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Micro-Morphological and Proximate Analysis of Sour Sop (*Annona muricata* L. of Annonaceae) Harvested from a Tertiary Institution Campus in the Niger Delta Region Nigeria

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ABSTRACT: This research investigated the micro-morphological and proximate analysis of sour sop (*Annona muricata* L. of Annonaceae), harvested from a tertiary institution campus in the Niger Delta Region. It is a perennial small tree commonly known as sour sop. Plant sample was collected fresh within the University of Port Harcourt Campus, ($4^{0}53^{1}30.12^{11}$North, and $6^{0}55^{1}39.6^{11}$East). The proximate analysis was done following the methods of AOAC Official methods of Analysis. The result revealed that *A. muricata* is glabrous and reach up to 8 ± 1 m tall. The petiolate foliar organs are simple, oblong, having acuminate apex, acute base with parted margins, pinnately veined and opposite in phyllotaxy; measuring up to 12.5 ± 4.5 cm in length and 4.6 ± 1.38 cm in width. The fruit is oval berry containing whitish pulp embellished with black shiny seeds, and spinous greenish fruit cover. The foliar epidermal study revealed presence of polygonal cells, trichomes and paracytic stomata which are amphistomatic. The proximate study showcased the following compositions: 9.27 ± 1.0 Carbohydrate (%); 5.38 ± 1.00 Protein (%); 2.00 ± 0.90 Lipid (%); 85.84 ± 1.74 Moisture (%) content; 0.58 ± 0.11 Fiber (%) and 0.89 ± 0.01 Ash (%). These information would assist for further delimitation of the species.

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The genus *Annona* L. has members known to be tropical trees with edible fruits, and belongs to the family Annonaceae (Nakasone and Paull, 1998). Despite their importance in nutrition, their identification may still be of a major constraint to consumers. Sour sop is a fruit lavishly consumed, it serves a palatable and delicious meal used as deserts and medicine. The family Annonaceae, is made of trees, shrubs or rarely Lianas which contain about 108 genera and 2,400 species (Chukwuka *et al.*, 2011) commonly called custard apple family or graviola family. They are found more in the tropical region with fewer number in the temperate biome (De Feo, 1992; Sulaiman *et al.*, 2012). Investigations on the vegetative and floral morphology of four out of the five species utilized had been reported (Folorunso and Olorode, 2006a). The taxonomic importance of their morphological features have been showcased by several workers, including (Metcalfe and Chalk, 1950, 1979; Adedeji and Illoh, 2005; Folorunso and Olorode, 2006b; Folorunso and Modupe, 2007; Abdulrahaman and Oladele, 2010). *Annona* species are multipurpose trees and have high nutritional value as food, medicinal and industrial products. Some members are cultivated for their edible fruits and often become naturalized beyond their native range (Wagner *et al.*, 2014); such as *Annona, Anonidium, and Asimina* etc. Some are Neotropical while others are Afro tropical and Indomalayan (Chatrou, 2005), coming from the native in Central America and in the

Northern part of South America (Hanett *et al*., 2001). It is well cultivated in Africa, mainly in the lowlands of Eastern and Western Africa, temperate and tropical Asia, Australia, North America, South Central Pacific Islands, the Caribbean and Mesoamerica (USDA-ARS, 2014). The fruits are heart shaped to oval dark green in color. It can be grown by micro propagation with its root stock, vegetative or clonally, in particular through various budding and grafting techniques on seedling stocks, however, *A. muricata* is commonly raised from seeds (Morton, 1987), and takes about 4 to 5 weeks to germinate after planting. It is cultivated by soaking the seeds in warm water overnight, the viability of seed is lost 6 months after harvest. There are two varieties of *Annona muricata*: the sweet sour sop, mostly often consumed and the sour sop, mostly for processing. They have high carbohydrate content which serves as excellent source of energy (International Centre for Underutilized Crops, 2002).

Furthermore, Olowokudejo (1990), provided useful data on the epidermal morphology, where it was revealed that only one type of stomata is present all together with some other characteristics. Olowokudejo (1990) reported presence of paracytic stomata while Folorunso (2014) findings revealed polygonal shaped cells in *Annona muricata*.

Also, the presence and absence of trichomes on the upper and lower surfaces of *A. muricata* distinguishes it from other species which can either be glabrous or pubescence on both surfaces (Olowokudejo, 1990), while Stace (1965) confirmed the opinion that trichomes can be influence by environmental factors. Stomata *A. muricata* are paracytic and elliptic, with a raised peristomatal rim and narrow aperture. Stomatal pore is visible Folorunso (2014). The ripe fruits of *A. muricata is rich in proteins, carbohydrate, vitamins and* fibre contents.

