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Proximate composition and phytochemical screening of root and leave extracts of *Annona senegalensis* Pers. in Aliero, Kebbi state, Nigeria

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Abstract
The roots and leaves of *Annona senegalensis* Pers were subjected to proximate and phytochemical analysis following the standard methods by Association of Official Analytical Chemists' (AOAC). The results of the proximate composition revealed that the root extracts contained 35.03% moisture, 5.77% ash, 23.13% lipids, 13.03% crude fiber, 7.67% crude protein, and 15.37% carbohydrate content. On the other hand, the leaf extracts contained 30.17% moisture, 6.73% ash, 7.67% lipids, 12.10% crude fiber, 21.87% crude protein, and 21.46% carbohydrate content. The phytochemicals detected in the root extracts were tannins, alkaloids, balsams, saponins, steroids, anthraquinones, carbohydrates, and terpenes, whereas flavonoids and cardiac glycosides were not detected. The leaf extracts contained tannins, alkaloids, saponins, steroids, carbohydrates, and terpenes. Cardiac glycoside was not detected in both the root and leaf extracts. Based on the results of this study, it is recommended that, GC-MS analysis should be carry out to determine and identify the total number of bioactive compounds in the root and leaf. The antimicrobial, antioxidant, and cytotoxicity study should be carry out to evaluate the antimicrobial efficacy as well as the toxicity of *Annona senegalensis* Pers.

Keywords: *Annona senegalensis Pers*, proximate analysis, phytochemical analysis

Introduction
African custard apple, scientifically known as *Annona senegalensis* Pers, is a seed vegetable that thrives in both dry and rainy seasons. Locally referred to as Gwandar Daji in Hausa land, this plant species is commonly found in the savanna regions, ranging from Senegal to Nigeria and even extending to the Central African Republic (Abdullahi *et al.*, 2011) [1]. *A. senegalensis* Pers, belong to the family *Annonaceae* and its commonly distributed indigenous plant in Aliero and Zuru local governments area of Kebbi State, Nigeria. The plant is of economic importance more than just using the flower, fruit, leaves, roots, feeding livestock and other practices in the two areas (Aliero and Zuru). The plant have high diverse characteristics such as high level of vitamin C, quality stable oil and the novel flavor of its flowers and fruits (Ellof *et al.*, 2018) [10]. *A. senegalensis* Pers. plant has been found essential in traditional medicine; the leaves have been used to cure smallpox, TB, and yellow fever (Ujowundu *et al.*, 2010) [25]. The root bark is effective against infectious infections, and the roots are used to treat conditions like difficulties swallowing, gastritis, snakebites, male sexual impotence, erectile dysfunction, and tuberculosis. The tree's juice is applied to the skin to treat chicken pox (Achu *et al.*, 2017). The flower is highly nutritious and has a pleasant flavour and sweetness. The nutritional value varies greatly, but the majority of varieties are high in proteins, carbohydrates, calcium, phosphorus, iron, thiamine, niacin, and riboflavin. Others are high in magnesium, ascorbic acid, and carotenoids (Yoa *et al.*, 2017) [29]. It has been discovered that heat-extracted seed oil is useful against several agricultural pests (Arthur, 2011) [3]. *Annona senegalensis* Pers. has been used in Guinea and Swaziland to treat malaria (Koyode *et al.*, 2010) [15].
Human kind has benefitted from *Annona senegalensis* as source of nutrition for more than 10,000 years (Nwonku, 2012) [20]. Due to the nutritional and medicinal value of this plant, the present study aimed to evaluate the nutritional and phytochemical profile of *Annona senegalensis* Pers found in Aliero community and Kebbi State at large through proximate and phytochemical analysis. The present study is important as it provides knowledge on the nutritional contents as well as the types of phytochemicals present in *Annona senegalensis* Pers found in Aliero community.

Materials and Methods
Description of study area
The research was carried out at Aliero local government area, Kebbi State, Nigeria. The local government covers a total of 412 square kilometres of land mass. The topography is flat and slightly undulating with compact, stony brown soil. It has a minimum temperature of 38 – 42^{0C} in the month of November. The temperature is low during the hamarttan (December-February) with mean temperature of 20–23^{0C} and relatively humidity ranges between 17-80% respectively (Lawal, 1999) [16]. Farming is the main occupation in Aliero community. People in the community cultivate crops like guinea corn, millet, ground nut, maize, sugarcane and pepper. Others are okro, tomatoes, potatoes and onions. *Annona senegalensis* Pers. is mostly used in this region as feed to animals and its fruit are used by children and adult, it is also serve as a source of firewood (Mollel *et al.*, 2004) [19].

