The Bioscientist Journal Vol. 12 (2): 127 – 136, March 2024
Journal of the Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria
Available online at *http://www.bioscientistjournal.com*
DOI 10.54117/the_bioscientist.v12i2.172

Full Length Research Article

COMPARATIVE PROXIMATE, PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY ON AQUEOUS EXTRACT OF *GONGRONEMA LATIFOLIUM* FRUIT AND LEAF.

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Article History: *Received: 12th July 2024, Accepted: 31st August 2024*

Abstract

Gongronema latifolium a tropical plant, has been extensively studied for its medicinal relevance and antioxidant properties. This research aims to investigate the comparative proximate, phytochemical, and antioxidant effects on aqueous extracts from *G. latifolium* fruit and leaf. The phytochemical and proximate composition of the fruit and leaf were assessed using standard analytical methods, while the antioxidant assays were conducted using colorimetric and spectrophotometric methods. Phytochemical analysis revealed the *Gongronema latifolium* fruit sample contain higher levels of total phenol (38.21±0.99), tannin (30.79±0.94) and flavonoid (29.78±0.64) than leaf samples. The proximate composition showed that *G. latifolium* leaf and fruit sample possessed significant carbohydrate and crude fat content, while the fruit sample contained higher ash content (4.47 ± 0.70%) compared to the leaf sample (1.80 ± 0.13%). In the antioxidant assay, the DPPH scavenging activity of the leaf extract (303.41µg/ml) had a lower EC_{50} value than the fruit extract (371.26µg/ml) while the fruit extract showed a higher IC_{50} (517.11 µg/ml) than the leaf extract (644.00µg/ml). The *in vitro* assay demonstrated strong antioxidant properties, with fruit extracts exhibiting higher activity than leaves. These findings suggest that *G. latifolium* fruit shows superior nutritional and antioxidant activity surpassing the leaf, making it highly recommended for managing diseases related to oxidative stress.

Keywords: *G. Latifolium*, Nutritive Value, Antioxidant Activity, Oxidative Stress.

Introduction

Given the possible health risk challenges in the world such as heart, liver, and kidney failure, which have been related to oxidative stress and high consumption of processed food, there is a need to investigate alternative therapies. Exploring natural antioxidants from plants may offer promising therapeutic potential in combating these detrimental processes. (Suleria *et al.,* 2020). Nigeria boasts a diverse range of plants and vegetables that serve as food, herbs, and traditional medicines (Obeta *et al*., 2021). One of suchmedicinal plant is *Gongronema latifolium*, also known as Bush Buck, which belongs to the family *Asclepiadaceae* (Iwu, 2014). It is grown locally in the southern part of Nigeria and is addressed with different names, such as "Utasi" by the Ibibios, Quas, and Efiks ethnic groups; "Utazi" by the Igbos; and "Arokeke" by the Yorubas (Aigbokhan, 2014). The leaves of *G. latifolium* are characterized by their broad, heart-shaped form and distinctive bitter-sweet taste when fresh, while while its oblong-lanceolate fruit splits open to release seeds with a slightly sweet taste used for culinary practices (Omodale *et al*.2017; Ojiako *et al*., 2013). Various parts of the *G. latifolium* Plants, including seeds, stems, roots, fruits, and leaves, have been utilized for treating and managing a range of ailments such as cough, intestinal worms, dyspepsia, dysentery, and malaria in communities across the West African region (Morebise, 2015; Balogun *et al*., 2016). The utility of *G. latifolium* is different in Senegal and Ghana, where the leaves are rubbed topically on the body joints of children to help them walk while the boiled extract of the fruit is used as a laxative (Mosango, 2022), promoting healthy living and general body healing (Agwarambo *et al.,* 2014). Based on previous studies, the whole plant *G. latifolium* exhibits the following herbal actions: analgesic, antitumor, broad-spectrum, anti-microbial, anti-sickling,development of novel functional dairy products (Damunupola *et al.*, 2014), anti-helminthic, antimalarial, antioxidant, antidiabetic, antihypertensive, antidiarrheal, antitussive, and nutritive value (Odukoya *et al.,* 2005; Nwonu *et al.,* 2023).

