

# Comprehensive Phytochemical and antioxidant profiling of *Tetrapleura tetraptera* fruit: A Nutritional and Bioactive Potential Assessment from Rivers State, Nigeria

## ABSTRACT

The fruit, *Tetrapleura tetraptera* belongs to the Mimosaceae family and is widely used in traditional remedies for the treatment of diseases and a popular seasoning spice in southern and Eastern Nigeria. The present study investigated the proximate analysis, phytochemical composition and antioxidant studies of ethanol extract of *Tetrapleura tetraptera* fruit consumed in Port-Harcourt city in Rivers State. The proximate and phytochemical compositions, including the antioxidant studies of ethanol extract of *Tetrapleura tetraptera* fruit were determined using standard methods. Proximate composition and calorific value of whole sample of *Tetrapleura tetraptera* fruit revealed that the (%) composition of moisture, ash, lipid, fibre, protein, carbohydrate and caloric value are 17.07, 17.17, 10.10, 5.26, 15.77, 34.62 and 1222.28kj/100g respectively. The qualitative phytochemical analysis of *Tetrapleura tetraptera* fruit showed that they contain relative amount of flavonoids, saponins, tannins, terpenoids, glycosides, alkaloids, amino acids, steroids and coumarins. Glycosides, alkaloids and phenolics were detected in very small concentration in the extract, flavonoids, coumarins, amino acids were found in moderate amounts while saponins, tannins, steroids and terpenoids were detected in very high amount in the extract. The *in vitro* antioxidant activities (TAC, FRAP and DPPH) of ethanol extract of *Tetrapleura tetraptera* fruit shows that the plant possessed high scavenging potency toward free radicals. The results of *In vitro* antioxidant studies; FRAP, DPPH and TAC suggest that *Tetrapleura tetraptera* fruit protects against lipid peroxidation by donating the radical chain reaction. The results demonstrated the strong antioxidant activities, nutritional and pharmacology potencies of the *Tetrapleura tetraptera* fruit.

Keywords: *Tetrapleura tetraptera*, Proximate compositions, Phytochemical, Antioxidant and Ethanol Extract.

## INTRODUCTION

The fruits of *Tetrapleura tetraptera* are an indispensable medicinal plant with proven effectiveness in managing a number of health conditions [1]. The fruits are green when still soft, but are shiny, glabrous, and dark purplish brown when ripe and ripening. About 15–25 cm long and about 4–5 cm wide (depending on size) with four longitudinal wing-like ridges nearly 2.5–3.0 cm wide [2]. Two of these wings are woody, while the other two are soft and are used for eating, drinking, and healing. The small black hard and flat seeds are hidden in pods [3]. “The

plant belongs to the Fabaceae family. It is locally called by different names: It is known as Aidan in English, Oshosho or Osakirisa in Igbo, Aridan in Yoruba, Dawo in Hausa” [4]. “The plant is valued in Eastern, Southern and Western Nigeria and beyond for its nutritional and pharmacological properties due to a number of active nutrients” [5]. “The fruits of this plant have a fragrant, characteristic – pungent, aromatic smell and taste [6], which is responsible for its insect repellent properties” [7]. “Hence, it is used as a popular spice in eastern and southern Nigeria. The fruits are used to remove pungent odour and slow the growth of fungi in cassava fufu” [8]. “Medicinally, the fruit is used to prepare soup or porridge for nursing mothers from the onset of labour to prevent postpartum contractions” [9]; [10] ) and as a lactation aid [11] ; [12] in nursing mothers. “It is also used in the treatment of spasms, leprosy, inflammation, flatulence, jaundice, malaria, diabetes mellitus in adults, rheumatism and molluscicide” [13]; [14]; [15]; [16] and [17].

Proximate analysis refers to the nutritional composition of a substance, which includes protein, lipids, moisture content, ash content, fiber and carbohydrates, which can vary depending on factors such as species, diet and environmental conditions. However, it is a method that determines the value of a macronutrient in food samples. It is a system used to measure the chemical composition or components of food such as moisture, ash, protein, fat and fiber. Information from proximate analysis of a substance helps support health and other important aspects by identifying the physical constituents of a sample. A distinct disadvantage of this process is that it only measures macronutrients, but cannot determine micronutrients such as vitamins and minerals in the substance.

