International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 4 Issue 06, June-2015

Proximate and Phytochemical Composition of the Pulp of Tetrapleura tetraptera Fruits Consumed in Abakaliki, Nigeria.

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***Abstract*— Tetrapleura tetraptera fruits have been used in ethnomedical practice in Nigeria for the treatment of diseases and as spices in food preparation. The proximate and phytochemical composition of the pulp of Tetrapleura tetraptera consumed in Abakaliki is determined. The percentage composition (%) on the basis of dry weight was Carbohydrate (40.12±2.57), Moisture (19.90±1.65), Ash (17.84±1.63), Crude Protein (14.10±1.77), Fats and Oil (8.69±1.82) and Crude Fibre (6.84±0.31). The qualitative phytochemical analysis revealed the presence of saponin, tannin, steroids, terpenoids in very high amount, alkaloids, flavonoid, resin in moderately high concentration while glycosides in trace concentration. The quantitative phytochemical analysis (%) based on dry weight were saponin (5.670±0.294), tannin (3.194±0.323), flavonoid (0.073±0.002), alkaloids (0.633±0.057), phenol (0.373±0.013) and glycosides (0.0072±0.0002). The appreciable abundance of these biologically essential compounds in this plant indicates the potential of the pulp of this fruit as a source of these phytochemicals, justifying the use of the fruit in traditional medicine.**

Key words: Tetrapleura tetraptera, Pulp, proximate composition, qualitative analysis, quantitative analysis, Abakaliki.

INTRODUCTION

The folks of Abakaliki extraction, South – East Nigeria, are known for their different cultures. Traditional home economics especially in the preparation of spicy cuisines is part of their common denominational heritage. One spice that special local dishes cannot serve without is *Tetrapleura tetraptera* fruit.

Tetrapleura tetraptera is a deciduous, single- stemmed, robust perennial tree with grey/brown, smooth/rough bark, of about 30 m high (1). The plant has yellow/pink to orange flowers with sessile, glaborous leaves (2), spreading branches and naturally distributed in the rainforest belt of West, Central and East Africa (3). The fruit of the plant is shiny, dark – brown, glaborous, slightly curved, with four (4) longitudinal, wings – like ridges and small, brownish – black seeds. Two wings are woody, while the other two filled with soft, sugary pulp, oily and aromatic. It is green when tender and darker brown when fully ripe (1). The plant belongs to the family of *Fabaceae* (formerly *Leguminoceae*: *Mimosaceae*) and locally called different names by different ethnic groups in Nigeria. It is known as Aidan in English, *Osakirisa* or *Oshosho* in Igbo, *Aridan* in Yoruba, *Dawo* in Hausa, *Abogolo* in Igala, *Osirisa* in Ikwo dialect.

The plant is valued in Eastern, Southern and Western Nigeria and beyond, for its nutritional and pharmacological properties due to a variety of active nutrients (4). The fruit of this plant has a fragrant, characteristic pungent, aromatic odour and flavour (3), which is responsible for its insect repulsive characteristics (5). Hence, it is exploited as a popular seasoning spice in Eastern and Southern Nigeria (6, 7). The fruit is also used to eliminate pungent odour and impede fungal growth in cassava fufu (8). Medicinally, the fruit is used to prepare soup or porridge for nursing mothers from the beginning of childbirth to prevent post-partum contraction (9, 10) and as lactation aid (11) in nursing mothers. It is also harnessed in the management of convulsions, leprosy, inflammation, flatulence, jaundice, malaria, adult onset diabetes mellitus, rheumatism and as a molluscide (3, 10, 12-15). Different solvents extracts of the fruit have been proved to have hypolipidaemic and hypokalaemic effects (16).

The continuous availability of the fruit is threatened by extinction as a result of overexploitation (17). This then begs for conservation and breeding efforts on this plant. Moreover, Uyoh *et al*. (17) reported highly significant differences in the nutrient composition of *Tetrapleura tetraptera* obtained from different localities in Cross River State, Nigeria. The objective of this research was to determine proximate and phytochemical composition of *Tetrapleura tetraptera* fruit sold in Abakpa Market, Abakaliki, Nigeria.

MATERIALS AND METHODS

Collection of Plant Material and Identification

Mature dried fruits of *T. tetraptera* were bought from a popular local dealer in Abakpa Market, Abakaliki, Ebonyi State, Nigeria. The fruit was identified in the Herbarium Unit of Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria. The fruits were dry cleaned, chopped and blended to powder.

