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Comparative evaluation of phytochemical, *in vitro* antioxidant activities and elemental composition of the fruit, leaves, and stem bark of *Tetrapleura tetraptera*

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ABSTRACT

Introduction: Nowadays, medicinal plants are widely accepted as a better option than synthetic medications due to their lower cost and little to no side effects. The current study aimed to compare the elemental composition, phytochemical profile, and antioxidant efficacy of *n*-hexane extracts derived from *Tetrapleura tetraptera* fruit, stem bark, and leaves.

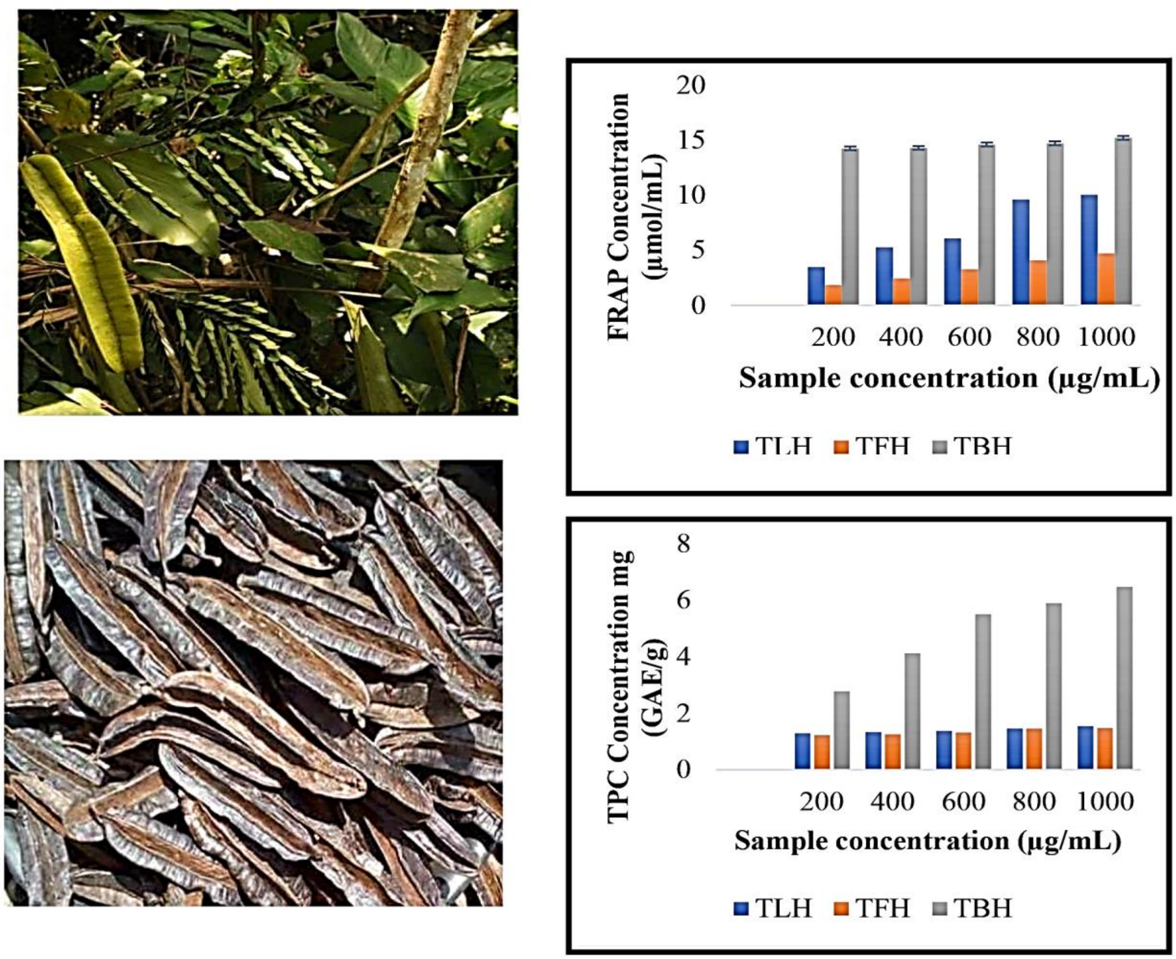
Methods: The fresh fruits, leaves, and stem bark of the plant were collected and dried. The dried samples were pulverized and exhaustively extracted with *n*-hexane. The extracts were screened for both qualitative and quantitative phytochemical constituents. Antioxidant parameters such as Ferric Reducing Antioxidant Property (FRAP) and 2,2-Diphenyl-1-picryl hydroxyl radical scavenging activity (DPPH) were also carried out. The elemental analysis of the various organs was also evaluated using standard protocols.

