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Ethnobotanical and chemical composition of "Achi" (*Brachystegia eurocoma*) and "Uhiokrihio" (*Tetrapleura tetraptera*)

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Abstract

Most indigenous plants in Nigeria exhibit a wide range of biological and physiological activities like anti-hypertensive, anti-inflammatory, anti-microbial, anti-diabetic and anti-carcinogenic. The aim of this study was to evaluate the chemical composition and ethnobotanical effect of "Achi" (*Brachystegia eurocoma*) and "Uhiokrihio" (*Tetrapleura tetraptera*). *Brachystegia eurocoma* and *Tetrapleura tetraptera* were processed raw and analyzed for chemical, phytochemicals, and bioactive substances using standard methods. The data obtained were analyzed statistically for mean and standard deviation using SPSS version 20. The result of this study showed that the flavonoids were 0.10 and 0.62%, glycoside 0.20 and 0.74%, saponins 0.81 and 3.40%, lycopene 0.21 and 0.03%, alkanoids 0.60 and 1.26%, tannins 0.11 and 2.56, and phenol 0.81 and 0.28% for *B. eurocoma* and *T. tetraptera* respectively. Conclusively, the ethnobotanical effect of *B. eurocoma* and *T. tetraptera* are of great importance for the healthy wellbeing of individuals and the communities that cultivate it should harness it and utilize it to boost their nutritional status.

Keywords: *Brachystegia eurocoma, Tetrapleura tetraptera*, ethnobotanical, phytochemicals, bioactive substances.

1. Background

Nigeria is blessed with indigenous plants of medicinal and nutritional importance. Some of these are used as condiments, flavouring agents and as a soup thickener. Apart from their thickening and flavouring properties in soup, these plants exhibit a wide range of biological and physiological activities, namely anti-hypertensive, anti-inflammatory, anti-microbial, anti-diabetic and anti-carcinogenic. The seeds of *Brachystegia eurocoma* are used in folkloric medicine to maintain body temperature, soften stool, and protect against colon and rectal cancer (Ndukwu, 2009). These plants are traditionally identified as harmless and are usually applied in the fight against long-standing diseases (Okwu and Okoro, 2006). Since the ancient days, the preventive role of medicinal plants has been known to mankind and has been passed to generations within human communities (Okwu and Okoro, 2006). Plants such as *Tetrapleura tetraptera* and *Brachystegia eurocoma* can also be classified as such because they exhibit medicinal properties (Uzomah and Ahuligwo, 1999).

Brachystegia eurycoma belong to the family of Caesalpiniaceae, and order fabaceae. *Brachystegia eurycoma* is known as "achi" in Igbo Land, "akalada" in Yoruba, "akpakpa or apaupan" in Ijaw, "dewen" in Benin (Keay et al., 2024). It is a large tree with irregular and twisted spreading branches. The seed has a roundish flat shape with brown colour and hard hull. The fruit ripens from September to January and is released by explosive mechanism (Enwere, 1998; Rahman et al., 2023). It flowers between April and May.

The fruits occur as broad lathery dark purplish brown pods containing between four and six brown shiny flat disc-like seeds. The plant also possesses a rough fibrous bark, which peels off in patches and often gives out brownish buttery exudates (Matic et al.,2018; Adesina et al., 2016).

Tetrapleura tetraptera, a flowering plant mostly found in the western part of Africa has an essential chemical composition that gives it nutritive value and medicinal capacities (Mensah et al., 2024). *Tetrapleura tetraptera* is a flowering-medicinal plant belonging to the Leguminosae family. It is a deciduous tree mostly found in the western part of Africa and is usually referred to as Aidan fruit in English (Adesina et al., 2016). Countries such as Ghana and Nigeria are common places where *T. tetraptera* can be obtained. It is known as "Prekese" and "Aridan" in the Twi and Yoruba languages of Ghana and Nigeria respectively (Adusei et al., 2019). *T. tetraptera* has a variety of chemical compositions that translate into its nutritive value. Significant amounts of various nutrients such as ash, fiber, proteins, carbohydrates, vitamins, and fats are present in *T. tetraptera* (Adesina et al., 2016; Oteng et al., 2020). The chemical composition of the plant may vary at different parts of its fruit. For example, the carbohydrate and mineral contents of *T. tetraptera* fruit among the seeds, pulp, and woody coats are significantly different (Adadi and Kanwugu, 2020).

The thrust of this research is to evaluate the ethnobotanical and chemical composition of *Brachystegia eurycoma* and *Tetrapleura tetraptera* flour.

2. Materials and Method

2.1 Materials

Brachystegia eurycoma and *Tetrapleura tetraptera* were purchased from Ogbete main Market, Enugu, Enugu State Nigeria. The plant material were identified and authenticated.

Plate 1: *Tetrapleura tetraptera*

Plate 2: *Brachystegia eurycoma*

2.2 Preparation of Samples

Mature seeds of *Brachystegia eurycoma* and mature dried fruits of *Tetrapleura tetraptera* were each weighed (500g). The fruits of *Tetrapleura tetraptera* were cleaned, chopped and blended to uniform powder, then the samples were milled with an electric blender before used for analysis. The seeds of *Brachystegia eurycoma* were soaked in water over night. The loosened testa were then peeled off and the decorticated seeds were ground into a uniform powder using the Thomas Wiley mill machine, dried and stored in airtight container.

2.3 Chemical analysis

Proximate analysis, mineral, vitamins, phytochemicals, and anti-nutrients properties were determined in triplicate using standard method.

