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CHEMOPROFILING AND *In vitro* ANTIOXIDANT POTENTIALS OF METHANOL-WATER EXTRACTS FROM NIGERIAN GINGER (*Zingiber officinale*)

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ABSTRACT

Ginger (*Zingiber officinale*) is one of the popular spices in Nigeria. It is highly utilised over the world as spice as well as for the treatment of inflammation, arthritis and other conditions involving reactive oxygen species (ROS), which are known to damage human cells. The proximate, phytochemical composition and antioxidant properties of the plant are reported in this work. Extraction by maceration was used for the pulverized rhizomes with methanol-water (50:50). Further partitioning of the extract was carried out using *n*-hexane, ethyl acetate and butanol into different fractions according to their polarity. The antioxidant activity was evaluated with 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, ferric reducing antioxidant power (FRAP) assay, nitric oxide radical scavenging activity (NOSA) and lipid peroxidation scavenging activity (LPSA). The proximate content was determined using conventional chemical methods. The FTIR analyses of *n*-hexane fraction of *Z. officinale* revealed some functional groups that may be present in two likely classes of secondary metabolites: terpenes and alkaloids. The ethyl acetate fraction produced flavonoid (121.31 mg/100 g; quercetin) and alkaloids because of the functional groups associated with them, while the butanol fraction with functional groups present revealed cardiac glycosides (4.88 mg/100 g; digoxin) and alkaloids (45.19 mg/100 g). The plant also contained reducing sugar (64.62 mg/100 g glucose), steroids (8.65 mg/100 g; cholesterol), tannins (56.82 mg/100 g; tannic acid) and phenolic compounds (110.64 mg/100 g; gallic acid) from the phytochemical analyses. The proximate analysis revealed that it has a high carbohydrate content (54.54%), but a relatively modest crude fibre (10.21%), moisture content (10.02%), total ash (9.30%), crude fat (8.80%) and crude protein (7.12%). The presence of these phyto-compounds could explain its customary use as oxidative stress inhibitor.

Keywords: Radical scavenging activity, Phytochemicals, Nutritional quality, *Zingiber officinale*.

INTRODUCTION

The extensive range of biological activities of secondary metabolites of plant origin have garnered a lot of attention in recent years. Consequently, the intake of functional foods has risen dramatically due to their safety and minimal side-effects. *Zingiber officinale* (ginger) is a bulbous plant primarily cultivated across Asia, Africa, South and Central America (Abdalla and Abdallah, 2018). It belongs to the family Zingeberaceae (Wagner, 1980), which comprises well over 1, 000 herbal plants. Other well-known members of the family include *Alpinia galangal* (galangal), *Elettaria cardamomum* (cardamom) and *Curcuma longa* (turmeric) as reported by Alolga *et al.* (2022). Historically, in addition to being used as a spice, ginger was also employed as medicinal herb in treatment of diseases.

Accumulated evidences have indicated that the ginger rhizome has enormous pharmacological activities like antimicrobial, anticonvulsant, analgesic, anti-inflammatory, anti-diabetic, anti-tumor, anti-cancer, anti-spasmodic, anti-allergic, anti-serotonergic, antioxidant, larvicidal and other advantageous properties (Kumar *et al.*, 2011; Kravchenko *et al.,* 2019). Furthermore, ginger improves blood circulation, and regulates blood pressure, hypertension and cholesterol which can lead to improved heart function (Ghayur *et al.*, 2005; Hasani *et al.*, 2019). These health benefits have been ascribed to its numerous bioactive phytochemicals, which include phenolic compounds, flavonoids, terpenes, proteins, carbohydrates, and minerals (Singh *et al.*, 2022).

Investigations have shown that rhizome consist of a number of antioxidant phytochemicals such as ascorbic acid, alkaloids, terpenoids, beta-carotene and polyphenols (Ghasemzadeh *et al.*, 2010). Studies have shown that there is a direct correlation between oxidative stress and a wide range of inevitable oxidative damages, leading to a broad range of pathological conditions such as

respiratory disorders, cardiovascular diseases, chronic kidney diseases, neurodegenerative diseases, and cancer diseases (Bekkouch *et al*., 2022). This highlights the scientific basis for continuing exploration of plants, such as ginger, for their potential antioxidant properties.