Results: The presence of alkaloids, flavonoids, tannins, saponins, terpenes, resins, and phenolswas revealed in the phytochemical data. The stem bark of *T. tetraptera* has higher concentrations of alkaloids and flavonoids than the leaf and fruit, according to a quantitative examination of the phytochemical elements of the plant. The stem bark had the highest Total Phenolic Content at 1000 μg/mL, whereas the leaves had the lowest TPC at 200 μg/mL.

At a concentration of 1000 µg/mL, the stem bark exhibited the greatest activity of total flavonoid (TFC) in the plant extracts, followed by the fruits and leaves. At 1000 µg/mL concentration, the FRAP test revealed the maximum activity in the stem bark. Generally speaking, when concentration rises, so do the TPC, TFC, and FRAP activities. Compared to the standard ascorbic acid (2.76 µg/mL), stem bark exhibited the highest level of DPPH antioxidant activity, with an IC_{50} of 13.34 µg/mL. The elemental analysis revealed that the stem bark had the highest concentrations of Ca, Mg, and Fe, followed by the fruit and leaves, in that order.

Conclusion: This research result has established that the plant, *T tetraptera* can be standardized and offer an alternative eco-friendly drug for immunity building. The stem barks are underutilized, however, its phytochemical studies and other investigations pointed to its potential in the cosmeceutical industries and medicine.

Keywords: Antioxidant activity, ethnomedicine, ferric-reducing assay, medicinal plant



Tetrapleura tetraptera

INTRODUCTION

People have employed medicinal plants and their extracts to treat a variety of ailments for thousands of years. Several different antitussives, antimalarial, antihypertensive, and analgesic medications are produced by these plants [1-2]. These therapeutic plants serve as significant starting points for the development of drugs that focus on a wide range of pharmacological targets, such as neurological diseases, cancer, malaria, and cardiovascular disease. In order to be categorized as a medicinal plant, a part of the plant or the entire plant must have medicinal qualities. Plant-based medications are cheaper and safer to use than conventional (synthetic) drugs, which often cause adverse side effects [3]. Plant derived medications include bioactive ingredients and extracts that have enormous potential for use in the development of unique and innovative methods for treating and preventing disease. These bioactive substances, which include steroids, cyanogenic glycosides, and essential oils, as well as tannins, flavonoids, alkaloids, phenols, phytates, and oxalates, can be employed as medications, be used as models for brand-new synthetic compounds, and taxonomic markers to identify novel compounds [2]. Plants play a crucial role in many scientific domains and are important to human survival. According to earlier studies, plant materials include several valuable natural compounds, such as coumarins, iridoids, alkaloids, and flavonoids [4]. The ethnopharmacology, phytochemistry, and biological activity of extensive collections of potential therapeutic plants from Africa and beyond have also been documented [5-7]. Compounds known as antioxidants prevent oxidation, a chemical process that can result in the production of free radicals. The regulation of physiological processes, cellular damage, and the etiology of brain disorders including dementia, stroke, and Parkinson's disease are all significantly influenced by free radicals [8, 9].