“Phytochemicals are chemical substances that occur naturally in plants” [18]. They are chemical compounds produced by plants in general to help them resist fungal, bacterial and plant viral infections as well as consumption by insects and other animals [19]. Some phytochemicals have been used as poisons and others as traditional medicine. Plants contain various types of chemical components such as flavonoids, glycosides, phenols, saponins, tannins, terpenoids, alkaloids, amino acids, steroids, coumarins and many others. Phytochemical analysis is essential for the identification of bioactive components in plants in order to develop new therapies and treatments. Phytochemical screening not only helps to reveal the components of plant extracts and the one that prevails over others, but also helps in the search for bioactive substances that can be used as a dietary supplement.

“An antioxidant is a substance that protects cells from damage caused by free radicals, which are unstable molecules produced by the body in response to environmental and other stresses” [20]. “These free radicals are unstable molecules created by the oxidation process during normal metabolism. Free radicals can increase the risk of inflammation and various health problems” [21]. They are sometimes called “free radical scavengers”. Free radicals may play a role in cancer, heart disease, stroke and other diseases of aging. The source of antioxidants can be natural or artificial. Some plant-based foods are believed to be rich in antioxidants. Plant antioxidants are a type of phytonutrients or plant nutrients. The body also produces some antioxidants known as endogenous antioxidants. Antioxidants that come from outside the body are called exogenous. Free radicals are waste products produced by cells when the body processes food and reacts to the environment. If the body cannot effectively process and remove free radicals, oxidative stress can occur. “Free radicals are also known as reactive oxygen species. When free radicals outnumber antioxidants, it can lead to a condition called oxidative stress. Factors that increase the body's production of free radicals can be internal, such as inflammation, or external, such as pollution, UV radiation, and cigarette smoke. Oxidative stress is associated with heart disease, cancer, arthritis, stroke, respiratory disease, immune deficiency, emphysema, Parkinson's disease, and other inflammatory or ischemic conditions” [20]. “The antioxidant is said to help neutralize free radicals in our body, and this is thought to promote overall health. The antioxidant acts as a scavenger of free radicals, thereby minimizing the harmful effects of free radicals on organisms” [4]. “Antioxidants are a major area of research due to their production as a pathophysiological response during times of imbalance against oxidative and antioxidant action. Antioxidants include beta-carotene, lycopene, vitamins A, C and E, lutein, selenium, manganese, zeaxanthin, glutathione, catalase, glutathione peroxidase, superoxide dismutase. Antioxidants can protect against cell damage caused by free radicals, known as oxidative stress. Activities and processes that can lead to oxidative stress include mitochondrial activity, excessive exercise, tissue trauma from inflammation and injury, ischemia and reperfusion injury, consumption of certain foods, especially refined and processed foods, trans fats, artificial sweeteners, additives, smoking, environmental pollution, radiation, exposure to chemicals such as pesticides and drugs, including chemotherapy” [8]. Such activities and exposures can lead to cell damage. This, in turn, can lead to an increase in enzymes that generate free radicals, excessive release of free iron or copper ions, disruption of electron transport chains. All of these can result in oxidative stress. Damage caused by oxidative stress is associated with cancer, atherosclerosis and vision loss. “According to studies, antioxidant act as radical scavenger, hydrogen donor, electron donor, peroxide

decomposer, singlet oxygen quencher, enzyme inhibitor, synergism and metal-chelating agents. Studies suggest that antioxidant cannot cure the effects of free radicals. At least not when antioxidants come from artificial sources" [12]. This raises questions about what free radicals are, and why they form. It is possible that free radicals are an early sign of cells already fighting disease, or that free radical formation is inevitable with age, without more data, it is impossible to understand the problem of free radicals fully. People interested in fighting free radical – related aging should avoid common sources of free radicals, such as pollution, and fried foods. They should also eat a healthful, balanced diet without worrying about supplementing with antioxidants.

