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EVALUATION OF CHEMICAL COMPOSITION, PROXIMATE STATUS AND ANTIOXIDANT ACTIVITY OF ETHANOL EXTRACT OF *Gongronema latifolium* (Benth) FRUIT

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Abstract

The purpose of the study was to evaluate the chemical composition, proximate status, and antioxidant activity of ethanol extract of *G. latifolium* fruit. The chemical composition was determined using spectrophotometric method. Moisture, ash, lipid, and fibre contents were determined using gravimetric method, while crude protein and total carbohydrate were determined using kjeldahl and difference methods respectively. Acute toxicity study was carried out with eighteen albino rats using lorke's method. The *in vitro* and *in vivo* antioxidant activities, reducing power and inhibition of lipid peroxidation were determined spectrophotometrically. The results of phytochemical composition obtained are flavonoids(39.32±0.88mgCE/g), phenols(37.50±1.41mgGAC/g), tannins(31.45±0.46mgTAE/g), oxalates(3.45±0.41mg/g), saponins (2.91±0.53%), alkaloids (0.23±0.06%), phytates (0.14±0.00%), beta carotene (0.12±0.03%), and lycopene (0.09±0.02%). Total carbohydrate, crude protein, lipid, moisture, ash, and crude fibre obtained are 64.59%, 10.07%, 9.20%, 8.62%, 4.96%, and 2.85% respectively. The fruit extract has antioxidant effect with EC_{50} 318.65μg/ml when compared with standard (263.56μg/ml) and showed inhibition of radicals with IC_{50} 1259μg/ml when compared with standard (306.84μg/ml). There was a significant increase in antioxidant enzymes activities and some biochemical parameters at 500mg/kg. However, decreases were recorded at doses ≤300mg/kg when compared to control. This finding suggests that *G. latifolium* fruit has active phytochemicals, good energy nutrients and antioxidant potentials at low doses of ≤300mg/kg.

Keywords: *Gongronema latifolium* fruit, Chemical constituents, nutritional composition, antioxidant enzymes.

INTRODUCTION

The plant, *Gongronema latifolium*, is of huge importance in food and medicine, it's nutritional and ethno medicinal uses are practiced in the southern part of Nigeria (Morebise, 2015). While much is known of the uses of the leaves, there is a need to find out the biochemical attributes of the fruits and to establish that the fruits can be consumed freely the same way as the leaves. This spicy plant belongs to the family of plants known as Apocynaceae (Osuagwu *et al*., 2013). *G. latifolium* has a characteristic sharp, bitter, and slightly sweet taste, especially when eaten fresh and can be found in tropical rainforest of West African countries (Balogun *et al.,* 2016). The local name of the plant is called "Utazi" by the Igbos, "Arokeke" by the Yoruba's and "Urasi" by the Efiks and the Ibibios (Balogun *et al.,* 2016).

The plant is therapeutically useful in the management of convulsion, stomach-ache, inflammation or rheumatoid pain, cough and can be taken as tonic to treat loss of appetite (Ciobanu *et al*., 2018). A decoction of the leaves or leafy stem is commonly used to prevent diabetes and high blood pressure and the boiled fruit in soup are eaten as laxative to prevent constipation (Osuagwu *et al*., 2013). Even though *G. latifolium* is widely known for its ethno medicinal and nutritional benefits especially its leaves, there is a need to find out the biochemical attributes of the fruits and the general aim of this research work is to evaluate the chemical composition, proximate status, and antioxidant activity of the ethanol extract of *G. latifolium* fruit.

MATERIALS AND METHODS

Collection and Identification of *G. latifolium* Fruit Sample

The fruit of *G. latifolium* was collected from Osete village in Umuchu, Aguata 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} was issued to the specimen.

Preparation of *G. latifolium* Fruit Sample

The fresh fruit was cut into pieces and completely air dried at ambient temperature to prevent fungal growth. The dried fruit was pulverized to a fine powder using an electrical grinder and stored in an air-tight jar container.

Preparation of Ethanol Extract of *G. latifolium* Fruit Sample

One kilogram (1kg) of the pulverized dried *G. latifolium* fruit was macerated in 10L of 70% ethanol and left for 24hours. After 24 hours, the sample mixture was filtered. The filtrate was concentrated at 60^{o}C using water bath (Memmert WTB). The crude extract of *G. latifolium* fruit was stored at 4^{o}C in refrigerator and used for further analyses.

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

Quantitative Analysis of Chemical Constituents

Alkaloids was determined by the method described by Harbone (1998); tannins were determined by the method described by AOAC (1998); oxalates were determined by the method described by Oke (1966); 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 Analyses of the Fruit Extract

Moisture content was determined by the method described by McDonald *et al*. (1996); crude protein was determined by the method described by Skoog *et al*. (1992); crude fat was determined by the method described by Perry *et al*. (1999); ash and crude fibre was determined by the method described by AOAC (1980) and AOAC (2000) respectively; carbohydrate content was determined by the method described by AOAC (1990).

