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## Comparative Evaluation of Bioactive Constituents and Antioxidant Capacity of *Tetrapleura tetraptera* Aqueous, Ethanolic and Methanolic Fruit Extracts

Onoriode Akeghware<sup>1,2,§</sup>, Oghenetega Jonathan Avwioroko<sup>3,4</sup>, Akpovwehwee Akporhwarho Anigboro<sup>1</sup> & Nyerhovwo John Tonukari<sup>1</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Science, Delta State University, Abraka, Nigeria; <sup>2</sup>Department of Biochemistry, Faculty of Basic and Applied Sciences, University of Africa, Toru-Orua, Bayelsa State, Nigeria; <sup>3</sup>Department of Biochemistry, Faculty of Basic Medical Sciences, Redeemer's University, Ede, Osun State, Nigeria; <sup>4</sup>Center for Chemical and Biochemical Research (CCBR), Redeemer's University, Ede, Osun State, Nigeria.

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<sup>§</sup>Corresponding Author: Onoriode Akeghware, Email: [akeghware.onoriode@delsu.edu.ng](mailto:akeghware.onoriode@delsu.edu.ng)

### Abstract

This study aimed at comparative analysis of bioactive constituents and *in vitro* antioxidant capacity of *Tetrapleura tetraptera*'s aqueous, ethanolic and methanolic fruit extracts. Coarsely ground dried matured fruits of the plant were extracted with distilled water, absolute ethanol and methanol, followed by filtration and complete evaporation at 50°C to produce the respective extracts for analysis of phyto-constituents, nitric oxide radical inhibition and total antioxidant capacity. The results revealed extraction yields of 14.75, 13.26 and 19.09% dry weight respectively and variation in the occurrence of the groups of bioactive compounds that were analysed. Also, the antioxidant assays showed moderate activities of the extracts relative to the standard compound (ascorbic acid) used. The inconsistency in the order of antioxidant activity in the different assays may be due to the variation of assay outcome with different components of the extracts in relation to the experimental method and condition. Thus, summation of the ranks of the IC<sub>50</sub>/EC<sub>50</sub> of antioxidant potentials obtained from both assays suggests that the overall capacity of the aqueous > ethanolic > methanolic fruit extract. Corroboration from this study is important in choice of extraction solvent during the utilization of this fruit against oxidative stress-related disorders. However, the use of more methods of evaluation is recommended for further study to provide a clearer order of these extracts' antioxidant activity.

**KEYWORDS:** *Tetrapleura tetraptera*, Antioxidant, Free Radical, Oxidative Stress, Bioactive Compounds.

### Introduction

There is increasing interest in plants for natural therapies due to the minimal side effects arising from their usage. The determination of the bioactive components of plant extracts is essential in order to ensure the reliability and repeatability of pharmacological and clinical research. It also helps to understand their bioactivities and possible side effects of the active compounds through toxicity evaluation protocol (Xiao *et*

*al.*, 2016; Anigboro *et al.*, 2019). Reactive oxygen species (ROS) play significant role in the pathogenesis and/or development of complications in numerous diseases including obesity, autoimmune disease, cancer, diabetes and cardiovascular diseases. These ROS induce damages to cells (that can lead to chronic illnesses of varying severity) via protein denaturation, peroxidation of lipids, membrane instability and DNA mutation (Fadeyi *et al.*, 2013; Aikin *et al.*,

