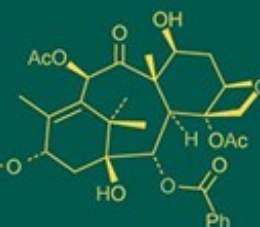
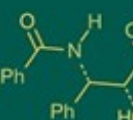


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Two methods, two chemical signatures: A comparative profile of decocted and macerated aqueous extracts of *Tetrapleura Tetraptera* (Aidan Fruit)

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ABSTRACT

Tetrapleura tetraptera (Aidan fruit), a West African culinary spice and medicinal plant, was extracted by decoction (500 g in 5,000 mL water, boiled 60 min) and maceration (1,000 g in 10,000 mL water, room temperature, 72 h), both at a 1:10 (w/v) plant-to-water ratio, to compare mineral, heavy-metal, phytochemical, proximate, and antioxidant profiles. Decoction yielded 4.43% extract versus 2.67% for maceration. Maceration recovered higher concentrations of calcium, magnesium, potassium, sodium, iron, zinc, copper, and manganese, while lead and cadmium were lower in macerated (0.33 and 0.030 mg/kg) than decocted extracts (0.44 and 0.039 mg/kg). Decoction produced higher alkaloids, flavonoids, tannins, saponins, phenols, and terpenoids, and greater DPPH, FRAP, and ABTS activity, reported as measured percentages/capacity since no reference antioxidant standard (e.g., Trolox, ascorbic acid) was used. Proximate composition differed by method. These findings demonstrate that extraction method significantly influences the nutritional and bioactive profile of *T. tetraptera* whole-fruit aqueous extracts.

Keywords: *Tetrapleura tetraptera*, Aidan fruit, Decoction, Maceration, Phytochemicals, Antioxidants

1. Introduction

Extracts of the plant have also been investigated for *anti-inflammatory* and other biological activities. (Ozaslan *et al.*)^[1] evaluated the cytotoxic and antiproliferative activities of the fruit extract on Ehrlich Ascites tumour cells. Medicinal plants contain organic compounds with diverse biological activities, including tannins, alkaloids, carbohydrates, triterpenoids, steroids and flavonoids (Edeoga *et al.*, 2005)^[2]. These naturally occurring plant constituents are collectively referred to as phytochemicals and may contribute to plant defence and characteristics such as colour, aroma and flavour (Jasiem, 2016)^[3]. Chelation is the binding of molecules or ions to metal ions to form coordination complexes. Chelating agents may influence the mobility and biological availability of metal ions (Flora *et al.*, 2010)^[4]. Previous studies have reported phytochemical constituents and antioxidant activity in *T. tetraptera* (Uyoh *et al.*, 2013)^[5]. Although *T. tetraptera* is traditionally prepared as a decoction, laboratory studies also use maceration and other extraction procedures. Extraction conditions can influence recovery because time, temperature, solvent-to-material ratio and matrix disruption affect mass transfer and compound stability (Adusei *et al.*, 2019)^[6]. However, direct comparison of aqueous decoction and aqueous maceration of the whole fruit under the same plant-material-to-water ratio remains limited. This study therefore compared the mineral, heavy-metal, phytochemical, proximate and antioxidant profiles of aqueous *T. tetraptera* whole-fruit extracts prepared by decoction and maceration.

2. Materials and Methods

2.1 Sample collection The whole fruits of *T. tetraptera* (50 samples weighing approximately 2.6 g each) were purchased in July 2026 from Okada Market, Ovia North-East Local Government Area, Edo State, Nigeria. The samples were botanically identified and authenticated by a plant taxonomist at the Department of Pharmacognosy, Igbinedion University, Okada, as *Tetrapleura tetraptera* (Fabaceae), Voucher No. IUO/17/156. The samples were placed in polyethylene bags and transported to the laboratory for processing and analysis.

2.2. Sample preparation

The whole fresh fruits of *T. tetraptera* were washed gently with tap water and then with distilled water, air-dried and crushed. The crushed fruits were pulverized into coarse powder using a mortar and pestle. The powdered sample was stored in airtight containers until required for further analysis.

