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Phytochemical, Elemental, Proximate, and Antinutrient Composition of Custard Apple Seed (Annona Reticulata) from Maiha Adamawa State, Nigeria

Ezekiel T. Williams [α], Kwaye Luka [σ] & Nachana'a Timothy [ρ]

Abstract- The Proximate, phytochemicals, elemental and anti-nutrients of *annona reticulata* seed collected from Michika were investigated. The Proximate, phytochemicals, and anti-nutrients were determined using standard methods, while the elemental composition was determined using Atomic absorption spectrophotometer (AAS) and flame photometer. The result of the proximate analysis shows the presence of Moisture (15.20± 0.02%), crude fibre (32.00 ± 0.01%), crude fat (30.34 ± 0.04 %), ash content (4.5 ± 0.00%), protein (17.12 ± 0.01%) and carbohydrate (37.91± 0.05 %) in the seed.. The result of the mineral composition showed Ca (14.40 ± 0.03 mg/ 100 g), Fe (0.32 ± 0.01 mg / 100g), P (46.26 ± 0.02mg /100g), Zn (0.85 ± 0.01mg / 100 g), Na (17.34 ± 0.02mg /100 g), Mg (18.18 ± 0.03mg /100 g) Se (0.12 ± 0.00 mg /100g) were present in the *annona reticulate* seed. The result of phytochemical test showed the presence of oxalates, phytates, lectins, saponins and glycosides in the annona reticulata seed. The proximate composition is an indication that the *annona reticulate* seed can be an important nutrient to both human and livestock, the phytochemical is an indication of bioactive component that is used as a source for drugs production and the mineral analysis showed that the *annona reticulate* seed had the potential to be used as a source of nutrient in human diet.

Keywords: phytochemical, elemental, proximate, anti-nutrient and annona reticulata.

I. Introduction

Plants serve as the sources of raw materials for diverse industrial products such as dyes, perfume, textile, fibre and building material. Plants provide a variety of resources that contribute to the fundamental need of both human being and animal such as food, clothing and shelter. Among plants of economic importance are medicinal plants. Plants have been utilized as therapeutic agents since time immemorial in both organized and unorganized forms. The healing properties of many herbal medicines have been recognized in many ancient cultures (Zhang, 2015). Medicinal plant has been the main sources of traditional herbal medicine among rural dwellers worldwide (Mustapha, 2016).

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Plant is a source of a large number of drugs comprising of different groups such as antispasmodics, emetics, anti-cancer, anti-microbial, anti-inflammatory, anti-malaria, anti-oxidant etc (Quilly *et. al.,* 2017; Williams *et al.,* 2019). A large number of plants are claimed to have the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. It is now believed that nature has given the cure of every disease in one way or another, as well as nutrition for both human and animals (Williams *et al*., 2019). Plants have been known to relieve various diseases in India, Africa, Panama and America. Medicinal plants are the richest bio-resources of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, Pharmaceutical intermediates and chemical entities for synthetic drugs (Akinwunmi and Omotayo, 2016; Du, 2016).

According to the world health organization, a medicinal plant is any plant which, in one or more of its organs contain substances that can be used for therapeutic purposes or which are pre cursors for chemo-pharmaceutical synthesis. Parts of such plant including leaves, roots rhizomes teams, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore contains chemical component that are medically active (Mohammed *et al.,* 2016; Williams *et al.,* 2020a).

*Annona reticulate*is semi–ever green and small deciduous tree from the plant family Annonaceae (Baskar *et al.,* 2007). It is well known for its fruit commonly called custard apple having flavored sweet and pleasant (Chang et al., 1993). The plant is native of Caribbean region and has also been spread across central and South America, Africa and Asia. *Annona* species are cultivated allover India for their edible fruit belonging to custard apple family. All parts of *annona* are used in natural medicine in the tropic (Maeda *et al*., 1993; Ogunwade and Olusegun, 2006; Pandy and Barve, 2011; Thang *et al*., 2013; Kaladhar *et al* 2014). It is considered to be a good source of natural, antioxidant for various diseases (Baskar *et al.*, 2007; *Bhalke et al*., 2011. *Annona reticulate* seed is often discarded after

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taking the pulp of the fruit. Research has shown that the custard apple seed is good source nutrition (Chang *et al*., 1998; Jirovetz *et al*., 1998; Joy *et al*., 2004). Since these nutrient composition vary not only with the varieties and species of plant but also with the environment in which the plant are grown. It is important to determine the nutritional composition of a given plant grown in different localities. In this part of the world the information on the nutritional composition of custard apple grown locally is not available. The purpose of the study is to investigate the nutritional composition of seed of custard apple grown in the study area.