2.4.1 Determination of Moisture Content

The moisture content of the sample was determined using the hot air oven method of (AOAC, 2010). Two gram (2g) of each sample (W_2) was weighed and put into a washed and dried previously weighed (W_1) petri-dish and placed in an oven at a temperature of 80ºC for 2 hours and at 105ºC until the weight was constant. The samples was cooled in a desiccator and weighed and the weight was recorded as (W_3). The weight loss obtained as the moisture content was calculated from the formula:

$$\% \text{ Moisture Content} = \frac{W_2 - W_3}{W_1 - W_1} \times 100 \qquad 1$$

Where W_1 = Initial Weight of empty crucible

W_2 = Weight of crucible + sample before drying

W_3 = Final weight of crucible + sample alter drying

2.4.2 Determination of Crude Fibre Content

This was determined by the method of (AOAC, 2010). In its determination, bottom flask and beaker was rinsed with clean water, dried in an oven at 100ºC for 5 minutes and cooled. The defatted sample after fat extraction was used. Two grams (2g) of the sample (W_1) was transferred into a 500mL flask and 200mL of pre-heated 1.25% H_2SO_4 was added and the solution was gently boiled for 30 mins, maintaining constant volume of acid by the addition of hot water. The residue obtained was washed 3 times with hot water and returned to the beaker. The 200mL of pre-heated 1.25% NaOH was added and boiled for another 30 min. This was filtered under suction and then was washed thoroughly with hot water and twice with ethanol. The residue was dried at 65ºC for about 4 hrs, weighed and recorded as (W_2). The residue was transferred into a crucible and placed in muffle furnace and ashed at 550ºC for 4 hours. It was cooled in a desiccators and weighed (W_3).

$$\% \text{ Crude Fibre Content} = \frac{W_3 - W_3}{W_1} \times \frac{100}{1} \qquad \text{-------------------} \; 2$$

Where W_3 = Weight of Crucible + sample before ignition

W_2 = Weight of crucible + ash after ignition

W_1 = Weight of sample

2.4.3 Determination of the Fat Content

The Solvent extraction method of (AOAC, 2010) was used. The extraction flask was washed with petroleum ether and then dried, cooled, weighed and recorded as (W_1). Two gram (2g) of the sample was weighed (W_2) into the extraction thimble. It was placed back in the Soxhlet apparatus. The washed flask was filled to about three quarter of its volume with petroleum ether (that has the boiling point range of 40-60ºC). The apparatus was then set-up and extraction were carried out for a period of 5 hours after which complete extraction was made. The petroleum ether was recovered leaving only oil in the flask at the end of the extraction. The oil in the extraction flask was dried in the oven, cooled and finally weighed (C). The fat content was expressed as a percentage of raw materials. The difference in weight of empty flasks and the flask with oil content was calculated as:

$$\% \text{ Ash Content} = \frac{W3 - W1 \times 100}{W2} \text{-----------------------------} 3$$

Where; W_2 = Weight of sample

W_1 = Weight of empty flask

W_3 = Weight of flask + oil

2.4.4 Determination Ash Content

The ash content of the sample was determined by the method of (AOAC, 2010). A silica dish was heated to about 60ºC and then cooled in a desiccator, weighed and recorded as (W_1). Two grams (2g) each of the sample was weighed (W_2) and put into the silica dish and then was transferred to the furnace. The temperature of the furnace was allowed to reach about 500ºC after placing the dish in it. The temperature was maintained until whitish-grey colour was obtained which was an indication that all the organic matter of the sample had been destroyed. The dish was then brought out from the furnace and was cooled in a desiccator, then re-weighed and recorded as (W_3). The percentage ash content was then calculated as:

$$\% \text{ Ash Content} = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \text{------------------------} 4$$

Where: A = Weight of empty dish

B = Weight of empty dish + sample before ashing

C = Weight dish + ash

2.4.5 Determination of Protein Content

The crude protein of the samples was determined by the Micro Kjeldahl technique as described by (AOAC, 2010). Two gram (2g) weight (W) of the sample was put into a Kjeldahl flask. Four grams (3g) anhydrous sodium sulphate and two (2g) of hydrated copper sulphate (catalyst) was added into the flask. Then 20mL of concentrated tetraoxosulphate (IV) acid (H_2S0_4) was added to digest the sample. The digestion was continued under heat until a clear solution was observed. The clear solution was cooled and made up to 100mLwith distilled water. A digest of about 5mL was collected for distillation. Also, 5ml of sodium hydroxide (NaOH) was put into the distillation flask and distillation was allowed to take place for some minutes. The ammonia that was distilled off was absorbed by boric acid indicator and this was titrated with 0.01M hydrochloric acid (HCl). The titre value (T) of the end point at which the colour changed from green to pink was taken. The crude protein was calculated as:

$$\% \text{ Crude Protein Content} = \frac{0.0001401 \times T \times 100 \times 6.25}{W \times 5} \text{------------------------------} 5$$

Where: T = Titre value

W = Weight of sample dried

2.4.6 Determination of the Carbohydrate

The Carbohydrate content of each sample was determined by difference. % Carbohydrate = 100 % - (protein + fat + fibre + ash + moisture) (AOAC, 2010).

2.5 Vitamin Composition of the Samples

The vitamin composition of each sample was determined to ascertain their nutritional quality. All analysis were done in triplicate.

2.5.1 Determination of Beta-carotene content

The beta-carotene concentration was determined using a colorimetric assay which developed by (Seo et al., 2005). Five hundred (500) mg of the dried sample were extracted twice using 5 mL chilled acetone, and the mixture was let to stand in an ice bath for 15 minutes with occasional shaking. The mixture was mixed vigorously for 10 minutes and centrifuged at 1370 x g for 10 min. The supernatants were pooled and filtered using Whatman filter paper No.42. The absorbance of the extract was read at 449 nm using a UV-Vis spectrophotometer. Three replicates for every fruit were measured.