Tetrapleura tetraptera Fruit

Preparation of Reagents

Preparation of 5 %w/v Ferric Chloride Solution

Ferric Chloride ($FeCl_3$, 2.5 g) was dispersed in 40 ml of distilled water and made up to fifty (50) ml after dissolution.

Preparation of Ammonium Solution

A stock concentrated ammonium solution (375 ml) was made up to 1000 ml using distilled water.

Preparation of 45 %v/v Ethanol

Forty-five (45) ml of ethanol (absolute concentration) was made up to 100 ml by addition of fifty (55) ml of distilled water.

Preparation of 0.5 %w/v Aluminium Chloride Solution

Aluminium chloride ($AlCl_3$, 0.5 g) was put in eighty (80) ml distilled water and made up to a total volume of 100 ml.

Preparation of Dilute Sulphuric Acid

To five (5) ml distilled water was added 10.4 ml of H_2SO_4 (Concentrated Sulphuric acid) and made up to a hundred (100) ml with distilled water.

Preparation of Lead Sub-Acetate Solution

A forty five (45) ml of 15 %w/v lead acetate was dissolved in twenty (20) ml of absolute ethanol and thirty-five (35) ml of distilled water.

Preparation of Wagner's Reagent

To 900 ml of distilled water were 2 g of I_2 (iodine) crystals and three (3) g of KI (Potassium Iodide) added and completed to 1000 ml.

Preparation of Molisch Reagent

To 100 ml of ethanol was 0.1 g of α – naphthol dissolved.

Preparation of 2%v/v Hydrochloric Acid

To 90 ml of distilled water, was 2 ml of concentrated HCl (hydrochloric acid) added and completed to 100 ml.

Proximate Analysis

Determination of the Percentage Crude Protein Content

The Kjeldahl method of AOAC (18) was used. A 0.5 g of the ground *T. tetraptera* fruit was measured into Kjeldahl flask, 10 g of Sodium Sulphate, Na_2SO_4 (to increase the boiling point) and 1 g Copper Sulphate, $CuSO_4$ (as catalyst) were added; then 20 ml of H_2SO_4 was added. This was digested by burning on a heating mantle until the solution turned bluish green. The bluish green solution was poured into a beaker and allowed to solidify for 24 hours. After cooled for 24 hours, the digested sample solidified and whitish colour obtained. Then, 200 ml of water (distilled) was added to dissolve the congealed sample and allowed further cooling for few minutes in the refrigerator. Addition of 60 ml of 40 %w/v NaOH and 2 pieces of Zinc metal was done. The mixture was poured into a round bottom flask of the distillation column. With a labelled conical flask, 100 ml 4 %w/v boric acid and 2 drops of screened methyl red as indicator, were added and placed on the receiving end of the distillatory apparatus. When the whole liquid in the receiver reached 200 ml on the conical flask, the distillation was stopped and the distillate was titrated against 0.1 M H_2SO_4 till the mixture became light pink coloured. The crude protein content was calculated as a percentage using the formula below:

$$\% \text{ Crude Protein} = \frac{100 \times Titre\ value \times Dilution\ factor \times 0.00141 \times 6.25}{Weight\ of\ the\ sample} \quad (1)$$

Determination of Percentage Moisture

The hot oven method of Pearson (19) was used. One (1) g of the ground sample was measured into a thoroughly washed and oven dried crucible. The sample in the crucible was put in the oven at 105˚C for 1 hour to dry and the weight was recorded. The sample was dried for another 30 minutes and cooled in desiccator. The weight was also taken. This was repeated till a constant weight was attained. Moisture content was then calculated thus:

$$\% \text{ Moisture} = \frac{W_2 - W_3}{W_2 - W_1} X\ 100 \quad (2)$$

where Weight of crucible = W_1, Weight of crucible + sample before drying = W_2, Weight of crucible + sample after drying = W_3

Determination of Percentage Crude Fibre

In the determination of crude fibre, the method of Joshyn (20) was used.