***In vitro* Antioxidant Activity**

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

ANIMAL STUDIES

Ethical Approval

All experimental protocol was approved by Animal Research Ethics Committee (aREC), Nnamdi Azikiwe University, Awka. The reference number obtained is NAU/AREC/2023/00067.

Purchase, Acclimatization and Feeding of Animals

The experiment followed the completely randomized design. A total of fifty-three (53) albino rats were purchased from Onyebuchi Farm, Ifite, Awka. These animals were acclimatized for 1week. They were fed with food and water.

Determination of LD_{50} of Extract

The acute toxicity study was conducted in accordance with Lorke's method (Lorke, 1983). In the first phase, nine (9) rats randomly divided into three (3) groups of three (3) rats per group were given 10, 100, and 1000mg/kg body weight orally (via a cannula), respectively. The rats were monitored for twenty-four (24) hours for any signs of adverse effect and mortality. In the second phase of the experiment, the procedure was repeated using nine (9) rats randomly divided into three (3) groups of three (3) rat each, given 1600, 2900 and 5000mg extract/kg bodyweight, respectively. The rats were monitored again for any signs of toxic effect and mortality.

Grouping of the Animal

Thirty-five (35) albino rats were divided into five (5) groups of seven (7) animals in each group. Group A, the normal control, was fed with food and water while Groups B, C, D, and E received 100, 200, 300, 500 mg/kg of the fruit extract respectively. The study was carried out for 28days.

Sacrifice and Blood Collection

The animals were anaesthetized with chloroform after being treated orally with the fruit extract and their blood were collected in a plain bottle via close cardiac puncture method and centrifuged for 10 minutes at 4000rpm.The serum obtained were used for further analyses.

***In vivo* Antioxidant Activity**

The effect of the fruit extract on antioxidant enzymes were determined using spectrophotometric method. Superoxide dismutase activity was determined by the increase in absorbance at 480nm described by the method of Sun and Zigma (1978); catalase activity was determined by the increase in absorbance at 620nm described by the method of Sinha (1972); glutathione peroxidase at 340nm was determined by the method described by Palgia and Valentine (1967); malondialdehyde activity at 532nm was determined according to the method of Buege and Aust (1978); reduced glutathione level at 412nm was determined according to the method of Exner *et al*. (2000).

Biochemical Analyses

Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin, total protein, creatinine, and urea were determined spectrophotometrically according to the method reported by Limbi and Hyde (2003).

RESULTS AND DISCUSSION

% Yield of Extract

The percentage yield of the crude extract of *G. latifolium* fruit used for further test analyses was 17.3%.

Acute Toxicity Study via Oral Administration

The acute toxicity study revealed that having administered lethal dose (LD_{50}) above 5000mg/kg body weight of the extract in the animals, there were no observations of any toxic effect throughout the period of the study and no death was recorded.

Quantitative Constituents of *G. latifolium* Fruit Extract

The phytochemical analyses revealed that *G. latifolium* fruit extract is rich in flavonoids, total phenols, tannins and low in alkaloids. In **Table 1**, the high content of flavonoids, total phenols, and tannins and fairly little quantity of alkaloids may confer therapeutic as well as industrial potentials on *G. latifolium* fruit extract. This result is in line with that reported by Osuagwu *et al*. (2013) and Offor *et al*. (2015); in their separate research, they observed higher concentration of flavonoids, phenols, tannins, and scarce quantity of alkaloids in the dried leaves of *G. latifolium.* The high increase in flavonoid and phenol contents recorded in the fruit of *G. latifolium* may provide antioxidant capacities to scavenge hydroxyl radicals protecting the cells against oxidative damage (Osuagwu *et al*., 2013; Ujong *et al*., 2022). Tannins extracted from *G. latifolium* fruit extract could inhibit food deterioration through inhibition of oxidative enzymes such as lipoxygenase (Eze and Nwanguma, 2013).

Table 1: Quantitative Compositions of *G. latifolium* Fruit Extract

Phytochemicals	Composition
Total Phenols (mgGAE/g)	37.50±1.41
Flavonoid (mgCE/g)	39.32±0.88
Phytate (%)	0.14±0.00
Oxalate (mg/g)	3.45±0.41
Saponin (%)	2.91±0.53
Alkaloid (%)	0.23±0.06
Tannin (mgTAE/g)	31.45±0.46
Beta Carotene (mg/g)	0.12±0.03
Lycopene (mg/g)	0.09±0.02

The values are mean and standard deviation for triplicate determination.