In Nigeria, ginger has a long history of cultivation (Ajayi *et al*., 2013) and is commonly used as flavouring agent. It is also used in the treatment of various illnesses including the common cold. Indeed, essential oils from rhizomes form part of natural products with a variety of metabolites that may represent a class of natural antimicrobials (Ekundayo *et al*., 1988; Abdullahi *et al*., 2020). Additionally, each species of medicinal plant has its own unique nutritional content and pharmacologically significant bioactive compounds. These nutrients, such as proteins, lipids, and carbohydrates, are necessary for all physiological activities and are especially important for supplying humans with the energy and other life-process needs. The phytochemical, proximate, and antioxidant characteristics of the *Z. officinale* were examined in the current study. The information will expand the corpus of currently existing information and provide new scientific support for its application in the pharmaceutical sector.

MATERIALS AND METHODS

Plant Collection and Processing

Fresh rhizomes of *Z. officinale* were procured from one of the main markets in Lagos, Nigeria. A taxonomically precise identification of the plant was made and the voucher specimen (Reference Number: LUH 8632) was deposited in the University herbarium at the Department of Botany, Faculty of Science. *Z. officinale* (900 g) was cut into tiny pieces, washed, air-dried for seven days and the dried plant material was pulverized.

Extraction of the Plant Materials

For extraction, 524 g of the pulverised plant material was weighed and extracted by maceration with 1,800 mL of methanol/water (50% v/v) mixture. Solvent partitioning using *n*-hexane, ethyl acetate and butanol fractions was sequentially performed as described by Osibote *et al.* (2020).

Proximate Composition Determination

Drying was used to ascertain the moisture content of the sample (Ayuba *et al*., 2011). Crude protein, carbohydrate, crude lipid, crude fibre and total ash compositions of the plant were analysed using the Association of Official Analytical Chemists standard methods (AOAC, 2006). The crude protein content was calculated as suggested (AOAC, 2006) and the crude lipid content was extracted using a soxhlet extractor and diethyl ether (AOAC, 2006). The crude fibers were determined using acid hydrolysis method. All tests were performed in triplicate.

Phytochemical Analyses

The preliminary phytochemical method applied in an earlier work (Osibote *et al*., 2020) was used to determine the phytochemicals of *Z. officinale*. Both qualitative and quantitative analyses were performed to determine the level of phytochemicals in the plant extract. Each analysis was carried out in triplicate. Alkaloid was quantified using the precipitation-gravimetric method (Harborne, 1998), and total phenolic compound concentration was estimated using the Folin Ciocalteau procedure (Singleton and Rossi, 1999). Total flavonoid was determined and expressed as Rutin equivalents (Ahmad *et al*., 2018). Reducing sugars, steroids, saponin, tannins, and cardiac glycosides were determined as previously described by El-Olemy *et al.* (1994).

Determination of Antioxidant Activity

The antioxidant activities of the crude extract of *Z. officinale* were investigated using five different assays**:**

1,1-Diphenyl-2-picrylhydrazyl (DPPH) Scavenging Activity

The DPPH technique was one of the methods used to assess the antioxidant activity of the crude methanolic extracts of *Z. officinale*. A 25-100 g/mL concentration of *Z. officinale* extract in water was added to 1 mL of 0.1 mM solution of DPPH in ethanol and absorbance measured with a UV-Visible Spectrophotometer at 517 nm. Lower absorbance of the reaction mixture indicated more free radical scavenging activity. The DPPH scavenging effect was calculated as follows:

$$\text{DPPH Scavenging effect (\%)} = [(A_0-A_1)/A_0] \times 100$$

Where:
$A_{0=}$ the absorbance for the control
A_1 = the absorbance in the standard sample or extract.
IC_{50} = concentration of the compounds that caused 50 % inhibition of DPPH radical formation.

