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Establishing some pharmacognostic standards for Nigerian *Musa x sapientum* L. leaf

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Abstract
Musa x sapientum is well known to Nigerian ethnomedicine and widely cultivated worldwide for its fruit. All the parts have medicinal application. The leaves collected from Abuja, Ibadan, Yenagoa and Owerri, Nigeria were subjected to microscopical examination and physic-chemical experiments in order to provide some pharmacognostic standards for it. The leaf possessed epidermal cells with straight anticlinal walls, bearing fewer stomata on the upper surface than the lower surface that showed numerous anomocytic stomata. The moisture content was (7.6% and 13.0%), water and ethanol soluble extractives (7.9% - 9.5% and 5.9% - 7.3%, respectively), total ash (5.5% - 14.0%), acid insoluble (0.5% - 2.7%) water soluble extractive (3.3% to 7.2%), sulphated ash (4.3% - 8.2%), stomata number and stomata index were 14 ± 0.80 and 16.00 ± 0.76, respectively. This will help to detect adulteration of the powdered sample.

Keywords: Adulteration; Standardization; *Musa sapientum*; Monograph.

INTRODUCTION

Basic requirements of reproducible quality, safety and efficacy are needed for medicines; these are achieved by processes of standardization which refer to confirmation of their identity and determination of quality and purity [1]. A collection of such standards of identity constitutes the monograph of such plant [2]. One major hindrance to the acceptance of herbal medicine is lack of adequate documented standards [3]. It is imperative therefore to determine standards right from the proper identification of the plants [4].

Musa x sapientum L. is an herbaceous plant of the family Musaceae. All parts of the plant have medicinal applications. The young leaves are placed as poultices on burns and other skin infections while the astringent ashes of the leaves are taken for dysentery and other ailments. The leaves are also used by the tribals of Western Ghats in India for bandaging cuts, blisters and ulcers [5, 6]. This study is aimed at providing some pharmacognostic standards for *Musa x sapientum* leaf.

EXPERIMENTAL

Plant collection and authentication. The leaves of *Musa x sapientum* were collected in different zones of the country: Ibadan representing West; Owerri representing East; Yenagoa, South-South and Abuja

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representing the Northern region of Nigeria. It was identified and the herbarium specimen (NDUP 150) deposited at the herbarium of the Department of Pharmacognosy and Herbal Medicine of the Faculty of Pharmacy, Niger Delta University, Bayelsa State. The leaves were powdered and stored until required for use.

The leaves of *Musa x sapientum* were cut into small pieces to facilitate the drying process at 40^{o}C in an oven and blended into a coarse powder.

Microscopic evaluation. The microscopic evaluation of powdered samples and anatomical sections were carried out using standard procedures. The upper and lower surfaces of fresh leaf were also prepared [7]. Stomata number and stomata index were determined. The coarse powder was cleared using chloral hydrate solution and then examined microscopically.

Determination of physicochemical constants

Moisture content. The moisture content was determined by loss on drying [8]. Powdered material (2 g) was accurately weighed to a known weight. The weighed powder was heated in the oven at 105^{o} C until a constant weight was attained. The moisture content was calculated with reference to the original weight of the powdered drug. This was carried out in twelve replicates for each of the samples from the different locations.

Total ash. Powdered plant sample (2 g) was weighed and ashed using a muffler furnace at 600°C until the sample turned into white ash at constant weight. The ash was weighed, and the percentage of the total ash calculated [9].

Acid insoluble ash. Dilute hydrochloric acid (25 ml) was used to wash the ash obtained from the determination of the total ash into a beaker and boiled for 5 minutes. This was filtered using an ashless filter paper and the residue was washed with hot water until the filtrate became neutral. The filter paper together with the residue was then dried and ignited to constant weight. The acid-insoluble ash was weighed, and the percentage of acid-insoluble ash was calculated. This was carried out in twelve replicates for each of the samples from the different locations [9, 10].