Tetrapleura tetraptera (Schumach. & Thonn.) Taub is a single-stemmed, sturdy, perennial tree that grows to a height of approximately 30 meters and belongs to the family Mimosaceae. The tree-like, strong, perennial plant has a single stem, thick, woody roots, and spreading branches in addition to dark green leaves. It has glabrous juvenile branches and a smooth or rough, grey-brown bark. The racemes of yellowish-pink flowers accompany the dark-brown, four-winged pods of the fruit. It is often found in tropical Africa's lowland forests. The fruit locally known as Aridan or prekese is made up of a luscious pulp and tiny, brownish-black seeds. It has an odor that is pleasant and distinctively pungent, which is linked to its ability to repel insects [10].

The goal of this study was to compare the antioxidant potential and the phytochemical and elemental composition of *n*-hexane extracts of leaves, stem bark, and fruit of *T. tetraptera*. Because medicinal plants are widely accepted today as being more effective than synthetic drugs and have fewer side effects, there is a great demand for natural antioxidants due to their lack of side effects. Many authors have studied the ethnomedicinal activity of the fruit extract of *T. tetraptera*, but little research has been done on the plant's stem bark.

METHODS

Chemicals and reagents: Analytical grade solvents such as methanol, n-hexane, chloroform, distilled water, ferric chloride, ammonia, Dragendorff's reagent, Mayer's reagent, sulphuric acid, glacial acetic acid, aluminum chloride, DPPH, potassium acetate (BDH Laboratory Supplies, Poole, England).

Plant collection and identification: Fresh fruits, leaves, and stem bark of *T. tetraptera* were collected from the herbal garden of the Forestry Research Institute of NigeriaThe plant samples were then carefully cleaned using tap water and then rinsed using distilled water. The cleaned samples were air-dried on a rack for 10 days to reduce the water content and later transferred into a cabinet dryer at a temperature of 35-40°C for 10 min to further reduce the moisture. The dried plant samples were pulverized, using the laboratory milling machine. The powder obtained was filtered through a fine (2 mm) mesh sieve to get rid of any leftover material, it was kept in a sealed glass container for further analysis.

Extract preparation: Four hundred grams (400 g) of each powdered sample were exhaustively extracted with analytical grade n-hexane and were subjected to 24-hour continuous shaking using a laboratory shaker. The extracts were filtered using Whatman No. 42 (125 mm) filter paper. A rotary evaporator was used to concentrate the filtrate *in vacuo*. The extract was kept in a refrigerator at 4˚C until utilized.

Qualitative analysis of phytochemical constituents: Using established protocols, the extracts were evaluated for the presence of numerous secondary metabolites, including anthraquinones, alkaloids, tannins, saponins, flavonoids, cardiac glycosides, and steroids [11-12].

Quantitative analysis of phytochemical constituents:

Total phenolic content estimation (Folin–ciocalteu method): Standard procedures were used in order to determine the extracts' total phenolic content [13, 14]. 1 mL aliquots of 50–500 µg/mL ethanolic gallic acid solution were mixed with 5 mL of diluted folin–ciocalteu reagent and 4 mL of 7.5% sodium carbonate to prepare the standard calibration curve.

Total flavonoid content estimation: This was done according to the technique written about in an article by Elbouny et al. [14], in which the aluminum chloride colorimetric method was employed to determine the presence of flavonoids. To prepare the calibration curve, a solution of quercetin was made in methanol at concentrations ranging from 20 to 100 µg/mL.

Ferric-reducing antioxidant property: The ferric-reducing property was evaluated by assessing the ability of extracts to reduce $FeCl_3$ solution. The test was performed according to standard procedures [15].

Determination of DPPH antioxidant activity: By using a slightly modified approach previously published the radical scavenging ability of the plant extracts against 2,2-Diphenyl-1-picryl hydroxyl radical (Sigma-Aldrich) was determined [14]. A positive control was employed, which was ascorbic acid. Based on the linear regression curve, the antioxidant activity of each sample was reported in terms of IC_{50}, or the micromolar concentration needed to prevent the generation of DPPH radicals by 50%. The following formula was used to compute the radical scavenging activity.