Despite the growing interest in natural antioxidants and bioactive compounds from plant sources, there is limited comparative information on the nutritional composition and antioxidant potential of the fruit and leaf of *G. latifolium.* Specifically, while both the fruit and leaf of *G. latifolium* are known to possess significant phytochemicals and antioxidant properties, there is a gap in the scientific literature regarding how their proximate compositions, phytochemical contents, and antioxidant activities compare. This lack of comparative data poses a challenge in maximizing the utilization of the plant's different parts for nutritional and therapeutic purposes.

The study aims to evaluate the proximate composition, phytochemical and *in-vitro* antioxidant potentials of aqueous extract of fruits and leaves *G. latifolium*; to contribute towards its proper usage in managing oxidative stress-related conditions in traditional or modern treatments

Materials And Methods

Collection and Identification of *G. latifolium* Fruit and leaf Sample

The fruits and leaves sample of *G. latifolium* were collected from Okochi's compound Umudunu village in Abagana, Njikoka L.G.A of Anambra state, Nigeria. The plant sample collected was taken to the Department of Botany Herbarium, Nnamdi Azikiwe University Awka, where it was identified and authenticated by Mr. Iroka Chisom, a taxonomist in the Department; a voucher specimen was deposited and a

voucher number NAUH-34^{D}and NAUH-34^{A}were issued to the specimen.

Preparation of G. latifolium Fruit and leaf Sample

The *G. latifolium* fruits were cut into slices and dried at room temperature for 3 weeks while the leaves were dried for 2 weeks to prevent fungal growth, then both samples were grounded separately into fine powder using an electrical grinder. The powdered sample was stored in an air-tight container till further analysis.

Preparation of Aqueous Extract of *G. latifolium* Fruit and leaf Sample

One Kilogram (1kg) of the pulverized samples of *G. latifolium leaves* and fruits, the aqueous extract was prepared by soaking 100g of each grounded sample separately in 1000ml of deionized water for 24hrs. After 24 hours, the sample mixtures were filtered, and concentrated at 60^{o}C using a water bath (Memmert WTB). The crude extracts were transferred into a sample bottle, stored at 4^{o}C in refrigerator and used for further laboratory test analysis.

Percentage Extract Yield=

Weight of $\frac{extract}{weight}$ of dried homogenized sample× 100 (Barros *et al.,* 2008).

Phytochemistry Analysis

Alkaloids was determined by the method described by Harborne (1998); Tannins were determined by the method described by AOAC (1995); Oxalates were determined by the method described by Iwuzor,2019; Phytates was determined by the method described by Young and Greaves (1999); Saponins was determined by the method described by AOAC (1990); Total flavonoid and total phenols was determined by the method described by Barros *et al*. (2008);Lycopene and beta carotene was determined by the method described by Fish *et al.* (2002) and Heinonen *et al.* (1990) respectively.

Proximate Analysis

The Crude fiber, crude protein, crude fat, Moisture and ash contents of *G. latifolium* fruit and leaf samples were determined in triplicates using the method described by AOAC (2004). The carbohydrate content was determined by difference method as reported by (Nwinuka *et al.*,2005). The total carbohydrate content was calculated as: Total Carbohydrate (%) = 100 - (% Moisture + % Ash + % Crude Fiber + % Crude Protein + % Fat).

***In vitro* Antioxidant Activity of the fruit and leaf extract**

DPPH free radical, reducing power and inhibition of lipid peroxidation were determined by the method described by (Barros *et al.,* 2008), Marzocco*et al.* (1998), and Oyaizu (1986) respectively.

IC_{50} and EC_{50} concentration

This was determined by the method of Barros *et al*. (2008) for the inhibition concentration The extract concentration providing 50% lipid peroxidation inhibition (IC_{50}) was calculated from the graph of antioxidant activity percentage against the extract concentrations, BHA was used as the standard. while the effective concentration was carried out using the method of Ebrahimzadem *et al.* (2014). The EC_{50} (concentration of sample at 50% RSA) was calculated from the graph of %RSA against the sample concentration.