Water soluble ash. This follow the same procedure as Acid insoluble ash but distilled water was used in place of hydrochloric acid. The weight of the insoluble ash was subtracted from the weight of the total ash to determine the water soluble ash [11]. This was carried out in twelve replicates for each of the samples from the different locations.

Sulphated ash. The same procedure for total ash was repeated for sulphated ash but the plant material was moistened with sulphuric acid prior to ashing [11].

Extractive values

Alcohol soluble extractive. Powdered plant (5 g) was weighed and 100 ml absolute ethanol added. The mixture was shaken for the first 6 hours and then allowed to stand for the next 18 hours. It was filtered and 25 ml of the filtrate was transferred to a weighed evaporating dish and evaporated to dryness at 105°C in an oven to constant weight [11]. This was carried out in twelve replicates for each of the samples from the different locations.

Water soluble extractive. This follows the same procedure as alcohol soluble extractive however chloroform water B.P. was used in place of ethanol [12].

Statistical analysis. Values were represented as Mean ± Standard Error of Mean (SEM). "One-way ANOVA with Turkey–Kramer Multiple Comparison Test was performed using GraphPad Prism version 5.01 for Windows, GraphPad Software, San Diego

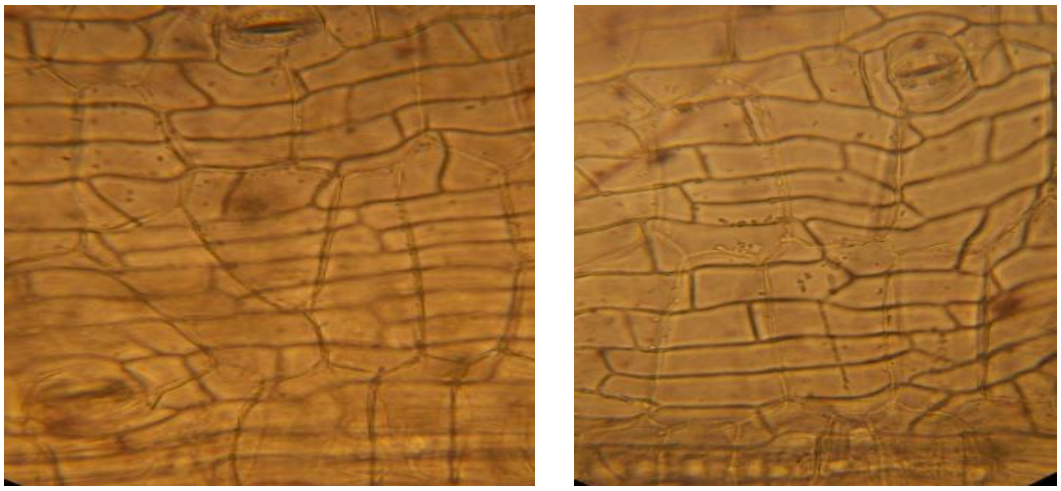
California USA, www.graphpad.com". P values < 0.05 were considered significant.

RESULTS

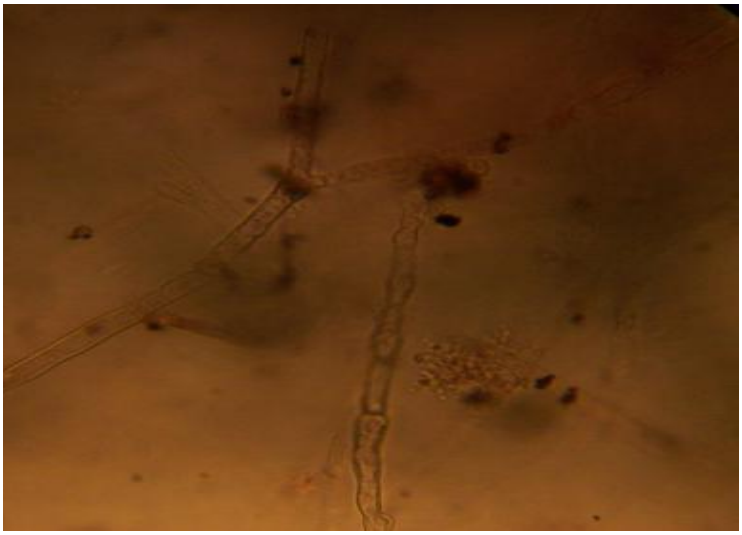
The microscopy of the upper, lower surfaces as well as that of the powder specimen of *Musa x sapientum* is as presented in Figures 1, 2 and 3, respectively while Tables 1 and 2 show its physicochemical constants and Quantitative microscopy, respectively.

