

COMPARATIVE STUDY ON THE BIOCHEMICAL PROPERTIES OF THE FRUITS OF SOME *ANNONA* SPECIES AND THEIR LEAF ARCHITECTURAL STUDY

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Abstract. Leaf architectural and biochemical study of four species of *Annona* found in Nigeria and characterized as important under-utilized species was conducted in search of intrageneric characters which may be of taxonomic value in the classification of the genus. Lamina balance, leaf form, leaf apex, leaf base, leaf margin, leaf texture were studied as the architectural features. Proximate analysis of mature and ripe fruits was also carried out to determine the nutritional status of the fruits. More intrageneric relationships among the *Annonas* have been reported along with characters of taxonomic value in the identification and classification of the genus. *A. reticulata* and *A. squamosa* appear to have better nutritional (protein) and shelf life.

Key words: biochemical, *Annona*, architectural, lamina, nutritional

INTRODUCTION

Annonaceae belong to the class *Magnolideae* in the order *Magnoliales*. Cronquist (1968), Mabberly (1987) and Brummit (1992) reported that the family consists of 2,050 species included in 125 genera and they are found mainly in the tropics. The family comprises woody trees, shrubs and lianes which are found in almost all vegetation types in the Neotropics. Some species of this family among other species constitute the shrub community in the old world tropics (Krishnan and Daviar, 1996).

Five species of the genus *Annona* have been selected as important under-utilized species (ICUC, 2002). The vegetative and floral morphological studies of four out of the five species categorised as important under-utilized have been reported by Folorunso and Olorode (2006 b).

The *Annonas* are generally consumed as fresh fruits but they are also widely in semi-processed and processed product (ICUC, 2002). *A. reticulata* fruit flavour is being considered the least tasty of cultivated *Annona*, the flesh is milk-white and sweet. The fruits and leaves of *A. muricata* are used in traditional medicine in tropical America and West Indies for their tranquilizing and sedative properties (Hasrat et al., 1997). The pulverized dried leaves and root of *Annona senegalensis* are applied to Guinea worm sores in Hausa land in Nigeria (Irvine, 1961). *A. squamosa* has many alkaloids such as glaucin and annonaine in different part of the plants (Kowalska and Puett, 1990).

Even though a number of studies had been carried out on the genus *Annona*, there has been no report on the comparative study of the biochemical properties of the fruits of *Annona*. The aim of this study is therefore to approach the leaf architectural features of the *Annona* species and compare the biochemical properties of the *Annona* fruits towards providing

additional features that may be of taxonomic value in the identification and classification of the genus.

MATERIAL AND METHODS

Four species out of the five species categorized as important under-utilized species, which are found in Nigeria were studied between 2000 and 2006 within the experimental garden of Obafemi Awolowo University Campus, Ile-Ife, Nigeria. They are *Ammona muricata*, *A. reticulata*, *A. senegalensis* and *A. squamosa*. All the species are readily available in the country especially in the South-West part of the country. Mature leaves and fruits were collected from the campus of Obafemi Awolowo University, Ile-Ife, Nigeria. They are:

- A. reticulata* (Custard apple)
- A. senegalensis* (Wild Soursop)
- A. muricata* (Soursop)
- A. squamosa* (Sugar apple).

For the purpose of the architectural study, sizable portions of the leaves were cut from the median portion of the matured and well-expanded leaves of the four different species. The cut parts were placed in a conical flask and 90% ethyl alcohol was added, it was placed on a hot plate to boil 15 minutes so that the leaves will decolorize i.e. the removal of chlorophyll. After 15 minutes the leaves were taken out and washed in 4-5 changes of water to remove traces of alcohol. The leaves were then transferred into 5% NaOH solution for further decolourization, boiled for 20 minutes on a hot plate after which they were rinsed in 4-5 changes of water to remove traces of alkaline solution. The leaves were then transferred into a conical flask containing 5% of domestic bleach for about 2 hours. After two hours the leaves were washed in 4-5 changes of water and stained in 1% aqueous safranin solution for 3-5 minutes, rinsed thoroughly with water to remove excess stain and then mounted with 25% glycerol for microscopic examination. The slides prepared from the leaves samples were examined with the aid of the microscope for the following anatomical features:

- (i) Veinlets endings: size and course
- (ii) Areole: developments, arrangement, shape and size. While whole leaves samples were viewed without the aid of microscope for the following features:
 - (i) lamina balance
 - (ii) leaf form, leaf apex, and leaf base
 - (iii) leaf margin
 - (iv) leaf texture
 - (v) petiole
 - (vi) venation
 - (vii) primary veins.

For the proximate analysis samples of ten matured and ripe fruits per treatment were homogenized in a warring blender for 1 minute. The carbohydrate content, crude fibre, crude protein, lipids (ether extract), moisture and nitrogen contents were determined as described in AOAC (1980).