Statistical Analysis

All chemical analysis and assays were performed in triplicate, unless otherwise indicated. Results were expressed as mean values ± standard deviation (SD), using Microsoft excel and Analysis of variance (ANOVA) followed by Duncan's test was performed to test for differences between

means by employing Kyplot (version 2.0 beta 15, c1997-2001, Koichi Yoshioka) statistical software.

Results

Phytochemical analysis results

Table 1 shows the result of the phytochemical constituents of *Gongronema latifolium* fruit and leaf samples. *Gongronema latifolium*fruit samplespossess high concentrations of Total Phenols (38.21±0.99mgGAE/100g), Tannins (30.79±0.94mgTAE/100g), Flavonoids (29.78±0.64%) than the leafwhich has (16.55±0.93mgGAE/100g), (10.60±1.03mgTAE/100g) and (5.46±0.74mgCE100/g) respectively. Comparatively, the leaf contains more Saponin (2.83±0.28%), Alkaloids (0.375±0.08%), Beta carotene (0.23±0.02 mg/100g), Phytate (0.145±0.04%), Lycopene (0.15±0.04 mg/100g) than the fruit. Moderate quantities of Oxalate were found more in the fruit (3.47±0.02%) and Saponin in the leaf (2.83±0.28mg/100g) while least level of concentrations of leaf contains more beta carotene (0.23±0.02 mg/100g), phytate (0.145±0.04%), lycopene (0.15±0.04 mg/100g) and alkaloids (0.375±0.08%) than the fruit.

Table 1: Phytochemical Compositions of *G. latifolium*Fruit and Leaf

PHYTOCHEMICALS	FRUIT COMPOSITION	LEAF COMPOSITION
Total phenol (mgGAE/100g)	38.21±0.99	16.55±0.93
Flavonoid (mgCE100/g)	29.78±0.64	5.46±0.74
Beta carotene (mg/100g)	0.15±0.04	0.23±0.02
Phytate (%)	0.15±0.01	0.145±0.04
Lycopene (mg/100g)	0.1±0.01	0.15±0.04
Oxalate (mg/100g)	3.47±0.02	2.17±0.21
Saponin (%)	2.40±0.73	2.83±0.28
Alkaloid (%)	0.25±0.03	0.375±0.08
Tanin (mgTAE/100g)	30.79±0.94	10.60±1.03

The values are Mean and standard deviation for triplicate determination

Proximate Analysis results

Table 2 shows the result of the proximate composition of *G. latifolium* fruit and leaf samples. *G. latifolium* fruit and leaf samples possessed significant carbohydrates with h the fruit at (67.01 ± 3.78%) and the leaf at

(66.44 ± 0.98%), Crude fiber content is slightly higher in the fruit (2.87 ± 0.08%) than in the leaf (2.22 ± 0.27%), and Crude fat content at the leaf at (8.47 ± 0.91%)is slightly higher than the fruit (8.22 ± 1.39.The leaf has a higher moisture content (9.98±0.11%) and crude protein (11.09±0.16%) than the fruit while the fruit contains more ash content(4.47±0.70%) compared to the leaf (1.80 ± 0.13%), these plant Samples contain a good source of carbohydrate which had the highest nutritional content followed by Crude protein, Moisture content, Crude fat, Ash content and Crude fibre had the least value.

Table 2: Proximate Compositions of *G. latifolium* Fruit and Leaf.

PARAMETERS	FRUIT	LEAF
Ash (%)	4.47±0.70	1.80±0.13
Moisture (%)	8.04±0.54	9.98±0.11
Crude Fibre (%)	2.87±0.08	2.22±0.27
Total carbohydrate (%)	67.01±3.78	66.44±0.98
Crude protein (%)	9.4±0.95	11.09±0.16
Crude Fat (%)	8.22±1.39	8.47±0.91

The values are mean and standard deviation for triplicate determination

Antioxidant results

Free radical scavenging activities of aqueous extract of *G.latifolium* fruit extract, leaf extract and BHA standard .

It is revealed in figure 1, that G. *latifolium* Fruit and leaf extracts competed favorably when compared to the BHA Standard.

