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The organic extracts from the leaves of *Ficus thonningii* Blume, *Jatropha tanjorensis* J.L Ellis and Saroja and *Justicia carnea* Lindley as potential nutraceutical antioxidants and functional foods

Kingsley Ikechukwu Ijoma[1]✉*, Vincent Ishmael Egbulefu Ajiwe[1]✉* And Stanley Chukwudindu Odinma[2]

[1]Department of Pure and Industrial Chemistry, Faculty of Physical Science, Nnamdi Azikiwe University, Awka, Nigeria
[2]Department of Industrial Chemistry, Faculty of Natural Science, Caritas University, Amorji-Nike, Enugu, Nigeria

ABSTRACT

The search for naturally occurring plant-based nutraceutical antioxidants is imperative in the management of arrays of diseases. Herein, the methods involved profiling antioxidant activities and nutritional composition of the methanolic extracts from the leaves of *Ficus thonningii* Blume (FTH), *Jatropha tanjorensis* J.L Ellis and Saroja (JTR) and Justicia carnea Lindley (JCN) using spectrophotometric techniques. In this sense, *J. tanjorensis* J.L Ellis and Saroja extract showed the highest total antioxidant capacity and strong DPPH radical scavenging activity with an IC_{50} of 10.490 ± 0.0320 µg.mL^{-1} when compared to ascorbic acid (IC_{50} of 114.140 ± 5.135 µg.mL^{-1}), while for the hydroxyl radical scavenging activity, *F. thonningii* Blume and *J. carnea* Lindl. extracts resembled IC_{50} values of 523.095 ± 13.629 and 503.733 ± 7.562 µg.mL^{-1}, respectively. A positive correlation was observed between the extracts and ascorbic acid ($p < 0.001$), as well. In addition, nutrients and minerals were present in appreciable quantities. The obtained results validate the assayed plants extracts as potential radical scavengers and promising nutraceutical antioxidants in ethnomedicine.

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1. Introduction

Plant-based nutraceuticals are potential antioxidants used in the management of different diseases including cancer, sickle cell disease, diabetes, Alzheimer's, cataract, and Parkinson's, etc. (Antwi-Boasiako et al., 2019; Shariff-Rad et al., 2020; Vona et al., 2021). Excessive production of reactive oxygen (RO) species weakens antioxidant defenses and thus creates an imbalance trend (Antwi-Boasiako et al., 2019). Redox stress triggers the activation of immune cells which release pro-inflammatory cytokines, ROS and nitrogen species that cause damage to biological molecules and induce imbalances in physiological and pathological pathways (Vona et al., 2021). Epidemiological and *in vivo* studies proved that dietary intake of antioxidants is a key strategy for health promotion by lowering oxidative stress (Watz, 2008). Currently, synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) are widely used in the food industry. However, restriction on synthetic antioxidants is being imposed because of their toxicity to the liver and carcinogenicity (Uzomba, 2022). Therefore, the development and utilization of more effective antioxidants of natural origins are desired and unavoidable.

Oxidative stress is reported to be associated with the development of several metabolic and chronic disorders particularly those causing cancer (Aminjan et al., 2019). Thus, the beneficial roles of antioxidants have been widely studied in the literature (Liu, 2019). Although the free radicals play an essential role in numerous biological processes, their excessive free quantities are usually quenched by the human natural antioxidant defense system, hence if this system cannot function properly, free radicals can lead to fatal consequences such as the destruction of the cell membrane, blockage of enzymatic actions and energy generation, prevention of cell division, lipid per oxidation, destruction of

✉Corresponding authors: Kingsley Ikechukwu Ijoma and Vincent Ishmael Egbulefu Ajiwe
Tel: +234-8144250562; Fax: +234-8173076500
E-mail address: ikechukwuijoma@gmail.com, vie.ajiwe@unizik.edu.ng, **doi: 10.30495/tpr.2023.1977670.1318**

deoxyribonucleic acid, etc. (Sharifi-Rad et al., 2020). Plant-based nutraceuticals have also shown excellent and remarkable characteristics as potential antioxidants widely accepted for the treatment and management of antioxidant-related diseases. However, it should be considered that antioxidants can function as prooxidants depending on the chemical/biological composition of the environment in which they are found and owing to the disparity in their activity and biological properties (Chen, 2018). It has been shown that there are significant differences between taking an antioxidant from food and administering an isolated compound as a supplement (Sharifi-Rad et al., 2020). The most commonly known natural resources of antioxidants, *e.g.*, polyphenols, ascorbate, tocopherols, and terpenoids are different organs of plant materials (Liu et al., 2018). It has been well-documented that a positive correlation exists between total phenols and antioxidant activity and the same trend is noted in many plant species involving natural compounds that possess reducing properties functioning through donating a hydrogen atom resulting in interruption of the free radical chain (Nurhidayah et al., 2017; Liu et al., 2018).