% inhibition = {[Ab-Aa] /Ab} x 100, where Aa represents the extract's absorption and Ab represents the blank sample's absorption.

Elemental Analysis: The elemental composition of the fruits, leaves, and stem bark of *T. tetraptera* was carried out using a standard analytical method [16], where the powdered samples were digested using nitric and perchloric acids. Potassium and Sodium were assessed using a Flame photometer, while magnesium, calcium, iron, copper, manganese, zinc, and phosphorus were determined from the filtered aliquots using an Atomic Absorption Spectrophotometer (Buck Scientific; 210VGP Model) (SearchTech British; FP640 Model). Each analysis was performed in triplicates.

Statistical analysis: Microsoft Excel software (2010) was used for statistical analysis. Three duplicates of each analysis were carried out.

RESULTS

Qualitative phytochemical screening: The results of the phytochemical screening revealed the presence of phenols, steroids, alkaloids, flavonoids, anthraquinones, tannins, and terpenoids except for saponin and tannin which were present only in the stem bark but absent in the fruit and leaves of the plant and cardiac glycoside which was completely absent in all (Table 1). The presence of these secondary metabolites qualifies the plant as a medicinal plant.

Table 1. Qualitative analysis of phytochemical constituents of *T. tetraptera*

Phytochemical constituents	Test	Fruit	Leaf	Stem bark
Alkaloid	Dragendorff	+	+	+
	Mayer	+	+	+
	Wagner	++	++	++
Saponins	Frothing	-	-	+
Glycoside	Keller-Killian	-	-	-
Flavonoids	Ammonia/H_2SO_4	+	+	+
	Aluminum solution	+	+	+
	Ethyl acetate/Ammonia	++	++	++
Anthraquinones	Borntrager	+	+	+
Terpenoids	Salkwoski's	++	+	++
Tannins	Ferric chloride	-	-	++
Steroids	Liebermann-Burchard	++	++	+

Interpretation: + Present; ++ Abundant; -ve Absent.

Quantitative phytochemical screening: The quantitative analysis of phytochemical constituents of *T. tetraptera* revealed the highest concentration of alkaloids and flavonoids in the stem bark as compared to the leaf and fruit (Table 2).

Table 2. Quantitative analysis of phytochemical constituents of *T. tetraptera*

Phytochemical constituents	Leaf (%)	Fruit (%)	Stem bark (%)
Alkaloids	12.7	20	33.5
Flavonoids	19	26.7	52.3
Saponin			9.1

Total phenolic content (TPC): At 1000 µg/mL concentration, the stem bark (TBH) had the greatest Total Phenolic Content (6.47 mg GAE/g), whereas at 200 µg/mL, the leaves (TLH) had the lowest TPC (1.28 mg GAE/g) (Fig. 1).