The analyses of proximate composition of the various parts of *Annona muricata* for the (fruit pulp, leaf, stem-bark, and root-bark) showed that the fruit had the highest moisture content followed by leaf, whereas the stem-bark had the lowest moisture content Agu and Okoli (2017). The usefulness of *Annona muricata* both as food sources and as medicine cannot be over emphasized. The need to add more information to the existing taxonomic relevance has created the interest to study the micro-morphological and proximate analysis of the sour sop, It is envisaged that the study would be an important source to further delimit the species. Hence, the objective of this study was to evaluate the micro-morphological and proximate analysis of sour sop (*Annona muricata* L. of Annonaceae) harvested from a tertiary institution campus in the Niger Delta Region Nigeria.

MATERIALS AND METHODS

Geographic Location: Plant sample (Plate 1) was collected fresh within the University of Port Harcourt Campus, ($4^{0}53^{1}30.12^{11}$North, and $6^{0}55^{1}39.6^{11}$East). The fruit is oval berry containing whitish pulp embellished with black shiny seeds, and spinous greenish fruit cover. Plate 1.

Plate 1: *Annona muricata*. Arrow showcased fruit

Morphological Studies: The meter rule was used to ascertain morphological measurements of plant parts such as: plant height from the root-collar to the terminal bud, the leaf length from the leaf tip to the petiole base and the leaf width across the leaf lamina, from one margin to another at the widest region.

Epidermal Studies: Fresh leaves collected were peeled chemically with nitric acid, and made to pass through alcohol solutions in the ratios of 50%, 75% and absolute alcohol for 5 minutes in each and thereafter stained with Safranin O, rinsed with distilled water and counter stained with Alcian blue for 5 minutes in each, rinsed again and mounted in aqueous glycerol solution placed on glass slide with coverslip following the method of Cutler (1977). Slides with good sections were placed on the stage, viewed and photo-micro graphed using Android Sony Camera on Monocular microscope.

Proximate Properties: The AOAC (1990) method was employed for the analysis of the proximate composition.

Carbohydrate (Cleg Anthrone Method): The formula used for carbohydrate analysis as shown in equation 1.

$$\%\ CHO\ as\ glucose\ = \frac{25\ ml\ x\ AS}{ASS\ Glucose} x\ 100 \quad (1)$$

Where AS = absorbance of sample, ASS absorbance of standard

Proteins (Kjeldahl method): The protein analysis was done using the formula shown in equation 2.

$$\%\ ON\ = \frac{TV\ x\ 1.4\ x\ 100}{1000\ \text{x}\ 20\ \text{x}\ 0.1} x100 \quad (2)$$

Where ON = Organic Nitrogen, TV = titre value, 1.4 = Nitrogen equivalent to the normalty of the HCl used in the titration is 0.1 N; 100 = the total volume of digest dilution; 100 = percentage factor; 0.1 g of the sample; 1000 = conversion from gram to milligram; 20 = integral volume of digits analyzed or distilled; 0.1 g = the weight of sample in gram digested

Lipid (Soxhlet Extraction Method): The % lipid composition was calculated using the formula in equation 3.

$$\%\ Lipid\ = \frac{WFE - WEF}{\text{WSE}} \text{x}\ 100 \quad (3)$$

Where WFE = weight of flask and extract; WEF = weight of empty flask; WSE = weight of sample extract

Moisture (Air Oven Method): The % moisture content was analyzed using the formula as shown in equation 4.

$$\%\ Moisture\ = \frac{WFS - WDS}{\text{WSU}} x100 \quad (4)$$

Where WFS = weight of fresh sample; weight of dry sample; WSU = weight of sample used

Crude Fiber: The % crude fiber composition was done using the formula in equation 5.

$$\%\ Crude\ Fiber\ = \frac{W2 - W3}{\text{W1}} x\ 100 \quad (5)$$

Where W1 = weight of sample extracted; W2 = weight of oven dried residue which was cooled and weighed; W3 = weight of dried residue heated and reweighed

Ash (Furnace Method): The % ash was calculated using the formula as shown in equation 6.

$$\%\ Ash\ = \frac{[WC\ + AS] - WC}{\text{WS}} x\ 100 \quad (6)$$

Where WC = weight of crucible; AS = ash sample; WS – weight of sample

RESULTS AND DISCUSSION

Morphological Study: The result revealed that *A. muricata* is glabrous and reach up to 8 ± 1 m tall. The petiolate foliar organs are simple, oblong, having acuminate apex, acute base with parted margins, pinnately veined and opposite in phyllotaxy; measuring up to 12.5 ± 4.5 cm in length and 4.6 ± 1.38 cm in width.