J. tanjorensis J.L Ellis and Saroja, *J. carnea* Lindl. and *F. thonningii* Blume respectively belong to the families of *Euphobiaceae, Acanthaceae* and *Moraceae* which are abundant in rainforest zones of West Africa (Dangarembizi et al., 2013; Onyeabo et al., 2017; Oladele et al., 2020). They are widely and frequently found in Southern Nigeria especially Southeast Nigeria and are used in ethnomedicine in the management and treatment of different ailments (Dangarembizi et al., 2013; Onyeabo et al., 2017; Oladele et al., 2020; Akintimehin et al., 2021). Recent reports show that *J. carnea* Lindl. is rich in antioxidant and minerals (Akintimehin et al., 2021). In addition, the antioxidant activity of *F. thonningii* Blume has been the subject of one of the previously reports (Dangarembizi et al., 2013), and the recent reports on *F. thonningii* Blume corroborate the relevant findings (Pougoue et al., 2020). It has also been reported that *J. tanjorensis* J.L Ellis and Saroja leaf is a rich source of essential nutrients and vitamins associated with antioxidant properties (Aiwonegbe et al., 2022).

Therefore, the main objective of this study is to profile the antioxidant activities of methanol extract of *F. thonningii* Blume, *J. tanjorensis* J.L Ellis and Saroja, as well as *J. carnea* Lindl. being used as ethnomedicine by ethnic Igbos of Eastern Nigeria in the management of arrays of diseases.

2. Experimental

2.1. Identification and authentication

The plants were collected from their natural habitat at Nnodo Amike-Aba (latitude: 6°24′51.9′′N, longitude: 8°07′34.1′′E) Ebonyi Local Government Area council, Nigeria. The plants' leaves were thereafter identified and authenticated by a taxonomist at the Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria.

2.2. Extraction

Extraction of the leaves was successively carried out with methanol. In this relation, two hundred grams (200 g) of the pulverized leaves of each plant were weighed and soaked in methanol for 72 h. The mixture was filtered, and the filtrate was heated in a water bath to reach one-tenth (1/10) of their initial volumes at 40 °C (Harborne, 1998). Each dried extract was then weighed and stored at 4 °C for further analysis.

2.3. Antioxidant assay

2.3.1. Scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH)radical

The DPPH free radical scavenging assay was carried out as described by Oyaizu (1986). A solution of varying concentrations (1000, 500, and 250 µg.mL^{-1}) of each crude extract and ascorbic acid was prepared and 1 mL of methanol solution of DPPH (0.1 mM) was added. The mixture was kept in the dark at room temperature (30 ± 2 °C) for 30 min and the absorbance was measured at 517 nm against a blank using a spectrophotometer (Mettler Toledo, Switzerland). The percentage of radical scavenging activity was calculated through the following formula.

DPPH radical scavenging activity (%) = $(A_1 - A_2/A_1) \times 100$ (Eqn. 1)

Where A_1 and A_2 respectively account for absorbance of control and the sample. The IC_{50} value (µg.mL^{-1}) is the effective concentration at which DPPH radicals were scavenged by 50% and the value was obtained by interpolation from logarithmic regression analysis.

2.3.2. Hydroxyl radical scavenging activity

The scavenging activity of the extract *vs.* hydroxyl radical was measured according to a previously described method by Mensor et al. (2001). Accordingly, various concentrations (1000, 500, and 250 µg.mL^{-1}) of each crude extract and ascorbic acid were prepared and 60 µL of $FeCl_3$ (1 mM), 90 µL of 1,10-phenanthroline (1 mM), 2.4 mL of 0.2 M phosphate buffer (pH 7.8) and 150 µL of H_2O_2 (0.17 M) were added, respectively. The mixture was then homogenized and incubated at room temperature for 5 min. Using a spectrophotometer, the absorbance was read at 560 nm against the blank. The percentage of hydroxyl radical scavenging activity was calculated using the following formula (Eqn. 2).

Hydroxyl radical scavenging activity (%) = $(A_1 - A_2/A_1) \times 100$ (Eqn. 2)

Where A_1 and A_2 respectively account for absorbance of control and sample. The extract concentration providing 50% inhibition (IC_{50}) was calculated and obtained by interpolation from logarithmic regression analysis.

2.3.3. Reducing power assay

The Fe^{3+} reducing power of the crude extracts was determined according to the method described by Oyaizu (1986). In accordance with this method, the

crude extracts and ascorbic acid (1 mL) of various concentrations (1000, 500, and 250 µg.mL^{-1}) were mixed with phosphate buffer (pH 6.6, 0.2M, 2.5 mL) followed by the addition of potassium ferricyanide (1%, 2.5 mL) to the mixture. A portion of the resulting mixture was mixed with $FeCl_3$ (0.1%, 0.5 mL) and the corresponding absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated a higher reductive potential of the extract.

2.3.4. Total antioxidant activity by ferric reducing antioxidant power (FRAP) assay

The FRAP assay was used to determine the total antioxidant activity based upon the reduction of ferric ions to the ferrous form in the presence of antioxidant compounds (Evans, 2009). The fresh FRAP reagent consists of 500 mL of acetate buffer (300 mM, pH 3.6), 50 mL of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) (10 mM), and 50 mL of $FeCl_3{\cdot}6H_2O$ (50 mM). For this assay, different concentrations of each crude extract (1000, 500 and 250 µg.mL^{-1}) and ascorbic acid were mixed with 2 mL of FRAP reagent and the relevant optical density was read after 180 s at 593 nm against the blank using a spectrophotometer.