Port-Harcourt city women often purchase these plant materials (*Tetrapleura tetraptera*) fruit for preparing of soup or porridge for nursing mothers from the beginning of childbirth to prevent post partum contraction and as lactation aid for nursing mothers. This plant material (*Tetrapleura tetraptera*) fruit has also been harnessed in the management of convulsions, inflammation, flatulence, malaria, diabetes mellitus and rheumatism. Besides these medicinal findings, there is little scientific evidence based on a thorough literature search and thus forms the basis of this research.

## **2. Material and Methods**

### **2.1 Plant Material**

The plant material are the fruits of *Tetrapleura tetraptera*.

### **2.2 Methods**

#### **2.2.1 Collection, Preparation and Extraction of plant fruit**

The plant material, (*Tetrapleura tetraptera*) fruit were purchased from Mile One market, Port-Harcourt, Rivers State, Nigeria. The fruit samples were identified and authenticated by Mr. Alfred Ozioko of the Bioresources Development and Conversation Programme (BDCCP), University of Nigeria, Nsukka, Enugu State. The specimen (*Tetrapleura tetraptera*) fruit voucher number is InterCEDD/085. They were thoroughly washed and air-dried for four weeks. The air-dried fruits were weighed using electronic weighing balance and milled with automatic electrical blender to powdered form and weighed (731.81g). The milled plant sample was later soaked for 72 hr at room temperature with 90% ethanol. After 72 hr, it was filtered using cheese cloth and then filtered through Whatmann 1 filter paper. The filtrate was concentrated using a water bath at a temperature of 47°C. After filtration, the filtrate was stored in an airtight container in a refrigerator until use.

#### **2.2.2 Proximate analysis of whole sample of *Tetrapleura tetraptera* fruit**

The whole sample of *Tetrapleura tetraptera* fruit were analyzed for proximate composition using standard analytical methods by [22].

### 2.2.2.1 Calorific Value Determination

Caloric value of whole sample of *Tetrapleura tetraptera* fruit was determined using the standard method [23].

Energy or Caloric Value (KJ/100g) = (Protein X 16.7) + (Lipids X 37.7) + (Carbohydrate X 16.7)  
[23]

### 2.2.3 Qualitative Phytochemical Screening of *Tetrapleura tetraptera* fruit

Phytochemical analysis of the *Tetrapleura tetraptera* fruits were carried out using the method described by [24] for the detection of saponins, tannins, phenolics, alkaloids, steroids, triterpenes, phlobatannins, glycosides and flavonoids.

**Alkaloids:** A known volume (500 $\mu$ l) of *Tetrapleura tetraptera* fruit extract, was added 1 ml (1% HCl) in a test tube. The mixture was heated for 20 minute, cooled and filtered. The filtrate was used in the following tests: 2 drops of Wagner's reagent was added to 1 ml of the extract. A reddish brown precipitate indicates the presence of alkaloids.

**Tannins:** A known volume (1000  $\mu$ l) of *Tetrapleura tetraptera* fruit extract, has 1 ml of freshly prepared 10% KOH added. A dirty white precipitate indicates the presence of tannins.

**Phenolics:** A known volume (1000  $\mu$ l) of *Tetrapleura tetraptera* fruit extract, was added 2 drops of 5% FeCl<sub>3</sub> in a test tube. A greenish precipitate indicates the presence of phenolics.

**Glycosides:** A known volume (500  $\mu$ l) of *Tetrapleura tetraptera* fruit extract, has 10 ml of conc. H<sub>2</sub>SO<sub>4</sub> added, the mixture was heated in boiling water for 15 minute. 10 ml of Fehling's solution was added and the mixture boiled. A brick red precipitate indicates the presence of glycosides.

**Saponins:** Frothing test: Distilled water (500  $\mu$ l) was added to 2 ml of extract in a test tube and was vigorously shaken for 2 minute. Frothing indicates the presence of saponins.

**Flavonoids:** In 3 ml of extract of *Tetrapleura tetraptera* fruit solution, 1 ml of 10% NaOH was added in a test tube. A yellow colouration indicates the presence of flavonoids.

**Steroids:** Salakowsti test: *Tetrapleura tetraptera* fruit extract (500  $\mu$ l), has 5 drops of concentrated H<sub>2</sub>SO<sub>4</sub> added in the test tube. Red colouration indicates the presence of steroids.