2.3. Sample extraction

Whole fruit of *Tetrapleura tetraptera* was used for both extraction procedures. For maceration, 1,000 g of the whole-fruit sample was mixed with 10,000 mL of distilled water and allowed to stand at room temperature for 72 h with frequent agitation. For decoction, 500 g of the whole-fruit sample was mixed with 5,000 mL of distilled water and boiled for 60 min. Both methods used a plant-material-to-water ratio of 1:10 (w/v). The extracts were filtered using Whatman No. 1 filter paper and concentrated to dryness in a hot-air oven at 40 °C for 48 h. The dried extracts were stored in desiccators until analysis (Adusei *et al.*, 2019) [6]. Extraction yield was calculated as:
$$\text{Yield (\%)} = \frac{\text{mass of dried extract}}{\text{mass of powdered plant material}} \times 100.$$
 The macerated extract yielded 26.74 g (2.67%), whereas the decocted extract yielded 22.17 g (4.43%). Values are mean $\pm$ standard deviation of three analytical determinations ($n = 3$). No inferential p -values are reported because the three determinations were analytical repeats from a single extraction batch per method.

2.4 Mineral and heavy-metal analysis

The dried extracts were homogenized, and 0.50 g of each sample was subjected to acid digestion using concentrated nitric acid and hydrogen peroxide. The digest was transferred quantitatively to a 50 mL volumetric flask and made up to volume with deionized water. Reagent blanks were prepared using the same procedure without sample, and samples were prepared in triplicate. Mineral and heavy-metal concentrations of calcium, magnesium, potassium, sodium, iron, zinc, copper, manganese, lead and cadmium were determined using an atomic absorption spectrophotometer (model N1100A; Shimadzu, Japan). Results were expressed as mg/kg dry extract.

AAS calibration and quality control: Calibration curves were prepared using a multi-element standard solution. Calibration concentrations were 0, 1, 2, 5 and 10 mg/L for trace elements and 0, 5, 10, 20 and 50 mg/L for major minerals. Calibration curves showed acceptable linearity (R (Edeoga *et al.*, 2005) [2] ≥ 0.995). The mineral elements were determined using atomic absorption spectrophotometry following calibration with appropriate elemental standards.

2.5 Qualitative phytochemical screening

Qualitative phytochemical screening was conducted using standard preliminary phytochemical procedures for alkaloids, tannins, saponins, terpenoids, steroids/phytosteroids, flavonoids, phenols and glycosides. The screening tests for the major phytoconstituents were carried out using standard qualitative procedures based on characteristic colour reactions, precipitate formation or persistent frothing (Trease *et al.*, 2002) [7]. Results were recorded as detected (+) or not detected (–).

2.5.1 Test for alkaloids - Dragendorff's test

Approximately 2 mL of the extract solution was placed in a test tube. A few drops of Dragendorff's reagent were added (Edeoga *et al.*, 2005) [2]. The formation of an orange, orange-red or reddish precipitate was recorded as positive for alkaloids. A negative reaction was recorded when no characteristic precipitate developed.

2.5.2 Test for flavonoids - Shinoda test

Approximately 2 mL of extract was placed in a test tube. A small quantity of magnesium turnings was added, followed by a few drops of concentrated hydrochloric acid. Development of an orange, pink or reddish colour indicated the presence of flavonoids. This type of magnesium/HCl reaction is commonly used as a preliminary flavonoid test (Edeoga *et al.*, 2005) [2].

2.5.3 Test for tannins - Ferric chloride test

Approximately 2 mL of extract was diluted with distilled water. A few drops of 1–5% ferric chloride solution were added. Formation of a blue-black, greenish or blue-green colour was taken as evidence of tannins/phenolic tannins (Edeoga *et al.*, 2005) [2].

2.5.4 Test for saponins - Froth test

Approximately 2 mL of extract was mixed with 5–10 mL distilled water. The mixture was vigorously shaken for approximately 30–60 s and allowed to stand. Formation of persistent foam that remained for several minutes indicated the presence of saponins. The froth test is a commonly used preliminary saponin test (Edeoga *et al.*, 2005) [2].

2.5.5 Test for phenols - Ferric chloride test

Approximately 2 mL of extract was treated with several drops of dilute ferric chloride solution. The development of a blue, green, violet or dark coloration indicated the presence of phenolic compounds (Edeoga *et al.*, 2005) [2].

2.5.6 Test for terpenoids - Liebermann–Burchard test

Approximately 2 mL of extract was mixed with approximately 1 mL acetic anhydride. Concentrated sulfuric acid was carefully added down the side of the test tube. Development of a reddish, pink or violet coloration was interpreted as positive for terpenoids (Edeoga *et al.*, 2005) [2].

2.5.7 Test for steroids - Salkowski test

Approximately 2 mL of extract was mixed with 2 mL chloroform. Approximately 1–2 mL concentrated sulfuric acid was carefully introduced down the side of the tube. Formation of characteristic blue, green or violet coloration at the interface was recorded as positive for steroids (Edeoga *et al.*, 2005) [2].