2.3.5. Total antioxidant activity by phosphomolybdenum assay

The total antioxidant capacities of the crude extracts were determined by the phosphomolybdenum method according to the procedure described by Prieto et al. (1999) and using ascorbic acid as a standard. The assay is based on the reduction of Mo(VI) to Mo(V) by the crude extracts and the subsequent formation of green phosphate/Mo(V) complex at acidic pH. An aliquot of 0.1 mL of various concentrations of crude extracts and ascorbic acid (1000, 500 and 250 µg mL^{-1}) were mixed with 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 765 nm against a blank. A typical blank contained 1 mL of the reagent solution and the appropriate volume of the solvent (0.1 mL) that was incubated under the same conditions. Ascorbic acid was used as standard and the antioxidant activity was expressed as ascorbic acid equivalent (AAE).

2.4. Nutritional and mineral analysis

For the evaluation of the nutritional constituents of the leaves of the assayed plants, the method described by Unuofin et al. (2017) was used with minor changes, whereas the mineral constituents of the extracts were analyzed using atomic absorption spectrophotometer (Bulk scientific, USA, model 210 VGP) after wet digestion.

2.5. Statistical analysis

For the antioxidant assay, each test was performed in triplicate and the results were expressed as mean ± standard error of the mean. The ANOVA, Student-Newman-Keuls (SNK) Posthoc test ($p < 0.001$), homogeneity of variance and descriptive statistics was carried out on each data from each of the crude extracts using Sigmaplot software version 14 for windows, while SAS version 9.4 for windows was used to estimate the nutritional and mineral compositions of the extracts using Tukey Posthoc analysis and was regarded as significant at $p < 0.05$. The IC_{50} was determined using logarithmic regression analysis on Microsoft Excel for Windows.

3. Results and Discussion

3.1. DPPH scavenging activity

The effect of antioxidants on DPPH is thought to be due to their hydrogen-donating ability (Unuofin et al., 2017). Our results suggested that the methanolic extract of *J. tanjorensis* J.L Ellis and Saroja showed strong antioxidant activity when compared to ascorbic acid (Fig. 1). α-Tocopherol as an identified compound in *J. tanjorensis* J.L Ellis and Saroja leaf, is known to scavenge DPPH radicals by its hydrogen donating ability (Rahman et al., 2015; Ijoma and Ajiwe, 2022). The results obtained in this study suggest that the extracts showed free radical scavenging activity by their electron transfer or hydrogen-donating ability since DPPH mechanism is based on single-electron transfer (SET) or hydrogen atom transfer pathways. Also, the DPPH scavenging activity for *J. carnea* Lindl. extracts and ascorbic acid increased in a concentration-dependent manner, while those of *F. thonningii* Blume and *J. tanjorensis* J.L Ellis and Saroja extracts were found to be independent of concentration (Fig. 1). This may probably be due to the presence of interfering impurities at different concentrations. The percentage inhibition of *J. tanjorensis* J.L Ellis and Saroja (10.490±0.0320 µg.mL^{-1}), *F. thonningii* Blume (892.258 ± 2.983 µg.mL^{-1}) and *J. carnea* Lindl. (207.649±3.695 µg.mL^{-1}) extracts were significantly different ($p < 0.001$) from ascorbic acid (114.140 ± 5.135 µg.mL^{-1}). From Fig. 1, it rapidly becomes apparent that ascorbic acid showed higher percent inhibition than *J. tanjorensis* J.L Ellis and Saroja leaves methanolic extract. Furthermore, the IC_{50} of the extract of *J. tanjorensis* J.L Ellis and Saroja was lower than ascorbic acid since the term IC_{50} represents the cumulative effect of a drug over a given range of concentration under study and not just the effect at one or two unique concentrations (Rahman et al., 2015).

3.2. Hydroxyl radical scavenging activity

From Fig. 2, the estimated IC_{50} for the extracts of *F. thonningii* Blume and *J. carnea* Lindl. were respectively 523.095 ± 13.629 µg.mL^{-1} and IC_{50} = 503.733 ± 7.562 µg.mL^{-1}, while ascorbic acid displayed an IC_{50} of 298.989 ± 14.462 µg.mL^{-1}. The IC_{50} for *J. tanjorensis* J.L Ellis and Saroja extract was not determined because at maximum concentration, the observed percent inhibition was 35.751 ± 0.002% and further dose variations did not yield an inhibition greater than 50% (Fig. 2). The percent inhibition of hydroxyl radical for ascorbic acid was significantly different ($p < 0.001$) from those of the