DISCUSSION

Total ash is a measure of the total amount of material remaining after ignition which may include physiological ash introduced from the plant tissue itself, and non-physiological ash, that originates from extraneous matter contaminating plant surface. Acid insoluble ash is a measure of the amount of silica present, mostly as sand and siliceous earth. Soluble extractives evaluate the amount of active constituents extractable with solvents from a given amount of powdered plant material [1]. The moisture content ranged between 7.6% and 13.0%. The lowest value came from the North (Abuja) and it was statistically significant ($p < 0.05$).

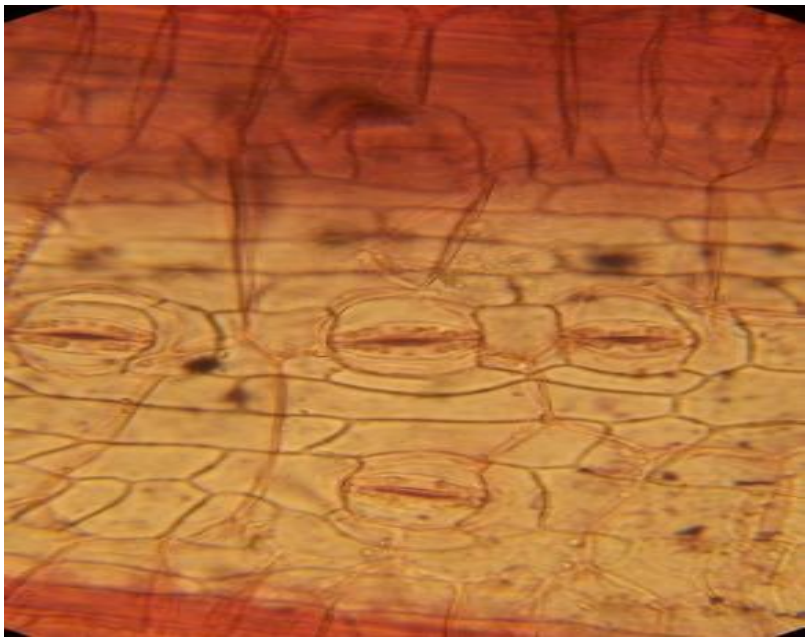


Straight anticlinal walls with anomocytic stomata xterised with oil globules



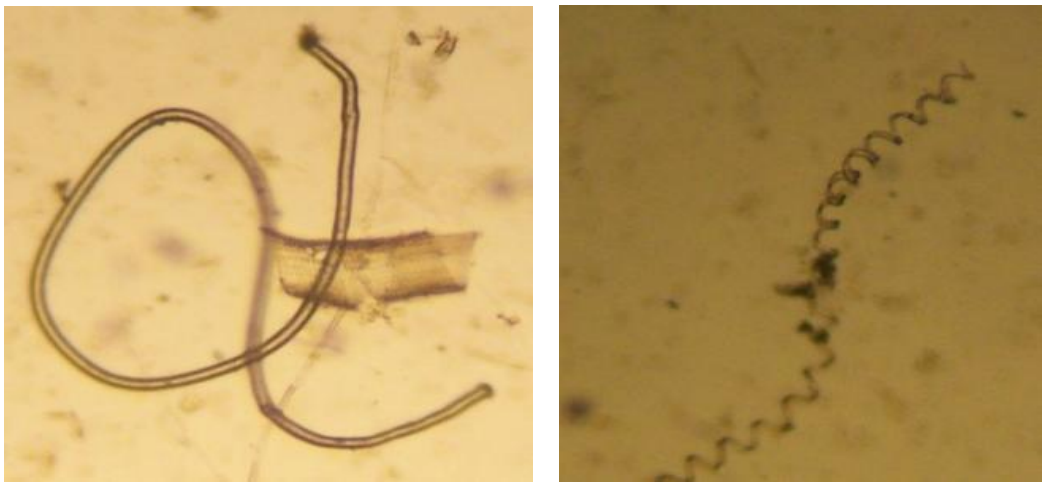
Uniserrate trichomes

Figure 1: Microscopy of the upper surface of *Musa x sapientum* leaf



Anomocytic stomata

Figure 2: Microscopy of the Lower surface of *Musa x sapientum* leaf



Fibres, vascular fibre

Figure 3: Microscopy of the powdered leaf of *Musa x sapientum*

Table 1: Physicochemical constants of *Musa x sapientum* leaf

Parameter	Location			
	Abuja	Bayelsa	Ibadan	Imo
Moisture content (%)	7.6 ± 0.94*a	11.0 ± 0.11	13.0 ± 0.81	13.0 ± 0.11
Water soluble extractive (%)	7.9 ± 0.79	9.2 ± 0.22	9.5 ± 0.41	9.0 ± 0.35
Ethanol soluble extractive (%)	6.5 ± 0.29	7.2 ± 0.52	5.9 ± 0.69	7.3 ± 0.78
Total ash (%)	5.5 ± 0.81*b	11.0 ± 0.33	14 ± 0.22*c	11 ± 1.20
Acid insoluble ash (%)	0.5 ± 0.02	2.6 ± 0.12	1.7 ± 0.20	2.7 ± 0.21
Water soluble ash (%)	3.3 ± 0.39*d	7.2 ± 0.85	3.8 ± 0.51*e	5.0 ± 0.71
Sulphated ash (%)	7.2 ± 0.65	8.2 ± 0.08	4.3 ± 0.09*f	7.7 ± 0.32