Ferric Reducing Power Assay (FRAP)
The procedure was carried out as reported by Asimi *et al.* (2013). Gallic acid was utilised as a control and the scavenging effect was calculated with the following equation:
FRAP scavenging effect (%) = $[(A_0-A_1)/A_0] \times 100$

Nitric Oxide Radical Scavenging Assay
Crude methanolic extract (10 mg/mL) of *Z. officinale* was serially diluted with distilled water to make concentrations of 25–100 g/mL using ascorbic acid standard (Panda *et al*., 2009). A 1 mL aliquot of various extract concentrations (25–100 g/mL) was mixed with 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline and the mixture was then incubated at 25 °C for 180 min. The extract was mixed with equal volume of freshly prepared Griess reagent. Control sample was prepared as described for the test sample but without the extract. The colour tubes contained methanol extracts at the same concentrations with no sodium nitroprusside. The reaction mixture was transferred in a volume of 15 mL to a 96-well plate. Using a microplate reader equipped with a UV/VIS TG 50 Plus UV-VIS, the absorbance was calculated at 546 nm (Molecular Devices, GA, USA). The percentage of nitrite radical scavenging activity of the methanol extracts and Ascorbic acid were calculated using the formula below:

$$\text{Nitric oxide scavenged (\%)} = \frac{(\text{control} - \text{test}) \times 100}{\text{control}}$$

Where control = absorbance of control sample and test = absorbance of the samples of extracts or standards

Lipid Peroxidation Assay
The lipid peroxidation method was carried out as described by Alvarez-Suarez *et al.* (2012). Supernatants after cetrifuging the mixture were collected and passed through a UV/VIS spectrophotometer at wavelengths of 515 nm/555 nm.

$$\text{Lipid Peroxidation (\%)} = \frac{(\text{control} - \text{test}) \times 100}{\text{control}}$$

Where control = absorbance of control sample and test = absorbance in the presence of the samples of extracts or standards.

Potassium Permanganate Assay (Total Antioxidant Capacity)
A 5 mmol/L solution of $KMnO_4$ was prepared and the solution was aliquotted into 100 mL portions and shaken before being transferred to a plastic plate containing the sample. A UV/VIS spectrophotometer was used to measure the absorbance of the mixture (optical density, OD, of 570 nm) after it had been warmed at 37 °C for 30 min in a water bath (Rocha-Guzman *et al*., 2013; Misso *et al*., 2020).

$$\text{Total Antioxidant Capacity (\%)} = \frac{(\text{control} - \text{test}) \times 100}{\text{control}}$$

Fourier Transform Infrared (FTIR) Spectroscopy Analysis
Chemical linkages (functional groups) were identified with FTIR spectroscopic analysis as described by Chandra (2019). Dried or concentrated column chromatography fractions were placed on the optical/platinum eye of the Bruker FTIR (Platinum ATR), pressed down by the doggle to make a thin film with a scan range of 4000 to 400 cm^{-1} and the infrared spectra region and peaks were recorded at room temperature.

Statistical Analysis
An analysis of variance was produced for all parameters that were tested in triplicate. The mean and standard error were used to express the results.

RESULTS

Fraction yield
The percentage of 50% methanol/water extract obtained from the plant materials, the *n*-hexane, ethyl acetate and butanol fractions obtained after the serial partitioning of crude extract are presented in Table 1. The yield from the *n*-hexane fraction was the lowest (1.125%), followed by that from the ethyl acetate fraction (4.467%).

Table 1: Yield of the crude extract and fractions obtained after serial partitioning with *n*-hexane, ethyl acetate and butanol.

Extract and fractions	Yield (g)	Percentage of yield
Crude extract	120	22.9
n-Hexane	1.35	1.125
Ethyl acetate	5.36	4.467
Butanol	8.55	7.125

Proximate Analysis

Proximate analysis of the rhizome showed that ginger contains mostly carbohydrates (54.54 %), crude fibre (10.21 %) and moisture (10.02 %) (Figure 1).