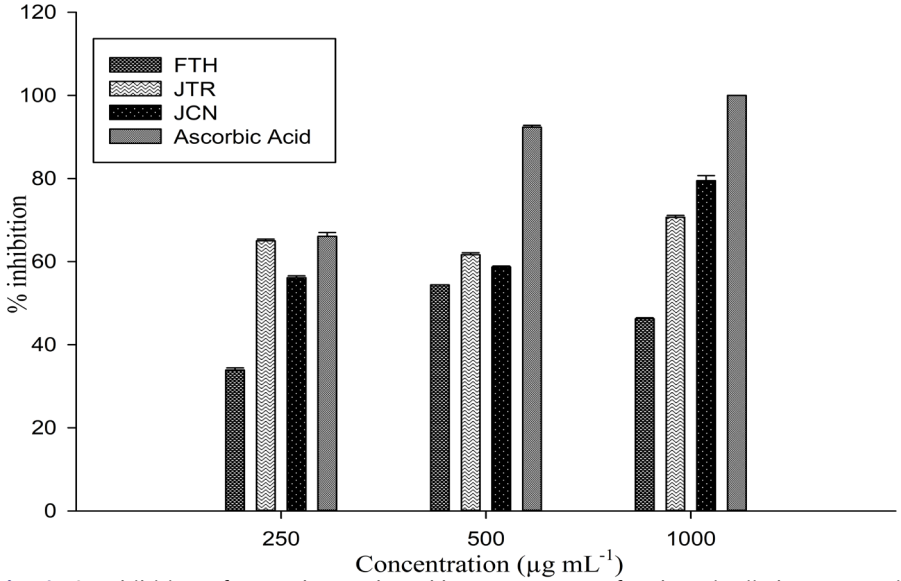

Fig. 1. % Inhibition of DPPH by methanol leaves extracts of *F. thonningii* Blume, *J. tanjorensis* J.L Ellis and Saroja, *J. carnea* Lindl. and ascorbic acid. FTH = *F. thonningii* Blume, JTR = *J. tanjorensis* J.L Ellis and Saroja, JCN = *J. carnea* Lindl.

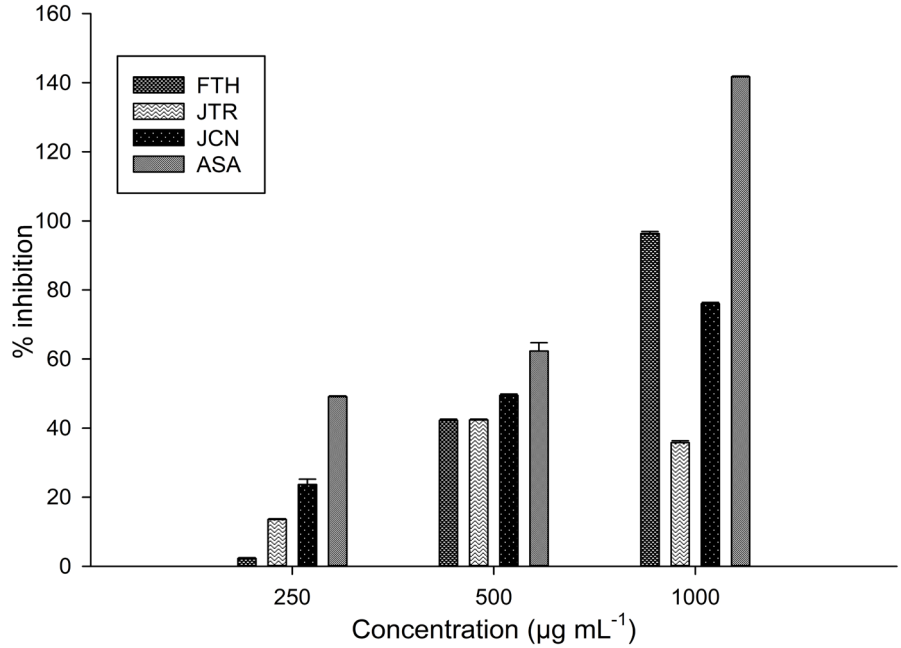

Fig. 2. % inhibition of hydroxyl radical by methanol leaves extracts of *F. thonningii* Blume, *J. tanjorensis* J.L Ellis and Saroja, *J. carnea* Lindl. and ascorbic acid. FTH = *F. thonningii* Blume, JTR = *J. tanjorensis* J.L Ellis and Saroja, JCN = *J. carnea* Lindl. ASA = Ascorbic acid.

extracts at all the studied concentrations. The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells and is a potent initiator of lipid peroxidation process (Sharifi-Rad et al., 2020). According to the literature, the mutagenic capacity of free radicals is due to the direct interactions of hydroxyl radicals with DNA, resulting in DNA breakdown (Sharifi-Rad et al., 2020). Hydroxyl radicals are formed by incubating Fe^{3+}/EDTA premixture with ascorbic acid and H_2O_2 at pH 7.4, causing 2-deoxy-ribose degradation and generating malondialdehyde-like product. The addition of the methanolic extracts of *F. thonningii* Blume, *J. tanjorensis* J.L Ellis and Saroja along with *J. carnea* Lindl. to the reaction mixture removes hydroxyl radicals and prevents further damage. These extracts showed appreciable hydroxyl radical scavenging activity when compared to ascorbic acid (Fig. 2) and could serve as potential antioxidant agents by inhibiting the interaction of hydroxyl radicals with DNA. The ability of the extracts to quench hydroxyl radicals might directly relate to the prevention of lipid peroxidation.