II. Materials and Methods

a) Sample collection and identification

The matured ripped unaffected fruit of custared apple (*Annona reticulata*) was collected from Michika Adamawa, identified and authenticate by Baba Yahaya Kirri in the Department of crop production Adamawa State University Mubi.

b) Sample preparation

The fruits were cut opened and the seed were removed then washed with distilled water and dried at room temperature and pulverized in to powder.

c) Chemicals and reagents

All Chemicals and reagents used were of analytical grade.

d) Preparation of extract

Twenty five grams (25 g) of powdered seed were extracted separately in a soxhlet apparatus and solvent were removed. The percentage yield was determined by following the method described by AOAC (2005): The yield percentage = weight of extract recovered x 100 / weight of dry powdered and the extract was used for phytochemical screening, proximate analysis, anti-nutrient and elemental composition.

e) Elemental Analysis

The mineral content of the sample were determined using atomic absorption spectrophotometer and flame photometer following the procedure described by AOAC (2003).

f) Phytochemical screening

Annona reticulata seed was tested for the presence of bioactive compounds. The phytochemicals of the seed samples were estimated following the procedure adopted by Williams *et al.,* (2020b).

g) Test for Terpenoids

Organic extract of 2 ml was dissolved in 2 ml of chloroform and evaporated to dryness. Concentrated sulphuric acid of 2 ml was added and heated for two minutes. A grayish color was observed.

h) Test for Flavonoids (Alkaline Reagent Test)

Extract of 200 mg was mixed with 2 ml of 2% solution of NaOH. An intense yellow colour formed which turned colorless on addition of few drops of diluted acid was observed.

i) Test for Steroids

To 2 ml of acetic anhydride was added 0.5 g of the sample followed by an addition of 2ml HR_2RSOR_4R. The color changed from violet to blue green was observed (AOAC, 2005).

j) Tests for tannin and phenolic compounds ferric chloride test

A small amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of phenolic compounds.

k) Test for Alkaloids

Extract of 200 mg was mixed with 10 ml of methanol. To 2 ml of the filtrate was added 1% HCl and then steamed. To 1ml of the filtrate was added 6 drops of Wagner reagent. Brownish-red precipitate was observed.

l) Proximate analysis

The proximate composition (moisture, crude fibre, crude fat, ash content, protein and Carbohydrate) of powdery sample of *Annona reticulata* was determined following the method described by AOAC (2003).

m) Anti-nutritional Content Analysis

The anti-nutrient contents (oxalates, phytates, lectins, saponins and glycosides) were determined using high performance liquid chromatography (HPLC) following the procedures adopted by AOAC (2003).

n) Statistical Analysis

All determinations were replicated three times and results were reported in mean (±) standard deviation.

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III. Results and Discussion

Table 1: The Result of the Elemental analysis of *Annona reticulata* seed (mg/100g)

Element	Concentration
Ca	14.40 ± 0.03
Fe	0.32 ± 0.01
P	46.26 ± 0.02
Zn	0.85 ± 0.01
Na	17.34 ± 0.02
Mg	18.18 ± 0.03
Se	0.12 ± 0.00

The elemental composition of *Annona reticulata* seed was presented in Table 1. The result revealed the presence of Ca, Fe, P, Zn, Na, Mg and Se. The concentration of elements ranged from 0.12 ± 0.00 mg/ 100 g to 46.26 ± 0.02 mg / 100g. Se (0.12 ± 0.00 mg / 100g) has the lowest concentration, while P (46.26 ± 0.02 mg / 100g) has highest concentration. This result corroborates with the study of Ubwa *et al* . (2015).