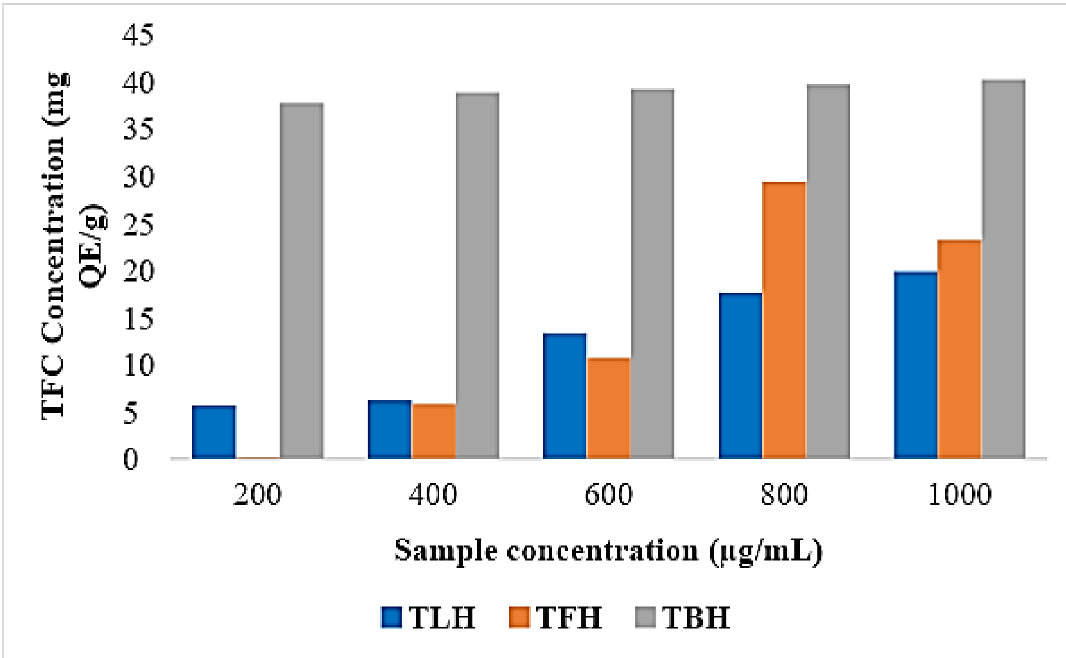


Figure 1. Total phenolic content (TPC) of samples

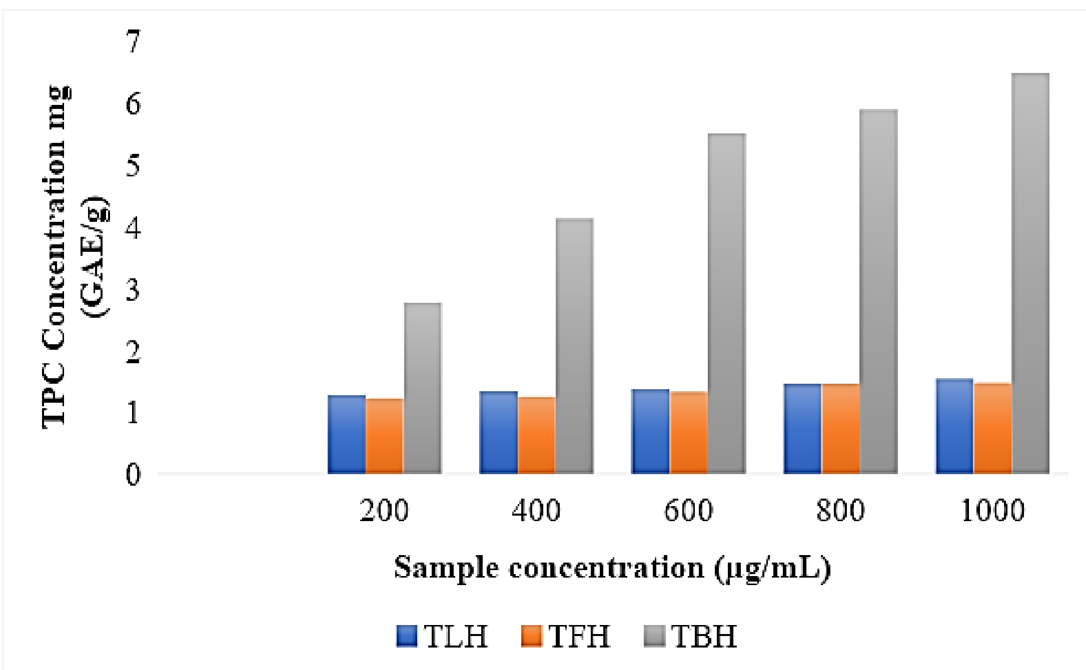


Figure 2. Total flavonoid content (TFC) of sample

Total flavonoid content (TFC): The total flavonoid (TFC) of the plant extracts showed the highest activity in the stem bark (TBH) (40.15 mg QE/g), followed by the fruits (TFH) (23.17 mg QE/g) and the leaves (TLH) (19.82 mg QE/g) at1000 µg/mL concentration (Figure 2).