2.5.2 Determination of Thiamine Content

The thiamine content of each sample was determined according to the flourimetric method of (AOAC, 2010). Five grams (5g) of each the sample was weighed into a 50mL conical flask, then 7.5mL of 0.2N

hydrochloric acid was added and the flask was heated to boiling point on the water bath for 30min, after which the flask containing the boiled sample was allowed to cool at room temperature. On cooling, the mixture was incubated overnight at a temperature of 37ºC after which it was placed in a 100mL volumetric flask and made up to the mark with distilled water. The mixture was filtered and the filtrate obtained was purified by passing it through the column of silicate. After purification, 10mL of the filtrate was pipetted into a 50mL conical flask and 5mL of acidified potassium chloride, 3mL of alkaline tenicyanide solution and 15mL of isobutanol were added to the flask to ensure the oxidation of thiamine to thiochrome. The flask was thoroughly shaken for 2min and the mixture was allowed to separate. After that, the alcohol layer was taken and two grams (2g) of anhydrous Sodium sulphate was equally pipetted into another 50mL stoppered volumetric flask. In addition, the standard solutions of thiamine was prepared from the stock and kept separately. Thereafter, 5mL of the sample solution obtained after the removal of isobutanol extract and that of the standard blank was pipetted into individual test tubes and 3mL of acidic sodium hydroxide was added to each test tube as a stabilizing agent. The flourescence of the sample solution (A) and the blank (B) was individually measured with the flourimeter set to the excitation wave-length 360nm and emission wavelength of 435nm, respectively. Then the reading of blank was subtracted from the reading of the sample solution to obtain an equation (C).

Thiamine content (mg/100g) = $\frac{C \times 1 \times 25}{A \times 5 \times B}$ ------------------------6

Where; C =Reading of sample solution – Reading of blank.

A = Reading of the standard solution.

B = Volume of the solution used for the test on the column.

2.5.3 Determination of Riboflavin Content

The riboflavin content of each sample was determined according to the flourimetric method of (AOAC, 2010). Five grams (5g) of each of the sample (A) was weighed into a 50mL conical flask and 20mL of 0.2N hydrochloric acid was added to the flask. The flask containing the sample and the acid solution was boiled on the water bath for 60min. After that, the mixture was filtered into a 100mL volumetric flask and made up to the mark with distilled water. Ten milliliters (10mL) of the filtrate was pipetted into a test tube and kept away from strong light. The standard solutions of riboflavin was prepared from the stock solution and 10mL of the standard solution was also pipette into another test tube and kept separately. One milliliter (1mL) of ascorbic acid was added to each of the test tube and the mixture was mixed thoroughly in each case. In addition, 0.5mL of acetic potassium permaganate solution was also added to each of the test tube and kept away for 5 min, followed by the addition of 0.5mL peroxide solution as a stabilizing agent. Thereafter, the fluorescence of the sample kept in the first test tube containing the sample filtrate was measured with the flourimeter set to excitation wavelength of 470nm and emission wavelength of 526nm and recorded (B). After the measurement, 10mL of sodium sulphate solution was added to each of the test tube and their flourescence measured within 10sec and recorded as blank reading (C). The riboflavin content of each sample in (mg/100g) was calculated from the formula:

Riboflavin Content (mg/100g) = $\frac{B \times 1}{C \times A}$ -------------------------7

Where; A = Weight of the sample

B = Reading of sample in the first tube - Reading of sample blank

C = Reading of the sample + standard solution in the second tube - reading of the sample + standard blank.

2.5.4 Determination of Vitamin C Content

The titration method of (AOAC, 2010) will be used to determine the vitamin C content of each sample. Two grams (2g) of each sample will be macerated in 50mL distilled water. To 50mL of the prepared sample,

equal volume of extraction solution (containing 15g of phosphoric acid, 40mL acetic acid, and up to 500 ml with distilled water) will be added. Few drops of indicator (0.1g thymol blue was dissolved in 10.75mL of 0.02N sodium hydroxide solution and diluted with 250mL distilled water) will be added to the aliquot and the resultant solution will be titrated with Indophenol standard solution to obtain a rosy pink solution (V). Standard solution will be prepared by dissolving 0.05g ascorbic acid in 45mL in the extraction solution and making up to 50mL and will be titrated with indicator. The vitamin C content of each sample will be calculated from the formula:

Standardization of indophenol = $\frac{0.002}{1000} mg\ ascobic\ acid$ (C)------------------8

Vitamin C content (mg/100g) = $20\ x\ (V) x\ (C)$

Where V = mL indophenol solution used.

C = mL ascorbic acid/mL indophenols.