Phosphorus (46.26 ± 0.02 mg/100g) is an important constituent of adenosine triphosphate and nucleic acid and is also essential for acid base balance, bone and tooth formation. Phosphorus deficiency can cause low appetite, irritation, numbness, bone pain, poor bone and tooth development. The concentration of magnesium recorded was 18.18 ± 0.03 mg/100 g. Magnesium is an important component of chlorophyll in plant. Mg is an important mineral element in connection with a circulatory disease such as heart disease (Tona *et al*., 2001).The value of sodium obtained in *annona reticulata* seed was found to be 17.34 ± 0.02 mg /100 g. Insufficient Na causes low blood pressure of the body. Na is important in the maintenance of osmotic balance between cell and the interstitial fluid. Calcium (14.40 ± 0.03 mg /100 g) is used in the construction, maintenance and normal function of the nerves and muscles (Williams *et al*., 2019). The concentration of Zn in *annona reticulatas* was found to be 0.85 ± 0.01 mg /100 g. Zinc is one of the important cofactor found in the structure of certain enzymes. It is involved in the normal function of the immune system. The value of Fe recorded was 0.32 ± 0.01mg/ 100 g. Iron is component of the cytochromes that function in cellular respiration. Selenium (0.12 ± 0.00 mg / 100 g) is an important co factor found in the structure of certain enzymes and are indispensible in numerous biochemical path way. It plays a critical role in metabolism and thyroid function and helps to protect the body from damage caused by oxidative stress. It also plays an important role in health of immune system.

Table 2: Result of the Phytochemical Screening of Annona reticulata

Phytochemical	Qualitative	Quantitative (mg/100g)
Tannins	+ + +	9.12 ± 0.01
Alkaloids	+ +	6.10 ± 0.00
Flavonoids	+ +	6.78 ± 0.01
phenols	+ + +	16.15 ± 0.02
Terpenoids	+	0.23± 0.01
Steroids	+	0.16 ± 0.01

+ = *present*
+ + = *moderate present*
+ + + = *highly present*

Table 2 contains the result of the phytochemical screening of *annona reticulata* seed. The result revealed the presence of tannins, alkaloids, flavonoids, phenols, terpenoids and steroids. The concentrations of the phytochemicals ranged from 0.16 ± 0.01mg / 100g to 16.15 ± 0.02 mg / 100 g. The lowest concentration recorded was Steroids (0.16 ± 0.01mg / 100/g), while highest was phenols (16.15 ± 0.02 mg / 100 g). This finding is in line with the report of Mariana *et al*. (2017), which describes the presence these phytochemicals.

Phenol (16.15 ± 0.02 mg / 100 g) was found to be highly present in the seed. It is used as defense against ultraviolet radiation or aggression by pathogens, parasites and predators as well as contributing to plant colors. Phenolic compounds are important for the quality of plant based foods, they are responsible for the color of red fruit, juices as well as wine and substrates for enzymatic browning and are also involved in flavor properties in particular, as stringency is ascribed to precipitation of salivary proteins by polyphenol, a mechanism possibly involve in defense against anti-nutrition effect (Ramadass and Subramanian, 2018).

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Tannins was highly present in the seed, the concentration was found to be 9.12 ± 0.01mg / 100g. It plays an important role as a source of energy. It serves as a protective to the plant. In the food industry tannins are used to carify wine, beer and fruit juice (Funatagawa *et al.,* 2004).

Flavonoid (6.78 ± 0.01mg / 100 g) was found to be moderately present in the seed. In general flavonoids can play an important role in decreasing disease risk through various physiologic mechanisms. Some of these include anti-viral, anti-inflammatory, cytotoxic, anti-microbial and antioxidant effect. Mechanisms responsible for improvement in heart disease risk include: improve endothelial function, decreased blood pressure and improvement in lipid sand insulin resistance (Pandey and Barve, 2011).

Alkaloids was moderately present in the seed the concentration was found to be 6.10 ± 0.00 mg / 100 g. It is used in pharmaceutical industries in the production of drugs (James, 2012).

Terpenoids was found present in the seed and the concentration was 0.23± 0.01mg / 100 g. It has biological activity as anti - platelet (Pandey and Barve, 2011).

The concentration of steroids in seed was 0.16 ± 0.01mg / 100 g. It plays an important role in the proper function of the human body.

Table 3: result of the proximate analysis of (Annona reticulata %)

Composition	Value
Moisture	15.20± 0.02
crude fibre	32.00 ± 0.01
crude fat	30.34 ± 0.04
ash content	4.5 ± 0.00
protein	17.12 ± 0.01
carbohydrate	37.91± 0.05

The result of proximate analysis of *annona reticulata* seed was presented in Table 3. The result showed that moisture, crude fibre, crude fat, ash, protein and carbohydrate were present in the seed. The ash content has the lowest value (4.5 ± 0.00 %) among all the nutrient composition while carbohydrate was the highest (37.91± 0.05%). This is an average when compared with that of other legumes ranging from 23% to 66% in groundnut though this value is higher compared with that of other legumes which have been reported to range between 15.00–16.00% (FAO/WHO, 2011; Ubwa *et al*., 2015).