Two (2) g of ground sample was measured and put in 250 ml conical flask, soaked in 200 ml of 1.25 %v/v H_2SO_4 and heated for 30 minutes on a hot plate. The resulted mixture was filtered and the residue washed with hot distilled water until no more acidic (used pH meter). The residue was re-soaked in 200 ml of 1.25 %w/v NaOH and heated again for another 30 minutes. The solution was filtered in a known weight of filter paper, dried in oven at 100 ˚C for 2 hours, cooled and reweighed.

$$\% \text{ Crude Fibre} = \frac{Weight\ of\ Fibre}{Weight\ of\ sample} X\ 100 \quad (3)$$

Weight of ash = Weight of Platinum Crucible + Ash – Weight of Platinum Crucible,

Weight of Fibre = Weight of Residue – Weight of Ash

Determination of Percentage Ash Content

The amount of ash in *Tetrapleura tetraptera* fruit was determined using the method of AOAC (18). One gram (1 g) of ground sample was measured to a dried platinum crucible of known weight and burnt at 550°C for 1 hour. It was heated until the ashing process completed. The ash was cooled in a desiccator and the weight determined. The ash content was then determined thus:

$$\% \text{ Ash} = \frac{W_2 - W_1}{1g} X\ 100 \quad (4)$$

W_1 = Weight of empty platinum crucible

W_2 = Weight of crucible + sample after ashing

Determination of Percentage Fat and Oil

Soxhlet extraction method of AOAC (18) was used. Five gram (5 g) of the sample was wrapped in a filter paper and put in a Soxhlet extractor. A heating mantle was applied below a conical flask with n-hexane inside, which aided in oil extraction. The system was recycled 8 – 9 times to achieve maximum yield of oil. At the end of the recycling, the extractor was disconnected and a distillation apparatus set up to separate the solvent (n-hexane) from the oil, as a way of solvent recovery. An empty beaker was weighed and the mixture containing oil and traces of the solvent after distillation was transferred into the weighed beaker and heated

to remove the remaining n-hexane leaving only the oil. This was cooled in desiccator and the weight of the beaker determined again.

$$\% \text{ (Oil and Fat)} = \frac{W_2 - W_1}{Weight\ of\ sample} X\ 100 \quad (5)$$

W_1 = Weight of empty beaker, W_2 = Weight of beaker + Oil,

$W_2 - W_1$ = Wight of Oil.

Determiation of Percentage Carbohydrate

The method of Pearson (19) was used. Thus, the carbohydrate content of the sample was determined by taking the sum of ash, protein, moisture, crude fibre, fat and oil from 100. That is,

% Carbohydrate = 100 - (%Ash + %Protein + %Moisture + %Crude Fibre + %Fat and Oil). (6)

Phytochemical Analysis of *Tetrapleura tetraptera* Fruit

The qualitative and quantitative distributions of primary and secondary metabolites differs from plant to plant and part to part (21). Tests for identification of phytochemicals in *Tetrapleura tetraptera* fruit pulp was carried out using standard protocols as reported in (22-24).

Qualitative Analysis

Test for Proteins

(a) Millon's Test

Two (2) drops of million's reagent was put to a small quantity of sample filtrate. Protein was indicated by the development of white precipitates

(b) Xanthoproteic Reaction Test

To five (5) ml of sample filtrate was added few drops of conc. HNO_3. Protein was indicated by development of yellow colour which changed when alkali was added.

(c) Biuret Test

A crystal of Copper Sulphate was added to 2 ml of the sample filtrate and 2 drops of potassium hydroxide added. A pink or purple colour showed the presence of proteins

(d) Picric Acid Test

Few drops of picric acid was put into small quantity of sample filtrate. Development of yellow colour precipitates showed the presence of protein in the sample.

Test for Alkaloids

To 5 ml of hydrochloric acid (2 %v/v) was added 1 g of the ground sample and boiled in a steam bath for 2 minutes. To 1 ml of filtrate that resulted from this treatment was added 2 drops of

(a) Wagner's reagent

(b) 1 % picric acid solution

Formation of turbidity or precipitate showed alkaloid presence.

Test for Saponins

One (1) g of the ground sample was put in 5 ml of distilled water and boiled for 5 minutes. The extracts from the treatment was filtered when still hot and used for subsequent steps below.

(a) Frothing Test

Four (4) ml of distilled water was used to dilute 1 ml of the extract filtrate. This was shaken vigorously, left to stand, and watched for steady persistent froth.

(b) Emulsion Test

The mixture from the frothing test was added 3 drops of olive oil and vigorously shaken. Formation of emulsion indicates the presence of saponins.