**Phlobatannins:** A known volume of (500  $\mu$ l) *Tetrapleura tetraptera* fruit extract, was added 1 ml of (1% HCl) in a test tube. A red precipitate indicates the presence of phlobatannins.

**Triterpenes:** A known volume of (500 µl) *Tetrapleura tetraptera* fruit solution, has 5 drops of acetic anhydride added with a drop of concentrated H<sub>2</sub>SO<sub>4</sub> and the mixture was steamed for 1 hour and neutralized with NaOH followed by the addition of 250 ml chloroform. A blue green colour indicates the presence of triterpenes.

**Phytosterols [25]: Liberman-Burchard's test:** A known volume of (500 µl) *Tetrapleura tetraptera* fruit solution, was added 50 mg which was dissolved in 2 ml acetic anhydride. Two drops of conc. H<sub>2</sub>SO<sub>4</sub> was added slowly along the sides of the test tube. An array of colour changes showed the presence of phytosterols.

**Xanthoproteic Reaction Test:** To five (5 ml) of sample filtrate was added few drops of Conc. HNO<sub>3</sub>. Protein was indicated by development of yellow colour which changed when alkali was added.

**Biuret Test:** A crystal of copper sulphate was added to 2 ml of the sample filtrate and 2 drops of potassium hydroxide added. A purple colour showed the presence of proteins.

#### **2.2.4 In Vitro Antioxidant activities of ethanol extract of *Tetrapleura tetraptera* fruit**

##### **2.2.4.1 Total Antioxidant Activity (TAC)**

Total antioxidant capacity of the extract was estimated using the phosphomolybdate method as reported by [26].

The total antioxidant capacity of the extract was determined with phosphomolybdenum method using ascorbic acid as the standard. The assay was based on the reduction of Mo (vi) to Mo (v) by the extract and the subsequent formation of a green phosphomolybdate (v) complex at acidic pH. 0.1 ml of the extract was mixed with 3 ml of reagent (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, absorbance of the aqueous solution of each was read at 695 nm against blank in a spectrophotometer. The blank solution contained 3 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions as the rest of the sample. The antioxidant capacity was expressed as the equivalent of ascorbic acid.

##### **2.2.4.2 FRAP (Ferric ion Reducing Antioxidant Power) Assay**

Ferric ions reducing power was measured according to the method of [27] with the slightest modification. In this method, to measure the reducing power of the extract, 2.5 ml phosphate

buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide solution was added to 1 ml of extract with various concentrations. After mixing and incubating the mixture at 50°C for 30 minute, the reaction was stopped by adding 2.5 ml of trichloroacetic acid (10%) solution to each sample. The samples were mixed and then centrifuged for 10 minute. The supernatant (2.5 ml) and 2.5 ml of FeCl<sub>3</sub> (0.1% W/V) were added to the mixture and was kept for 10 minute. The absorbance at 700 nm was measured in a spectrophotometer as the reducing power. The higher absorbance of the reaction mixture indicated the increased reducing power. Ascorbic acid was used as positive control. All tests were performed in triplicate.

#### **2.2.4.3 DPPH Free Radical Scavenging Assay**

DPPH (2,2- diphenyl – 1-picrylhydrazyl) Free Radical Scavenging Assay was carried out using the method of [28]. In the DPPH assay based on the scavenging ability of stable radical DPPH, fresh methanolic solution of DPPH (1 ml) was added to 2 ml of each of the methanol extract solutions with a concentration range of 2.5 – 100 µg/ml. The final DPPH was 0.1 mM. The mixture was shaken and incubated for 30 minute at room temperature in the dark and then absorbance was measured at 517 nm using a UV-Spectrophotometer. Methanol was used as a blank and ascorbic acid (Sigma, USA) was used as a positive control. All tests were performed in triplicate.

The activity was calculated based on the following equation:

$$\% \text{ Inhibition} = 100 \times \frac{(A_0 - A)}{A_0}$$

Where **A<sub>0</sub>** was the absorbance of the control reaction (containing no test compound) and A was the absorbance of the test compound.

#### **2.2.5 Statistical Analysis**

The biochemical data obtained from the study were analysed using IBM statistical product and service solutions (SPSS) version 26, and the results were expressed as Mean ± Standard deviation. Statistical difference between means were obtained using one-way analysis of variance (ANOVA), followed by Post Hoc Multiple Comparison Test (PHMCT). (P < 0.05) was considered statistically significant.