RESULTS AND DISCUSSION

The summary of the architectural features of the *Ammona* species are shown in Table 1. The lamina balance is largely symmetrical in all species. Leaf shape is largely elliptic in the species except in *A. muricata* where it is obovate. In *A. muricata* and *A. squamosa*, leaves

apices are acute, leaf apex attenuate in *A. reticulata* but obtuse in *A. senegalensis*. Leaf base obtuse in *A. muricata* and *A. senegalensis* but acute in *A. reticulata* and *A. squamosa*. Leaf margin is entire in all the species, and similarly, leaf texture in all *Ammonia* species is coriaceous. Venation is of the pinnate, camptodromous, and eucamptodromous type in all the species. Primary vein order is moderate in *A. reticulata* and *A. squamosa*, it is weak in *A. muricata* and stout in *A. senegalensis*. The course of the primary vein order is largely straight lacking any noticeable curvature. Veinlet ending branched once in *A. muricata* and *A. senegalensis*, it is branched twice in *A. reticulata* and none in *A. squamosa*. Areole shape is polygonal in *A. reticulata* and *A. muricata*, but is quadrangular-shaped in *A. senegalensis* and triangular-shaped in *A. squamosa*. Areole is well-developed in *A. senegalensis* and *A. squamosa* while is imperfect in *A. reticulata* and lacking in *A. muricata*. Areole is randomly arranged in *A. muricata* and *A. squamosa*, oriented in *A. senegalensis* and showing no preferred orientation in *A. reticulata*.

Table 1
Summary of the Architectural Features of the Species of *Ammonia*

S/N	Architectural features	<i>A. reticulata</i>	<i>A. muricata</i>	<i>A. senegalensis</i>	<i>A. squamosa</i>
1	Lamina balance	symmetrical	symmetrical	symmetrical	symmetrical
2	(i) Leaf shape (ii) Leaf apex (iii) Leaf base	elliptic attenuate acute	Obovate Acute obtuse	elliptic obtuse obtuse	elliptic acute acute
3	Margin	entire	entire	entire	entire
4	Texture	coriaceous	coriaceous	coriaceous	coriaceous
5	Petiole	normal	normal	inflated	inflated
6	Venation	pinnate, camptodromous, and eucamptodromous	pinnate, camptodromous, and eucamptodromous	pinnate, camptodromous, and eucamptodromous	pinnate, camptodromous, and eucamptodromous
7	Primary vein order (a) size (b) course	1.92% - moderate straight and branched	0.75% - weak straight and branched	2.6% - stout straight and branched	1.75% - moderate straight and branched
8	Veinlet ending	branched twice	branched once	branched once	none
9	(a) Areole Development (b) Arrangement (c) Shape (d) Size	imperfect no preferred orientation polygonal very large(>3mm)	lacking randomly arranged polygonal large (3mm)	well-developed oriented quadrangular- shaped very large(>3mm)	well-developed randomly arranged triangular-shaped large (2mm)

The proximate data of the fruits of the *Ammonia* species studied are shown in Table 2. Protein was highest in *A. reticulata* ($0.85 \pm 0.07\%$) and lowest in *A. muricata* ($0.3 \pm 0.00\%$). Crude fibre content of *A. squamosa* was the highest ($1.25 \pm 0.07\%$) followed by *A. reticulata* ($1.05 \pm 0.21\%$) and lowest in *A. muricata* ($0.40 \pm 0.00\%$). Ash content of *A. reticulata* is the highest ($1.65 \pm 0.01\%$) and lowest in *A. senegalensis* ($0.95 \pm 0.07\%$). All the fruits of the *Ammonia* species showed high moisture content with *A. muricata* having the highest value ($91.76 \pm 0.03\%$) followed by *A. senegalensis* ($82.09 \pm 0.13\%$) and lower in *A. squamosa* ($67.86 \pm 0.11\%$). Carbohydrate content of *A. reticulata* is the highest ($27.92 \pm 0.48\%$) and lowest in *A. muricata* ($6.39 \pm 0.02\%$). The nitrogen content is also highest in *A. reticulata*

($0.14 \pm 0.1\%$), followed by *A. squamosa* ($0.11 \pm 0.00\%$) and in *A. muricata* ($0.05 \pm 0.00\%$) it was lowest.

Table 2

Showing the Proximate Analysis of the Fruits of the species of *Ammona*

<i>Ammona</i> species	% Protein	% Crude fibre	% Fat	% Ash	% Moisture	% CHO	% Nitrogen
<i>A. muricata</i>	0.30±0.00	0.40±0.00	-	1.16±0.01	91.76±0.03	6.39±0.02	0.05±0.00
<i>A. reticulata</i>	0.85±0.07	1.05±0.21	0.50±0.21	1.65±0.01	68.04±0.34	27.92±0.48	0.14±0.01
<i>A. squamosa</i>	0.70±0.00	1.25±0.07	1.25±0.07	2.80±0.00	67.86±0.11	23.84±1.50	0.11±0.00
<i>A. senegalensis</i>	0.40±0.00	0.60±0.00	0.60±0.00	0.95±0.07	82.09±0.13	15.76±0.06	0.06±0.00