2.5.8 Test for glycosides - Fehling's test

Approximately 2 mL of extract was treated with Fehling's solution. After heating, the development of a brick-red precipitate was interpreted as evidence of reducing sugar released from glycosidic constituents (Trease *et al.*, 2002) [7].

2.5.9 Test for cardiac glycosides — Keller–Kiliani test

Approximately 2 mL of extract was mixed with about 1 mL glacial acetic acid containing a trace of ferric chloride. Concentrated sulfuric acid was carefully introduced along the side of the test tube. The appearance of a reddish-brown ring at the interface, with a bluish-green colouration in the upper acetic acid layer, was recorded as positive for cardiac glycoside (Edeoga *et al.*, 2005) [2]. The Keller–Kiliani reaction is commonly used for preliminary cardiac glycoside screening.

2.5.10 Test for anthraquinones - Bornträger's test

Approximately 2 mL of extract was subjected to an alkaline anthraquinone screening reaction. The extract was partitioned with an appropriate non-polar solvent where required. Development of a pink, red or reddish-violet colour in the alkaline layer indicated the presence of anthraquinone derivatives (Trease *et al.*, 2002) [7].

2.5.11 Test for phlobatannins - Hydrochloric test

Approximately 2 mL of extract was heated with dilute hydrochloric acid. Formation of a red-coloured precipitate or deposit was interpreted as positive for phlobatannins. A similar acid-heating precipitation approach has been reported for phytochemical screening (Edeoga *et al.*, 2005) [2].

2.6 Quantitative phytochemical analysis

Quantitative phytochemical determinations were performed in triplicate for total alkaloids, total flavonoids, total tannins, total saponins, total phenols, terpenoids and total phytosteroids. Gallic acid was the reference standard for total phenolics.

2.6.1 Quantitative determination of total phenolic content - Folin–Ciocalteu test

A series of gallic acid standards (0, 20, 40, 60, 80 and 100 $\mu\text{g/mL}$; $y = 0.0071x + 0.0342$, R (Edeoga *et al.*, 2005) [2] = 0.998) was prepared. Approximately 0.5 mL of appropriately diluted extract was transferred into a test tube, followed by 2.5 mL of diluted Folin–Ciocalteu reagent. Absorbance was measured at approximately 725–765 nm using a UV–visible spectrophotometer (Spectrumlab 752S, Shanghai

Lenslight Technology Co., Ltd., Shanghai, China). A reagent blank was prepared using the extraction solvent instead of the sample (Mensah *et al.*, 2024; Singleton *et al.*, 1965) [8, 9].

2.6.2 Quantitative determination of total flavonoid content - Aluminium chloride method

Flavonoids form a coloured complex with aluminium chloride. Quercetin was used as the reference standard of 20–100 µg/mL. A calibration curve was prepared ($y = 0.0005x - 0.0063$; R (Edeoga *et al.*, 2005) [2] = 0.996). Approximately 1 mL of extract was mixed with 1 mL of aluminium chloride solution, 0.1 mL potassium acetate and 1.5 mL ethanol. The mixture was allowed to react for approximately 20–30 min at room temperature. Absorbance was measured at approximately 415 nm (Adusei *et al.*, 2019; Zhishen *et al.*, 1999) [6, 10].

2.6.3 Quantitative determination of alkaloids - Gravimetric method

Total alkaloids were determined gravimetrically (Olorundare *et al.*, 2020) [11]. Approximately 5.0 g of dried extract/sample was transferred into a suitable flask and 200 mL of 10% acetic acid in ethanol was added. The mixture was covered and allowed to stand for approximately 4 h and then filtered. The filtrate was concentrated on a water bath to approximately one-quarter of its original volume. Concentrated ammonium hydroxide was added slowly until precipitation was complete, and the mixture was allowed to stand to permit the alkaloid precipitate to settle. The precipitate was collected on a pre-weighed filter paper and washed with dilute ammonium hydroxide solution to remove residual soluble impurities. The filter paper containing the precipitate was dried in an oven at approximately 60 °C, cooled in a desiccator and weighed. Drying, cooling and weighing were repeated until a constant weight was obtained. The mass of alkaloid was determined from the difference between the final mass of the filter paper plus dried precipitate and the initial mass of the filter paper. Total alkaloids (%) = $[(W_2 - W_1)/W] \times 100$, where W_1 is the mass of the empty filter paper, W_2 is the mass of the filter paper plus dried alkaloid precipitate, and W is the initial mass of sample used.