Test for Steroids and Terpenoids

To 1 g of powder was added 9 ml of ethanol. The solution was refluxed for some time followed by filtration. Boiling of the filtrate was carried out in a water bath to concentrate it to 2.5 ml. Five (5) ml of hot water was added to the concentrated extract, allowed for 1 hour and filtered to remove waxy materials. The filtrate now was subjected to further extraction using chloroform (2.5 ml) via separating funnel. The chloroform extract (0.5 ml) was dried over water bath and further boiled in a water bath for 10 minutes using 3 ml conc. H_2SO_4. Development of grey colour indicated terpenoids presence in the sample.

Test for Reducing Sugar

To 5 ml of distilled water was added 0.1 mg of sample extract and vigorously shaken. The filtrate was used for Fehling's and Benedict's tests:

(a) Fehling's Test

Fehling's solutions 1 and 2 were added to 1 ml of filtrate. The resulted mixture was subjected to heating for few minutes using water bath. Reducing sugar was indicated by development of brick red coloured precipitate.

(b) Benedict's Test

Two (2) ml of Benedict's solution was put in 1 ml of the extract filtrate. The resulted mixture was heated using water bath for 5 minutes. Development of rusty brown coloured precipitates showed the presence of reducing sugar.

Test for Starch

Iodine Test

One (1) drop of iodine solution was added to 0.1 g of sample extract. Development of blue-black colouration showed starch presence.

Test for Resins

(a) Precipitation Test

About 15 ml of ethanol (96 %v/v) was used to extract 0.2 g of the ground sample. The extract from the ethanol extraction was added to 20 ml distilled water in a test tube and observed for precipitates formation, which indicated positive result for resins.

(b) Colour Test

Extraction of 0.12 g of sample was done with chloroform, with the extract concentrated to dryness. Equal mixture of acetone (3 ml) and concentrated hydrochloric acid (3 ml) was used to re-suspend the residue with the resulted mixture heated for 30 minutes in a water bath. Presence of resins is indicated by development of pink colour which changed to magnet red.

Test for Tannins

One (1) ml of distilled water was mixed with 0.5 ml of extract followed by addition of Ferric Chloride solution (2 drops). Development of blue colour showed the presence of gallic tannins while green black colour indicated the presence of catecholic tannins.

Test for Glycosides

Hydrolysis of glycosides gives two products, glycones (sugar) and aglycones or genine (non-sugar). Presence of glycosides in a solution is characterised by reddish brown colour in between two layers and bluish green colour at the upper layer. The extract solution was put in glacial acetic acid and, ferric chloride and conc. H_2SO_4 added drop wise. Presence of glycosides was indicated by development of reddish brown colour at the junction of the two layers and bluish green colour at the upper layer.

Test for Flavonoids

A quantity 0.2 g of the powdered plant material was mixed with ethylacetate (10 ml) and boiled for 3 minutes using water bath. The resulted mixture was allowed to cool, subsequently filtered with the filtrate subjected to further tests below:

(a) Ammonium Test

One (1) ml dilute ammonia solution was added to 4 ml of filtrate and mixed thoroughly. The mixture was left standing to separate into layers and flavonoids presence was indicated by the development of yellow colour at the ammoniacal layer.

(b) 1 %w/v Aluminium Chloride ($AlCl_3$)Solution

To 4 ml portion of filtrate was added 1 ml $AlCl_3$ solution (1 %w/v). Development of yellow colour in the $AlCl_3$ layer after separation into layers showed flavonoids presence.

Test for Cyanogenic Glycosides

An alkaline picrate colorimetric method was used. One gram (1 g) of the processed *T. tetraptera* was dispersed in 150 ml of distilled water in a conical flask. An alkaline picrate paper was suspended over the mixture and held in place by a rubber bung (stopper).Care was taken to avoid the paper touching the surface of the mixture. The arrangement was allowed to stand for 18 hours (over-night) at room temperature. The picrate paper was examined for colour change from yellow to orange as a positive test for the presence of cyanogenic glycoside.

Quantitative Phytochemical Analysis

Determination of Saponin

The amount of saponins contained in the fruit pulp was determined according to description contained in (25).