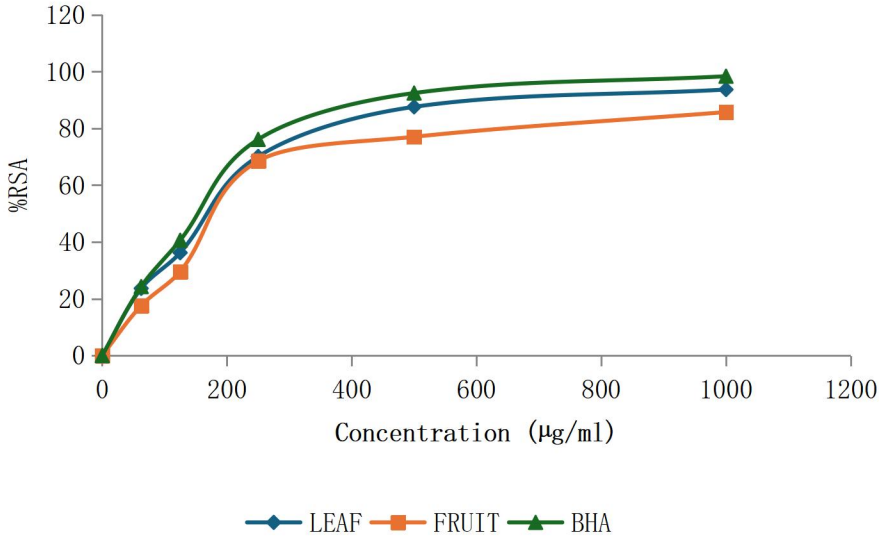

Figure 1: Free radical scavenging activities of *G. latifolium* fruit extract, leaf extract and BHA Standard

Free Reducing Power of aqueous extract of *G. latifolium* against BHA Standard

The *G.latifolium* fruit extract competed favourably with the BHA standard, while the *G.latifolium* leaf is observed to be slower than the fruit and BHA Standard, as shown in In figure 2.

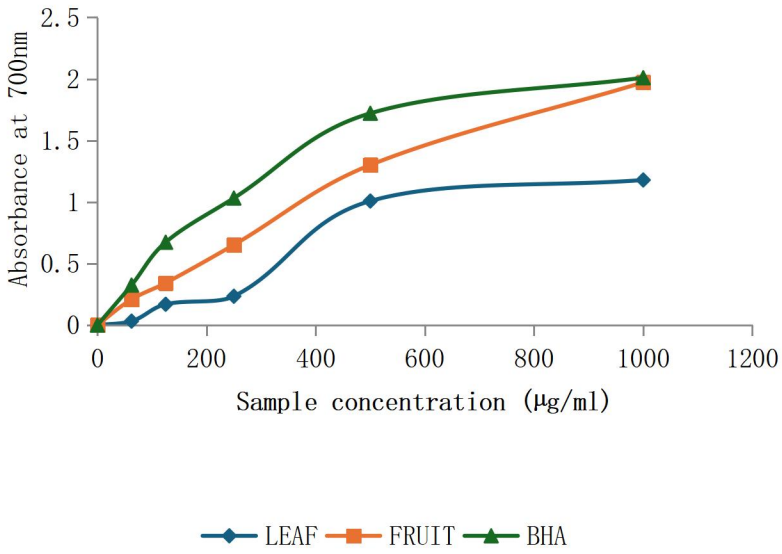

Figure 2: Free Reducing Power of *G. latifolium* fruit extract, leaf extract and BHA Standard

Lipid peroxidation of *G.latifolium* extract against BHA standard

In figure 3, *G.latifolium* Fruit shows a higher activity than the leaf extract when compared to BHA Standard.

