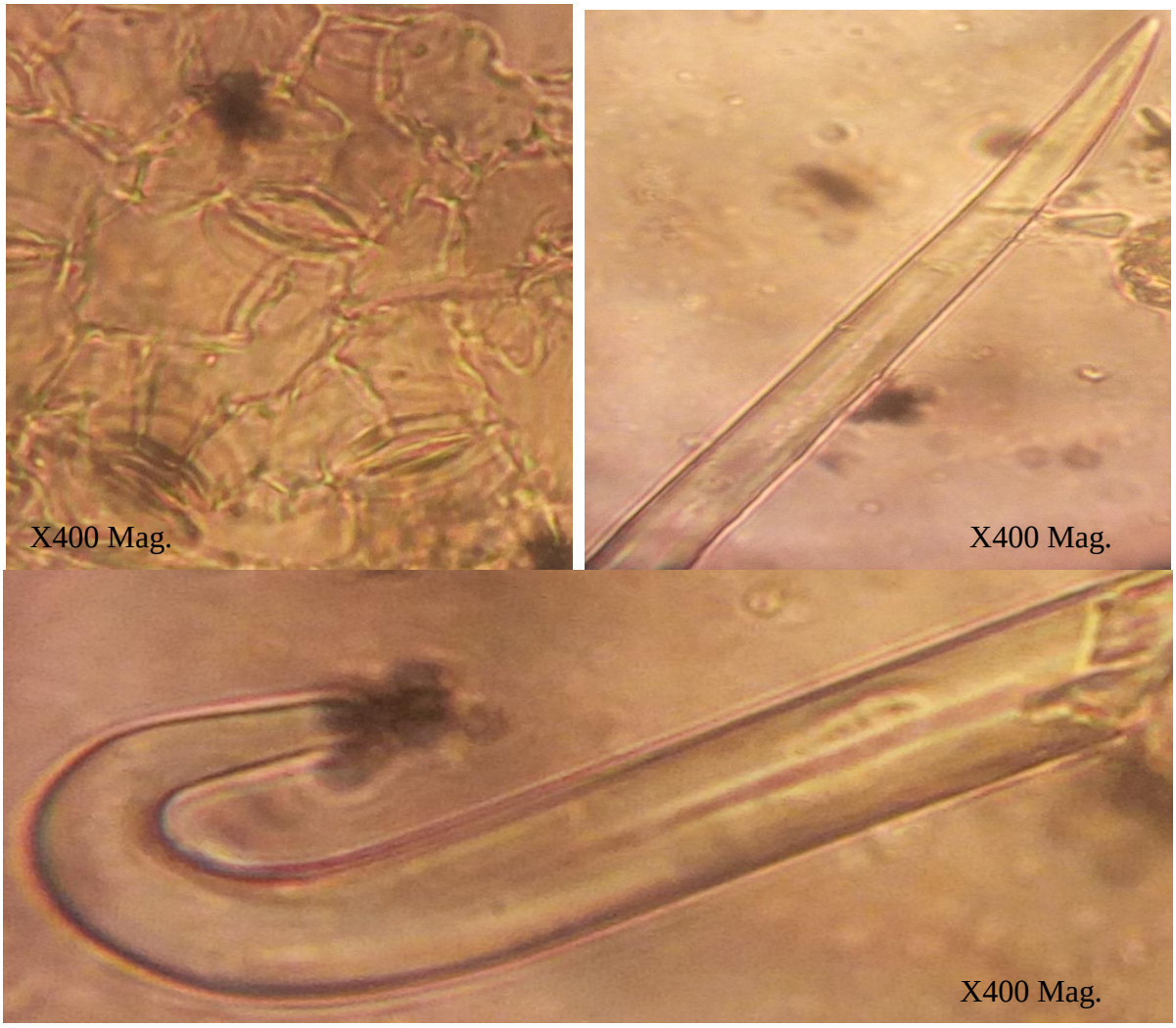


Figure 7: powdered leaf microscopy of *A. camansi* Blanco showing fragments of epidermal cells (A), anomocytic stomata (B), trichome (C).

The average values of the stomata number and stomata index were 12.4 and 28.5, respectively (Table 2).

Table 2: Quantitative microscope of *Artocarpus camansi.*

Parameter	Mean	Standard deviation	Minimum value	Medium value	Maximum value
Stomata number	12.4	2.1604	9	13	15
Epidermal cell	31.100	3.542	28	29.50	37.00
Stomata Index	28.483	3.039	22.920	29.635	31.710

The value of the moisture content (11.1%) was found to be within the limit for powdered herbal drugs which is between 8 and 12 %.[19] Above this range, chemical constituents which determine the biological activities of medicinal plants can be adversely affected due to deterioration and enzymatic activity. The aesthetic value of the drugs is also compromised.[25] Moisture content therefore plays a critical role in the storage of these plant drugs.[26] From this study, the values of the total ash, acid insoluble, water soluble and sulphated ash were found to be 10.0, 2.0, 4.1 and 7.3%, respectively, the value of the acid insoluble ash being half of water soluble ash value (Table 3). The presence of siliceous matter is determined by water soluble ash. The value of total ash in this study falls below the maximum 14% while the value for acid insoluble ash is the exact value specified by the European pharmacopoeia.[12] Determination of extractive values is vital to in the assessment of the quality and eventual safety of plant drugs. It is an indication of the identity of the classes of phytoconstituents contained in medicinal plants. It also helps in adjudging an exhausted plant material. The value of the water soluble extractive (6.4%) was 30% in excess of the alcohol soluble extractive (4.5%) (Table 3). It therefore shows that any values greater than these may suggest an adulterated A. camansis leaf material.

Table 3: Physicochemical parameters of *Artocarpus camansi* Blanco.

Parameter	% Mean	Standard Error of mean	% Minimum value	% Medium value	% Maximum value
Moisture content	11.078	1.415	9.900	10.320	13.580
Total ash	10.048	0.344	9.430	10.055	10.570
Acid insoluble ash	2.010	0.686	1.10	2.130	2.710
Water soluble ash	4.108	1.252	3.180	3.580	6.360
Sulphated ash	7.298	0.284	6.900	7.360	7.690
Water soluble extractive	6.394	0.377	5.870	6.405	7.110
Alcohol soluble extractive	4.494	1.039	2.380	4.510	5.770

CONCLUSION

This report has provided some standards, and it is expected to aid the identification of the leaf of *A. camansi*, that will eventually ensure its safety and therapeutic efficacy in drug development.

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