

AN EVALUATION OF *LUFFA AEGYPTIACA* MILL PLANT LEAVES (CUCURBITACEAE)

Abubakar, Abba¹. Abdurra'uf, R. Idris¹, Salamatu Suleiman¹ and Suleiman M.D¹.

¹Depart. of Bio. and Microbio, School of Applied Sciences, Nuhu Bamalli Polytechnic Zaria, Kaduna State.

Correspondence: abubakarabba056@gmail.com.

Abstract

*This study is aimed at contributing more elaborate ways for authenticating *Luffa aegyptiaca* Mill (Cucurbitaceae) plant by studying the leaves through the use of Light microscope to undertake Pharmacognostic evaluations of *Luffa aegyptiaca* Macroscopical, Microscopical, Physical Constants, Preliminary Phytochemical Screening and Thin Layer Chromatography were employed. Studies of the leaf revealed the presence of anomocytic stomata and covering trichomes at the lower and upper surfaces. Stomatal number and stomatal index; 14.09 ± 1.35 and 6.3 ± 0.12 , vein islet and Veinlet termination; 9.6 ± 0.09 and 21.8 ± 0.07 were observed and recorded respectively. Physical constant studies on the leaf showed moisture content of $10.36 \pm 0.12\%$, Ash value of $13.3 \pm 0.21\%$, Acid insoluble ash $9.03 \pm 0.12\%$, alcohol and water extractive values; $2.07 \pm 0.13g$ and $4.73 \pm 0.7g$ respectively. Preliminary Phytochemical screening of the leaf, revealed the presence of alkaloids, flavonoids, saponin, tannin, anthraquinone, cardiac glycoside, coumarins and carbohydrates. Thin Layer chromatography of the leaf methanol extract showed 12 spots of variable colors with P-Anisaldehyde and 3 spots with ferric chloride spraying reagents these are indications for the presence of the above mentioned secondary metabolites as observed during phytochemical screening. These characteristics and parameters add courage and value to the community usage of the plant for medicinal purpose traditionally. For the research is an important factor to detect adulteration and could help in the classification and identification in plants taxonomy.*

keywords: Evaluation, *luffa aegyptiaca*, cucurbitaceae.

Introduction

The evaluation of plant materials and their derived products has always been an important part of the professional expertise of the pharmacists and analysts; however the nature and the degree of this evaluation have changed (Abdurrahman, 2010). Initially it was considered sufficient to authenticate the plant material by comparison with a standard botanical description or monograph, but later it was realized that for the detection of adulterations this practice has to be complimented with both a microscopical analysis and confirmatory characteristic test for the classification of the Phytochemicals it contained therein. These resulted in the extension of the evaluation procedures in to areas of estimation and identification of these constituents, characters, structures and requirements that drugs should conform to a phytochemical as well as morphological monograph (Brain and Turner, 1975, Kumari S A *et al*, 2018.). These characters like; stomata, trichomes, epidermal cells are distributed at the surface aerial part of the plants, having various functions and are extremely variable in their prevalence across plant, location on plant organs, density and forms, therefore, their morphology and structure can be used as taxonomic markers in the infrageneric classification of the genus (Banerjee *et al*, 2004: Sauban *et al*, 2016). The Stomata, epidermal cells and particularly the trichomes in the family Cucurbitaceae vary from unicellular to multicellular, conical to elongated, smooth to ridges, with or without flattened disk at base and cytolithic appendages, thin to thick walled, curved at apices to blunt, trichomes micromorphology in the family Cucurbitaceae were found significant taxonomically (Muhammad *et al*, 2011, Sauban *et al*, 2016).

Luffa aegyptiaca (Soso in Hausa) is a plant of multi-purpose importance whose usefulness cuts across virtually all areas of life (Ani, E. *et al* 2020). The plant is an annual herb climbing or prostrate up to 6m high with pentagonal stems, small yellow flowers and cylindrical club-like fruit with a network of woody vascular bundles under the thin scale. Tendrils are present at 90° to the leaf petiole at nodes. Leaves are simple palmately lobed or palmately compound. The flowers are unisexual, with male and female flowers on different flowers have interior ovaries. The fruit is often a kind of modified berry called pepo (Schaefer *et al.*, 2011, Sunita VR. 2018). *Luffa cylindrical* (Linn) M. Roam (Synonyms; *L. aegyptiaca* ex Hook) is widely distributed in the tropics. Decoction of the leaves and roots are abortifacient (Neuwinger, 1996). The juices from the fresh leaves are used to treat *Candidiasis*. The leaves are used for better and rapid cicatrization of wounds (Sashikala *et al.*, 2009). In Chinese pharmacopoeia the fibrovascular bundle of the dry ripe fruits is listed for the treatment of paralytic disease, in folk medicine it is used against chronic bronchitis as an antitussive (Neuwinger, 1996). While in northern Nigeria the leaves are squeezed and rubbed to cure rheumatics' pains and the early growing fruits are slide and kept to ferment, the filtrates cures pneumonia in children, while the dry matured fruit is used as sponge.