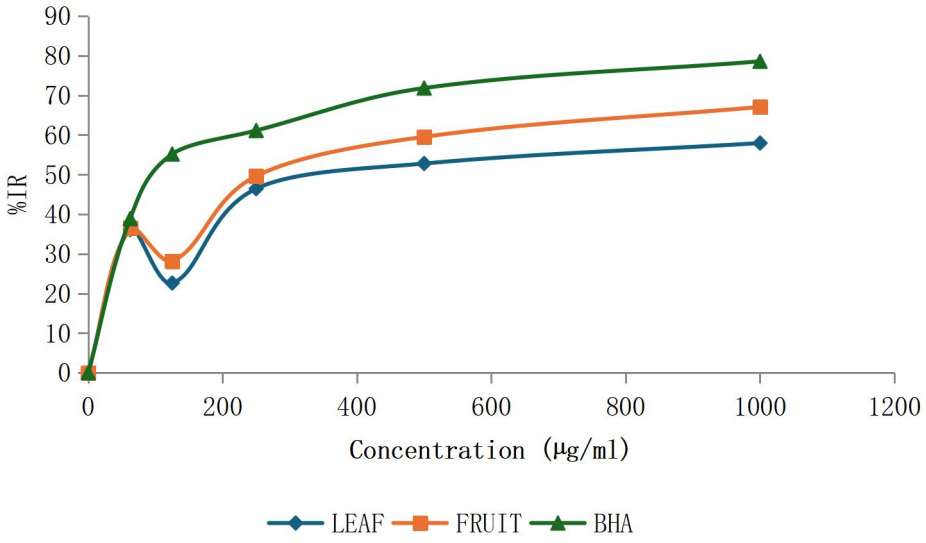

Figure 3: Lipid peroxidation of the fruit extract, leaf extract of *G.latifolium* and BHA standard

Result on EC_{50} and IC_{50} of the Aqueous extract of *G.latifolium* of fruit extract, Leaf extract and BHA Standard.

The result showed that BHA (standard) has the lowest IC_{50} value (307.89µg/ml), indicating it is the most potent in inhibiting the target followed by the *Gongronema latifolium* Fruit (517.11µg/ml) and leaf extract (644.00µg/ml),While the *Gongronema latifolium* leaf extract(303.41µg/ml) has a lower EC50 value than the fruit extract(371.26µg/ml), suggesting that the leaf extract may be more effective in inhibiting the target compared to the fruit extract, but less potent than the standard BHA(264.38µg/ml).

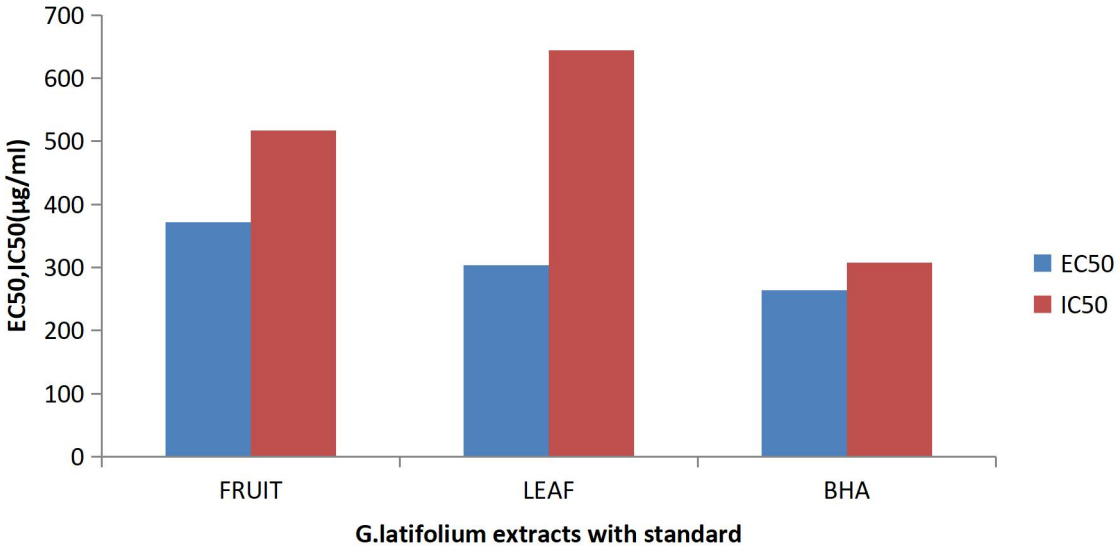

Figure 4: EC_{50} and IC_{50} of the fruit extract, Leaf extract and standard BHA