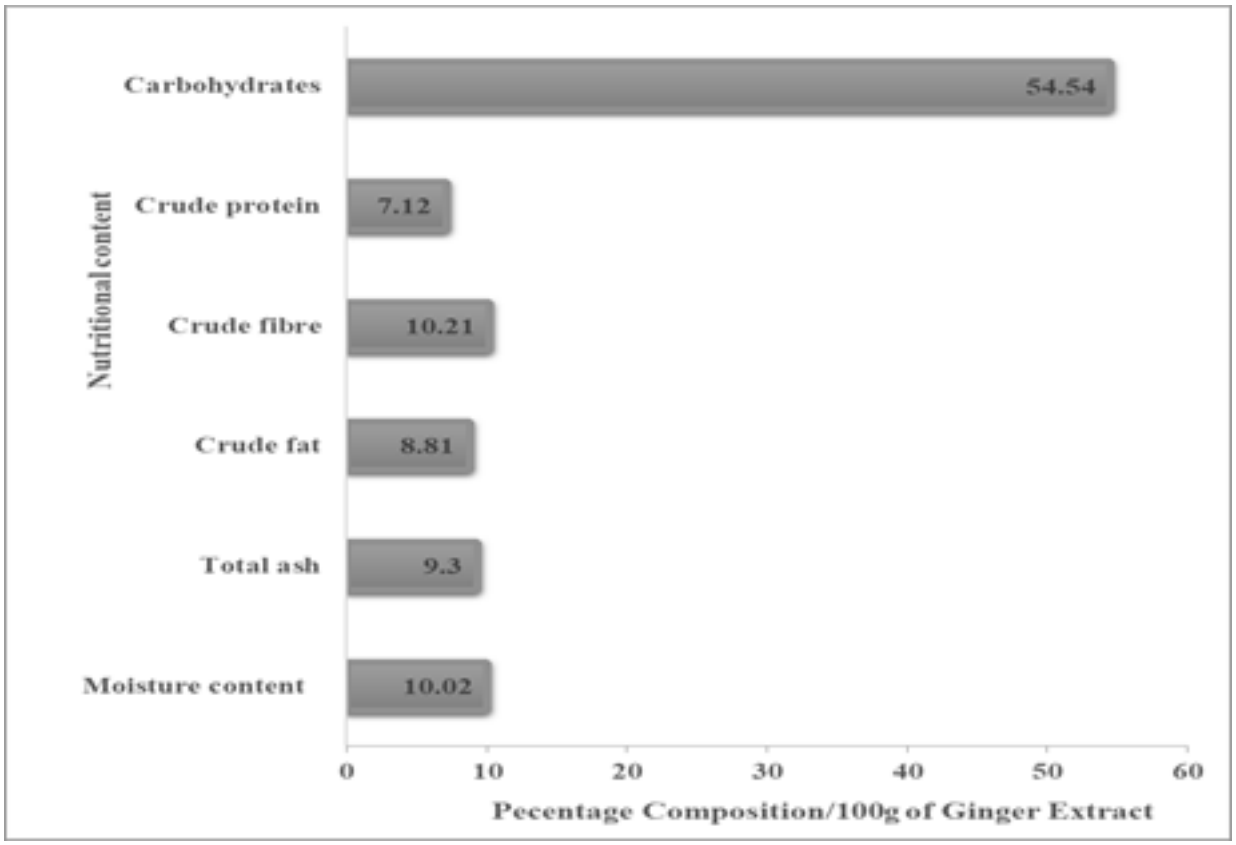

Figure 1: Nutritional composition of *Z. officinale*.

Qualitative Phytochemical Screening of *Z. officinale*

Table 2 outlines the phytochemical properties of *Z. officinale*. Some of the constituents found present are alkaloids, reducing sugars, cardiac glycosides, tannins, terpenoids, triterpenoids, steroids, phenolic compounds and flavonoids. However, saponins and anthraquinones were not detected in the extracts.

Table 2: The results of qualitative phytochemical screening of *Z. officinale*

Phytochemical Components	Tests	Inference
Alkaloids	Mayer's	+
	Dragendorff' s	+
	Wagner's	-
Saponins	Frothing	-
Reducing Sugar	Fehling's	+
Anthraquinones	Borntrager's	-
Cardiac glycosides	Keller Killani's	+
Terpenoids	Liebermaan-Burchard	+
Triterpenoids	Liebermaan-Burchard	+
Steroids	Salkowski's	+
Phenolic Compounds	Lead acetate	+
Tannins	Ferric chloride	+
Flavonoids	Shinoda's	+

(+): Present, (-): Absent

Table 3: Phyto-constituents of *Zingiber officinale* rhizome using quantitative analysis.

Phytoconstituents	**(mg/100 g of Dry sample)**rhizomes
	Z. officinale
Alkaloids	45.19
Saponins	-
Reducing Sugar	64.62
Cardiac glycosides	4.88
Steroids	8.65
Tannins	56.82
Phenolic compounds	110.64
Flavonoids	121.31
Anthraquinones	-

Antioxidant activity

The IC_{50} of the extract of *Z. officinale* is depicted in Table 4. The total anti-oxidant capacity of the plant was 61.67 mg/100 g whilst the total flavonoid was 65.8 mg/100 g and total phenol was 42.99 mg/100 g.