3.3. Reducing power

It was observed that the results of the reducing power of the analyzed extracts, as shown by their absorbance at 700 nm, correlates with the increase in concentrations (Fig. 3). There was a very significant difference ($p < 0.001$) between the reducing power of the extracts and ascorbic acid. Reducing power is usually attributed to the presence of reductants, whose antioxidant activity involve breaking free radical chains through hydrogen atom donation. Using the reducing power assay, the presence of reductants in *F. thonningii* Blume, *J. tanjorensis* J.L Ellis and Saroja, and *J. carnea* Lindl. extracts reduced the Fe^{3+}/ferricyanide complex to the Fe^{2+}/ferrous form. Therefore, the reducing power of each extract can be evaluated by measuring the formation of Perl's Prussian blue at 700 nm. The reducing ability of the extracts was in the range of 0.48 ± 0.001 to 0.84 ± 0.00 µm Fe(II)/g, respectively (Fig. 3). As can be seen in this figure, all the extracts showed a good reducing power capacity which was concentration-dependent. Our results are consistent with those reported previously by Rahman et al. (2015). Here, it can be assumed that the antioxidant activity and reducing power capacity of the extracts were likely due to the presence of antioxidant-based nutraceutical.

3.4. Ferric reducing antioxidant power (FRAP)

The observed increase in absorbance for FRAP assay correlates with the increase in concentration (Fig. 4). The FRAP values at 1000 µg mL^{-1} for *F. thonningii* Blume, *J. tanjorensis* J.L Ellis and Saroja, and *J. carnea* Lindl. were found to be 365.411 ± 0.791 mg $AAEg^{-1}$, 406.328 ± 1.524 mg $AAEg^{-1}$ and 335.0904 ± 0.791 mg $AAEg^{-1}$, respectively. The FRAP value of ascorbic acid was significantly different ($p < 0.001$) from those of the analyzed extracts at various concentrations. The FRAP assay is based on the rapid reduction of ferric-tripyridyltriazine (Fe^{3+}-TPTZ) by antioxidants present in the extracts thus forming ferrous-tripyridyltriazine (Fe^{2+}-TPTZ) complex which is evident from the formation of a blue colored product (Evans, 2009). The FRAP assay is a widely used assay in the evaluation of antioxidant activities of a variety of samples since its reaction is rapid. The results of the FRAP assay are shown in Fig. 4 which suggest that the reducing ability of the assayed extracts was within 0.018 to 0.041 nm. Accordingly, extracts of *J. tanjorensis* J.L Ellis and Saroja showed the highest FRAP value indicating comparatively an excellent antioxidant power.

3.5. Phosphomolybdenum assay

Phosphomolybdenum assay is based on the reduction of phosphate-Mo(VI) to phosphate-Mo(V) by the extracts and subsequent formation of a bluish-green colored phosphate/Mo(V) complex at an acidic pH. The phosphomolybdenum method is routinely applied in the laboratory to evaluate the total antioxidant capacity of plant extracts (Prieto et al., 1999). The antioxidant potential of *F. thonningii* Blume, *J. tanjorensis* J.L Ellis and Saroja, and *J. carnea* Lindl. was deduced from their ability to induce the reduction of Mo(VI) to Mo(V) by the antioxidant-rich methanolic extracts and subsequent formation of a green phosphate/Mo(V) complex at acidic pH. Antioxidant activity of plant-based nutraceutical depends on the presence of bioactive compounds mainly polyphenols, carotenoids, and vitamin E and C. Hence, the concentration of the bioactive compounds should correlate with viability in antioxidant activity. Thus, a higher concentration of antioxidant extracts shows higher antioxidant activity. In the present study, the reducing ability of the extracts was over the range 0.083 ± 0.00-0.067 ± 0.00 µm green phosphate/Mo (V) and all the extracts showed promising total antioxidant activity (Fig. 5). The results of phosphomolybdenum assay for ascorbic acid was significantly different ($p < 0.001$) from those of the studied extracts. The phosphomolybdenum assay at the maximum concentration for the methanolic extracts from the leaves of *F. thonningii* Blume, *J. tanjorensis* J.L Ellis and Saroja, and *J. carnea* Lindl. was calculated as 10.617 ± 0.111 mg $AAEg^{-1}$, 26.759 ± 0.0535 mg $AAEg^{-1}$ and 18.179 ± 0.0535 mg $AAEg^{-1}$, respectively (Fig. 5). The results obtained from the phosphomolybdenum assay denote that *J. tanjorensis* J.L Ellis and Saroja extract possess better antioxidant capacity. The observed increase in absorbance for phosphomolybdenum assay didn't correlate with the increase in the concentration of the extracts which could most probably be attributed to the interference of impurities at varying concentration.

3.6. Nutritional and mineral composition

Plants are invaluable sources of ethnomedicine and their nutritional properties are important for the improvement of the human immune system (Vignesh et al., 2021). Nutritional deficiencies are associated with a wide variety of disorders and diseases, *e.g.*, blindness, anemia, preterm birth, stillbirth, cretinism, cardiovascular