Chemicals and Reagents
All the chemicals used were of analytical grade and were obtained from various sources. The solvent were re-distilled before use. Solution and reagents were prepared with double distilled water.

Sampling and Sample Preparation
The leaves and roots of *Annona senegalensis* Pers. (Gwandar masar) in Hausa language were collected from Sabiyal, Aliero area in Kebbi State, Northern Nigeria. Fresh leaves and roots were washed with water to remove dust and other foreign particles and shade-dried for 7 days. The plant samples were crushed separately into smaller pieces using sterile mortar and pestle and further reduced to powder by sieving to remove large particles. The powder was collected in a sterile container and stored in a cool dry place for further analysis.

Laboratory Analysis
Kjeldahl method was used to determine the nutritional and proximate composition of *Annona senegalensis* Pers. of leaves and roots. Crude fat was extracted by the Soxhlet method with N- hexane (40 - 60°^{C}) for 8 hours. Crude protein content, carbohydrate, crude fiber, ash and moisture were determined as per the standard protocols by (Usman *et al.,* 2020 [24]; Muhammad *et al.,* 2023).

Extraction Techniques
The extraction was carried out by cold maceration technique as described by (Junaid *et al.,* 2011) [14]. A sterile conical flask was used to soak 40g of the ground leaf and root samples in 350ml of distilled water for 24 hours. The mixture was then filtered through sterile filter paper (Watman no. 1) before being concentrated in a water bath at 100ºC to remove remaining water. For further research, the resulting extract was subsequently kept in a refrigerator at a temperature of -4ºC.

Phytochemical analysis
Following the procedures outlined by Trease and Evans (2002) [9] and Hassan *et al*. (2009) [13], phytochemical screening was done to check for the presence of tannins, alkaloids, balsams, saponins, steroids, flavonoids, anthraquinones, glycosides, resins, carbohydrates, and tarpenes.

Test for Tannins (Ferric Chloride Test)
In order to detect the presence of tannins, a small amount of water extract was diluted with water in a 1:4 ratio and a few drops of a 10% ferric chloride solution were added. Tannins were identified by a blue or green colour.

Test for Alkaloid
The alkaloids was detected following the standard procedure used by (Usman *et al.,* 2020) [24]

Test for Balsams
To 0.5g of each extracts, 2 drops of an alcoholic ferric chloride solution were added. Appearance of dark green colour indicates the presence of Balsams.

Test for Saponins
0.5cm^3 of each extract received 10ml of distilled water added to it. In the test tube, the contents were forcefully shaken for two minutes. The presence of saponins is indicated by foaming or bubbling.

Test for Steroids
2 drops of concentrated H_2SO_4 were added to 1cm^3 of the extract. A reddish brown color indicates the presence of steroids.

Test for Flavonoids
In a test tube, a few drops of sodium hydroxide (NaOH) solution was added to each of the extracts. The presence of flavonoids was identified by a golden colour that turned colourless when dilute acid was added.

Test for Free Anthraquinones
In a different test tube, 0.5g of the powdered sample was taken, 10ml of chloroform was added, and the test tube was shaken again for 5 minutes. The extracts were then filtered and shaken once more. The presence of free anthraquinones is indicated by a brilliant pink colour in the upper aqueous layer.

Test for Cardiac Glycosides
5mL of each plant extracts, 2mL of glacial acetic acid, and a few drops of ferric chloride solution was placed in a test tube. Alongside of the test tube, 2ml of concentrated H2SO4 was added. The presence of glycosides was shown by the formation of a brown ring at the contact (Riaz *et al*., 2018).

Test for Carbohydrates (General Fehling Test)
Carbohydrate was detected using Fehling Test.

Test for Terpenes (Liebermain-Burchard Test)
1ml of concentrated sulphuric acid was introduced down the test tube wall after the initial portion of the extract's chloroform solution was combined with 1ml of acetic anhydride to create a layer. Terpenes are present when a reddish violet colour develops.

Proximate Analysis
Micro Kjeldah method was used to analyse the amount of carbohydrate, protein, ash content, moisture, crude lipid, and available energy in *Annona senegalensis* roots and leaves were determined in percentage.