2.6.4 Quantitative determination of tannins - Folin–Denis spectrophotometric method

Approximately 0.5 g of dried extract was extracted with 75 mL of distilled water. The mixture was filtered, and a 1 mL aliquot of the filtrate was transferred into a test tube, to which Folin–Denis reagent was added followed by saturated sodium carbonate solution. Absorbance was measured at approximately 760 nm. Tannic acid was used for reporting as mg tannic acid equivalents per g extract (20–100 µg/mL) (Chataika *et al.*, 2021) [12].

2.6.5 Quantitative determination of saponins - Vanillin–sulfuric acid colorimetric approach

Saponins react with vanillin in the presence of sulfuric acid to produce a coloured complex whose absorbance is proportional to saponin concentration. Diosgenin was used as the standard reference, and a calibration curve was prepared using a diosgenin concentration of 0–100 µg/mL⁷. A measured aliquot was transferred into a test tube. Vanillin reagent was added followed by sulfuric acid reagent. Absorbance was measured at approximately 560 nm⁷.

2.7.6. Energy value - Atwater calculation

The calorific value of the extract was estimated from the proximate composition using the conventional Atwater factors (AOAC, 2019) [13]: protein = 4 kcal/g, carbohydrate = 4 kcal/g and fat = 9 kcal/g.

2.8 Antioxidant assays

Antioxidant capacity was assessed using DPPH radical-scavenging, 211 FRAP reducing-power and ABTS radical-scavenging assays. DPPH

2.6.6 Quantitative terpenoid determination - Gravimetric method

Total terpenoids were determined gravimetrically. A known mass of dried extract was dissolved in methanol and subjected to liquid–liquid extraction with chloroform. The terpenoid-containing fraction was separated, evaporated and dried to constant weight.

2.6.7 Quantitative steroid determination - Liebermann–Burchard test

For steroid/phytosteroid estimation, a colorimetric Liebermann–Burchard-type assay was used. Cholesterol was used as the standard reference, and a calibration curve was prepared using a cholesterol concentration of 10–80 µg/mL⁷.

2.7 Proximate analysis

Moisture, ash, crude protein, crude fat, crude fibre, carbohydrate and energy value were determined in triplicate using procedures described by AOAC International [13]. Carbohydrate was calculated by difference. Energy value was estimated using Atwater factors of 4 kcal/g for protein, 4 kcal/g for carbohydrate and 9 kcal/g for fat (AOAC, 2019) [13].

2.7.1 Moisture content - Oven drying method

A moisture dish was cleaned, dried and placed in an oven at approximately 105 °C. It was cooled in a desiccator and weighed. About 2–5 g of the dried aqueous extract was added. The dish plus sample was weighed and dried at 105 °C until approximately constant weight. Then it was cooled in a desiccator and weighed again (AOAC, 2019) [13].

2.7.2 Ash content - Dry ashing method

Approximately 2–5 g of extract was weighed into a previously ignited, cooled and weighed crucible. The crucible was placed in a muffle furnace and ashed at approximately 550 °C until a light grey/white ash was obtained. It was cooled in a desiccator and then the crucible containing the ash was weighed (AOAC, 2019) [13].

2.7.3. Crude protein — Kjeldahl method

The Kjeldahl method determines nitrogen, which is then converted to crude protein using an appropriate nitrogen-to-protein conversion factor. In this study, crude protein was calculated as total nitrogen $\times$ 6.25. Approximately 0.5–1.0 g of extract was weighed, concentrated sulfuric acid and an appropriate digestion catalyst were added, and the sample was digested until the solution became clear. The digest was cooled and diluted with distilled water, after which excess alkali was added. The liberated ammonia was trapped and titrated with standardized acid. A reagent blank was included (AOAC, 2019) [13].

2.7.4. Crude fat - Soxhlet extraction method

A Soxhlet extraction was used. Approximately 2–5 g of sample was weighed and placed in a suitable extraction thimble. Petroleum ether was added to the Soxhlet apparatus. The receiving flask containing the extracted lipid was dried, cooled in a desiccator and weighed (AOAC, 2019) [13].

2.7.5. Crude fibre - Crude fibre digestion method

A conventional crude-fibre procedure involves sequential digestion. Approximately 1–2 g of defatted sample was weighed. It was digested with standardized dilute sulfuric acid under controlled conditions. It was filtered and washed thoroughly. The dried residue was weighed and ashed in a furnace. It was cooled and reweighed (AOAC, 2019) [13].

$$\text{Carbohydrate (\%)} = 100 - [\text{moisture (\%)} + \text{ash (\%)} + \text{crude protein (\%)} + \text{crude fat (\%)} + \text{crude fibre (\%)}]$$

activity was measured after incubation for 30 min in the dark at room temperature using DPPH at 1 mg/mL, with absorbance measured at

approximately 517 nm. FRAP reagent was prepared from 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM FeCl₃ in a 10:1:1 ratio, followed by incubation at 37 °C and absorbance measurement at approximately 593 nm; results were expressed as $\mu\text{mol Fe}^{2+}$ equivalents/g dry extract. For ABTS, the radical solution was diluted to an absorbance of 0.70 ± 0.02 at 734 nm and activity was measured at approximately 734 nm. No reference antioxidant standard was documented in the study records; activities are therefore reported as measured percentages or capacity, and this is stated in the Limitations (Xiao *et al.*, 2020; Prior *et al.*, 2005) [14, 15].