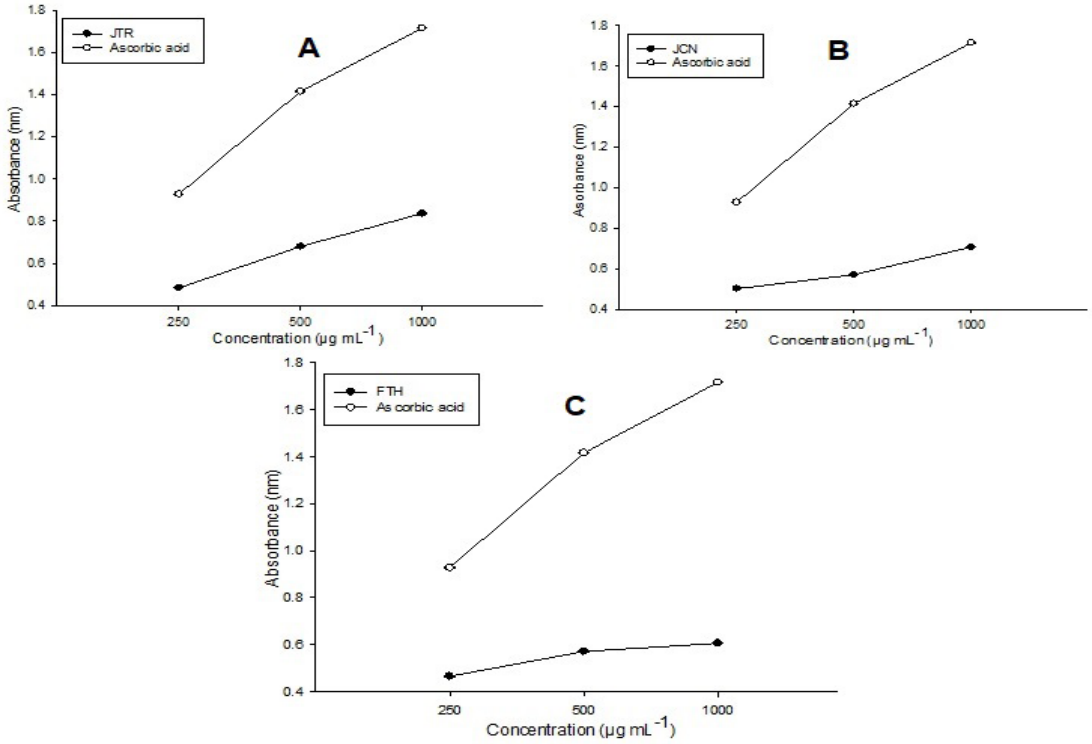

Fig. 3. Absorbance of methanol leaves extracts of *J. tanjorensis* J.L Ellis and Saroja (**A**), *J. carnea* Lindl. (**B**) and *F. thonningii* Blume (**C**) in comparison to ascorbic acid in reducing power assay. FTH = *F. thonningii* Blume, JTR = *J. tanjorensis* J.L Ellis and Saroja, JCN = *J. carnea* Lindl.

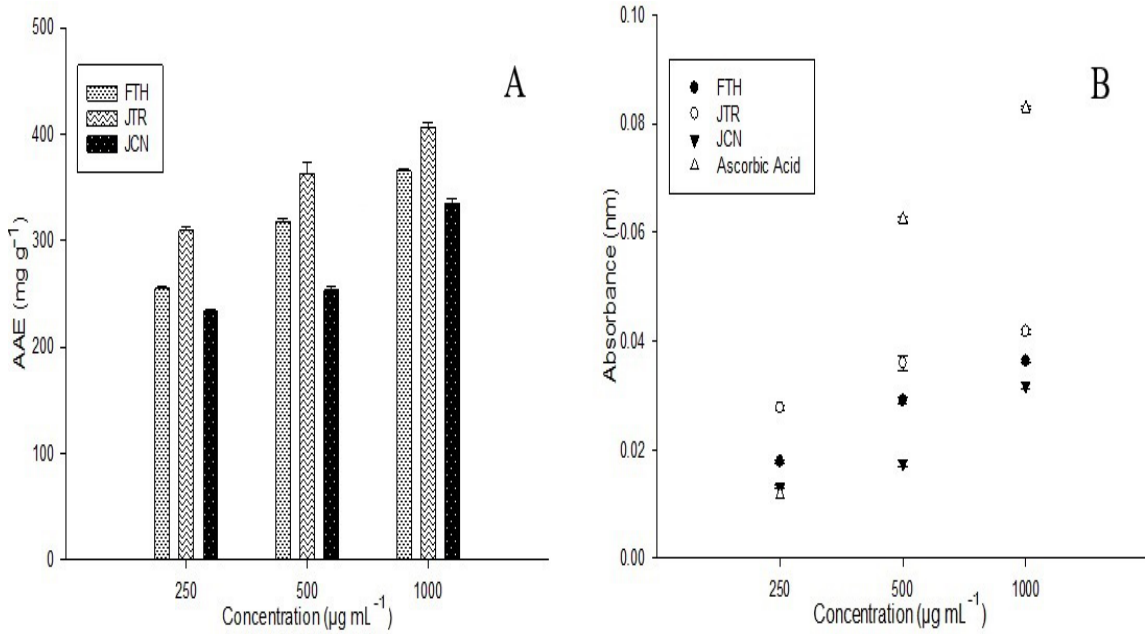

Fig. 4. Ascorbic acid equivalent (**A**) and absorbance (**B**) of methanol leaves extracts of *F. thonningii* Blume, *J. tanjorensis* J.L Ellis and Saroja, and *J. carnea* Lindl. in comparison to ascorbic acid in FRAP assay. FTH = *F. thonningii* Blume, JTR = *J. tanjorensis* J.L Ellis and Saroja, JCN = *J. carnea* Lindl.