### **3. Results**

#### **3.1: Proximate Analysis of the whole sample of *Tetrapleura tetraptera* fruit**

The proximate analysis of the whole sample of *Tetrapleura tetraptera* fruit as presented in table 1 revealed that it has moisture content ( $17.07 \pm 5.53$ ), protein content ( $15.77 \pm 3.11$ ), crude fibre ( $5.26 \pm 0.89$ ), crude oil ( $10.10 \pm 0.50$ ), ash content ( $17.17 \pm 0.82$ ) and carbohydrate content ( $34.62 \pm 5.84$ ) respectively.

**Table 1: Proximate Analysis of the whole sample of *Tetrapleura tetraptera* fruit**

S/N	Proximate composition %	Composition (%)
1	Moisture Content	$17.07 \pm 5.53$
2	Protein Content	$15.77 \pm 3.11$
3	Crude Fibre	$5.26 \pm 0.89$
4	Crude oil	$10.10 \pm 0.50$
5	Ash Content	$17.17 \pm 0.82$
6	Carbohydrate Content	$34.62 \pm 5.84$

Results are expressed as Mean  $\pm$  SD; (n=3).

### 3.1.1 Caloric Value of the whole sample of *Tetrapleura tetraptera* fruit

Table 2 shows that the caloric value of the whole sample of *Tetrapleura tetraptera* fruit was 1222.39 kJ/100g.

**Table 2: Caloric Value of the whole sample of *Tetrapleura tetraptera* fruit**

Sample	Caloric value (KJ/100g)
<b>Whole Sample of <i>Tetrapleura tetraptera</i> fruit</b>	$1222.39 \pm 79.61$

Results are expressed as Mean  $\pm$  SD; (n=3);

### 3.2: Qualitative phytochemical composition of ethanol extract of *Tetrapleura tetraptera* fruit

The phytochemical constituents of *Tetrapleura tetraptera* fruit extract as presented in table 3 showed that they contain relative amount of flavonoids, glycosides, phenolics, saponins, tannins, terpenoids, alkaloids, amino acids, steroids and coumarins. Glycosides, alkaloids and phenolics were detected in very small concentration. Flavonoids, coumarins and amino acids were found in moderate amounts while saponins, tannins, steroids and terpenoids were detected in very high amount in the extract.

**Table 3: Qualitative phytochemical composition of ethanol extract *Tetrapleura tetraptera* fruit**

Phytochemical Constituents	Extract composition
<b>Phenolics</b>	+
<b>Saponins</b>	+++
<b>Tannins</b>	+++
<b>Flavonoids</b>	++
<b>Coumarins</b>	++
<b>Anthocyanins</b>	N.D
<b>Steroids</b>	+++
<b>Glycosides</b>	+
<b>Amino acid</b>	++
<b>Phlobatannin</b>	N.D
<b>Alkaloids</b>	+
<b>Terpenoids</b>	+++

#### Keys

- + Present in low concentration
- ++ Present in moderate concentration
- +++ Present in high concentration
- N.D Not Detected

### 3.3 *In vitro* antioxidant activities of ethanol extract of *Tetrapleura tetraptera* fruit

The Tables below shows the results of antioxidant activities of ethanol extract of *Tetrapleura tetraptera* fruit.

#### 3.3.1: FRAP ASSAY

The Ferric Reducing Antioxidant Power (FRAP) of *Tetrapleura tetraptera* fruit extract are shown in table 4 below. The higher the concentrations of the extract, the higher the absorbance recorded. FRAP measures the reducing potency of the extract (*Tetrapleura tetraptera*) and standard antioxidant. However, higher absorbance indicates higher reducing potency.

**Table 4: FRAP ASSAY**

FRAP ASSAY
Absorbance at 700 nm

Concentration ( $\mu\text{g/ml}$ )	Ethanol extract of <i>T. tetraptera</i>	Ascorbic acid
20	$0.35 \pm 0.03$	$0.37 \pm 0.02$
40	$0.36 \pm 0.05$	$0.38 \pm 0.04$
60	$0.46 \pm 0.04$	$0.47 \pm 0.03$
80	$0.54 \pm 0.04$	$0.56 \pm 0.02$
100	$0.57 \pm 0.03$	$0.60 \pm 0.06$

(Results are expressed as Mean  $\pm$  S.D); Standard = Ascorbic Acid; n=3.