2.5.5 Determination of Vitamin E

Vitamin E content of each sample was determined using the method of (AOAC, 2010). A mixture of the sample (1g), absolute alcohol (10ml) and 1mL alcoholic Sulphuric acid (20 ml) was refluxed for 45 minutes in a condenser and cooled for 15minutes. Unsaponifiable matter in the mixture was extracted with dimethyl ether. The extracts evaporated at a low temperature and the residues obtained were dissolved in 10ml absolute alcohol. Absolute alcohol (5ml) and 1ml Conc. HNO3 was added to aliquots of the samples and standards (0.3-3.0mg vitamin E). The mixture was evaporated in a water bath (Techmel and techmel, Texas, USA) at 900C for 3 minutes from the time the alcohol started boiling. A series of standard solutions of known concentrations was determined with reference to their absorbance from which average was recorded. Its absorbance was measured at 290 nm using a UVIKON XL spectrophotometer (North Star Scientific Leeds, UK) against a blank containing 5 ml absolute alcohol and 1ml conc HN03 and treated in a similar manner. Vitamin E content was calculated using the formula below:

$$\text{Vitamin E (mg/100g)} = \frac{\text{Absorbance of sample x dilution factor x gradient}}{\text{Weight of sample}} \quad \text{---------9}$$

2.6 Mineral Composition of the Samples

The mineral composition of each sample was evaluated to ascertain their nutritional quality. Prior to the analysis, the minerals was extracted from each sample according to the dry ash extraction method of (AOAC, 2005). Two grams (2g) of each sample was weighed into the ashing dish and ignited in a muffle furnace at 550°C until a greyish colouration was obtained. The dish containing the samples in each case was removed from the furnace after ashing and the resulting ash was dissolved by the addition of 5mL of hydrochloric acid. The dish containing the ash solution was placed in a sand bath for 10 min after which the solution was carefully filtered into a 100mL volumetric flask and made to the mark with distilled water. The extracts produced in each case was stored separately in small bottles at room temperature (29±2ºC) and then was used individually for mineral analysis. All determinations were carried out in triplicate samples.

3.6.1 Determination of Potassium Content

The potassium content of each sample was determined using the method of (AOAC, 2010). Two grams (2g) each of sample was digested with 2.5mL of 0.03N hydrochloric acid (HCL). The digest was boiled for 5 min, allowed to cool to room temperature, transferred to 50ml volumetric flask and was made up to the mark with distilled water. The resulting digest was filtered with ashless Whatman No.1 filter paper. The filtrate from each sample was analysed for potassium content using the atomic absorption spectrophotometer (Buck Scientific Atomic Absorption Emission Spectrophotometer Model 205, Manufactured by Nowalk, Connecticut, USA) using standard wavelengths. The real values was extrapolated from the respective standard curves and the values obtained was adjusted for HCl-extractability for the respective ions.

2.6.2 Determination of Phosphorus Content

This was done according to the method of (AOAC, 2010). The phosphorus content of each sample was determined using the simultaneous inductively coupled plasma optical emission spectrophotometer. Two grams (2g) of each sample and 8 mL of 65 % HNO3 was added to 2 mL of 30 % H_2O_2 and the mixture was burned in the microwave milestone at 200°C for 15 min. This process was complemented with the addition of 50mL of distilled water and the sample was removed from the container. All the reagents and the sample were prepared in double distilled water. The phosphorus content of each sample was measured at the wavelength of 214.9 nm.

2.6.3 Determination of Zinc Content

The zinc content of each sample was determined using the method of (AOAC, 2010). Two gram (2g) of each sample was digested with 2.5mL of 0.03N hydrochloric acid (HCL). The digest was boiled for 5 min, allowed to cool to room temperature, transferred to 50mL volumetric flask and was made up to the mark with diluted water. The resulting digest was filtered with ashless Whatman No.1 filter paper. The filtrate from each sample was analysed for zinc content using an atomic absorption spectrophotometer (Buck Scientific Atomic Absorption Emission Spectrophotometer Model 205, Manufactured by Nowalk, Connecticut, USA) using standard wavelengths. The real values was extrapolated from the respective standard curves and the values obtained was adjusted for HCl-extractability for the respective ions.

2.6.4 Determination of Iron Content

The iron content of each sample was determined using the atomic absorption spectrophotometer according to the method of (AOAC, 2010). Two gram (2g) of the extract was pipetted into a fifty milliliters (50mL) volumetric flask and made up to mark with distilled water. Six (6) drops of the glycolic acid with two milliliters (21nl) of 7N ammonia solution were added to the flask. The absorbance of the solution was read with the spectrophotometer at 45nm. The standard solutions of iron (Oppm, 2ppm, 4ppm, 6ppm and 10ppm) was prepared from the stock solution and their absorbance measured at the same wavelength. The values of the absorbance of the standard solutions obtained was used to plot the calibration curve, from which the concentration of iron in each sample was calculated from the formula:

$$\text{Iron Content (mg/l00g)} = \frac{\text{Curve reading x Ash Dilution x 100}}{\text{Weight of the sample x } 10^3} \quad \text{--------10}$$

2.6.5 Determination of Calcium Content

The calcium content of the samples was determined according to EDTA versente complex metric method of (AOAC, 2010). Two milliliters (2mL) each of the sample (s) was pipetted into fifty milliliter (50mL) flask and 10mL of distilled water for dilution (DF) and a few drops of 4N (M.WH) sodium Hydroxide solution were added to the flask with a small amount of meroxide indicator to give the solution a pink colour. Thereafter, titration reading (T.R) was done on the content of the flask using 0.02 (N EDTA) (ethylene diamine tetra acetic acid) until the end point of the coloration was obtained. The percentage calcium content of each sample was calculated as shown:

$$\text{Calcium Content (mg/100g)} = \frac{\text{T.R x N (EDTA) x DF x M.WH x 100}}{10^6 \text{ x 5 x 2 x variance}} \quad \text{---------------------11}$$