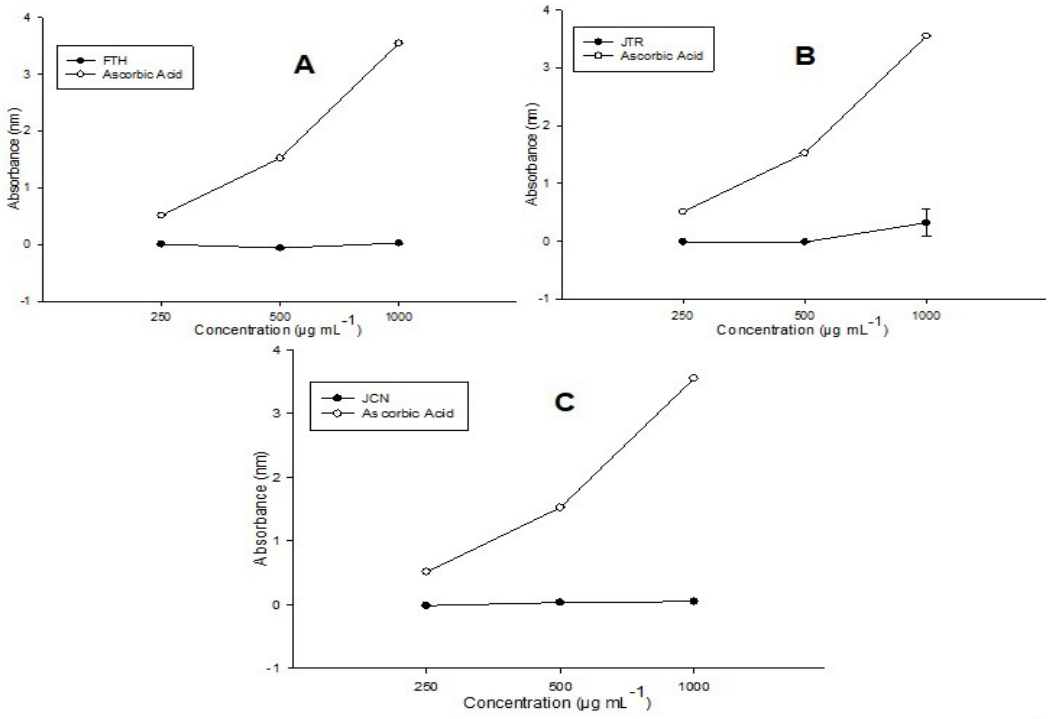

Fig. 5. Absorbance of *F. thonningii* Blume (**A**), *J. tanjorensis* J.L Ellis and Saroja (**B**), and *J. carnea* Lindl. (**C**) methanol leaves extracts in comparison to ascorbic acid in phosphomolybdenum assay. FTH = *F. thonningii* Blume, JTR = *J. tanjorensis* J.L Ellis and Saroja, JCN = *J. carnea* Lindl.

diseases, inflammation, hypertension, cancer, obesity and diabetes mellitus (Vignesh et al., 2021) and plants are known to mitigate them through different pathways including nutritional, antioxidant, antimicrobial, anti-diabetic, anti-hypertensive, anti-inflammatory and anti-cancer pathways (Awuchi et al., 2020; Mahdavi and Mohammadhosseini, 2022). Some natural antioxidant supplements including essential oils are nutritional and functional foods having greater target activity as potential medicine and sources of nutrients that perform basic nutritional role (Vignesh et al., 2021; Okpala et al., 2023). Previous reports have shown that essential oils exhibit antioxidant-based pharmacological properties due to their chemical constituents (Oloyede et al., 2021). Hence, the antioxidant and nutritional properties of the assayed plants could be attributed to the presence of antioxidant-based phytochemicals of pharmacological relevance contained in the plants leaves extracts as well as their nutritional and micronutrient compositions.

From Table 1, it could be inferred that the concentrations of protein in the assayed plants were not statistically significant ($p > 0.05$). Previous reports on *F. thonningii* suggested the presence of crude proteins, ash and moisture contents, crude fat, and higher content of crude fiber (Osowe et al., 2021), while higher carbohydrate content of *J. tanjorensis* J.L Ellis and Saroja was corroborated by Bello et al. (2008). Also, high content of carbohydrate has been reported for *J. carnea* Lindl. by Orjiakor et al. (2019), though our study presents a higher moisture content probably because of seasonal variation during the sample collection step. The moisture content of *F. thonningii* Blume, *J. tanjorensis* J.L Ellis and Saroja, and *J. carnea* Lindl. leaves was estimated as 20.12 ± 0.45, 70.44 ± 0.11, and 46.78 ± 0.45% DW, respectively.

From Table 2, the concentration of iron, sodium and zinc in the assayed plant part was found to be statistically significant ($p < 0.05$). Micronutrients' supplementations are the trend in the management of ailments (Osowe et al., 2021). Hence, the mitigation offered by the supplementation of extracts of *F. thonningii* Blume, *J. tanjorensis* J.L Ellis and Saroja, and *J. carnea* Lindl. are of utmost relevance in curtailing severity in antioxidant-related pathologies. Previous research demonstrated that *J. carnea* Lindl. improves hematological and biochemical indices in male Wistar rats (Orjiakor et al., 2019). Therefore, the nutritional and micronutrient profiling of its extracts showed that *J. carnea* Lindl. also functions via the nutritional pathway to mitigate severe disease complications as evidenced by Table 1 and Table 2.