*Data expressed as mean ± SEM (n=12), *p<0.05 (*a between Abuja and Ibadan, Bayelsa, Imo, *b between Abuja and Ibadan, Bayelsa, Imo, *c between Ibadan and Bayelsa, Imo, *d between Abuja and Bayelsa, Imo, *e between Ibadan and Bayelsa, *f between Ibadan, Abuja, Bayelsa, Imo).*

Table 2: Quantitative microscopy of *Musa x sapientum* leaf

Parameter	
Stomata number	9.00 – 14.00 – 18.00 (14.00 ± 0.80)
Stomata Index	13.00 – 16.00 - 24.00 (16.00 ± 0.76)

Data expressed as mean ± SEM (n=20)

Water and ethanol soluble extractives ranged from 7.9% - 9.5% and 5.9% - 7.3%, respectively and were not statistically different among the regions. Total ash ranged from 5.5% - 14.0%, the least value being from Abuja, and it was statistically significant ($p < 0.05$). Also, the highest value was from Ibadan specimen and it was also statistically significant ($p < 0.05$). Acid insoluble ash ranged from 0.5% and 2.7% and the differences were not significant among the regions. Water soluble extractive ranged from 3.3% to 7.2% with the least from Abuja which was significantly lower ($p < 0.05$) than the values obtained from Bayelsa but approximately same as those from the rest regions. The highest value was obtained from Bayelsa. Sulphated ash ranged from 4.3% and 8.2%, with Ibadan being significantly lower ($p < 0.05$) than the values from other regions (Table 1). The Stomata number and Stomata Index were 14 ± 0.80 and 16.00 ± 0.76, respectively (Table 2).

The microscopy showed straight almost rectangular epidermal cells with straight anticlinal walls, bearing fewer stomata on the upper surface than the lower surface that showed numerous anomocytic stomata.

Conclusion. The data obtained for *Musa x sapientum* can be employed for proper identification of the plant. It will also help to detect adulteration of the powdered sample.

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