### 3.3.2: Total Antioxidant Capacity (TAC)

The Total Antioxidant Capacity (TAC) of crude extract of *Tetrapleura tetraptera* fruit are shown in table 5. The higher the concentrations of the extract, the higher the absorbance recorded. However, The  $\text{IC}_{50}$  of crude extract of *Tetrapleura tetraptera* was 2.49 at a correlation of 0.988, while Ascorbic acid was 4.98 at a correlation of 0.958. Since, the lower the  $\text{IC}_{50}$ , the higher the antioxidant capacity. Therefore, antioxidant capacity of ethanol extract of *Tetrapleura tetraptera* fruit possess higher antioxidant capacities when compared to that of the standard (Ascorbic acid).

**TABLE 5: Total Antioxidant Capacity (TAC)**

Total Antioxidant Capacity		
Absorbance at 695 nm		
Concentration ( $\mu\text{g/ml}$ )	Ethanol extract of <i>T. tetraptera</i>	Ascorbic acid
20	$0.09 \pm 0.01$	$0.20 \pm 0.03$
40	$0.14 \pm 0.04$	$0.27 \pm 0.04$
60	$0.21 \pm 0.06$	$0.29 \pm 0.03$
80	$0.25 \pm 0.07$	$0.32 \pm 0.02$

<b>100</b>	0.29 ± 0.05	0.36 ± 0.06
IC <sub>50</sub>	2.49	4.98

**(Results are expressed as Mean ± S.D); Standard = Ascorbic Acid; n=3.**

### 3.3.3: DPPH Radical Scavenging Activity

Table 6 shows the DPPH Radical Scavenging Power of ethanol extract of *Tetrapleura tetraptera* fruit. The higher the concentration of the extract, the higher the % inhibition of DPPH radical. Thus, at the highest concentration of 100 µg/ml, the best inhibition of DPPH free radicals were observed. The IC<sub>50</sub> for crude extract of *Tetrapleura tetraptera* was 2.12 at a correlation of 0.623, while IC<sub>50</sub> for ascorbic acid was 1.67 at a correlation of 0.797. The results shows that the percentage inhibition has direct relationship with the concentrations of the extract. The lower the IC<sub>50</sub>, the higher the antioxidant capacity and the higher the IC<sub>50</sub>, the lower the antioxidant potential. Hence, The Ascorbic acid (the standard) possessed higher antioxidant capacities when compared to that of the organic substance (*Tetrapleura tetraptera*) fruit.

**Table 6: DPPH Radical Scavenging Activity**

DPPH Assay		
Absorbance at 517 nm		
Concentration (µg/ml)	% Inhibition of ethanol extract of <i>T. tetraptera</i>	% Inhibition of Ascorbic acid
<b>20</b>	13.33%	8.33%
<b>40</b>	10.00%	13.89%
<b>60</b>	16.67%	22.22%
<b>80</b>	36.67%	44.44%
<b>100</b>	56.67%	61.11%
IC <sub>50</sub>	2.12	1.67

**Standard = Ascorbic Acid; n=3.**

## 4. Discussion

Proximate parameters of the whole sample of *Tetrapleura tetraptera* fruit showed a significant differences ( $p < 0.05$ ) for ash, crude fibre, crude protein, fats and oil, moisture and carbohydrate as presented in (table 1) above. According to this study carbohydrate content ( $34.62 \pm 5.84$ ) had the highest value while crude fibre ( $5.26 \pm 0.89$ ) had the lowest content in the assayed fruit. Carbohydrates are substance which produce polyhydroxyl aldehydes or ketones on hydrolysis.