Carbohydrate (37.91± 0.05 %) provide energy, stored energy, build macro molecules and spare protein and fat for use. Glucose energy is stored as glycogen, with majority of it in muscle and liver. The ash content of seed was found to be 4.5 ± 0.00 % which plays an important role in the relief of fever, arthritis, constipation, fluid retention and bladder problems. It is also used as tonic (Zida *et al.,* 2016).

Table 4: The Result of the Anti - nutrient Analysis of the Annona reticulate seed

Anti - nutrient	Concentration (mg/100g)
Oxalates	2.12 ± 0.01
Phytates	9.45 ± 0.02
Lectins	0.20 ± 0.01
Saponins	6.12 ± 0.02
Glycosides	7.13 ± 0.01

The result of the anti- nutrient analysis of annona reticulate seed was shown in Table 4. The result revealed that oxalates, phytates, lectins, saponins, and glycosides were present in the seed.

The presence of these secondary metabolites in plants produces some biological activity in man and animals and it is responsible for their uses as herbs in primary health care (Ramadass and Subramanian, 2018). These compounds also serve to protect the plant against infections by microorganisms, predations by insects and herbivores, while their odor and flavor are responsible for their pigments (Galeane1, *et al.*, 2017).

The results indicate that phytates (9.45 ± 0.02 mg / 100 g) have the highest value while lectins (0.20 ± 0.01 mg / 100 g) has the lowest value. Phytates serve as a store of cat ions, of high energy phosphoryl groups, an chelating free ion, as a potent natural anti- oxidant it also bind mineral in digestive track, making them less available to the body but when it is in required amount it can help in the prevention of chronic disease (Mueller, 2001). Glycosides (7.13 ± 0.01 mg/ 100 g) are used in preventing optimal exploitation of the nutrients present in a food and decreasing the nutritive value.

The concentration of saponins in the seed was 6.12 ± 0.02mg /100 g. Saponins were recognized as anti- nutrient constituents due to their adverse effect such as growth impairment and reduce their food intake due to the bitterness and throat- irritating activity. In addition saponins were found to reduce the bioavailability of nutrients and decrease enzymes activity

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and it affect protein digestibility by inhibit various digestive enzymes such as tryps in and chymotrypsin (Linner, 2005). Saponins are attracting considerable interest as a result of their beneficial effect in humans. Recent evidence suggests that saponins possess hypocholesterolemic, immunostimulatory and anti-carcinogenic properties. In addition it reduces the risk of heart diseases in humans. Saponins rich food are important in human diet to control plasma cholesteros preventing peptic ulcer, osteoporosis and to reduce the risk of heart disease. Saponins are used as adjuvants in viral (e.g quillaja saponaria- 21) and bacterial vaccine (e.g quillaja saponins) application (Williams et al., 2018). A high saponin diet can be used in the inhibition of dental caries and plate late aggregation in the treatment of hypercalciuria in humans and as an anti-dote against acute lead poisoning (Rwarinda, 2016).

Oxalates was found to be 2.12 ± 0.01mg /100 g in the seed. It plays an important role in the formation of kidney stone and urinary tract when the acid is excreted in the urine (Nachbar *et al.,* 2000).

The concentration of lectins in the seed was 0.20 ± 0.01mg / 100g. It has the ability to bind carbohydrate and also have potent in vivo effect. When consumed in excess, they can cause three primary physiological reactions: they can cause severe intestinal damage disrupting digestion and causing nutritive deficiency (Timothy, 2018; Tona, 2001). It can also bind to erythrocytes, simultaneously with immune factors causing hem agglutination and anemia (Fereidoon, 2014)Generally these anti-nutritional factors can easily be reduced to the tolerable limit by proper simple technics such as soaking, cooking and frying (Williams *et al.,* 2019).

IV. Conclusion

From the study, it can be concluded that the *annona reticulata* seed analysed have great potential as nutrient sources, considering their proximate chemical composition and mineral composition. Thus the *annona reticulata* seeds can be used as food in narrowing the nutrients supply deficit that is prevalent in many developing countries. Furthermore, the proximate composition indicates the potential of the plant seed as sources of the important nutrients needed by the body. The mineral analysis of the *annona reticulata* seed indicated that the seed is rich in minerals considered. This indicates that *annona reticulata* seed could also be a source of minerals in diet as well as drugs in pharmaceutical industries.

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