Twenty (20) g of the ground sample was dissolved in 100 ml of aqueous ethanol (20 % v/v) using a conical flask. The resulted mixture was concentrated using a water bath under continuous stirring for 4 minutes at 55 ˚C. This was filtered, with the residues further extracted in 200 ml of the ethanol. The extracts were pooled and concentrated to 40 ml using water bath operating at 90 ˚C. Recovery of the aqueous layer was done by transferring the concentrate to a separating funnel (250 ml) with the addition of 20 ml diethylether (to remove impurities) vigorously mixed. The layer containing the ether was thrown away with repeat of the process of purification. Separation of the recovered layer was achieved by addition of 60 ml n-butanol (absorbs saponins). The bottom layer was discarded and the supernatant containing n-butanol was recovered. Ten (10) ml of NaCl (5 %w/v) was used to purify the extract by washing it two times. The lower layer discarded again and the upper layer recovered, which was concentrated in a water bath. This was further dried to constant weight in oven.

$$\% \text{ Saponin} = \frac{W_2 - W_1}{Weight\ of\ Sample} X\ 100 \qquad (7)$$

W_1 = Weight of empty crucible, W_2 = Weight of crucible + sample after oven drying.

Determination of Total Phenols

The total phenolic constituent of the sample was determined by spectrophotometric method.

Two gram (2 g) of ground sample was defatted for 2 hours by adding 100 ml of diethylether in a soxhlet apparatus. Fifty (50) ml of diethylether was added to the defatted sample and heated for 15 minutes for phenolic compounds to be extracted. Five (5) ml extract was transferred to 50 ml Erlenmeyer flask followed by the addition of 10 ml distilled water. One (1) ml standard phenol solution prepared in like manner. Two (2) ml ammonium hydroxide (NH_4OH) solution was added followed by the addition 5 ml conc. amylacohol to each test tube. The mixture was brought to 50 ml mark and allowed to stand for 30 minutes to develop colour. This was measured spectrophotometrically at 505 nm wavelength.

$$\text{Dilution Factor (DF)} = \frac{Volume\ of\ flask\ (50\ ml)}{Volume\ of\ extract\ (5\ ml)} = 10$$

$$\% Phenol = \frac{100\ x\ AS\ x\ CS\ x\ VF\ x\ DF}{Wt\ x\ AP\ x\ 1000\ x\ VE} \qquad (8)$$

AS = Absorbance of sample solution, AP = Absorbance of standard phenol solution, CS = Concentration of Standard Phenol solution, VF = Total volume of extract, Wt = Weight of sample used, VE = Extract volume analysed,
DF = Dilution factor used.

Determination of Tannin

The amount of tannin contained in the sample was estimated according to (26).

A 0.5 g of ground sample was dissolved in 50 ml of distilled water contained in 250 ml conical flask. This was stirred using magnetic stirrer for 1 hour, with 5 ml of filtrate dispensed into a 50 ml volumetric flask.

A measured weight of tannic acid (0.1 g) was dissolved in 100 ml of distilled water and 5 ml of the tannic acid solution pipetted out into 50 ml volumetric flask. A blank sample was set up by using 5 ml of distilled water. One (1) ml Folin-Dainas reagent was put in each and 2.5 ml of sodium carbonate (saturated Na_2CO_3) solution added. Fifty (50) ml distilled water was added to dilute the mixture, and the absorbance of the dark color, which developed, was measured in a spectrophotometer after incubation at 25 ˚C for 90 minutes. The absorbance was measured at a wavelength of 760 nm with a reagent blank at zero.

$$\% \text{ Tannin} = \frac{AT\ X\ VF\ X\ DF}{AS\ X\ VE\ X\ WS} X\ 100 \qquad (9)$$

AT = Absorbance of tannic solution, *AS* = Absorbance of sample solution

VF = Total volume of filtrate, *VE* = volume of extract analysed
DF = Dilution factor, *WS* = Weight of sample.

Determination of Flavonoid
The flavonoid content of *Tetrapleura tetraptera* fruit was determined according to (27).
A measured weight of the sample (10 g) was repeatedly extracted using 100 ml of methanol (80 % w/v) at room temperature for 30 minutes. The resulted mixture was separated by filtration using a 125 pore-size filter paper (Whatman No. 42). The filtrate was evaporated to constant weight on a crucible using water bath. Percentage flavonoids were calculated thus:

$$\% \text{ Flavonoid} = \frac{W_2 - W_1}{Weight\ of\ sample} X\ 100 \quad (10)$$

w_1 = Weight of empty crucible, w_2 = Weight of crucible + sample after oven drying.