2021; Abankwah *et al.*, 2025). However, antioxidants are capable of neutralizing their activities in biological systems (Nakagawa *et al.*, 2002; Ahmad and Ahmed, 2006; Fadeyi *et al.*, 2013; Tonukari *et al.*, 2013, 2015; Anigboro *et al.*, 2018, 2024). *Tetrapleura tetraptera* is a flowering medicinal plant of the Fabaceae family that is found predominantly in the rain forest part of Africa (Adesina *et al.*, 2016). It is commonly known as aridan and evenwe amongst the Yorubas and Urhobos, respectively in Nigeria, and prekesse in Ghana. The matured fruits of this plant is utilized in herbal medicine formulation and as an ingredient in food (Odesanmi *et al.*, 2009). Preliminary investigation of the phytochemicals of *T. tetraptera* fruit peel extract has been reported (Abii and Amarachi, 2007; Erukainure *et al.*, 2017). The plant's parts have diverse medicinal purposes which include application in treatment of convulsion, obesity, inflammation and jaundice (Ojewole and Adewunmi, 2004) which may be linked majorly to its possession of antioxidant property (Badu *et al.*, 2012; Famobuwa *et al.*, 2016). The choice of solvent is very important when considering factors like solubility and safety of the extraction process/product (Kumar *et al.*, 2023; Lee *et al.*, 2024). Although, water and ethanol are commonly used traditionally for medicinal plant's preparations, methanol and other non-consumable solvents are also employed in the laboratory for extraction and isolation of different classes of compound (Zhang *et al.*, 2018; Anigboro *et al.*, 2019, 2022, 2024). Water is the safest and most environmentally friendly polar extraction solvent. It is cost-effective and can be employed (without special equipment) to extract water-soluble components including saponins, phenolic compounds, lipids and fatty acids, carbohydrates, minerals and vitamins. However, due to the high vapourisation energy of water, the process of drying aqueous extracts is usually tedious and disadvantaged by the duration involved (Kumar *et al.*, 2023; Jin *et al.*, 2023). Ethanol can efficiently extract various bioactive compounds like phenolics, terpenoids, lipids and fatty acids. It has the advantage of being a preservative and safe for both traditional and industrial applications (Azwanida, 2015). Methanol, a more polar solvent with higher solubility than ethanol, is also very effective for extraction (Spigno *et al.*, 2007; Carrera *et al.*, 2012), though its use is limited by its *in vivo* toxicity. The type and nature of the bioactive compounds obtained during extract preparation is influenced by the polarity and solubility of the extraction solvent used, which ultimately affect the intended therapeutic application (Spigno *et al.*, 2007; Gonfa *et al.*, 2020; Lee *et al.*, 2024). Thus, different solvents are used for extraction to increase chance of obtaining a variety of extracts, providing knowledge of which to choose for future utilization base on the yield, type/amount of specific class of compound and desired pharmacological property. This process is labour- and cost-intensive, necessitating the need for scientific evaluation and documentation of findings of this nature for future use. With the wide and extensive use of *T. tetraptera* in both nutritional, prophylactic and therapeutic applications, assessing the variation with these commonly used extraction solvents is useful for decision making in future

research and applications. Thus, this study is centered on solvent-dependent variation in the bioactive constituents and *in vitro* antioxidant capacity of *T. tetraptera*'s aqueous, ethanolic and methanolic fruit extracts.

## Materials and Methods

### Reagents and chemicals

Glass-distilled water and analytical grade chemicals/reagents were used for the various experimental analysis in this study.

### *Tetrapleura tetraptera* authentication and preparation of fruit extracts

Matured fruits of *Tetrapleura tetraptera* (Schumach. and Thonn.) Taub. were obtained from a site at Oginibo, Delta State. The plant specimen was authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Nigeria and voucher number (UBH-T472) was deposited in the herbarium. The standard cold maceration protocol modified by Anigboro *et al.* (2019) was used for the fruit extracts' preparation. Coarsely ground dried matured fruits were soaked in distilled water and absolute ethanol and methanol for 48 hrs, followed by filtration and evaporation to dryness over water at 50 °C to obtain the respective extracts. The extraction yield was calculated using the equation (1) below.

$$\text{Extraction yield} = \frac{\text{Weight of conc fruit extract}}{\text{weight of ground dried fruits}} \times 100 \dots(1)$$

### Qualitative determination of bioactive constituents

The qualitative analysis of the *T. tetraptera*'s fruit extracts was carried out as described by Njoku and Obi (2009), and Prabhavathi *et al.* (2016) to check for the presence of saponins, tannins, terpenes, steroids, alkaloids, thiols, phlobatannin, cardiac glycosides, reducing sugars and proteins.