Carbohydrates are widely distributed in plant and animal tissues. They are produced during photosynthesis in plants and some micro-organisms. They serve as structural and supportive elements in the cell walls of micro-organisms, plants and animals such as cellulose and chitin. It also serve as lubricants between skeletal joints example hyaluronic acid. It is essential for stability of plasma level and prevents easy degradation of body protein to obtain energy. The high content of carbohydrate revealed by this study suggests that the plant provides the body with efficient and easy access of energy. The result of this study is in agreement with the earlier research done by [29] who reported that carbohydrate had the highest composition in 20 accessions of *Tetrapleura tetraptera* obtained from different locations in Cross River State, Nigeria. The result of this study is also in tandem with [30] who reported that the percentage carbohydrate had the highest proximate composition of the pulp of *Tetrapleura tetraptera* consumed in Abakiliki, Nigeria. Calories of food represent all the energy that the human body can produce during metabolism and are expressed in kilojoules per 100 grams. In this study, the caloric value of *Tetrapleura tetraptera* fruit was found to be 1222.39 kJ/100 g.

“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 micro-organisms. The higher the moisture content of a substance, the more is susceptible to spoilage by microbial action. 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. This findings is in consonant with the work done by” [30]. “The moisture content of the fruits of *Tetrapleura tetraptera* (17.07%) was similar in percentage composition when compared to” [30] whose moisture content was  $(19.90 \pm 1.65 \%)$ .

Ash content is an indication of mineral constituents of *Tetrapleura tetraptera* fruits. A high content of ash in food means a high content of mineral components. Minerals affect water balance, bone health and body metabolism. The results of the whole sample of *Tetrapleura tetraptera* fruits were not consistent with the findings of [29], [16] and [31]. Ash content was observed higher  $(17.17 \pm 0.82)$  than  $(2.86 - 4.8\%)$  reported by [29], 9% by [16] and 10.5% according to [31]. The difference in these values can be partly attributed to the choice of analytical methods and the variation of soil micronutrients at the sampling site.

Fibre content is the roughage content of the food substance. It is one of the basic nutrients of the body. Its presence in food helps reduce the risk of constipation, high blood pressure, diabetes, cardiovascular disease, cancer, obesity and improves bowel movement. These findings are not consistent with the documentation of [29], [16], [32] and [33] respectively. The

percentage of crude fiber in this study ( $5.26 \pm 0.89$ ) was higher than 2.79 as reported by [33] for *Tetrapleura tetraptera*.

“Lipids are a class of cellular biomolecules that are poorly soluble in water and other polar solvents, while highly soluble in nonpolar organic solvents. They are structural components of cell membranes. It also acts as the main fuel reserve for the body's metabolism. Their chemical structure and composition gives them a higher heat content than in the case of carbohydrates and proteins. When adipose tissues are deposited around vital organs, they serve as protection against mechanical damage. They are an important part of the outer protective shell of many organisms. In warm-blooded animals, they form an insulator where they are stored in the subcutaneous tissue. These findings contradict the work of” [16]. The crude oil content ( $10.10 \pm 0.50$ ) was higher than 4% and 5.6% as reported by [16].

Proteins are indispensable agents of biological function. They were the first biologically active macromolecules that were assembled before the cell was formed. Proteins fulfill a number of important functions: hormonal, structural, enzymatic and transport in all organisms. It is an indicator of the calorific value of food. Therefore, any plant food that fails to supply up to 12% of its caloric value from protein is generally not considered a good source of protein [29]. Very similar to this finding are the results [30] whose protein percentage is comparable to the % protein result of this research finding. However, this result contradicts the documentation done by [31] and [29]. The percentage protein composition was ( $15.79 \pm 3.11$ ), which is higher than 5.48-7.8% by [29] and 5.0% by [31].

The ethanolic extract of *Tetrapleura tetraptera* was subjected to phytochemical screening to determine the presence or absence of any specific bioactive substance. Phytochemical screening of *Tetrapleura tetraptera* fruit extract showed that they contain relative amounts of flavonoids, glycosides, phenols, saponins, tannins, terpenoids, alkaloids, amino acids, steroids and coumarins. Glycosides, alkaloids and phenolics were detected in the extract in very low concentration. Flavonoids, coumarins, amino acids were found in moderate amounts, while saponins, tannins, steroids and terpenoids were found in very high amounts in the extract. The detected phytochemical compounds have medical importance and pharmacological therapy. This result was in agreement with [34] who studied flavonoids, tannins and alkaloids in many plants and found that these chemical components were found in sufficient quantities in the studied plants. This finding is also consistent with the results of [30] whose phytochemical screening has almost all the phytochemical content discovered in this study.