Determination of Moisture Content
Apparatus
Hot air oven, weighing balance, crucibles and dessicator.

Procedure: Cleaned empty crucibles were dried in an oven at 80^{0C} for about 30 minutes, two (2g) grams of the samples were weighed with weighing balance, a dried-cleaned crucible with the grounded weighed sample (2g) before drying was weighed as (W_1), place in a hot dry oven at 105^{0C} for 24 hours, and cooled in desiccators after removing from the oven. The crucibles together with the sample after drying were weighed as (W_2). It was then returned into the oven and dried further for another 24 hours under 150ºC and reweighed until the weight was constant (Hassan *et al.,* 2009) [13].

Calculation
% Moisture = $[W_1\text{-}W_2/W_1 – W_o] \times 100$
Where, W_1= Weight of the sample + weight of empty crucible before drying
W_2 = Weight of sample + empty crucible after drying
W_o = weight of empty crucible

Determination of Crude Protein
Digestion
2.0g of the dried (ground) sample was transferred into a micro-Kjeldahl flask and digestion tablets were added, 20ml

of HNO_3 and selenium tablets were added to the sample mixtures of the micro-Kjeldahl flask and heated using a digestion block (heater) in a fumed cupboard continuously until the nitrogen present in the sample reduces to ammonium sulphate

Distillation
The digest was diluted to 50cm^3 with distilled water. 10cm^3 of the sample aliquot, 40cm^3 of the distilled water and 20cm^3 of 40% NaOH were transferred into a micro Kjeldahl flask. The distillate was collected into a flask containing 10cm^3 of boric acid and few drops of methyl orange indicator which gives a green colour distillate.
$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + H_2O_{(g)} + Na_2SO_{4\,(aq)}$
$NH_3 + H_3BO_3 \rightarrow NH_4^+{}_{(aq)} + H_2BO_3^-{}_{(aq).}$ (AOAC, 1995).

Titration: The distillate content in the flask was titrated against 0.01N H_2SO_4
The colour changed from green to purple at the end point. The titre values were recorded and the average titre value was calculated. This was used to determine the percentage nitrogen.

Titration: $H_2BO_3^-{}_{(aq)} + H^+{}_{(aq)} \rightarrow + H_3BO_{3\,(aq)}$

Calculation
If the titre value was found to be (TV), then the concentration of protein can be calculated as follows:

$$\% \text{ N} = \frac{TV \times NA \times 0.014 \times DF}{Vol\ of Aliquot \times sample\ weight} \times 100$$

$$\% \text{ Crude protein (g)} = \text{C.F} \times \% \text{ Nitrogen}$$

Where
Sample weight = 2g
Vol. of Aliquot = 10ml
Conversion Factor C.F = 6.25
TV= Titre value
NA= Normality of acid
DF= Dilution factor

Determination of lipid content
Procedure
In an oven set at 105 to 110ºC, a 250ml extraction flask was dried. After that, it was given time to cool in a desiccator. The material was weighed into a designated thimble at a weight of two grams (2g). The cotton wool was covering the thimble's porous mouth. The 250 ml dried extractor flask was then filled with 200 ml of N- hexane. The apparatus was put together after the covered porous thimble was put in a condenser. It took around five to six hours to retrieve it. Carefully removing the porous thimble, the N-hexane was collected in the top container for later reuse. The dried, empty crucible into which the material had been extracted had its weight measured as (W1) after it had been transferred from the thimble. The sample-containing crucible was dried in an oven at 105–110°C for one hour, chilled in a desiccator, and its weight was recorded as (W2) (AOAC, 1995).

Calculation
% of lipid = $[W_1\text{-}W_2/W_1 – W_0] \times 100$
Weight of empty crucible = W_0
Weight of crucible + grounded extracted sample (oil) before drying = W_1
Weight of empty crucible + weight of extracted sample (oil) after drying = W_2

Determination of Ash Content
2g of the sample was weighed and a muffle furnace was used at 500^{0}C for 3 hours. The sample was cooled in the desiccators and was weight again until the dried ash was obtained (Hassan *et al.*, 2009) [13].