Materials and methods

Microscopy of the *Luffa aegyptiaca* leaf

The lower peel from the lower leaf epidermis of a fresh leaf of *Luffa aegyptiaca* was carefully removed cleaned with chloral hydrate in a water bath for 15 minutes. The cleared peels were placed on a cleaned glass slide and covered with cover slide. A few drops of glycerol were added to the edges of the glass slide cover. The same procedure was observed for the upper surface of the *Luffa aegyptiaca* leaf and then drawn at $\times 10$ magnification.

The measurement of the stomatal index of *Luffa aegyptiaca* was made using microscope equipped with camera lucida (Model-wild M11, Manufactured by Wild Heerbrugg Switzerland). The prepared samples were examined at both low magnification and high magnifications; the superimposed squares drawn were marked \times for stomata and marked 0 dash for epidermal cell WHO 1998.

Determination of physical constant of *Luffa aegyptiaca* leaf

The determination of physical constants of *Luffa aegyptiaca* leaf was achieved by adopting the procedures described by Brain and Turner, (1994), Kokate, (1993), for the determination of moisture contents, Total ash value, acid insoluble- ash value, the extractive values; like water and alcohol extractive values.

Phytochemical screening of *Luffa aegyptiaca* leaf

The preliminary phytochemical screening was carried out on the methanol extract of the leaf for the presence of Alkaloids, Flavonoids, tannins, Saponins, Anthracene, Cardiac glycoside and Coumarins using methods described by Evans (2009).

Thin Layer Chromatography of methanol extracts of *Luffa aegyptiaca* leaf

The Thin Layer Chromatography profile of the extract (methanol) was obtained by spotting the extract on the TLC plates and developed in ethyl acetate/ methanol/ water (8: 1:1) and Butanol: Acetic acid: Water (4: 1:1). The plates were sprayed with sulphuric acid (10% H₂SO₄, 90% H₂O); Anisaldehyde/sulphuric acid (0.5% Anisaldehyde, 5% H₂SO₄, glacial acetate 10%, alcohol 84.5%) and ferric chloride (Ferric chloride 2%, alcohol 98%) as detecting reagents, followed by heating at 105°C in a Fisher scientific Oven. The chromatograms were scanned under ultraviolet light accordingly.

The Results

The result of the microscopical examination of the leaf reveals the presence of covering uniseriate trichomes at both lower and upper sides with average sizes of 89.3 μm and 60.5 μm , polygonal epidermal cells and Anomocytic Stomata at the lower and upper surfaces measured an average size of 5.5 μm and 4.2 μm respectively.

Stomatal average numbers obtained were 174.4 - 205.1 - 235.9 and 78.1-91.6 - 105.7, for both lower and upper surfaces. The lower Stomatal Index number was 16.20 - 14.09 - 11.98 and 7.25 - 6.3 - 5.36 for the upper Surface. The Vein Islet number was 8.16 - 9.6 - 11.04 and Vein Termination number of 18.53 - 21.8 - 25.07.

Various microscopical features were observed at both lower and upper surfaces of the leaf comprising of; anomocytic stomata, covering trichomes of viable cell number, straight walled epidermal cells and calcium oxalate crystal.

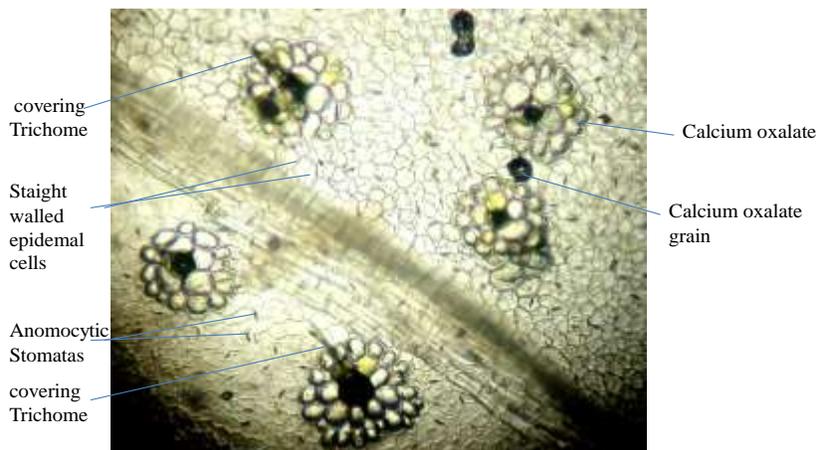


Plate I: An Upper epidermal peel showing trichomes, stomatas, calcium Oxalate crystal, Calcium Oxalate grain and staight walled epidermal cells. X100