The data recorded in this study are sufficient to establish the taxonomic and evolutionary relationships among the four species of *Ammona* (Folorunso and Olorode, 2006a). Earlier in the study of this genus, evidence of common evolutionary origin in the *Ammona* species was reported. According to them, there are intrageneric relationships among the *Ammonas*. Intrageneric relationships were similarly noted among the *Ammona* species in this work. These characters which are typical of the *Ammonas* include symmetrical lamina balance, entire margin, coriaceous leaf texture and pinnate venation that are camptodromous / eucamptodromous and may be of taxonomic value in the identification and classification of the genus as reported by Folorunso and Olorode (2006b). Veinlet ending which are largely branched in all species except in *A. squamosa* can be employed to delimit the species of *Ammona*. Areole development would also delimit the *Ammonas*. It is well developed in both *A. senegalensis* and *A. squamosa*, it is imperfect in *A. reticulata* and lacking in *A. muricata*. Areole arrangement and shape are other good delimiting characters in *Ammona*.

All the fruits of *Ammona* species showed high moisture contents. This is typical of fleshy fruits (Ereifej et al., 1997). This high moisture fruit content indicates that the fruits are highly susceptible to degradation when not properly stored. *A. squamosa* has the lowest moisture content ($68.04 \pm 0.34\%$) and thus the longest shelf-life. *A. reticulata* has the highest protein content ($0.85 \pm 0.07\%$) and the highest nitrogen content ($0.14 \pm 0.01\%$), thereby representing the best source of protein intake, when compared with the other three species. It could be used as a protein supplement in countries where protein deficient diet is common and also processed to be used as animal feed. The crude fibre content is highest in *A. squamosa* and lowest in *A. muricata* ($0.40 \pm 0.00\%$). This is of interest as dietary fibre is considered to be helpful in maintaining good health (Davidson et al., 1975). In humans, a higher fibre diet exerts beneficial effects by aiding water retention during passage of food along the gut and thereby producing larger and softer faeces. A higher fibre diet is associated with reduced incidence of cancer of the colon, cardiovascular disease and diabetes mellitus (Daryl et al., 1990). In herbivores such as ruminants, fibre is the source of energy after it is digested by micro-organisms to acetate, propionate and butyrate, which are absorbed into the bloodstream (Daryl et al., 1990). *A. reticulata* also has the lowest fat content ($0.50 \pm 0.21\%$) and high in *A. senegalensis* ($0.60 \pm 0.00\%$) and absent in *A. muricata*. fat serves as an efficient source of energy both directly and potentially when stored in adipose tissue (Daryl et al., 1990). It serves as thermal insulator in the subcutaneous tissues and around certain organs. Combinations of fat and protein are important cellular constituents (Christie, 1982). *A. squamosa* has the highest ash content ($2.80 \pm 0.00\%$), which implies higher mineral contents than all other species. *A. reticulata* has the highest carbohydrate content ($27.92 \pm 0.48\%$). The carbohydrate is broken down during cellular respiration and converted into glucose - a major fuel of the mammal tissues (Passmore and Eastwood, 1986). In all, *A. reticulata* and *A.*

squamosa fruits are the most nutritious because they contain less moisture and a high percentage of protein, fat, nitrogen, crude fibre, ash and carbohydrate.

In conclusion, more intrageneric relationships among the *Annonas* were revealed in this study. Primary vein order, veinlet endings, areole development, areole arrangement, areole shape, and size have been reported along with other characters to be of taxonomic value in the identification and classification of the genus. *A. reticulata* and *A. squamosa* appear to have more nutritional (protein) and shelf-life than *A. muricata* and *A. senegalensis*.

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REZUMAT

STUDIUL COMPARATIV AL PROPRIETĂȚILOR BIOCHIMICE ALE FRUCTELOR ȘI AL ARHITECTURII FRUNZELOR LA CÂTEVA SPECII DE *ANNONA*

Studiul arhitecturii frunzelor și conținutului biochimic al fructelor la patru specii de *Annona*, descoperite în Nigeria și caracterizate ca specii inferioare importante a condus la căutarea unor caractere intragenerice, care pot avea valoare taxonomică la clasificarea genurilor. Proporția frunzei, forma frunzei, apexul frunzei, baza frunzei, marginea frunzei, textura frunzei au fost studiate ca și trăsături arhitecturale. Analize imediate au fost făcute la maturarea și coacerea fructelor pentru a determina starea de nutriție a acestora. Cele mai multe relații intragenerice la *Annona* au fost corelate cu caractere cu valoare taxonomică în identificarea și clasificarea genurilor. S-a emis ipoteza că speciile *A. reticulata* și *A. squamosa* au o capacitate nutrițională superioară (inclusiv prin prisma proteinelor) și o păstrare mai bună a fructelor.