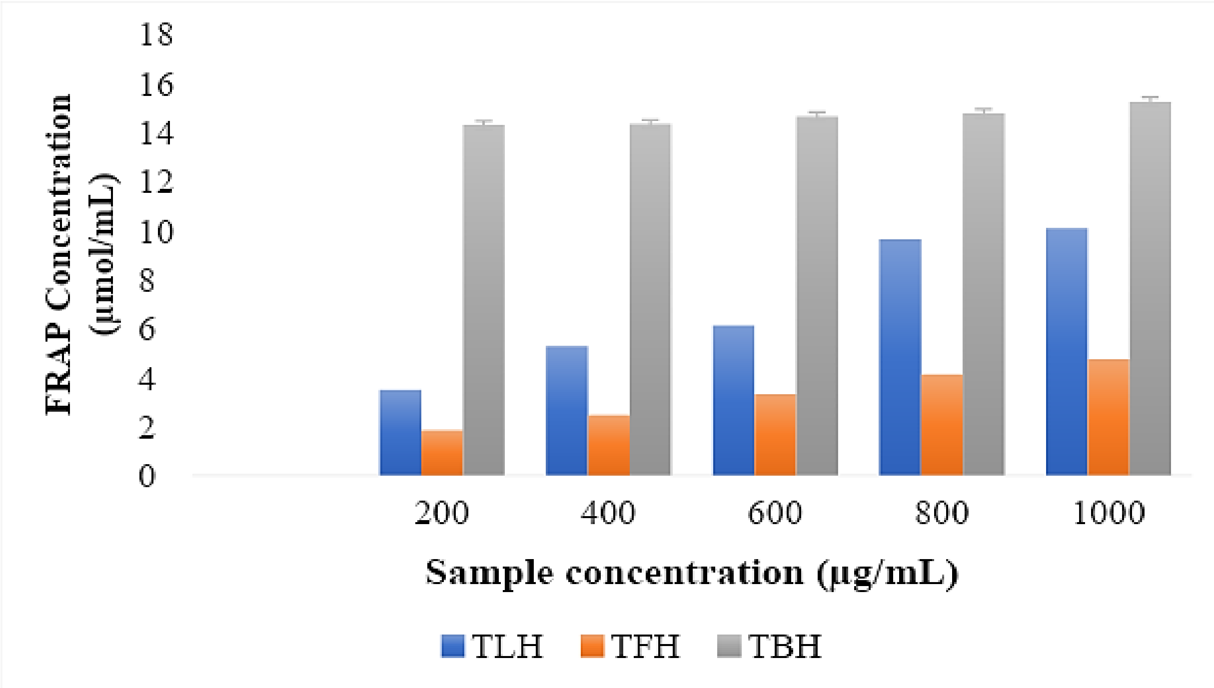


Figure 3. Ferric reducing antioxidant power (FRAP) of samples.

Ferric reducing antioxidant power assay (FRAP): The FRAP result showed the highest activity in the stem bark (TBH) (15.22 µmol/mL) at 1000 µg/mL concentration (Fig. 3). In general, the TPC, TFC, and FRAP activities increase as the concentration increases (Fig. 1-3).

Table 3 shows the DPPH free radical scavenging activity of *T. tetraptera* extracts. The stem bark was found to be most active with IC_{50} of 13.34 µg/mL when compared with the standard, ascorbic acid 2.76 µg/mL.

Table 3. DPPH radical scavenging % inhibition of *T. tetraptera*

Plant parts	% Inhibition
Ascorbic acid	2.76 ± 0.07
Stem bark	13.34 ± 1.43
Leaves	449.59 ± 149.70
Fruit	7815 ± 309.01

Values represent Mean ± SEM.