Calculation
% of Ash content = $[W_1\text{-}W_2/W_1 – W_0] \times 100$
W_0 = weight of empty crucible
W_1 = weight of sample fresh
W_2 = weight of dried sample

Determination of Crude Fiber
A beaker was filled with 2g of the sample. After adding 20ml of 10% H2SO4 and 100ml of distilled water, the mixture was placed on a hot plate for 30 minutes to heat up. The sample was scraped into the beaker using a spatula after being filtered with a muslin cloth and washed with hot water. The base content was subsequently removed from the sample by heating it once more with NaOH for 30 minutes, filtering it, and then rinsing it under hot water. It was allowed to drain and the residue was scraped into a pre-weighed crucible (W_1). It was then put into a muffle furnace to dry for 2hrs at 600^{0}c and allowed in a desiccator to cool and weighed as (W_2). The percentage was then calculated as:

Calculation
% Crude fibre = $[W_1\text{-}W_2/W_1 – W_0] \times 100$

Determination of Carbohydrate
The soluble carbohydrate, also known as the nitrogen free extract (NFE). N.F. E = 100% - (%Moisture +%Ash +% Crude fat +%Fibre +%Crude protein) is how it is obtained rather than being directly determined.
N. F. E. referred to percentage carbohydrates, such as starches, sugars, and some hemicelluloses (Hassan *et al.*, 2009) [13].

3.9 Statistical Analysis
The data obtained were expressed as mean and standard deviation (SD) using SPSS version 21.0 and bar-chart was used to interpret the significance difference between the proximate composition of roots and leaves of *Annona senegalensis* Pers.

Results and Discussion
Phytochemical Screening
The phytochemicals detected in the root extracts include; tannins, alkaloids, balsams, saponins, steroids, anthraquinones, carbohydrates, and terpenes, whereas flavonoids and cardiac glycosides were not detected. The leaf extracts contained tannins, alkaloids, saponins, steroids, carbohydrates, and terpenes. Cardiac glycoside was not detected in both the root and leaf extracts. The qualitative phytochemical result was in agreement with the works of (Foong & Hamid, 2012) [12] and (Vijayameena *et al.*, 2013) [26] which reported tannins, alkaloids, balsams, steroids, anthraquinones, carbohydrates and terpenes in *Annona*

squamosa while flavonoids and cardiac were not detected. This research is also in agreement with the finding of (Banjo, 2006 [6]; Barker *et al.*, 1998 [8]; Hatt *et al.*, 2003 [21]; Finke, 2007 [11]: Ramos-Elorduy *et al.*, 1997) [22] which reported the absence of cardiac glycosides, anthraquinones and balsams in the leaf extract of *Annona senegalensis.* However, other phytochemicals like tannins, alkaloids, steroids, flavonoids and terpenes were reported present in leaf extract. The result of phytochemical analysis was presented in the table 1.

Table 1: Results of the Phytochemical screening of root and leaf of *A. senegalensis* Pers.

1.	Tannin	+	+
2.	Alkaloids	+	+
3.	Balsams	+	-
4.	Saponins	+	+
5.	Steroids	+	+
6.	Flavonoids	-	+
7.	Anthraquionones	+	-
8.	Cardiac glycosides	-	-
9.	Carbohydrates	+	+
10.	Terpenes	+	+

Key:
+ = Detected
- = Not detected

Proximate Analysis

Proximate Analysis of Roots

The results of the proximate analysis for root part were presented in table 2 below. Moisture content was found to be (35.03 ± 0.15), ash (5.77 ± 0.15), lipid (23.13 ± 0.15), crude fibre (13.03 ± 0.06), crude protein (7.67 ± 0.15) and Carbohydrate (15.37 ± 0.10) of roots of *Annona senegalensis* were determined.

Table 2: Results of the proximate compositions of root of *Annona senegalensis* Pers.

S.N	Proximate Compositions	Concentration (%) ± sd
1.	Moisture	35.03 ± 0.15
2.	Ash	5.77 ± 0.15
3.	Lipid	23.13 ± 0.15
4.	Crude fibre	13.03 ± 0.06
5.	Crude protein	7.67 ± 0.15
6.	Carbohydrate	15.37 ± 0.10