2.8.1 DPPH procedure- DPPH radical scavenging assay

The DPPH radical (2,2-diphenyl-1-picrylhydrazyl) is sufficiently stable to be used directly as a reagent, and it imparts an intense violet colour to its solution. When an antioxidant transfers a hydrogen atom or an electron to the radical, the violet colour fades as DPPH is converted to its reduced form, and the resulting drop in absorbance is used to quantify scavenging activity. DPPH solution was prepared at a concentration of 1 mg/mL. The extract was mixed with the DPPH solution and incubated for 30 min in the dark. The absorbance was measured at 517 nm using a UV-visible spectrophotometer. Each concentration was performed in triplicate (Xiao *et al.*, 2020) [14]. Radical-scavenging activity (%) = $\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$, where A_{control} is the absorbance of the radical solution (Blois, 1958) [16].

2.8.2 FRAP procedure- FRAP assay

The working FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ prepared in 40 mM HCl, and 20 mM FeCl₃ in a ratio of 10:1:1. The extract was reacted with the freshly prepared FRAP reagent and incubated at 37 °C. Absorbance was measured at approximately 593 nm in triplicate, and the results were expressed as $\mu\text{mol Fe}^{2+}$ equivalents/g dry extract (Xiao *et al.*, 2020; Benzie *et al.*, 1996) [14, 17].

2.8.3 ABTS procedure- ABTS radical cation decolorization assay

The ABTS assay measures the ability of antioxidants to quench the blue-green ABTS radical cation (ABTS^{•+}). When antioxidants react with ABTS^{•+}, its colour decreases. The reduction in absorbance is used to estimate antioxidant activity. The ABTS radical cation (ABTS^{•+}) was generated by reacting ABTS with potassium persulfate, and the mixture was allowed to stand in the dark for approximately 12–16 h. The resulting radical solution was diluted to obtain an absorbance of 0.70 ± 0.02 at 734 nm. The extract was then reacted with the working ABTS solution, and absorbance was measured at approximately 734 nm in triplicate. ABTS radical-scavenging activity was expressed as percentage scavenging (Xiao *et al.*, 2020; Re *et al.*, 1999) [14, 18].

2.9 Statistical analysis

All determinations were performed in triplicate and results are expressed as mean $\pm$ standard deviation. The three determinations available for each extraction method were analytical measurements of a single prepared extract rather than three independently prepared extraction batches. They therefore describe analytical repeatability and do not provide independent extraction-level replicates for inferential comparison between methods. Accordingly, no inferential *p*-values are reported. Independent extraction batches would be required before statistical testing of differences between extraction methods.

3. Results

3.1 Mineral and heavy-metal composition

The extraction yield and mineral and heavy-metal composition of the decocted and macerated extracts are presented in Table 1. Decoction produced a higher extraction yield (4.43%) than maceration (2.67%). The macerated extract had higher measured concentrations of calcium, magnesium, potassium, sodium, iron, zinc, copper and man-

ganese, whereas lead and cadmium concentrations were lower than in the decocted extract.

Table 1. Extraction yield and mineral and heavy-metal composition of decocted and macerated aqueous extracts of *T. tetraptera* whole fruit.

Element	Decoction (mg/kg)	Maceration (mg/kg)
Extraction yield (%)	4.43	2.67
Ca	431.87 $\pm$ 3.31	516.27 $\pm$ 3.61
Mg	188.23 $\pm$ 1.91	217.07 $\pm$ 2.25
K	688.03 $\pm$ 3.65	746.77 $\pm$ 4.10
Na	98.10 $\pm$ 1.30	114.20 $\pm$ 1.60
Fe	28.79 $\pm$ 0.37	34.61 $\pm$ 0.42
Zn	10.05 $\pm$ 0.21	12.44 $\pm$ 0.28
Cu	2.25 $\pm$ 0.07	2.82 $\pm$ 0.08
Mn	6.57 $\pm$ 0.15	8.45 $\pm$ 0.19
Pb	0.44 $\pm$ 0.02	0.33 $\pm$ 0.02
Cd	0.039 $\pm$ 0.003	0.030 $\pm$ 0.002

Values are mean $\pm$ standard deviation of three analytical determinations (*n* = 3). No inferential *p*-values are reported (see Section 2.9).