Table 4: Reducing activities and IC_{50} antioxidant potential of the crude extract of *Z. officinale*.

Anti-Oxidant Assay	Activity				IC_{50} Antioxidant Potential
	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL	
DPPH					59.93
FRAP	0.05	0.16	0.24	0.38	11,613.95
NOSA	28.49	54.54	65.76	77.34	52.15
Lipid Peroxidation Scavenging Activity	23.81	34.57	42.29	51.68	95.11

Functional Groups of the Active Components in Rhizome Extracts of *Z. officinale*

The FTIR spectral analysis of the *n*-hexane fraction of *Z. officinale* showed the characteristic functional groups of terpenes and alkaloids (Table 5). Also, the FTIR analysis of the ethyl acetate fraction showed functional groups that are characteristics of flavonoids and alkaloids. The FTIR spectral classification of the butanol fraction indicated also, functional groups that are characteristics of cardiac glycosides and alkaloids. Many functional groups were detected in the various *Z. officinale* fractions, including C = O, C = C, CH, OH, and C-N (Table 5).

DISCUSSION

This report indicated the dried and crushed rhizomes of *Z. officinale* produced a 22.9% extraction yield. The proximate analysis revealed that ginger can be regarded as an herb that is rich in energy because of its high carbohydrate content. An appreciable amount of crude fibre, moisture, total ash, crude fat and protein were also present. Although, this finding compared favourably with those described by Ajayi *et al.* (2013), it was less than the ash, fat, crude fibre and carbohydrate levels published by other authors (Taoheed *et al*., 2017; Awonegan *et al*., 2022). These outcomes showed that the plant had nutritional qualities, indicating that it may help meet human needs for food and energy.

Recently, plant secondary metabolites have garnered a lot of attention due to their extensive spectrum of biological functions. The detection of phytochemicals in ginger rhizomes in this study indicates their potential use as a source for the development and synthesis of pharmaceuticals. These bioactive components, including terpenes and terpenoids, have been linked to powerful antioxidants and antibacterial activities (Kazlowska *et al*., 2010; Anh *et al*., 2020). Even though there may be minor variations in the phyto-components of the *Z. officinale* reported in different studies due to the polarity of the solvent used, the geo-location of the plant and the plant part used for extraction (Sulaimana *et al*., 2014; Sharif and Bennett, 2016), the high concentration of phenols and flavonoids found in the crude extract of the rhizome variants examined in this study is compatible with the conclusions of Yusuf *et al*. (2018).

Table 5: Bands and functional groups of the classes of secondary metabolites of the *n*-hexane, ethyl acetate and butanol fractions of *Zingiber officinale* rhizome.

Fractions	Bands present	Functional groups assigned	Likely classes of compounds	Appearance	Classes of secondary metabolites identified
n-Hexane	3272.6cm-1	OH stretching	Alcohol	Strong, Broad	Terpenes
	2922.2cm-1	OH stretching	Carboxylic Acid	Strong, Broad	
	2322.1cm-1	C-H stretching	Alkane	Broad	
	1722 cm-1	C=O stretching	Aliphatic Ketone	Strong	Alkaloids
	1617.7 cm-1	C=C stretching	Cyclic Alkene	Medium	
	1408.9 cm-1	O-H Bending	Carboxylic Acid	Medium	
	1371.7 cm-1	O-H Bending	Phenol	Medium	
	1241.2 cm-1	C-N Stretching	Amine	Medium	
	1032.5 cm-1	C-N Stretching	Amine	Medium	
Ethyl-acetate	3280.1 cm-1	OH stretching	Alcohol	Strong, Broad	Flavonoids
	2914.8 cm-1	C-H stretching	Alkyne	Strong, Sharp	
	2847.7 cm-1	C-H stretching	Alkyne	Strong, Sharp	
	1599.0 cm-1	C=C stretching	Cyclic Alkene	Medium	
	1416.4 cm-1	OH stretching	Carboxylic Acid	Medium	
	1364.2 cm-1	O-H Bending	Phenol	Medium	
	1289.7 cm-1	C-O stretching	Aromatic Ester	Strong	Alkaloids
	1140.6 cm-1	C-O stretching	Aliphatic Ether	Strong	
	1021.3 cm-1	C-N Stretching	Amine	Medium	
	790.2 cm-1	C=C Bending	Alkene	Medium	
Butanol	3239.1 cm-1	OH stretching	Alcohol	Strong, Broad	glycosides
	2926.0cm-1	OH stretching	Carboxylic Acid	Strong, Broad	
	1736.9cm-1	C=O stretching	Esters	Strong	
	1602.8 cm-1	C=C stretching	Conjugated Alkene	Medium	
	1416.4 cm-1	O-H Bending	Carboxylic Acid	Medium	
	1312.0 cm-1	O-H Bending	Phenol	Medium	Alkaloids
	1226.3 cm-1	C-O stretching	Vinyl Ether	Strong	
	1002.7 cm-1	C-N Stretching	Amine	Medium	