T.R = Titration reading

N (EDTA) =Normality of EDTA

M.WH = Molecular weight

S = Sample weight

DF = Dilution factor

2.6.6 Determination of Magnesium Content

The magnesium content of each sample was determined using the atomic absorption spectrophotometer described by (AOAC, 2005). Five milliliters (5mL) of the extract in each case was pipetted into a fifty milliliters (50mL) volumetric flask. Then, ten milliliters (10mL) of silicon chloride solution ($SiCl_2$) was added to the flask. The content of the flask was then diluted to volume with distilled water and the absorbance was measured with the spectrophotometer after 30 min at 540nm. In addition, a set of standard solutions of magnesium (Oppm, 2ppm, 4ppm, 6ppm and l0ppm) were prepared from the stock solution and their absorbance measured individually at the same wavelength. The values of the absorbance of the solution obtained was used to plot the calibration curve, from which the magnesium content of each sample was extrapolated. The magnesium content of each sample was calculated from the formula:

$$\text{Magnesium content (mg/100g)} = \frac{\text{Curve reading x Ash Dilution x 100}}{\text{Weight of sample x } 10^3} \text{-------------------12}$$

2.7 Phytochemical Composition

2.7.1 Determination of the Total Phenol Content

Total phenolic content of each extract was measured as described in (Trease and Evans, 1989). Briefly, 0.1 ml of extract was mixed with 0.1 ml of Folin Ciocalteu reagent and 1.5 ml of distilled water. After 5 minutes 0.3 ml of 20% (w/v) Na2CO3 was added. The mixtures were vortexed and left at room temperature for 30 min in the dark. Absorbance was measured at 765 nm (Specord 200 Analytic Jena, Germany). Total phenols were calculated from a gallic acid standard curve and reported as mg gallic acid equivalents (GAE) /g of dried weight. The linearity range of the calibration curve was 0.05 to 0.50 mg/ml.

2.7.2 Determination of flavonoid

The flavonoid content was determined spectrophotometrically using AlCl3 reagents (Harbone, 1983). One ml of extract was mixed with 4 ml of distilled water and 0.3 ml of 5% NaNO2 (w/v). The mixtures were vortexed, and left at room temperature for 5 minutes. After that, 0.3 ml of 10% AlCl3 (w/v) was added, vortexed and left for 6 minutes. Two ml of 1 M NaOH and 2.4 ml distilled water were added. Absorbance was immediately measured at 510 nm. Total flavonoids were calculated from a catechin hydrate standard curve and reported as mg catechin hydrate equivalents (CAE)/g of dried weight. The linearity range of the calibration curve was 0.01 to 0.08 mg/ml.

2.7.3 Determination of Saponin Content

The saponin content of each sample was determined using the method of (AOAC, 2010). Saponin extract of each sample was done using acetone and methanol. Crude lipid content of each sample was extracted with acetone while methanol was used to extract saponin. Two gram (2 g) of each sample in triplicate (S) was folded in filter paper and put in thimble and was extracted by influxing in a Soxhlet extractor. Extraction was done with acetone in a 250 mL capacity round bottomed flask containing 100 mL methanol, fitted to the extractor and the extraction was sustaining for another 3 h. The weight of flask was taken before (B) and after extraction (A) to note the change in weight. Methanol was recovered by distillation after the second extraction and the flasks was oven-dried and allowed to cool at room temperature and weighed. The saponin content was calculated using the formula;

$$\text{Saponin (mg/100g)} = \frac{A-B \times 100}{Sm} \text{------------------13}$$

Where A= mass of flask and extract

B = mass of empty flask

2.7.4 Determination of Alkaloids

The alkaloids content of each sample will be determined using the method of (AOAC, 2005). About five gram (5 g) of each sample will be weighed into a 250 mL beaker, and 200 mL of 20% acetic acid in ethanol will be added and will be covered to stand for 4 h. This will be filtered and the extract will be concentrated

using a water bath to evaporate one-quarter of the original volume. The concentrated ammonium solution will be added drop-wise to the extract until the precipitation will be completed. The entire solution will be allowed to settle and the precipitate will be collected by filtration, after which it will be weighed and recorded.

2.8 Anti-Nutrient Compounds

2.8.1 Determination of Phytate Content

The phytic acid content of each sample was determined using the method of (AOAC, 2005). Four grams (4 g) of each sample was soaked in 100 mL of 2% HCl for 3 h and then filtered. Then 25 mL of the filtrate was dispensed into a conical flask and 5 mL of 0.3 mL ammonium thiocyanate solution was added as indicator. Thereafter, 53.5 mL distilled water was added to the mixture to give it a proper acidity and this was titrated with standard iron III chloride solution, which contains about 0.00195 g (1.95 g) of iron per millimeter until a brownish colour persisted for 5min.

2.8.2 Determination of tannin

The tannin content of each sample was determined using the method of (AOAC, 2005). Two grams (2g) of the sample was poured into a beaker containing 50 mL of distilled water and heated to 60°C. Then it was filtered and the residue was discarded. Then ten millilitres (10mL) of 4% copper acetate solution was added to the hot filtrate and boiled for another 10 min. The precipitate was filtered and the filtrate was discarded. The residue was dried using a filter paper and then dried sample was scraped from filter paper into a pre-weighed crucible. The weight was recorded as (W). The crucible containing the sample was incinerated in a muffle furnace at 550°C, cooled in a desiccator and was reweighed as W_1. The difference between the weight of sample before ashing and the ash residue after incineration represents the tannin content.