### Quantitative analysis of bioactive constituents

More so, quantification of the *T. tetraptera* fruit extracts' tannin (Singleton and Rossi, 1965), protein (Lowry *et al.*, 1957), reducing sugar (Miller, 1959), alkaloid (Shamsa *et al.*, 2008), phytate (Vaintraub and Lapteva, 1988), was conducted using the respective standard procedures as reported by Anigboro *et al.* (2022). Calibration graphs of tannic acid, bovine serum albumin, glucose, atropine and dipotassium hydrogen phosphate dilutions were used to deduce the amounts of the above compounds, respectively in mg/g equivalent (dry weight) of extract.

### *In-vitro* Evaluation of Antioxidant Activity

The *in vitro* antioxidant activity of the different fruit extracts was assessed using nitric oxide radical (NO<sup>•</sup>) inhibition at 0.25 – 4.00 mg/ml concentrations using the standard methods of Marcocci *et al.* (1994). A mixture of sodium nitroprusside (2.00 ml; 10 mM), phosphate buffered saline (0.50 mL; 10 mM; pH 7.4) and the samples (0.50 mL) were incubated (25°C; 150 mins). The incubated solution (0.5 mL) is mixed with sulfanilic

acid (1.0 ml; 0.33 %) and naphthylethylenediamine dichloride (1.0 mL; of 0.1%) sequentially with incubation at the above temperature again for 5 and 30 mins, respectively. Similar preparations with ascorbic acid (reference antioxidant compound) and distilled water in place of the extract solution served as the standard and control, respectively. The optical densities of the control (OD<sub>control</sub>) and standard/extract (OD<sub>test</sub>) were recorded spectrophotometrically at 540 nm, while the percentage inhibition of NO<sup>•</sup> radical by the samples was calculated with the equation (2) below:

$$\text{Radical Inhibition (\%)} = \left( \frac{\text{OD}_{\text{control}} - \text{OD}_{\text{test}}}{\text{OD}_{\text{control}}} \right) \times 100 \dots\dots\dots(2)$$

Also, assessment of total antioxidant capacity (TAC) was done as reported by Prieto *et al.* (1999) by measuring the optical densities of incubated (95°C; 90 minutes) mixtures of the samples (0.10 ml) and 1.0 ml of the working reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4.0 mM ammonium molybdate), at 695 nm against blank. The inhibitory/effective concentrations (IC<sub>50</sub>/EC<sub>50</sub>) at which 50% activity or 0.5 absorbance were observed, were determined graphically.

**Statistical analysis**

The data obtained were analysed by One-way Analysis of Variance and Tukey post hoc test using **SPSS (version)**. The results were expressed as Mean ± Standard deviation, (n = 3) and compared at 95% confidence level, while standard calibration and results graphs were plotted using Microsoft Office Excel and GraphPad Prism packages, respectively.

**Results and discussion**

Across all fields of bioscience, *in vitro* studies are basically employed to unravel the basis through which biological processes take place in cells. In this study, the result of the extraction as well as the qualitative screening of biological constituents of *T. tetraptera*'s aqueous, ethanolic and methanolic fruit extracts (Table 1) revealed yields of 14.75, 13.26 and 19.09% dry weight of ground fruit, respectively. In all extracts, tannins, alkaloids and reducing sugars were confirmed present at varying degrees whereas terpenes and steroids were not. Saponins, phlobalanin and proteins were detected only in the aqueous extract, cardiac glycosides were found only in the ethanolic extract, while phenols and thiols were present in both the aqueous and methanolic extracts.