Discussion

The rising popularity of natural foods has led to the food industry's interest in incorporating natural antioxidants amongst products, despite synthetic antioxidants having some adverse effects (Fereidoon and Ying, 2010; Nalgonda, 2012), to delay lipid oxidative degradation, thereby improving quality and economic nutrition. Phytochemical composition results in Table 1 showed that total phenol (38.21±0.99%), tannin (30.79±0.94%), and flavonoid (29.78±0.64%) content were significantly higher in *G. latifolium* fruits samples than in leaves. Consumption of food rich in phenolics over time helps to prevent and treat diabetes, cancer, osteoporosis, cardiovascular disease, neurodegenerative illnesses, and other conditions (Rakesh *et al*., 2021)**.** Flavonoids exhibit a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angiogenic, antidiabetic, analgesic, anti-allergic, cytostatic, and antioxidant properties (Owoyele *et al*., 2008). Tannins have anti-diarrhea properties, astringent activities, and exert physiological effects like enhanced blood clotting, decreased blood pressure, and reduced serum lipid level (Chang *et al.,* 2001; Sylveste*et al*., 2009), *which* relate to findings that *G. latifolium* is enriched with varieties of flavonoids, saponins, alkaloids, and steroidal phytochemicals that exhibit prominent pharmacological actions such as hypoglycemic, hypolipidemic, cytotoxic, antioxidant, antimicrobial, and potent antileukemic activity (Moinuddin, 2022). Terpenoids, including β-carotene and lycopene, are found in *Gongronema latifolium* samples. These compounds exhibit antioxidant properties and may contribute to the plant's anti-inflammatory effects (Erukainure *et al*., 2019). The presence of these high-concentration phytochemicals gives an insight into the medicinal properties of *Gongronema latifolium* leaves and fruits. A comparison shows that *G. latifolium* fruit possesses a higher phytochemical composition when compared to the leaves, which goes in line with the result by Onwukeme *et al.* (2023), who showed that the fruits of *G. latifolium* are more potent than leaves due to a higher concentration of flavonoids, tannins, and phenols.

Proximate analysis revealed that both samples possess significant carbohydrate, crude fiber, and crude fat content, while the leaf has a higher moisture content and crude protein content, and the fruit extract contains more ash content, as seen in Table 2. The *G. latifolium* fruit at 67.01±3.78% and the leaf at 66.44±0.98% show a high carbohydrate content, indicating that it is a good source of energy because carbohydrates are polar compounds that are readily converted into glucose (Lawal *et al*., 2018). The result shows that *Gongronema latifoliumleaf* contains more crude protein (11.09±0.16%) when compared to the fruit (9.4±0.95%). The presence of protein content in the leaves shows that vegetables are useful for hormone formation, enzyme production, tissue repair, and regulation of body processes (Abu *et. al.,* 2014). In Gongronema *latifolium*, the leaf contains slightly more moisture content compared to the fruit, which revealed that moisture content is significantly used for the determination of the stability and quality of foods; materials with less moisture content stay longer (Onwuka, 2005; Isaac and Ekpa, 2009). G. *latifolium* fruit has a moderate ash content of 4.47±0.70%, while the leaf has a lower ash content of 1.80±0.13%. revealed that mineral elements speed up metabolic processes and improve growth and development (Opara *et al*., 2018). Crude fat content indicates both the fruit and leaf of *Gongronema latifolium* have comparable total lipid levels, with the fruit at 8.22±1.39% and the leaf at 8.47±0.91%

contributing to the energy content, which closely aligns with Glew *et al. (*2004) which states that fat content is a good source of energy for the body. The fruit of *Gongronema latifolium* has a slightly higher crude fiber content of 2.87±0.08% when compared to the leaf at 2.22±0.27%. Crude fibre represents the indigestible plant material, and higher amounts of dietary fibre contribute significantly to nutrient intakes since fibre lowers cholesterol level in the body, thus decreasing the risk of cardiovascular diseases (Aremu *et al., 2019*), which shows that both fruit and leaf extracts have nutritional value and may serve medicinal and culinary purposes (Offor and Uchenwoke, 2015).