2.8.3 Determination of Oxalate

The permanganate titration method described by (AOAC, 2005) was used. A 5 g weight of each sample (W) was suspended in 100 mL of distilled water and 5 mL of 6mHCl was added. The mixture was digested by heating at 100°C for an hour. It was cooled and filtered. Then the pH was adjusted by adding 2 drops of methyl red indicator followed by drop wise addition of concentrated aqueous ammonia solution (NH40H) until a faint yellow colouration was obtained, at pH between 4-4.5. The mixture was heated to 90°C in a water bath, cooled and filtered (to remove ferrous ion precipitates). The filtrate was again heated 90°C and 10 mL of 5% $CaCl_2$ solution was added with constant steering. It was allowed to cool and then allowed to stay overnight in the refrigerator (5°C) the mixture was centrifuged at 3000 rpm for 15 minutes. The supernatant was decanted and the precipitate was dissolved in 10 mL of 20% H_2SO_4. The solution was made up to 100 mL with distilled water and was titrated against 0.05 $KMnO_4$ solutions to a faint pink colour which persisted for 30 seconds. The oxalate content was given by the relationship that 1mL of 0.05m $KMnO_4$ solution = 0.00225g oxalate.

Calculation of oxalate content

$$\% \text{ oxalate content} = \frac{100 \text{ x titre x } 0.00225}{W} \text{-----------------14}$$

Where W = weight of sample use.

2.9 Statistical Analysis

The data generated was subjected to one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS, version 20) software, and significant means was separated using the Turkey's Least Significance Difference (LSD) Test at $p<0.05$.

3. Results and Discusssion

3.1 Proximate composition of the sample

Table 1. Proximate composition of achi (*Brachystegia eurycoma)* and Uhiokrihio (*Tetrapleura tetraptera)%.*

Samples	Protein	Ash	Fat	Fibre	Moisture	CHO
B. eurycoma	8.29±0.08	3.63±0.28	9.21±0.48	2.68±0.14	8.28±0.24	67.74±0.38
T. tetraptera	7.67±0.28	3.62±0.46	5.23±0.91	4.28±0.86	10.76±0.18	64.44±0.38

Values are mean ±standard deviation of 3 replications.

Protein: The crude protein content for both *B. eurycoma* and *T. tetraptera* were 8.26 and 7.67 respectively. The values compared favourably with the protein values reported for *B. eurycoma* (8.75%) Ajayi et al.(2014) and also the protein value for *T. tetraptera* protein value (7.64%) of *T. tetraptera* as reported by *Edak et al.* (2013). Ene-Obong (1992) reported that diet is nutritionally satisfactory if it contains high calorie value and a sufficient amount of protein. Ali (2010) and Effionf et al. (2009) also stated that any plant food that provide about 12% of their calorie value from protein are considered good sources of protein as it helps in building body mass and directly involved in the chemical processes essential for life.

Ash: The ash content of *Brachystegia eurycoma* and Uhiokrihio (*Tetrapleura tetraptera* were 3.63 and 5.23 respectively. The ash content of *Brachystegia eurycoma* is lower than the ash content of 5.00 Ajayi (2014) and 4.09% Edat et al (2013). The proportion of ash content is a reflection of the mineral content present in the food materials (Omotoso, 2005; Nnamani et al., 2009).

Fat: The fat content was 9.21 and 5.23. *Brachystegia eurycoma* had the higher value while *Tetrapleura tetraptera* had the least value (5.23%). The value obtained were higher than the previous reports Ajayi et al. (2014) 4.49% on *Brachystegia eurycoma*. Dietary lipids are responsible for carrying nutritionally essential fat soluble vitamins and excess fat consumption has been implicated in cardiovascular disorder such as artherosclerosis, cancer and aging (Anita et al.,2006).

Crude fibre: The crude fibre content of *Brachystegia eurycoma* was (2.68%) and that of *Tetrapleura tetraptera* was (4.28%) which varied with 17.20% Ajayi et al (2013). It increase stool bulk in the gastrointestinal tract. Fibre helps in the maintenance of human health and has been known to reduce cholesterol level of the body (Bello et al 2008). A low fibre diet has been associated with heart disease, cancer of the colon and rectum, varicose veins, obesity, appendicitis, diabetes and even constipation (Umerah et al.,2019).

Moisture content: The moisture content obtained for both seeds were 8.28% and 10.76% for *Brachystegia eurycoma* and *Tetrapleura tetraptera* respectively. The result of *Brachystegia eurycoma* is in line with *Brachystegia eurycoma* (10.60%) as reported by Ajayi et al (2014). High amount of moisture in food makes them vulnerable to microbial attack, hence spoilage. The moisture content of any food is an index of its water activity (Frazie, 1998) and is used as a measure of stability and susceptibility to microbial contamination (Davey, 1989). These moisture contents also mean that dehydration would increase the relative concentration of other food nutrient and therefore improve the shelf life or preservation of the food. There is also need to store the seeds in cool condition if they would be kept for a long period without spoilage especially in the tropics where wastage of crops is estimated to be high due to high moisture content.

Carbohydrate content: The carbohydrate content obtained for both samples were 68.44 and 67.94% for *Brachystegia eurycoma* and *Tetrapleura tetraptera* respectively. Carbohydrate provides energy to the cell in the body, particularly the brain which is the only carbohydrate dependent organ in the body (Effiong et al., 2009). Carbohydrate is necessary for maintenance of the plasma level, it spares the body protein from

being easily digested and helps to prevent the using up of protein, The high carbohydrate content observed in this study suggest high calorie energy.

3.2 Mineral composition of the samples (mg/100g)

Samples	Ca	Mg	K	P	Na	Fe	Zn
B. eurycoma	6.53±0.12	1.61±0.10	0.24±0.25	1.23±0.11	0.03±0.14	12.53±0.19	2.86±0.12
T. tetraptera	138.24±0.34	60.21±0.13	49.30±0.21	50.34±0.1	26.46±0.10	15.10±0.03	3.44±0.11

Calcium: The calcium content for both samples were 6.53 and 138.24mg/100g Calcium and phosphorus are associated with each other for the growth and maintenance of bones, teeth and muscles (Okaka et al., 2006).