Determination of Alkaloid
The alkaloid content in the sample was determined using (22) method.
Five (5) g of ground sample was dissolved in 200 ml hydrochloric acid in ethanol (10 %v/v) using 250 ml beaker. This was covered and kept for 4 hours. The mixture was separated again by filtration with the volume of the filtrate reduced to 25 % of original volume using water bath. Pigments and other unwanted materials were removed by shaking it with 100 ml chloroform in separating funnel. The free alkaloids were precipitated with the addition of excess ammonia. The resulted solution was also separated by filtration using 125 mm size filter paper (Whatman No. 42), with the resulted filtrate evaporated to dryness at constant weight using oven.

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{Weight\ of\ sample} X\ 100 \quad (11)$$

w_1 = Weight of empty crucible, w_2 = Weight of crucible + sample after oven drying.

Determination of Cyanogenic Glycosides
An alkaline picrate colorimetric method was used. One gram (1 g) of the processed *T. tetraptera* was dispersed in 150 ml of distilled water in a conical flask. One (1) ml standard cyanide solution was prepared in the same manner. An alkaline picrate paper was suspended over the mixture and held in place by a rubber bung (stopper).Care was taken to avoid the paper touching the surface of the mixture. The arrangement was allowed to stand for 18 hours (over-night) at room temperature. Sixty (60) ml distilled water was used to elute the picrate papers, the following day. Absorbance was read spectrophotometrically against reagent blank at 540 nm wavelength.

$$\% \text{ Cyanogenic glycosides} = \frac{AS\ x\ CS\ x\ DF}{Wt\ x\ AT}\ x\ 100 \quad (12)$$

AS = Absorbance of sample solution, CS = Concentration of cyanide solution, AT = Absorbance of cyanide solution, DF = Dilution factor, Wt = Weight of sample.

Statistical Analysis
Experimental data were determined in triplicates from three different fruits and results shown using charts and tables as mean ± standard deviation. One- way ANOVA (analysis of variance) via SPSS package, version 16 was used for data analysis.

RESULTS AND DISCUSSION

Proximate Composition
Profiling of proximate parameters of the pulp of *Tetrapleura tetraptera* fruit revealed a significant differences ($p<0.05$) for ash, crude fibre, crude protein, fats and oil, moisture and carbohydrate as shown in figure 1 below. According to this study, carbohydrate content (40.12±2.57) had highest value while crude fibre (6.84±0.31) had the lowest content in the assayed fruit.
Carbohydrate provides energy to body cells, especially the brain cells that solely depend on glucose (component of carbohydrate) for its function (28). It is also essential for stability of plasma level and prevents easy degradation of body protein to obtain energy. Hence, it is the primary source of energy for organisms. The high content of carbohydrate revealed by this study suggests that the plant provides the body with efficient and easy access energy. The result of this study is in agreement with earlier research by (17), who reported that carbohydrate had the highest composition in 20 accessions of *T. tetraptera* obtained from different locations in Cross River State, Nigeria.
The moisture content of the test fruit (19.90±1.65%) was higher than the values (7.37% - 10.64%) reported by (17). This could be attributed to the fact that the fruits were freshly harvested as reported by the dealer. The amount of moisture contained in a food is indicative of water activity of the food. Therefore, it is used to determine food's susceptibility and stability to spoilage microorganisms. The moisture content of the fruit is low when compared to other fruits and this proves its seeming resistance in nature to antimicrobial degradation, thereby improving its shelf-life (29). However, moisture plays a helpful role in food digestion, weight loss, better productivity at work and relief of fatigue.
The ash content was observed to be higher (17.84±1.63%) than 2.86 – 4.8% reported by (17), 9% by (14) and 10.5% by (30). The differences in these values may be attributed partially to the choice of analytical methods and the variation in soil micronutrients at the site of collection. High ash content in foods denotes high mineral content. Minerals function in water balance, bone health and body metabolism.
The percentage crude fibre in the present study (6.84±0.31%) was higher than 2.79 – 4.81% reported by (17) and 4.5% by (14), but lower than 7.76% and 8.75% reported by (31) and (32) respectively in *T. tetraptera*. The crude fibre content of the test fruit is comparable to that of mango fruits (5.428 – 7.380%) as reported by (33). Fibre is one of the essential body nutrients. Its presence in foods help to lower risks of constipation, high blood pressure, diabetes, cardiovascular disease, cancer and obesity. It is worthwhile to mention that, high value reported in this study is not a disadvantage to the usage of this plant since the fruits are utilized as spice and flavouring agent and not consumed directly.