The values were represented as Mean ± Standard deviation

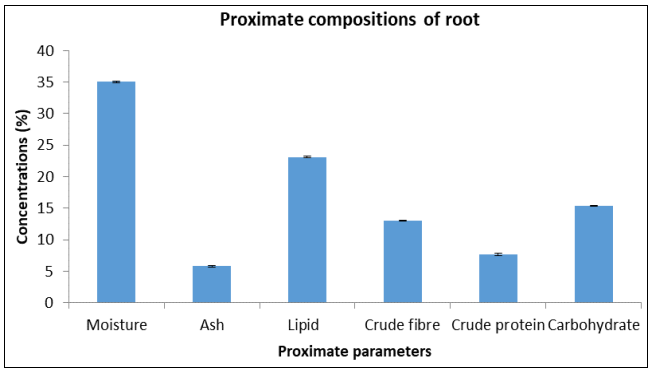

Fig 1: Graphical representation of the Proximate Compositions of root of *A. senegalensis*

Proximate Analysis of Leaves

The results of the proximate analysis were presented in table 3 and figure 2. The moisture content was found to be (30.17 ± 0.19), ash (6.73 ± 0.21), lipid (7.67 ± 0.15), crude fibre (12.10 ± 0.10), crude protein (21.87 ± 0.15) and Carbohydrate (21.46 ± 0.10) of leaves of *Annona senegalensis* Pers. were determined.

Therefore, the result of proximate compositions in both root and the leaf are in disagreement with finding of (Mubarak *et al.*, 2022) [18] which analysed the proximate compositions of flower of *A. senegalensis*. The study reveals the presence of moisture 7.67%, carbohydrate 76.96%, ash 7.33%, crude protein 8.37 %, crude lipid 4.17% and crude fiber 3.17%. One more study by (Yisa *et al.*, 2010) [28] on proximate composition of seed of *A. senegalensis* revealed that the sample consisted of approximately 12.20% moisture, 12.10% ash, 24.00% fat, 17.60% crude fiber, 8.80% crude protein, and 25.3% carbohydrates. The variation in proximate compositions could be attributed to several factors, such as the utilization of different sample part used, specifically the flowers and the seed of *Annona senegalensis*. Additionally, discrepancies could arise from variations in the plant's location, experimental methods employed, and the specific reagents used during the analysis. These factors collectively contribute to the observed differences in the obtained values.

Table 3: Results of the Proximate Composition of Leave of *Annona senegalensis* Pers.

S.N	Proximate Composition	Concentrations (%) ± sd
1.	Moisture	30.17 ± 0.19
2.	Ash	6.73 ± 0.21
3.	Lipid	7.67 ± 0.15
4.	Crude fibre	12.10 ± 0.10
5.	Crude protein	21.87 ± 0.15
6.	Carbohydrate	21.46 ± 0.10

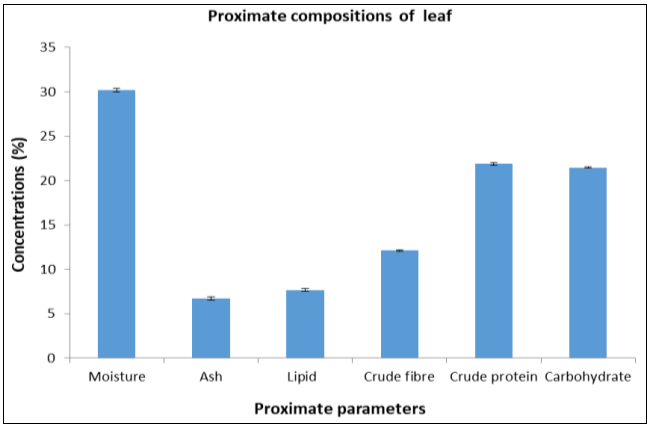

Fig 2: Graphical representation of the Proximate Compositions of leaf of *A. senegalensis*

Conclusion

The proximate composition analysis of *Annona senegalensis* Pers (roots and leaves) revealed significant variations in the nutritional composition between the two plant parts. The roots exhibited higher moisture, ash, lipids, crude fiber, and carbohydrate content compared to the leaves. In contrast, the leaves had higher crude protein content. Phytochemical screening of the root and leaf extracts indicated the presence of various bioactive compounds. Both root and leaf extracts contained tannins, alkaloids, saponins, steroids, carbohydrates, and terpenes. Flavonoids and cardiac glycosides were not detected in either extract. The study recommends further analysis, specifically GC-MS analysis,

to identify and quantify the total number of bioactive compounds present in the root and leaf extracts of Annona senegalensis Pers. Additionally, antimicrobial, antioxidant, and cytotoxicity studies are suggested to assess the potential antimicrobial efficacy and toxicity of the plant.

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