The yield obtained in the above result is in direct proportion to the polarity of the extraction solvents used, suggesting the presence of polar compounds in the plant's fruit. This finding is in alignment with the reports of Al-Fatimi *et al.* (2007) and Gonfa *et al.* (2020). However, the lowest yield of the aqueous extract may be due to losses associated with its prolonged extraction process. The variation in extraction yield was also reflected in the qualitative outcome of the extraction. The compounds detected are elemental in the plant's diverse pharmacological activities, including antioxidant potential (Okechukwu *et al.*, 2022; Anyamele *et al.*, 2023; Chi *et al.*, 2024; Mensah *et al.*, 2024; Abankwah *et al.*, 2025). For example, saponins are known to boost immunity and improve respiratory health, while terpenoids, alkaloids and tannins are useful for soothing inflammation (Okechukwu *et al.*, 2022; Ekeanyanwu *et al.*, 2024).

**Table 1: Qualitative Bioactive Constituents of *T. tetraptera*'s Aqueous, Ethanolic and Methanolic Fruit Extracts.**

<i>Tetrapleura tetraptera</i> 's Extract	Yield (% DW)	Bioactive Compounds										
		Saponins	Phlobatannins	Cardiac glycosides	Tannins	Terpenes	Alkaloids	Steroids	Proteins	Reducing Sugars	Thiols	
<b>Aqueous</b>	14.75	++	+	-	++	-	+	-	+	+	+	+
<b>Ethanolic</b>	13.26	-	-	+	+	-	+	-	-	+	-	-
<b>Methanolic</b>	19.09	-	-	-	+	-	+	-	-	+	+	+

**KEY:** (++) = Moderately Present; (+) = Present; (-) = Not Detected. The outcomes are presented based on the colour intensity observed in the reaction.

Variation in the solubility of different classes of compounds in different solvents results to difference in amounts obtained during extraction. Quantitatively, tannin was statistically the same in the ethanolic and methanolic extracts (p > 0.05) but significantly higher in the aqueous extract than the other two (p < 0.05). More so, significantly highest amounts of proteins and reducing sugars were measured in the aqueous, while the ethanolic extract recorded the lowest amounts (p < 0.05). There

was no significant difference in the amount of phytates in all extracts examined, while total alkaloid was the same only in the aqueous and ethanolic extracts (p > 0.05), with significantly higher amount detected in the *T. tetraptera*'s methanolic fruit extract (p < 0.05) (Table 2). The quantitative result is in consonant with the qualitative outcome with very slight inconsistency (Table 1) probably as a result of the method's sensitivity.

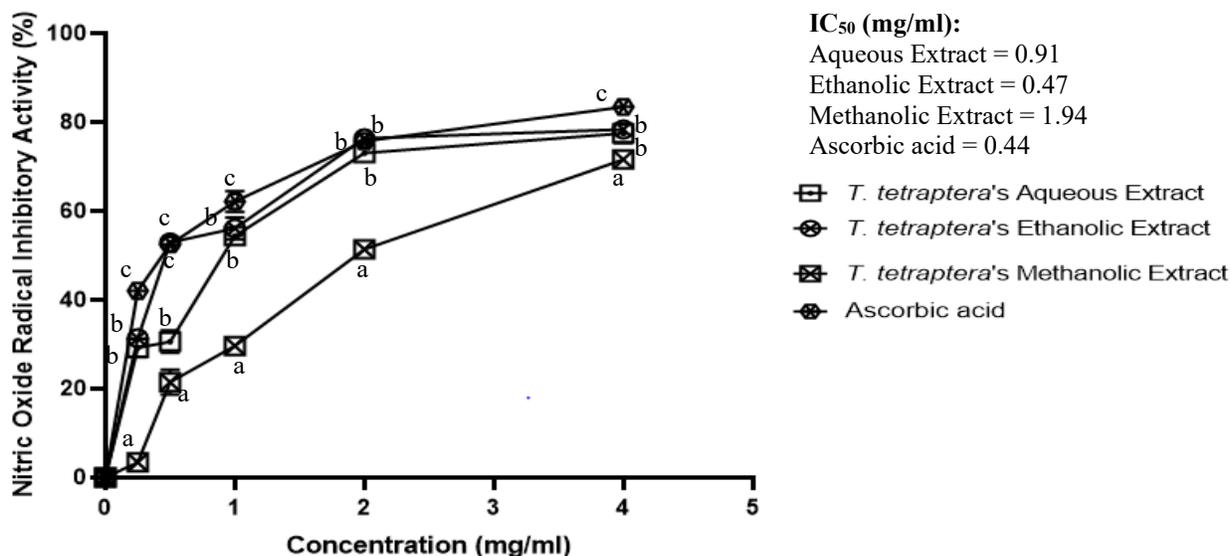
**Table 2: Quantitative Bioactive Constituents of *T. tetraptera's* Aqueous, Ethanolic and Methanolic Fruit Extracts.**