The Ferric Reducing Antioxidant Power (FRAP) assay is a new method used to assess reduced ferric ion concentration. This assay is based on the redox antioxidant reaction that measures the reducing power of extract and standard antioxidant [35]. Higher absorbance indicates higher reducing potency. In this study, increased concentrations showed increased absorbance of free radicals. In this study, increased concentrations indicated increased absorption of free radicals. Thus, at a concentration of 100 µg/ml, the highest reduction efficiency was achieved in the extract. This study is also in agreement with the findings of [36] that showed an increased antioxidant activity across the concentrations when compared with ascorbic acid in the FRAP analytical assessments. The findings of [10] is in tandem with this study documenting that the FRAP assay also showed an increase in percent inhibition with a corresponding increase in concentration. Substances with high antioxidant capacity tend to release their electrons and capture reactive oxygen species and free radicals. This potency can remove many diseases from the body and thus individuals can live healthily.

The Total Antioxidant Capacity (TAC) assay is also referred to as the phosphomolybdenum (PM) assay. The TAC assay is a quantitative method used to investigate the rate of the reduction reaction between antioxidants, oxidants, and a molybdenum ligand, providing a direct estimate of the antioxidant's reducing capacity. In this study, the higher the concentration of *Tetrapleura tetraptera* fruit extract, the higher the absorbance. The antioxidant activity depends on the presence of its bioactive compounds, especially phenols, flavonoids, triterpenes, alkaloids and coumarins. This suggests that the concentration of bioactive compounds present in the extract is important to demonstrate antioxidant activity. Thus, a higher concentration of the extract shows a higher antioxidant activity. The IC<sub>50</sub> of crude extract is 2.49 at a correlation of 0.988, in comparison to the IC<sub>50</sub> value of ascorbic acid (4.98 µg/ml) at a correlation of 0.958. The extract showed good antioxidant activity, which increased with increasing concentration when compared to the standard ascorbic acids

.The DPPH method is a spectrophotometric procedure used to analyze the free radical activity of natural compounds [37]. The antioxidant activity of a substance can be expressed as its ability to scavenge DPPH free radicals. For DPPH radical scavenging activity, the higher the concentration, the higher the free radical inhibition %. This is because the extract has a greater tendency to donate electrons to scavenge free radicals. The IC<sub>50</sub> for crude extract was 2.12 at a correlation of 0.623 in comparison to the IC<sub>50</sub> value of ascorbic acid (1.67 µg/ml) at the correlation of 0.797. This findings is in line with [10] whose work showed that DPPH radical scavenging activity assay revealed an increase in percentage inhibition with a corresponding

increase in concentration. The results of this study are in agreement with [38], whose findings revealed that *Parkia biglobosa* leaf extracts exhibited considerably potent radical scavenging activity against the DPPH radical and the observed effect was dose-dependent, with the highest activity occurring at extract concentration of 250 µg/ml.

## 5 Conclusion

The study investigated the proximate composition, phytochemistry and antioxidant potentials of the extract of *Tetrapleura tetraptera* fruit consumed in Port-Harcourt city, Rivers State to be the power house of nutritional and pharmacological properties, bioactive compounds, with high antioxidant capacities. Ethanol extract of the fruit of *Tetrapleura tetraptera* contain contain relative amount of flavonoids, glycosides, phenolics, saponins, tannins, terpenoids, alkaloids, amino acids, steroids, coumarins, with adequate nutrients and significant radical scavenging activities. However, this study is in support of ethnomedical uses and could be evaluated for other pharmacological activities.

**Ethical approval:** The Department of Biochemistry, Madonna University, Nigeria, Elele, Rivers State approved the use of animals for this research study. All the experiments has been examined and approved by the appropriate ethics committee.

## Authors' Contributions

This work was carried out in collaboration among all authors. Authors MNN, NFG and UOA designed the study, wrote the protocol and supervised the work. Author AJW and AB performed the statistical analysis. Authors CSO, EBA and MOE managed the analyses of the study. Author MNN wrote the first draft of the manuscript, managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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