Elemental analysis: The result of the elemental analysis carried out using atomic absorption spectrophotometry is presented in Table 4. The fact that Ca, Mg, K, Cu, Fe, and Zn are present indicates their role as necessary nutritional components. The highest Ca, Mg, and Fe concentrations were found in the stem bark followed by the fruit and the leaves respectively. This shows that different parts of plants take up and absorb various nutrients from the soil in varying amounts.

Table 4. Mineral composition of *T. tetraptera* stems, bark, leaves and fruits on when dry.

Elements	Quantity (mg/100g)		
	Stem bark	**Leaves**	**Fruit**
Calcium	3.55±0.56	0.43±22.29	0.45±12.61
Magnesium	1.81±0.83	0.99±0.04	0.71±0.48
Copper	0.02±11.11	0.19±1.37	0.09±7.12
Iron	1.08±11.70	4.02±1.94	2.37±1.84
Zinc	0.28±4.88	0.32 ±1.79	0.27 ±7.23
Potassium	4.89±00	4.89±00	4.89+00

Values represent Mean ± SEM

DISCUSSION

Alkaloids are basic and naturally occurring organic compounds with at least one nitrogen atom.Thisis a vastly diversified group of naturally occurring compounds generated through secondary metabolism (does not include nitrogen in an amide bond or peptide). They are frequently isolated from plants, although various mammals, insects, marine invertebrates, and other microbes have also been shown to contain them [17, 18]. Our present study established a comparison evaluation of the phytochemical, *in vitro* antioxidant activities and elemental composition of the various parts of *T. tetraptera*. The fruit, stem bark, and leaf extracts of the plant, *T. tetraptera*, all contain alkaloids, flavonoids, anthraquinones, terpenoids and steroids which indicates that the plant has therapeutic qualities. Saponins play a critical role in the stimulation of mucous membrane defensive factors thereby exerting its defensive factors in gastric ulceration [19-20].

Early studies have shown that saponins affect fish, Oncomelania snails, and insects biologically, in addition to having hemolytic and antibacterial properties. It has been established that saponins are risk-free, environmentally beneficial, and microbially degradable [21-22].

The culinary and medicinal industries make extensive use of tannins' antioxidant qualities. Tannins have received a lot of attention due to their antioxidant properties, which include fighting cancer, osteoporosis, and cardiovascular disease [23]. Several biological activities of tannins have been reported [24]. These include antibacterial [25-26], antiviral [27-28], antiparasitic [29-30], anti-inflammatory [31-32], antidiarrheal [33-34], and antioxidant [23].

The present antioxidant activity result showed that *T. tetraptera* is rich in antioxidants which may protect the human cells against free radicals and thereby play a significant role in the treatment of heart diseases, cancer, and other diseases. The stem bark had the highest concentrations of TPC, TFC, and FRAP.

This study reported that the *T. tetraptera* plant, especially the stem bark possesses the highest concentrations of tannins flavonoids, phenolics, and FRAP. It has been shown that flavonoids and phenols have antiplatelet, anticancer, anti-inflammatory, and antioxidant properties [35].

Many fruits and vegetables, including tomatoes, berries, and grapes, contain phenolic compounds. By lowering the chance of developing metabolic diseases like type 2 diabetes mellitus, phenolic compounds can improve one's health [36]. Numerous research has examined their impact on antioxidant, anti-inflammatory, anti-aging, and antiproliferative activities [37-39].

Flavonoids have been associated with several health-promoting advantages, making them crucial for a broad variety of applications in nutraceuticals, pharmacology, medicine, and cosmetics. This is because they have potent anti-oxidative, anti-inflammatory, antimutagenic, antibacterial, anti-carcinogenic, and vascular properties. They also have the therapeutic capacity to alter important cellular enzyme activities and scavenge free radicals [40].