Bioactive Compounds	Amounts in <i>Tetrapleura tetraptera's</i> Extracts (mg Equivalent/g Dry Weight)		
	Aqueous	Ethanolic	Methanolic
Tannins (TAE)	7.13±0.25 <sup>a</sup>	6.20±0.12 <sup>b</sup>	6.32±0.16 <sup>b</sup>
Proteins (BSAE)	39.27±1.53 <sup>a</sup>	23.97±2.10 <sup>b</sup>	30.90±2.31 <sup>c</sup>
Reducing Sugars (GE)	160.81±0.33 <sup>a</sup>	151.44±0.26 <sup>b</sup>	156.52±1.12 <sup>c</sup>
Alkaloids (AE)	13.99±0.06 <sup>a</sup>	13.98±0.09 <sup>a</sup>	14.83±0.04 <sup>b</sup>
Phytates (PE)	41.03±0.02 <sup>a</sup>	41.05±0.00 <sup>a</sup>	41.03±0.02 <sup>a</sup>

Values are presented as Means ± Standard Deviation (n = 3). Values with different superscript alphabets on the same row, differ significantly (p < 0.05). TAE, BSAE, GE, AE and PE represent: Tannic acid, Bovine serum albumin, Glucose, Atropine and Dipotassium phosphate Equivalents, respectively.

In this study, estimation of the antioxidant activity was done by spectrophotometric measurements of colour changes. The result of the NO• scavenging activity of *T. tetraptera's* fruit extracts alongside the standard compound (Figure 1) showed that the methanolic extract had the lowest antioxidant activity while the standard compound possessed the highest activity (p<0.05) though it was not different from that of the ethanolic extract at 0.50 and 2.00 mg/ml concentrations (p>0.05). The ethanolic

extract's antioxidant activity was statistically the same with the aqueous except at 0.05 mg/ml where the former was significantly higher. Higher pharmacological activity is usually indicated by a lower value of IC<sub>50</sub>/EC<sub>50</sub>. The IC<sub>50</sub> showed that the NO• scavenging activity of Ascorbic acid > Ethanolic > Aqueous > Methanolic extract with values of 0.44, 0.47, 0.91 and 1.94 mg/ml, respectively



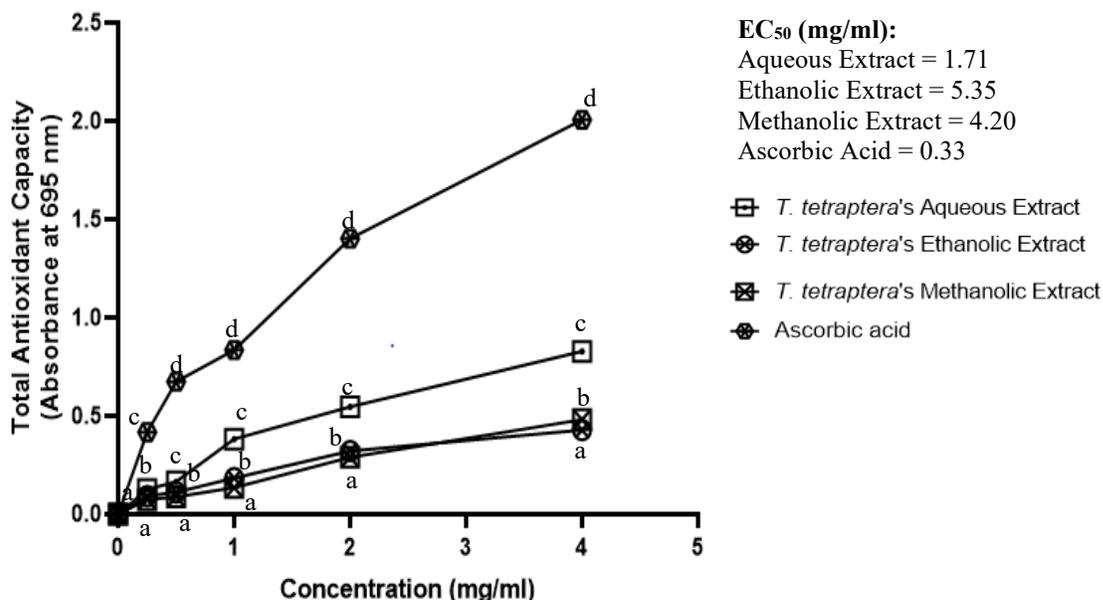
**Figure 1: In vitro Nitric Oxide Radical Inhibitory Activity of *T. tetraptera's* Aqueous, Ethanolic and Methanolic Fruit Extracts.** Values are means ± standard deviations (n = 3). Data points (along a common concentration value) containing a similar attached letter are statistically the same (p > 0.05).