Magnesium: The magnesium content obtained for both samples were 1.61 and 60.21 mg/100g for *Brachystegia eurycoma* and *Tetrapleura tetraptera* respectively. Magnesium is a compound of chlorophyll and it is an important content in connection with Ischemic heart disease and calcium metabolism in bones (Elegbede, 1995). *Brachystegia eurycoma* had lower value than *Tetrapleura tetraptera*.

Zinc: The zinc content obtained for both samples were 2.86 and 3.45mg/100g for for *Brachystegia eurycoma* and *Tetrapleura tetraptera* respectively. Zinc is involved in normal functioning of immune system and is associated with protein metabolism (Umerah and Nnam, 2019).

Iron: The iron content obtained for both samples were 12.52 and 15.10 mg/100g for *Brachystegia eurycoma* and *Tetrapleura tetraptera* respectively. Iron is an essential trace element for hemoglobin formation, normal functioning of central nervous system and in the oxidation of carbohydrate, protein and fats (Asaolu, 2007).

Potassium: The potassium content content obtained for both samples were 0.24 and 49.80mg/100g for *Brachystegia eurycoma* and *Tetrapleura tetraptera* respectively. Potassium is primarily an intercellular cation, in large part this cation is bound to protein and with sodium influences osmotic pressure and contribute to normal Ph equilibrium.

Copper: The copper content obtained for both samples were 0.10 and 3.29 mg/100g for for *Brachystegia eurycoma* and *Tetrapleura tetraptera* respectively. Copper and iron are present in the enzyme cytochrome oxidase involved in energy metabolism. Copper deficiency is of little concern since it is widely distributed in other types of food. Copper makes up approximately 0.9g of the body. It can be found in some enzymes that are crucial to oxygen reactions and the way iron is metabolized. It also colors hair and skin, and helps from the protectives shield around nerve fibres.

Sodium: The sodium content obtained for both samples were 0.03 and 26.64mg/100g for for *Brachystegia eurycoma* and *Tetrapleura tetraptera* respectively. Sodium is normally consumed in the form of salt. It is essential in the regulation of water content and in the maintenance of osmotic pressure of the body fluid. It also aids in the transport of CO_2 in the blood. However, sodium is one of the minerals whose intake is considered at actor in the etiology of hypertension, hence its low intake is encouraged.

Phosphorus: The phosphorus content obtained for both samples were 1.23 and 50.78mg/100g for for *Brachystegia eurycoma* and *Tetrapleura tetraptera* respectively. With both an extracellular and intracellular distribution, phosphorus functions as a structural component of bones and teeth and DNA/RNA and enables the bipolarity of lipid membranes and circulating lipoproteins. Metabolically, phosphorus functions in critical pathways to produce and store energy in phosphate bond (ATP), buffer blood, regulate gene transcription, activate enzyme catalysis, and enables signal transaction of regulatory pathways affecting a variety of organ functions ranging from renal excretion to immune response.

3.3 Vitamin Composition of the Samples

Samples	Vit C	Riboflavin	Thiamin	Niacin	Vit A	Vit E
B. eurycoma	8.16 ±0.23	0.53±0.08	0.08±0.25	0.13±0.11	3.28±0.14	2.91±0.14
T. tetraptera	0.31±0.34	0.08±0.13	0.09±0.21	0.07±0.16	1.93±0.10	5.67±0.14

Riboflavin: The values of riboflavin content of both samples were 0.53 and 0.08mg/100g for *Brachystegia eurycoma* and *Tetrapleura tetraptera* respectively. Like thiamine, riboflavin acts as a coenzyme in the breakdown of fats, proteins, carbohydrate, and other nutrients. It also helps fatty acid reduction and also necessary for catabolism of nutrients in the liver. Furthermore, it assists eye and skin maintenance.

Vitamin A: The value for vitamin A content of both samples were 3.28 and 1.93mg/100g for *Brachystegia eurycoma* and *Tetrapleura tetraptera* respectively. Vitamin A exists in plants as the precursor of carotenoids family. It functions in the immune system in the modulation of diverse pathways in the expression of mucins and keratins, lymphopoession, cytokine production, neutrophil maturation and function, the functional expression of natural killer cells, monocytes and macrophagis, T and B lymphocytes and immunoglobin production.

Vitamin C: The value for vitamin C content of both samples were 81.16 and 0.31mg/100g for *Brachystegia eurycoma* and *Tetrapleura tetraptera* respectively. Vitamin C is vital for the body performances. The antioxidants properties of vitamin C stabilizes foliate in food and in blood plasma. A common feature of vitamin C deficiency is anaemia. Its deficiency also impairs the normal formation of intercellular substances throughout the body, including collagen, bone matrix and tooth dentine. Thiamine plays a central role in cerebral metabolism. Its deficiency results in dry and wet beriberi, lactic acidosis and internicke-korsakoff syndrome.

Vitamin E: The value for vitamin E content of both samples were 2.91 and 5.67mg/100g for *Brachystegia eurycoma* and *Tetrapleura tetraptera* respectively. Vitamin E has potential in providing protection from free radicals and products of oxygenation. It works in conjuction with other antioxidation and nutrients to quench free radicals. It also inhabit lipoxygenation, an enzyme responsible for the formation of pro-inflammatory ,leukotriene (Anon, 2002).

Thiamine: The value for thiamine content of both samples were 0.08 and 0.09% for *Brachystegia eurycoma* and *Tetrapleura tetraptera* respectively. *Brachystegia eurycoma* contains the lower value 0.08% while *Tetrapleura tetraptera* contains the higher value 0.09%. These values are lower than the USDA (1.5mg/100g).