Plate II: A Lower Epidermal Peel of *L. aegyptiaca* leaf Showing Covering Trichomes, anomocytic Stomata and Polygonal Epidermal cells X100

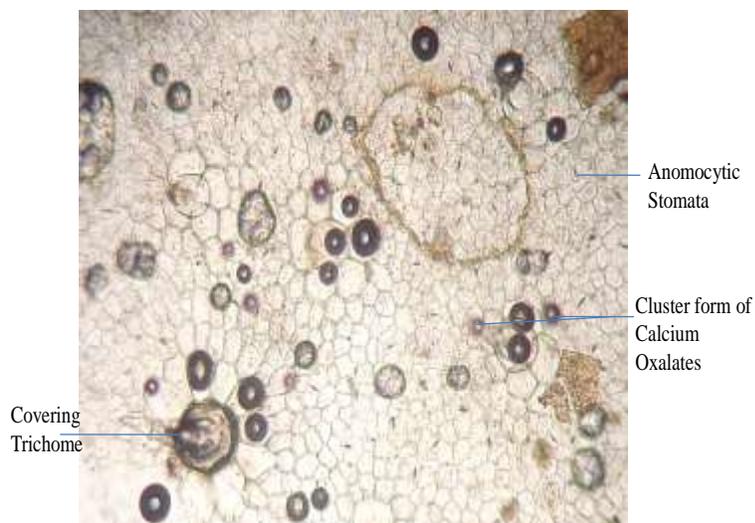


Plate III: *L. aegyptiaca* leaf Upper Epidermal peel Showing Anomocytic Stomata, Covering Trichome, Calcium Oxalate grain and Calcium Oxalate Cluster X100

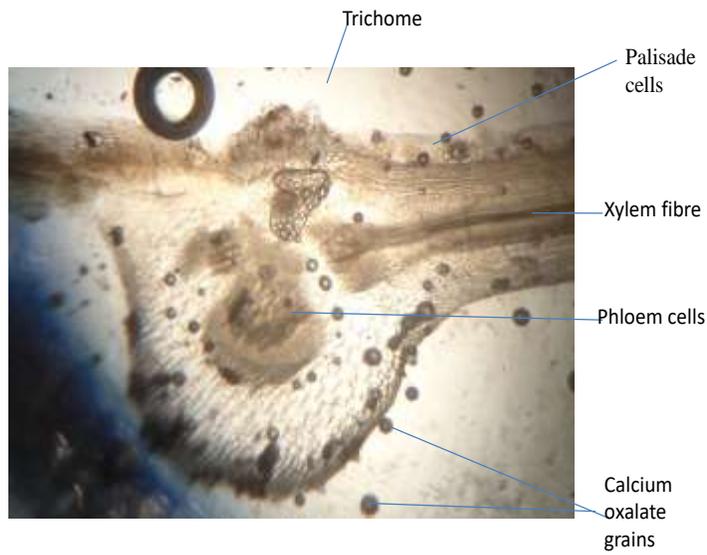


Plate IV: Transverse sections of *L. aegyptiaca* leaf through the midrib $\times 100$

Table 1: Quantitative Values

Parameter	Lower Surface	Upper Surface
Stomatal number	174.4 - 205.1 - 235.9	78.1-91.6 - 105.7
Stomatal Index	16.20 - 14.09 - 11.98	7.25 - 6.3 - 5.36
Vein islet	8.16 - 9.6 - 11.04	
Veinlet –termination	18.53 - 21.8 - 25.07	

Number of count (n) = 9

Quantitative Parameters

Evaluative parameters	Mean Value(% w/w ± SEM
Moisture content	10.36 ± 0.1
Total ash	13.3 ± 0.2
Acid insoluble ash	9.03 ± 0.1
Alcohol soluble extractive	2.07 ± 0.1
Water soluble extractive	4.73 ± 0.7

Table3: Results of Preliminary Phytochemical Tests of *L. aegyptiaca* Leaf

Test/Reagent used		Test/Reagent used	
ALKALOIDS TEST	INFERENCE	TEST FOR TANNINS /PHENOLIC COMPOUNDS	INFERENCE
Dragendoff’s reagent	+	Ferric chloride test	+
Mayer’s reagent	-	Bromine water	+
Wagner’s reagent	+	Lead sub-acetate	+
Picric acid	+	TEST FOR CARBOHYDRATES	
Dragendoff’s reagent	+	Molish test	+
TEST FOR FLAVONOID		TEST FOR FREE ANTHRAQUINONE	
Shinoda test	+	Bontrager’s test	-
Sodium hydroxide test	+	TEST FOR CARDIAC GLYCOSIDE	
Ammonium test	+	Kella-killiani	+
TEST FOR SAPONIN		Salkowski’s	+
Frothing test	+		
Hemolysis test	+		

+ indicate presence

- indicate absence

Thin Layer Chromatographic Analysis of Methanol extract of *L. aegyptiaca* Leaf

Chromatographic analysis of the extracts of *L. aegyptiaca* parts methanol that extract shows 14 different spots at different points on the TLC plate with Anisaldehyde spraying reagent, 10 spots with sulphuric acid and Ferric chloride, the chromatograms are presented on plates while the Rf values were calculated and presented.