The fat content (8.69±1.82%) was lower than 11.79 – 21.71 % reported by (17) but higher than 4% and 5.6% reported by (14) and (30) respectively, and similar to 8.5% reported by (31) in the same plant. Fats give palatability to foods, serve as storage and transport forms of metabolic fuel, serve as thermal/electrical insulators for subcutaneous tissues, emulsifier for drug preparation and forms structural components of biomembranes. Essential fat-soluble vitamins are processed (transported) by dietary lipids and consumption of much fats are known to cause cardiovascular diseases such as atherosclerosis, cancer and ageing (34).

The percentage composition of protein was 14.10±1.77% which is higher than 5.48 – 7.8% reported by (17) and 5.0% by (30) in this plant. Protein is an index of food's calorific value. Hence, any plant food that cannot supply up to 12 % of its caloric value from protein is generally not considered as a good source of protein (17, 35). Protein is very essential for healthy growth in children, repair and maintenance in adult, production of immunoglobulins for body defence, production of enzymes and increase muscle mass.

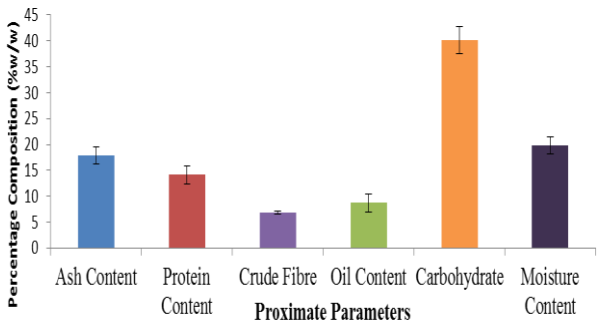

Figure 1: Proximate Composition of the pulp of *Tetrapleura tetraptera* Fruit

Qualitative Phytochemical Analysis

The ground sample was subjected to screening to establish the presence or absence of some specific active principles. The phytochemical screening of the pulp of *T. tetraptera* fruits showed that tannins, phenolic compounds, saponins, alkaloids, steroids, glycosides, resins, terpenoids and flavonoids were present and could be considered responsible for its varied biological and pharmacological properties as shown in table 1. Saponins, tannins, steroids, terpenoids and carbohydrates were present in very high concentration while glycosides showed weak positive reaction. The result of the qualitative phytochemical analysis of *T. tetraptera* fruit is at par with previous reports by (36) who studied flavonoids, tannins and alkaloids in many plants and found that, these chemical constituents were found in sufficient amounts in the studied plants.

Table 1: Qualitative Phytochemical Screening of the Pulp of *T. tetraptera* Fruit.

S/N	Phytochemical Constituent	Experimental Method	Relative Presence
1	Carbohydrates		
a.	Carbohydrates	Molisch's Test	+++
b.	Polysaccharide	Iodine Test	-
c.	Reducing Sugar	Benedict's Test Fehling's Test	+++ +++
d.	Reducing Monosaccharide	Barfoed's Test	+++
e.	Pentose Sugar	Bial's Test	
f.	Ketose Sugar	Seliwanoff's Test	+++
2.	Protein		
a.	Protein	Biuret's Test	+++
b.	Protein	Picric acid Test	+++
c.	α-amino acid	Ninhydrin Test	++
d.	Aromatic amino acid	Xantheoprotein Test	+
3.	Saponins	Frothing Test Emulsion Test	+++ +++
4.	Tannin	Ferric Chloride Test	++
5.	Flavonoids	Ammonium Test	+++
6.	Glycosides		+
7.	Alkaloids	Picric acid Test Wagner's Test	+ +
8.	Steroids		+++
9.	Terpenoids		+++
10.	Resins	Precipitation Test Colour Test	++ ++
11.	Oil	Filter Paper Test	++

+ = Present in trace concentration, ++ = Present in moderately high concentration, +++ = Present in very high concentration, - = Absent.

Quantitative Phytochemical Analysis

The result of the quantitative phytochemical composition of the pulp of *T. tetraptera* fruit presented in figure 2 showed that flavonoids, tannins, saponins, glycosides, phenols and alkaloids were present. The test fruit exhibited higher quantities of saponins (5.670±0.294%) and tannins (3.194±0.323%) but possess trace amount of glycosides (0.0072±0.0002%).