The abundance of phenolic compounds and flavonoids detected herein supports the effectiveness of extracts from the plant in the management of degenerative ailments linked to the presence of reactive oxygen species (Yonbawi *et al*., 2021). Phenols are integral elements of plants that serve a variety of biological purposes, like antioxidant activity because of their capacity as free radicals scavenging ability through their OH groups (Morales *et al*., 2008). Previously, Yousfi *et al*. (2021) had found a strong link between the total phenolic content and antioxidant activity of *Z. officinale* extracts. Likewise, flavonoids are believed to enhance the antioxidant activities of plants (Yusuf *et al*., 2018; Bekkouch *et al*., 2022).

The mean IC_{50} value across all assays in this investigation was found to be 11,749.8 g/mL. This result agrees with that of Abbas *et al*. (2021) that reported the IC_{50} value of the ginger extract examined in their investigation as 1166.7 g/mL. We might hence surmise that *Z. officinale* extract possessed remarkable superoxide radical scavenging capabilities, inhibition of NO production and lipid peroxidation. It has been established that an excessive amount of ROS causes lipid peroxidation via enzymatic (LOX-catalyzed) and non-enzymatic (iron-dependent) processes, which induces cell death. Lipid peroxidation has been connected to conditions like kidney impairment, atherosclerosis, Parkinson's disease and asthma (Mylonas and

Kouretas, 1999). Thus, cells are shielded from oxidative damage by a broad endogenous antioxidant system, like enzymatic antioxidants and non-enzymatic antioxidants (metabolites, vitamins or their analogues and minerals).

Fourier transformed infrared (FTIR) analysis was used to pinpoint the functional groups present in the partitioned fractions (*n*-hexane, ethyl acetate and butanol) from the crude extract. The functional groups assigned peaks in the FTIR measurements matched the phytochemicals found in the *Z. officinale* extracts. The *n*-hexane fraction contained some functional groups which are linked to alkaloids and terpenoids, flavonoids and alkaloids were recognized in the functional groups identified in the ethyl acetate fraction; cardiac glycosides and alkaloids were also linked to the functional groups present in the butanol fraction. This significant scientific evidence supports the idea that secondary metabolites play a central role in plant bioactivities. Similar distribution pattern of FTIR spectra were observed by Aye (2020).

The phytochemicals found in this study support the bioactivities of the plant and are known for their strong medicinal values. Phytochemicals with their nutritional, biological and pharmacological properties are well-documented (Adeyemo *et al.*, 2020).

CONCLUSION

The results from the present study showled that *Z. officinale* contains a high carbohydrate and fibre content, making it an effective source of nutrients and energy supplements. In addition, high phenolics, flavonoids and alkaloids contents were found in the extract. As demonstrated by the free radical DPPH, FRAP, TAC and lipid peroxidation scavenging activity, these phytochemicals possess significant antioxidant potentials. The FTIR analyses of *n*-hexane, ethyl acetate and butanol fractions of *Z. officinale* revealed the presence of many functional groups which are attributed to the various secondary metabolites. These secondary metabolites are linked to the various bioactivities of the plant providing good protection against oxidative damage in human body. Further studies are needed to explore these plants as alternatives to prevent chronic diseases.

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CONFLICT OF INTEREST

The authors disclose that there is no potential conflict of interest.

CONTRIBUTION OF THE AUTHORS

OEA designed and supervised the project, AOA and OEA interpreted the data and wrote the initial draft of the manuscript; OAS and NNJ did the experimental. All the authors read and approved the final submission.

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