DPPH radical scavenging ability is widely used as an index to evaluate the antioxidant potential of medicinal plants (Kumbhare *et al.,* 2012). In this result, both the *G.* latifolium fruit and leaf aqueous extracts competed favorably when compared to the BHA Standard, as shown in Figure 1. Ebrahimzadeh *et al.* (2010) revealed that the higher the total phenolic content, the better the DPPH radical scavenging activity. Similar results occurred during the findings of the current investigation, which indicate that the plant extracts of V. amygdalina, *P. quineense*, and *G. latifoluim* possess notable free radical scavenging properties and antioxidant capacity, which may be attributed to their high phenolic profile (Osuagwu *et al., 2023*). It was shown that the reduction in the absorbance of DPPH radicals caused by the phenolic compound or other phytochemical content resulted in the scavenging of the radical by hydrogen or electron donation and was seen as discoloration from violet to yellow (Meir *et al*., 1995).In Figure 2, among the examined *G. latifolium aqueous* extracts, fruit extracts had the highest reducing power than leaf extracts when compared to the BHA Standard. The existence of reductones from sample extracts is the key to their reducing power, and these exhibit their antioxidant activities through the action of breaking the free radical chain by donating a hydrogen atom (Elekofehinti *et al.,* 2013), which aligns with the fact that the higher the reducing power, the greater the antioxidant activity (Pakade *et al.,* 2013).Flavonoids, tannins, and phenolics are potent antioxidants in lipid systems, where they reduce oxidative modifications of membranes by restricting the access of oxidants to the bilayer and the propagation of lipid oxidation in the hydrophobic membrane matrix. The result in Figure 3 showed the plant extract of *G.latifolium* fruit has higher activity than the leaf aqueous extract, which displayed an effective inhibition of lipid peroxidation, and this could also be attributed to the presence of phenolic compounds (Khalili *et al.,* 2013). This distinguishing ability explains the role of phenolic compounds in mediating membrane lipid peroxidation arising from free radical species (Veeru *et al.,* 2009). A possible mechanism by which they confer protection against Fe^{2+}-induced lipid peroxidation in this homogenate can be attributed to the presence of flavonoids, tannins, and phenolics found in these plants, which are well known to be chelator compounds, that may form redox-inactive complexes with Fe^{2+}, rendering this pro-oxidant unavailable for the Fenton reaction (Elekofehinti *et al.,* 2013; Ani *et al.,* 2020). However, in-vitro antioxidant assays demonstrated that both extracts exhibited significant free radical scavenging, lipid peroxidation, and reducing power, with the fruit extract showing superior activity.The lower the IC_{50} and EC_{50} values of an extract, the more potent the substance is in terms of its antioxidant capacity or effectiveness in inhibiting the target activity. Meyer *et al.,* 2019). In Figure 4, the result illustrates that

BHA (standard) has the lowest IC_{50} value (307.89µg/ml), indicating it is the most potent in inhibiting the target, followed by the *Gongronema latifolium* fruit (517.11µg/ml) and leaf extract (644.00µg/ml). While the *Gongronema latifolium* leaf extract (303.41µg/ml) has a lower EC_{50} value than the fruit extract (371.26µg/ml), suggesting that the leaf extract may be more effective in inhibiting the target compared to the fruit extract but less potent than the standard BHA (264.38µg/ml), These results showed that the fruit and leaf extracts may exert potency near that of the standard BHA due to their high active antioxidant phytochemicals, which closely align with Osuagwu *et al.* (2013), where they reported that the fruit of *G. latifolium* was more potent than the leaves which helped in scavenging free radicals and reducing oxidative stress.

Conclusion

Gongronema latifolium, commonly known as "Utazi"is a crucial plant in Nigerian traditional medicine and cuisine. The phytochemical analysis of its fruits and leaf extracts revealed high concentrations of saponins, tannins, phenols, and flavonoids in the fruits, while the leaf had the lowest concentrations. Samples of fruit and leaves had notable concentrations of fibre, crude fat, and carbohydrates. These findings imply that *Gongronema latifolium fruit* may have both nutritional and therapeutic uses, with the extract's antioxidant efficacy contributing to its potential as a natural remedy for oxidative stress-related disorders. Further research is needed on *In-vivo* studies to evaluate the efficacy of *G. latifolium* extracts in treating specific oxidative stress-related disorders like liver diseases, cardiovascular diseases, and diabetes.

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