Micronutrients are responsible for the production of hormones and enzymes and are needed for normal growth and development, while their deficiency leads to a notable reduction in energy levels, mental clarity and overall capacity (WHO, 2022). The burden of micronutrient deficiency lies on children and pregnant

Table 1
Nutritional compositions of leaves of *F. thonningii* Blume, *J. tanjorensis* J.L Ellis and Saroja, and *J. carnea* Lindl. Individual readings (n = 3) were averaged and presented with ± SEM.

Assayed plants	Moisture (% DW)	Ash (% DW)	Crude fat (% DW)	Crude fiber (% DW)	Crude protein (% DW)	Carbohydrate (% DW)	Calorific value (kcal 100g^{-1})
FTH	20.12±0.45^{a}	8.28±0.11^{a}	2.24±0.08^{a}	24.97±0.62^{a}	5.83±0.23^{a}	22.75±0.32^{a}	134.48±0.22^{a}
JTR	70.44±0.11^{b}	6.32±0.07^{b}	2.53±0.42^{a}	25.93±0.12^{a}	5.78±0.03^{a}	27.09±0.48^{b}	154.24±0.23^{b}
JCN	46.78±0.45^{c}	3.45±0.21^{c}	1.97±0.12^{c}	18.65±0.22^{c}	5.34±0.03^{a}	18.54±0.77^{c}	113.25±0.12^{c}

*Superscripts a, b, and c represent various levels of significance as obtained from SAS. Similar letters in the same column indicate that data is not statistically significant (p > 0.05) while different letters in the same column show statistical significance (p < 0.05). Similar letters with * are statistically significant (p < 0.05). FTH = *F. thonningii* Blume, JTR = *J. tanjorensis* J.L Ellis and Saroja, JCN = *J. carnea* Lindl.

Table 2
Mineral compositions of leaves of *F. thonningii* Blume, *J. tanjorensis* J.L Ellis and Saroja, and *J. carnea* Lindl. Individual readings (n = 3) were averaged and presented with ± SEM.

Assayed plants	Calcium (mg 100g^{-1} DW)	Potassium (mg 100g^{-1} DW)	Iron (mg 100g^{-1} DW)	Sodium (mg 100g^{-1} DW)	Zinc (mg 100g^{-1} DW)
FTH	22.86±0.03^{a}	13.54±0.02^{a*}	0.22±0.03^{a}	6.17±0.03^{a}	4.02±0.03^{a}
JTR	34.54±0.03^{b}	12.76±0.12^{a}	9.98±0.78^{b}	8.43±0.04^{b}	8.77±0.09^{b}
JCN	23.65±0.01^{a}	11.93±0.04^{a*}	10.69±0.56^{c}	3.65±0.15^{c}	12.87±0.02^{c}

*Superscripts a, b, and c represent various levels of significance as obtained from SAS. Similar letters in the same column indicate that data is not statistically significant (p > 0.05) while different letters in the same column show statistical significance (p < 0.05). Similar letters with * are statistically significant (p < 0.05). FTH = *F. thonningii* Blume, JTR = *J. tanjorensis* J.L Ellis and Saroja, JCN = *J. carnea* Lindl.

women in low-income and middle-income countries (WHO, 2022). Therefore, the use of the extracts from *F. thonningii* Blume, *J. tanjorensis* J.L Ellis and Saroja, and *J. carnea* Lindl. in the management of arrays of diseases by ethnic Igbos in Eastern Nigeria is attributed in part to the presence of micronutrients contained in their leaves extracts. Some diseases such as sickle cell anemia are marked by relative energy shortage. This metabolic irregularity drastically increases the energy requirement and reduces the availability of nutrients for growth and development in children and for maintaining adequate muscle mass in adults (Nartey et al., 2021). Hence, incorporating extracts or consuming leaves of the assayed plants may probably fill in the gap for these nutrient deficiencies.

4. Concluding remarks

Different antioxidant assays vary in terms of their structures and constituents, nature of their performance as well as experimental conditions. Hence, to assess the antioxidant potential of plant extracts, a single analytical-based method is not enough. Moreover, the time taken to initiate free radical formation, the polar/non-polar nature of antioxidants as well as their action mechanisms remarkably vary. A simple perusal of the literature also demonstrates that the pathophysiology of a large number of diseases displays overwhelming nutritional implications not limited to higher energy, nutrient requirements, nutrient deficiencies along with growth abnormalities. Hypothetically, to address increased energy expenditure, nutrition interventions could be a valid mechanism. Hence, in low-income countries, attention to nutritional care is increasingly felt to be an important aspect of supportive management for several diseases. Considering the deleterious effect of reactive oxidizing species and the (end)-products of their oxidative reactions, antioxidant therapy plays a vital role in the management and treatment of various diseases, thus representing targets for antioxidant therapeutics. In short, the present report suggests that *F. thonningii* Blume, *J. tanjorensis* J.L Ellis and Saroja, and *J. carnea* Lindl. could be considered as potential sources of nutraceutical antioxidants and functional foods. However, future studies should consider *in vivo* and clinical trials of the extracts of the assayed plants and their characterized bioactive compounds.

Conflict of interest

The authors declare that there is no conflict of interest.

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