3.2 Qualitative phytochemical screening

Alkaloids, flavonoids, tannins, saponins, phenols, terpenoids and glycosides were detected in both extracts, as shown in Table 2. Steroids and cardiac glycosides were detected in the decoction but not in the maceration, while anthraquinones and phlobatannins were not detected in either extract.

Table 2. Qualitative phytochemical screening of decocted and macerated aqueous extracts of *T. tetraptera* whole fruit.

Phytochemical	Decoction	Maceration
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Phenols	+	+
Terpenoids	+	+
Steroids	+	–
Glycosides	+	+
Cardiac glycosides	+	–
Anthraquinones	–	–
Phlobatannins	–	–

Key: + = Present; – = Not detected.

3.3 Quantitative phytochemical composition

The quantitative phytochemical composition of the two extracts is presented in Table 3. The decocted extract had higher measured concentrations of total alkaloids, flavonoids, tannins, saponins, phenols and terpenoids than the macerated extract, and total phytosteroids were detected only in the decocted extract.

Quantitative phytochemical results are expressed as mean $\pm$ SD. Where applicable, phenolics are reported as mg gallic acid equivalents per g extract (mg GAE/g), flavonoids as mg quercetin equivalents per g extract (mg QE/g), tannins as mg tannic acid equivalents per g extract (mg TAE/g), saponins as mg diosgenin equivalents per g extract (mg DE/g), and phytosteroids as mg cholesterol equivalents per g extract (mg CE/g). Values are reported as determined by the stated gravimetric or spectrophotometric method. Where a parameter has no verifiable reference standard, no equivalence unit is assigned (see Limitations).

Table 3. Quantitative phytochemical composition of decocted and macerated aqueous extracts of *T. tetraptera* whole fruit.

Phytochemical	Decoction	Maceration	Higher measured concentration
Total alkaloids	18.59 ± 0.17	16.72 ± 0.19	Decoction
Total flavonoids	24.53 ± 0.18	21.38 ± 0.21	Decoction
Total tannins	16.39 ± 0.12	14.83 ± 0.14	Decoction
Total saponins	12.76 ± 0.12	11.26 ± 0.12	Decoction
Total phenols	21.91 ± 0.17	19.47 ± 0.18	Decoction
Terpenoids	10.93 ± 0.11	9.18 ± 0.10	Decoction
Total phytosteroids	7.47 ± 0.11	ND	Decoction

Values are mean ± standard deviation of three analytical determinations ($n = 3$).

ND = Not detected.

3.4 Proximate composition

The proximate composition of the two extracts is presented in Table 4. The macerated extract had higher ash, crude protein, crude fat and energy values, whereas the decocted extract had higher moisture, crude fibre and carbohydrate values.

Table 4. Proximate composition (% dry weight) and energy value of decocted and macerated aqueous extracts of *T. tetraptera* whole fruit.

Parameter	Decoction	Maceration
Moisture (%)	6.26 ± 0.08	5.49 ± 0.08
Ash (%)	5.79 ± 0.07	6.93 ± 0.09
Crude protein (%)	10.55 ± 0.13	12.30 ± 0.12
Crude fat (%)	4.94 ± 0.08	5.71 ± 0.08
Crude fibre (%)	8.39 ± 0.08	7.54 ± 0.08
Carbohydrate (%)	64.08 ± 0.44	62.02 ± 0.45
Energy (kcal/100 g)	342.98 ± 0.23	348.67 ± 0.25

Values are mean ± standard deviation of three analytical determinations ($n = 3$). Energy is expressed as kcal/100 g.

No inferential p -values are reported.