Finally, the assessment of the TAC of *T. tetraptera's* fruit extracts alongside the standard compound (ascorbic acid) (Figure 2) revealed that the activity of the ethanolic extract was significantly higher than that of the methanolic from 0.50 to 2.00 mg/ml concentrations, the reverse was observed at 4.00 mg/ml (p < 0.05), while both extracts had similar activity at 0.25 mg/ml (p > 0.05). From the EC<sub>50</sub>, the TAC of Ascorbic acid > Aqueous > Methanolic > Ethanolic extract, with values of 0.33, 1.71, 4.20 and 5.35 mg/ml respectively. Antioxidants counter the activities of ROS and reactive nitrogen species (RNS)

through free electron donation (Moukette *et al.* 2015; Anigboro *et al.* 2018, Kadiri *et al.*, 2020; Anigboro *et al.* 2021; Oborirhovo *et al.* 2023). The general performance of plant extracts in carrying out the pharmacological activities is based on the presence of the bioactive constituents (Rebaya *et al.*, 2015; Anigboro *et al.*, 2019; Aikins *et al.*, 2021; Anigboro *et al.*, 2022; Abankwah *et al.*, 2025). However, the quantity of antioxidant compounds (such as alkaloids, tannins and phytates) in the fruit extracts (Table 2) is not perfectly correlated with the order of antioxidant activity obtained in this

study. The variation may be due to the dependence of the assay outcome on different specific components of the extracts in

relation to the experimental methods and conditions.



**Figure 2: In vitro TAC of *T. tetraptera's* Aqueous, Ethanolic and Methanolic Fruit Extracts.** Values are means  $\pm$  standard deviations ( $n = 3$ ). Data points (along a common concentration value) containing a similar attached letter are statistically the same ( $p > 0.05$ ).

## Conclusion

The pharmacological property of medicinal plants is a reflection of their phytoconstituents which may vary in types and amounts, depending on the extraction solvent and experimental protocol employed. Analysis of the *T. tetraptera* fruit extracts in this study revealed solvent-dependent variations in extraction yield, type and amount of compound detected, together with the antioxidant capacity. Although, there was variation in the order of extracts' IC<sub>50</sub>/EC<sub>50</sub> of antioxidant potential in the different assays, summation of their ranks suggests the overall capacity of the aqueous > ethanolic > methanolic extract. The type of extraction solvents used varied the yield, phyto-constituent and antioxidant potential which ultimately influence the efficiency of therapeutic usage that are derived from the pharmacological property. Corroboration from this study is important in choice of solvent during the utilization of this fruit. However, there is need to employ more antioxidant assay protocols in order to reveal a clearer outcome of the order of antioxidant activity.

## Conflict of Interests

The authors declare no known competing interests that could influence this work.

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