The values reported for saponins is higher than 0.54% reported by (36) and 0.44 – 0.80% by (17) in the same plant. Kannabiran *et al*. (38) studied the antibacterial activity of saponin extracted from the leaves of *Solanum tricobatum* and reported that, there is a linear relation between the saponin content and antibacterial activity. However, (39) reported that saponins have the ability to combine with cholesterol, imparts bitter taste and cause haemolytic activity in water-based solutions. Present study indicated higher saponin content, which might be accountable for the antimicrobial and therapeutic values of the plant, as reported by some researchers.

Tannin was also found at high quantity in the test fruit. Tannins are known to be anti-nutrients in diets, are responsible for astringency and poor taste of food, and drink (40). Tannins interact with proteins forming insoluble complexes, thereby reducing its bioavailability (41). Studies by (42) and (43) showed that tannins are used in the treatment of diarrhoea, dysentery and urinary tract infections. Hence, high content of

tannins in this plant may be responsible for utilization of the fruit in treating gastrointestinal disorders.

The phenolic content (0.372633±0.0126%) was higher than 0.05 – 0.12% reported by (17) in 20 accessions but similar to 0.42% reported by (37). Tawaha *et al.* (44) established a close association between phenolic content and antioxidant activity of some Jordanian Plant species studied. These researchers concluded that phenolic compounds were the major antioxidant machinery in the plant species studied. The finding of the present study is in line with previous reports that there is a linear relationship between antioxidant activity and total phenolic content, due to appreciable quantity of phenols in the fruit. This suggests that phenolic compounds significantly contribute to the antioxidant potential of the fruit of the plant as reported by some investigators.

The alkaloids content (0.633±0.057%) was lower than 1.73% and 1.28% reported by (17) and (37) respectively in the same plant. Alkaloids have the most therapeutic significance among plant chemical constituents (17). It possesses analgesic, antispasmodic and antibacterial properties (45). Chemical fractionation of methanolic extract of stem bark of *Holarrhena pubescens* by (46) indicated that antibacterial activity of the plant was mainly associated with alkaloids. The present study shows that *T. tetraptera* may be a potential source of alkaloids justifying the exploitation of this plant in orthodox medical practice.

The flavonoid content in the test fruit (0.073± 0.002%) was lower than 1.63 – 3.84% and 0.82% respectively reported by (17) and (37). Flavonoids play antioxidative role against liver tumours, toxins, viruses and other microorganisms, allergies and inflammation (17). Flavonoids are powerful in protecting blood vessels particularly the tiny capillaries responsible for distribution and transport of nutrients and oxygen to cells and have been shown to delay cataracts development in diabetic patients (45). Flavonoids are important antioxidants that are significant in the removal of oxidative stress. The major drawback with the consumption of synthetic antioxidants is the side effects associated with in vivo utilization (47). Even though, the value of flavonoids reported here was lower than some reports, the plant fruit could be a potential source of flavonoids.

The cyanogenic glycosides reported in the present study (0.0072±0.0002%) were lower than 3.64 – 5.25 mg/kg reported by (17) in 20 accessions of the same plant. The presence of glycosides in the test fruit may be responsible for why the fruits are not consumed raw, but (31) revealed that glycosides can be reduced by cooking.

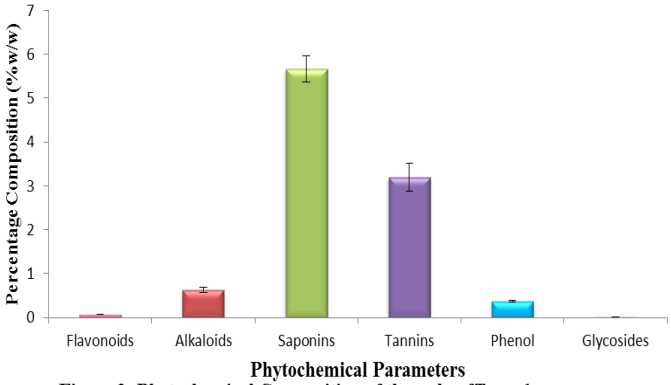

Figure 2: Phytochemical Composition of the pulp of *Tetrapleura tetraptera* fruit

CONCLUSION

The present study, which investigated the proximate and phytochemical compositions, established the fruits of *Tetrapleura tetraptera* marketed in Abakaliki to be a powerhouse of nutrients and phytochemicals, as it contained appreciable quantities of carbohydrates, proteins, fibre, ash, phenols, flavonoids, saponins, tannins, steroids and alkaloids. Therefore, the potential of this plant in promoting good health owing to considerable amounts of secondary metabolites and nutrients (therapeutic potential) cannot be overemphasized.

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