Table 1: Morphological Characteristic of *Annona muricata* L.

Characters	***Annona muricata* L.**
Habit	Small tree (Shrub)
Duration	Perennial
Root	Tap root and shallow rooted.
Stem Description	Woody habit, glabrous and grows up to 8 ± 1 m tall.
Leaf type	Petiolate foliar organs are simple.
Leaf venation type	Pinnately veined
Phyllotaxy	Opposite
Leaf outline or shape	Oblong with acuminate apex and acute base
Leaf margin	Even and parted
Length of leaf (cm) 13 cm	
Range of leaf length	8 to 17 cm
Mean standard deviation of leaf length	12.5 ± 4.5 cm
Breadth of leaf (cm) 6 cm	
Range of breadth of leaf	3 to 6 cm
Mean standard deviation of leaf width	4.6 ± 1.38 cm
Flower description	Numerous, actinomorphic, yellowish green.
Fruit description	A large berry with spinous exocarp.

The foliar epidermal study revealed presence of polygonal cells, trichomes and paracytic stomata which are amphistomatic. Plates 2a and b. The description of *Annona muricata* used here conforms to those described by Hutchinson and Dalziel (1958). The epidermal study revealed presence of trichomes, paracytic stomata and polygonal cells which are amphistomatic, in line with the works of Olowokudejo (1990) and Folorunso (2014). The morphological properties described here concorded to those showcased by several workers, including (Metcalfe and Chalk, 1950, 1979; Adedeji and Illoh, 2005;

Folorunso and Olorode, 2006b; Folorunso and Modupe, 2007; Abdulrahaman and Oladele, 2010). The moisture content is largest at ranges of 84.10 to 87.58 % followed by carbohydrate, 8.27 to 10.27 % and the protein at 4.38 to 6.38 %, supported by Agu and Okoli (2017), also, the high carbohydrate composition conformed to that of International Centre for Underutilized Crops, 2002. All these justify the fact that sour sop is an all-round season's energy food.

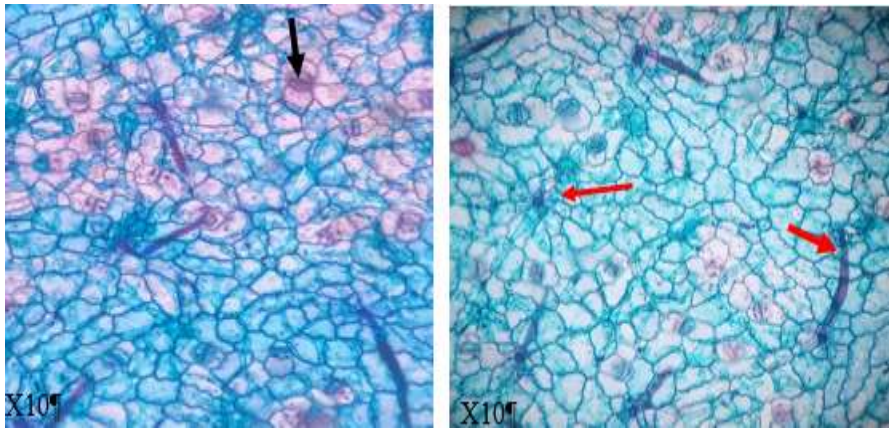

Plate 2a: *Annona muricata* adaxial Epidermis; 2b: *A. muricata* abaxial epidermis
Red arrows revealed glandular and linear Trichomes while black arrow showcased stoma.

Table 2: Quantitative Proximate Composition of *Annona muricata* L.

Proximate substances	Quantity analyzed in %
Carbohydrate	9.27 ± 1.0
Proteins	5.38 ± 1.00
Lipids	2.00 ± 0.90
Moisture content	85.84 ± 1.74
Fiber	0.58 ± 0.11
Ash content	0.89 ± 0.01

Conclusion: Annona muricata L. is a good food and medicine source for healthy body nourishment. A very clear background of types of trichomes, polygonal shaped cells and stomatal complexes are contributions made.

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