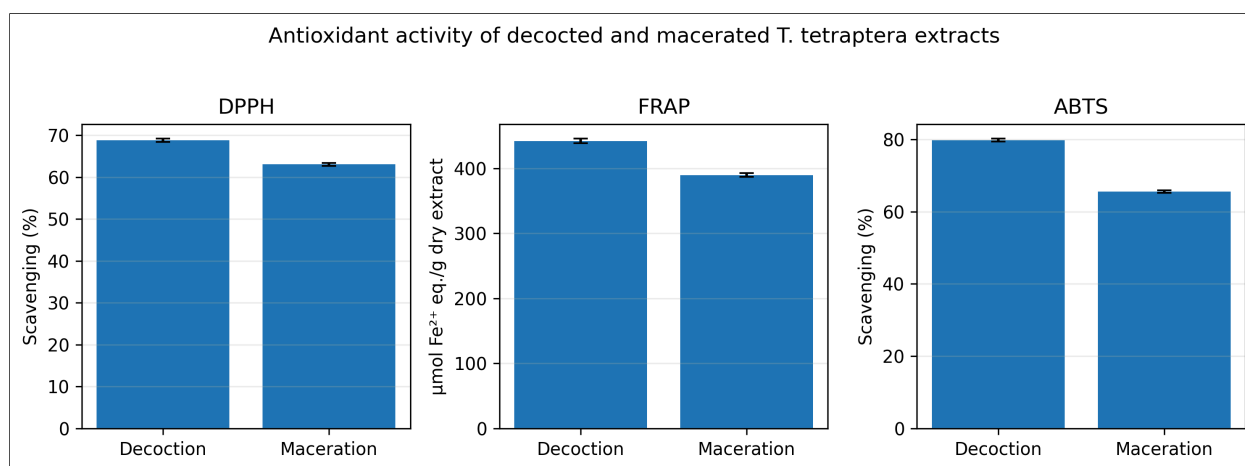
3.5 Antioxidant activity

The antioxidant activity of the two extracts is presented in Table 5 and Figure 1. The decocted extract showed higher measured DPPH and ABTS radical-scavenging activity and greater FRAP reducing capacity than the macerated extract.

Table 5. Antioxidant activity of decocted and macerated aqueous extracts of *T. tetraptera* whole fruit at 1 mg/mL.

Assay	Decoction	Maceration
DPPH scavenging (%)	68.79 ± 0.38	63.07 ± 0.34
FRAP (μmol Fe ²⁺ equivalents/g dry extract)	442.20 ± 3.34	389.67 ± 2.85
ABTS scavenging (%)	79.79 ± 0.37	65.51 ± 0.33

Values are mean ± standard deviation of three analytical determinations ($n = 3$). No inferential p -values are reported. FRAP is expressed as μmol Fe²⁺ equivalents/g dry extract.

**Figure 1.** Antioxidant activity of decocted and macerated *T. tetraptera* whole-fruit aqueous extracts. Error bars represent standard deviation ($n = 3$). No significance markers are shown because the replicates were analytical repeats from a single extraction batch.

4. Discussion

The present study showed that the extraction procedure influenced the measured mineral, phytochemical, proximate and antioxidant profiles of *T. tetraptera* whole-fruit aqueous extracts. The macerated extract contained higher measured concentrations of calcium, magnesium, potassium, sodium, iron, zinc, copper and manganese, whereas the decocted extract showed higher measured values for the quantified phytochemicals and antioxidant assays. Previous studies have also demonstrated that the chemical profile of *T. tetraptera* varies with fruit part and extraction solvent (Adusei *et al.*, 2019) [6].

The higher measured mineral concentrations in the macerated extract may be related to its substantially longer contact period. Maceration

lasted 72 h compared with 60 min of boiling, providing a much longer period for diffusion of soluble ionic constituents from the plant matrix. A second possible explanation is that boiling may promote interactions between mineral ions and matrix components such as tannins, phytates and oxalates, potentially reducing the fraction that remains dissolved after filtration. Heat-induced changes in proteins and pectic materials could also alter the retention or release of mineral ions. These mechanisms are plausible explanations rather than directly demonstrated mechanisms in the present study.

When mineral concentrations are normalised to the yield obtained from a fixed quantity of starting material, the picture changes. Decoction produced 4.43% dried extract compared with 2.67% for maceration, so despite its lower mineral concentration per gram of

dried extract, decoction delivered a greater total mineral mass per 100 g of starting fruit powder for every element measured; for example, calcium recovery was approximately 1.91 mg per 100 g of powder for decoction versus 1.38 mg for maceration. This indicates that the higher mineral concentrations reported for the macerated extract reflect a more mineral-dense extract rather than greater absolute mineral extraction from the starting material, and the two findings are not in conflict once extraction yield is taken into account.

Lead and cadmium concentrations were lower in the macerated extract than in the decocted extract. The measured values were 0.33 ± 0.02 and 0.030 ± 0.002 mg/kg, respectively, in the macerated extract, compared with 0.44 ± 0.02 and 0.039 ± 0.003 mg/kg in the decocted extract. For comparison, the manuscript uses the (WHO) [19] proposed maximum amounts of 10 mg/kg for lead and 0.3 mg/kg for cadmium in dried medicinal plant materials. All four measured values are below those reference limits. These limits are presented as comparative reference values and should not be interpreted as a complete safety assessment.