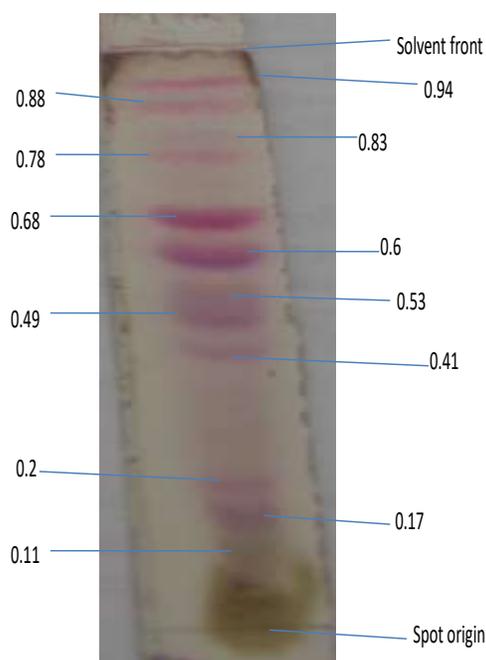


Plate V: The chromatograms of *L. aegyptiaca* leaf methanol extract developed in ethylacetate/methanol/water (EtOAc/MeOH/H₂O) 8:1:1 and spray with anisaldehyde/sulphuric acid, Showing spots and their Rf values.

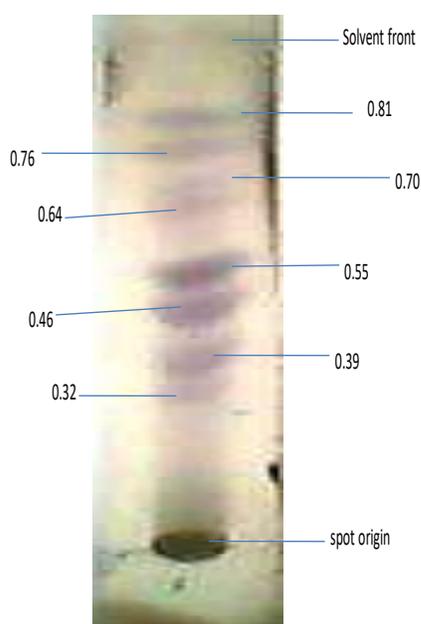


Plate vi: The chromatograms of *L. aegyptiaca* leaf methanol extract developed in ethylacetate/methanol/water (EtOAc/MeOH/H₂O) 8:1:1 and spray with Sulphuric acid showing spots and their Rf values.

DISCUSSIONS

This study was able to observe and establish the presence of Polygonal epidermal cells, an amphistomatic with anomocytic stomata, multicellular covering trichome on both the lower and upper epidermal layers, although these features were more numerous at the lower sides, beside that was the form of trichomes surrounded by an outgrowth of endolymphs containing calcium oxalate. Biran *et al.*, (2010) reported the presence of anisocytic type of stomata and different types of trichomes at both lower and upper surfaces of a family member *Lagenaria siceraria*.

The numerous parameters observed at both the lower and upper epidermis peels adopt the plant in its physiological activities to withstand adverse conditions with regards to pest attack, excess rainfall and dry seasons, as such found to be cosmopolitan year in year out available for local community that use it traditionally to treat different illness. It has been noted that Cucurbits are mostly dietary vegetables and fruits containing a large range of secondary metabolites that belong to different chemical families and possess distinct biological activities (Rizvi, 2009).

Quantitative microscopy indicated that stomatal number could be a good criterion for identification of the plant, likewise other distinguishing parameters observed include the varying sizes of the trichomes on both sides, moisture content found to be 10.5 ± 0.3 in this research was similar to that of some family members such as *L. acutangula* with 13.0 (Gowtham, 2012), *Lagenaria siceraria* (10.04) Pradhan (2013), *L. cylindrica* (9.4) Dairo *et al.*, (2007). The fruit fibrous material were employed as adsorbent in the removal of brilliant green from aqueous effluent via batch studies. (Olaseni *et al* 2014).

CONCLUSIONS

Epidermal features of species (*Luffa aegyptiaca*) plant can be distinguished from other cucurbitaceae through the parameters of these Pharmacognostic proceedings, Result obtained from Chromatography could also be an additional reference in the plant identification.likewise certain common taxochemical makers of the family.

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