The origin, development, and advancement of tumors have been demonstrated to be inhibited by flavonoids, which are also known to have antioxidant properties [41]. There have been reports linking flavonoid consumption to a decrease in coronary heart disease [42]. Other biological activities that flavonoids perform in addition to acting as antioxidants include defense against platelet aggregation, free radicals, bacteria, viruses, hepatotoxins, inflammation, cancers, ulcers, and allergies [43]. Both humans and plants use phenols as antioxidants [44]. Haslam [45] draws attention to the growing interest in the possibility of treating illnesses by simply increasing dietary consumption of foods abundant in plant phenolics including tannins and flavonoids, as well as antioxidants like vitamins E, C, -carotene, and carotenoids. The ability of the *n*-hexane extract of *T. tetraptera* bark to scavenge DPPH radicals indicates its antioxidant activity.

In addition, the elemental analysis showed an abundance of mineral elements than the leaves and fruits. The mineral elements Calcium, Magnesium, and Potassium present in abundant in the stem bark showed that *T. tetrapleura* could play a significant role in the body's metabolism. This edible species can be made in supplements because trace elements supplements are beneficial in the treatment of type 2 diabetes mellitus, prevention of hypoglycemia, corticosteroid-induced hyperglycemia, molybdenum deficiency, and others.

In addition to providing food for both plants and animals, mineral elements also play other significant roles in the ecosystem. It has long been established that inorganic chemical elements are crucial for nourishment and serve as significant structural elements in cellular functions [46]. When used medicinally for a therapeutic effect, the amount of trace elements and active ingredients in medicinal plants have a significant influence on the body's metabolism [47].

The elements Fe^{+2}, K^{+}, Mg^{+2}, Na^{+}, Ca^{+2}, Co^{+2}, Mn^{+2}, Zn^{+2}, and Cu^{+2} is considered essential, and Ni^{+2} and Cr^{+3} is possibly essential, and Cd^{+2}, Pd^{+4}, and Li^{+1} is considered non-essential elements of the human body.

Zinc is an essential micronutrient, which makes it an important component of the plant life cycle [48]. Copper is another important micronutrient for plants [49]. It is involved in several metabolic activities, including the scavenging of superoxide, hormone signaling, electron transport during photosynthetic reactions, mitochondrial respiration, and cell wall metabolism. One of the critical micronutrients for plant growth and development is iron. The synthesis of DNA, chlorophyll, respiration, photosynthesis, hormones, and nitrogen-fixing are just a few of the biological processes in which it takes part [50-51].

CONCLUSION

In addition to being used for timber, *T. tetraptera* may also be used in traditional medicine. Most of its leaf components, including the seeds, fruit, and leaves, have been used in traditional medicine in well-known ways, while the stem bark has received less attention from pharmaceutical and phytochemical companies.

This research has established through the investigation of this plant's phytochemistry and antioxidant properties that *T. tetraptera* has industrial potential, especially for use in pharmaceuticals. The efficacy of the fruit and stem bark extracts of *T. tetraptera* in its bioactivities may be attributed to its potential antioxidant activity. Based on the findings, stem bark was more effective as an antioxidant. The stem bark of this plant should not be neglected as could be explored for further biological studies and chemical evaluation. It is recommended that further research be done on the biological actions of active ingredients and isolation.

List of abbreviations: Ca: Calcium, Mg: Magnesium, K: Potassium, Cu: Copper, Fe: Iron, Zn: Zinc, TPC: Total Phenolic Content, TFC: Total flavonoid content, DPPH: 2,2-diphenyl-1-picrylhydrazyl, FRAP: Ferric reducing antioxidant power.

Competing interests: The authors declare that there are no competing interests.

Authors' contributions: All authors performed the experiment and data analyses. OYB wrote the first draft of the manuscript. IOL and IAA reviewed and edited the manuscript. OYB and IOL contributed to the conceptualization, project administration, and financial support. IOL guided the experiment and revised the manuscript. All authors approved the final version of the manuscript.

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