The decocted extract showed higher measured concentrations of total alkaloids, flavonoids, tannins, saponins, phenols and terpenoids, whereas total phytosteroids were not detected in the macerated extract. The qualitative absence of steroids and cardiac glycosides in the macerated extract is consistent with the greater extraction of these relatively non-polar constituents under heated conditions. Steroids and aglycone portions of cardiac glycosides are generally less soluble in cold water, while heating can increase solubilization and disrupt cell-wall and membrane structures. This interpretation is supported internally by the quantitative finding that phytosteroids were not detected in the macerated extract, but the qualitative tests remain preliminary screening methods rather than definitive identification.

The antioxidant results followed the phytochemical pattern: the decocted extract had higher DPPH scavenging, FRAP reducing capacity and ABTS scavenging than the macerated extract. (Adusei *et al*) [16] reported antioxidant activity in aqueous extracts of *T. tetraptera*. The higher measured phenolic and flavonoid values observed in the decocted extract provide a plausible chemical basis for its higher measured antioxidant activity, although correlation or mechanistic causation cannot be established from the present dataset alone. DPPH, FRAP and ABTS are in-vitro chemical assays and should not be interpreted as direct evidence of therapeutic efficacy (Xiao *et al.*, 2020) [14].

The proximate composition also differed between extraction methods. The higher ash value in the macerated extract is directionally consistent with its higher measured mineral concentrations and provides an internal cross-check. The lower moisture value in the macerated extract may also be favourable for storage stability because lower residual moisture generally reduces conditions that support microbial growth and some deterioration processes; however, actual shelf stability was not measured in this study. Published values for *T. tetraptera* vary with plant part, location and preparation (Nwoba, 2015) [20]. Several limitations should be considered. The present work was based on a single extraction batch for each method, with three analytical determinations per measurement; therefore, the analytical replicates do not capture between-batch extraction variability. The material was obtained from a single market and collection period, so seasonal and geographical variation was not assessed. Individual phytochemicals were not identified chromatographically, antioxidant activity was assessed only by chemical in-vitro assays, and bioavailability was not evaluated. Future work should use independent extraction batches, complete quantitative calibration data, reference antioxidant standards and chromatographic characterization. The instrument-specific LOD and LOQ values could not be recovered from the available laboratory records and therefore were not reported.

Practically, decoction is a traditional household preparation of *T. tetraptera*, and the present results show that it produced higher measured phytochemical and antioxidant values under the analytical conditions used. Maceration produced a more mineral-concentrated extract per gram, together with lower measured lead and cadmium con-

centrations, which may be relevant where a small, concentrated dose is preferred or where minimising heavy-metal carry-over per gram of extract is the priority; however, on a whole-fruit basis, decoction recovered more total mineral mass as well as more phytochemical content and antioxidant activity. These findings should be interpreted within the limitations of the study and do not establish clinical superiority or therapeutic efficacy.

5. Conclusion

This study shows that decoction and maceration are not interchangeable methods for preparing aqueous *Tetrapleura tetraptera* extracts: each produces a chemically distinct product suited to a different purpose. Maceration yields an extract that is more concentrated in essential minerals per gram and lower in lead and cadmium, making it preferable where a compact, low-metal mineral supplement is the goal. Decoction, the traditional household method, produced higher measured phytochemical content and antioxidant activity, and its considerably higher extraction yield meant it delivered more total mineral mass from the same quantity of fruit despite being less mineral-dense per gram, an outcome that provides a biochemical rationale for the way Aidan fruit has always been prepared and consumed. Taken together, these findings indicate that extraction method is not a neutral procedural choice but a determinant of the nutritional and bioactive identity of the final product, and that phytochemical or antioxidant data generated by one method should not be assumed to represent the other. Researchers, herbal-medicine practitioners and food processors working with *T. tetraptera*, and with traditionally prepared plant materials more broadly, should specify and standardise extraction method as a matter of course. Confirming these findings with independently replicated extraction batches, validated antioxidant reference standards and chromatographic identification of individual compounds is the logical next step toward a definitive, statistically supported comparison.

Data availability

The data generated and/or analysed during the present study are contained within this article. Additional laboratory records are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare no conflict of interest.

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Author contributions

Esumai Ifeoma Victory: conceptualization, sample collection, extraction, laboratory analyses, data curation and manuscript preparation. Onovughe Faith Avwerosughene and Helen Kwipnchep Njoya: supervision, methodological guidance and critical review of the manuscript. Osimen-Eboziegbe Uche Christina: contribution to the study and critical review of the manuscript. All authors read and approved the final manuscript.

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