Niacin: The value for niacin content of both samples were 0.13 and 0.07% for *Brachystegia eurycoma* and *Tetrapleura tetraptera* respectively. *Brachystegia eurycoma* contains the higher value 0.08% while *Tetrapleura tetraptera* contains the lower value 0.07%.

3.4: Phytochemical composition of the samples (%)

Samples	Flavonoids	Glycoside	Saponins	Lycopene	Alkanoids	Tannins	Phenol
B. eurycoma	0.10 ±0.03	0.20±0.11	0.83±0.23	0.21±0.01	0.60±0.14	0.81±0.26	0.75±0.20
T. tetraptera	0.62±0.42	0.74±0.31	3.40±0.18	0.03±0.16	7.56±0.23	6.88±0.70	5.45±0.67

Flavonoids: The value for flavonods content of both samples were 0.10 and 0.62% for *Brachystegia eurycoma* and *Tetrapleura tetraptera* respectively. Flavonoids enhance the effects of vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes, allergies and inflammations. They protect blood vessels especially the tiny capillaries

that carry oxygen and nutrients to cells and are believed to slow down the development of cataracts in person who have diabetes mellitus .

Glycoside content: Glycoside value for both samples were 0.2 and 0.74% for *Brachystegia eurycoma* and *Tetrapleura tetraptera* respectively. Glycosides were not detected on Brachystegia eurycoma according to Ajayi et al., (2014).

Saponin: Saponin value for both samples were 0.83 and 3.40% for *Brachystegia eurycoma* and *Tetrapleura tetraptera* respectively. Saponin are produced by plants as a deterrence mechanism to stop attack by foreign pathogens, making them natural antimicrobials. They have the ability to bind with cholesterols, bitterness and heamolytic activity in aqueous solution (Asouzu and Umerah. 2020A) like phenol, the presence of saponin in *Tetrapleura tetraptera* might contribute to some reported medicinal properties of the plant.

Lycopene: Lycopene value for both samples were 0.21 and 0.03% for *Brachystegia eurycoma* and *Tetrapleura tetraptera* respectively.

Alkaloids: Lycopene value for both samples were 0.21 and 0.03% for *Brachystegia eurycoma* and *Tetrapleura tetraptera* respectively. They are the most therapeutic significant plant substances pure isolates of alkaloids and their synthetic derivatives are used as basic medicinal agents because of their analgelsic, anti-spasmotic and anti-bacterial properties (Stay, 1998; Hasaranraj et al., 2009). The presence of appreciable concetration of alkaloids in *T. tetraptera* explains their use in traditional medicine.

Tannin: Tannins value was higher in *T. tetraptera* 6.88% and *Brachystegia eurycoma* 0.81%. Tannins are generally regarded as dietary anti-nutrient for their astringent taste and poor palatability of foods and drinks (Chikezie et al., 2008). They usually form insoluble complexes with proteins, thereby interfering with their bioavailability (Enujiugba and Agbede, 2000). Tannins are useful in the treatment of intestinal disorders such as diarrhea and dysentery and urinary tract infections (Fahey, 2005). Akimpelu and Onakoya (2006), this explains the use of the plant in the treatment of gastrointestinal disorders.

Phenol: Phenol value was higher in *T. tetraptera* 6.88% and *Brachystegia eurycoma* 0.81%. Phenolic compounds are potent water soluble antioxidant and free redical scavengers which prevent axidative cell damage and have strong anti-cancer activity (Asouzu and Umerah, 2020B; Del-Rio et al., 1997).

3.5 The anti-nutrient composition of *T. tetraptera* and *Brachystegia eurycoma*.

Samples	Cyanide	Oxalate	Phytate
B. eurycoma	0.10 ±0.0	0.28±0.03	0.49±0.05
T. tetraptera	Trace	0.31±0.01	0.23±0.03

Phytate content: The phytate content were 0.49% and 0.23% for *T. tetraptera* 6.88% and *Brachystegia eurycoma* respectively. The two samples were low when compared with those reported for some commonly consumed tropical legumes, cowpea, *vigna ungiculata* (2.0-2.9%), pigeon pea (*Cajanus cajan*) (2.0-2.4%) and African yam beans, *Sphenostylus tenocarpa* (2.4%) (Oboh, 2006).

Oboh et al.(2003) reported that phytate has the ability to chelate divalent minerals and prevent their absorption. Phytic acid has complicated effect in human system including indigestion of food and flatulence.

Cyanide content: The level of cyanide in both samples were 0.01 and trace for *Brachystegia eurycoma* and *T. tetraptera.* Respectively. These two samples would be non-toxic when ingested due to its very small amount. Thus, the results revealed that cyanide composition of both samples were generally low such that the cyanide content was below the lethal dosage of 6% approved by standard (National Agency for Food and Drug Administration and Control, NAFDAC) in Nigeria (Blessing *et al*., 2011).

Oxalate content: The oxalate value for *Brachystegia eurycoma* and *T. tetraptera* were 0.28 and 0.31% respectively. The amount of oxalate reported in this study was well below the range of values that would adversely affect their nutritional values or cause any of the toxic effects associated with the anti-nutrients. This could mean that the two samples will not affect human nutrition if consumed in large quantity and is therefore an advantage to the consumers.

Conclusion

The study confirmed that *Brachystegia eurycoma* and *T. tetraptera* can be utilized in the management of some illness because of their appreciable concentration of some phytochemicals which are active ingredients in the manufacturing of drugs in pharmaceutical industry.

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