Proximate Analyses of *G. latifolium* Fruit Extract

The proximate analyses revealed that the fruit extract is rich in carbohydrate and low in ash and crude fibre content. The results of the proximate analysis of *G. latifolium* fruit extract are shown in **Table 2.** In comparison, a report by Asaolu *et al*. (2012) and Mgbeje *et al*. (2019) revealed protein and carbohydrate as the major constituent in the

leaves of *G. latifolium* respectively. The variation in composition could be as a result of variation in environmental factor, geographical location, soil nutrient, method of cultivation, seasonal variation, or procedures in extraction and preparation (Mgbeje *et al*., 2019).The amount of protein in the fruit may be used as a protein supplement for patients with protein deficiency diseases (Mgbeje *et al*., 2019).Vegetables with high moisture content are prone to spoilage (Gomez *et al*., 2022). The fruit extract indicates moderate moisture content which could minimize degradation by microorganisms during storage (Adeniran *et al*., 2021). The fruit extract contains lesser quantity of fat, which could aid control of hypertension and prevent obesity (Uhegbu *et al*. (2011). Ash content is a measure of the mineral content and the fruit extract of *G. latifolium* contain low amount of mineral element. Mgbeje *et al*. (2019) have also reported that *G. latifolium* leaves have low amount of mineral content when compared with other selected tropical vegetable plants.

Table 2: Proximate Compositions of *G. latifolium* Fruit Extract

Parameters	Compositions
Ash (%)	4.96±1.18
Crude Fibre (%)	2.85±0.15
Moisture (%)	8.62±0.02
Total carbohydrate (%)	64.59±1.56
Crude protein (%)	10.07±0.88
Total lipids (%)	9.20±0.60

The values are Mean and standard deviation for triplicate determination.

In vitro Antioxidant Assay

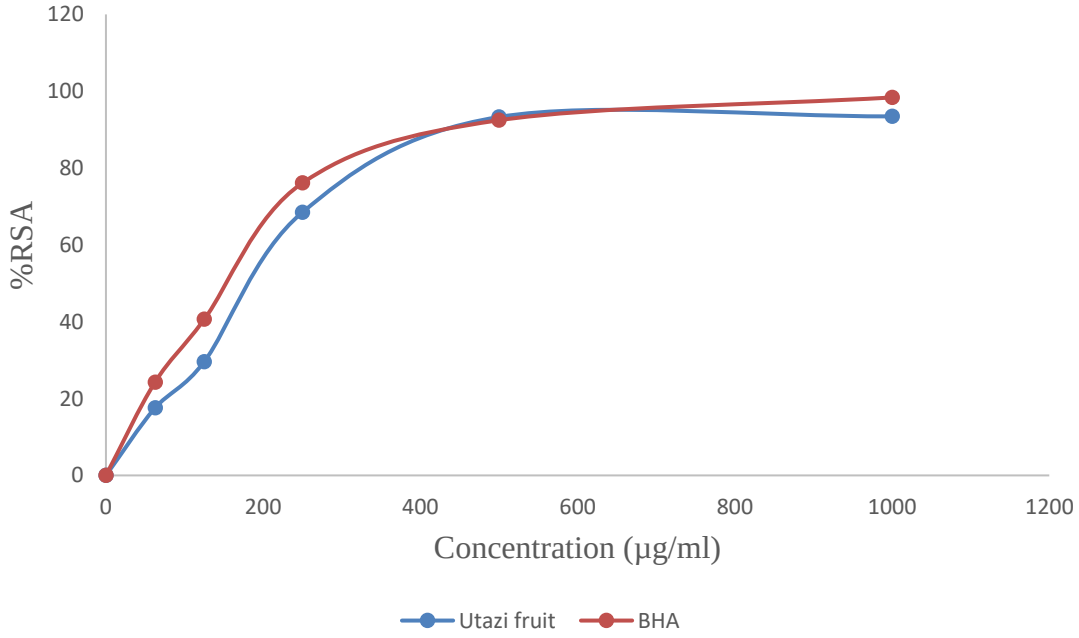

Figure 1: Free radical scavenging activities of *G. latifolium* fruit extract

The antioxidant activity of *G. latifolium* fruit extract increased with increasing concentration and competed favorably with BHA standard. The EC_{50} value of the fruit extract was higher when compared to the BHA standard. The result in **Figure 1** revealed that the fruit extract may act as free radical scavengers to diminish the deep violet colour of DPPH at 517nm which is an indication of their antioxidant potentials (Agwaramgbo *et al*., 2014). The potency could be due to the high phenols or other phytochemicals in the fruit extract possessing electron and hydrogen donating properties. Higher total phenol content leads to better DPPH radical scavenging activity (Ebrahimzadeh *et al.*, 2010). This result is in line with that reported by Emeka *et al*. (2015), who observed that the *in vitro* free radical scavenging activity of *G. latifolium* leaves increased with increasing concentration against DPPH test.

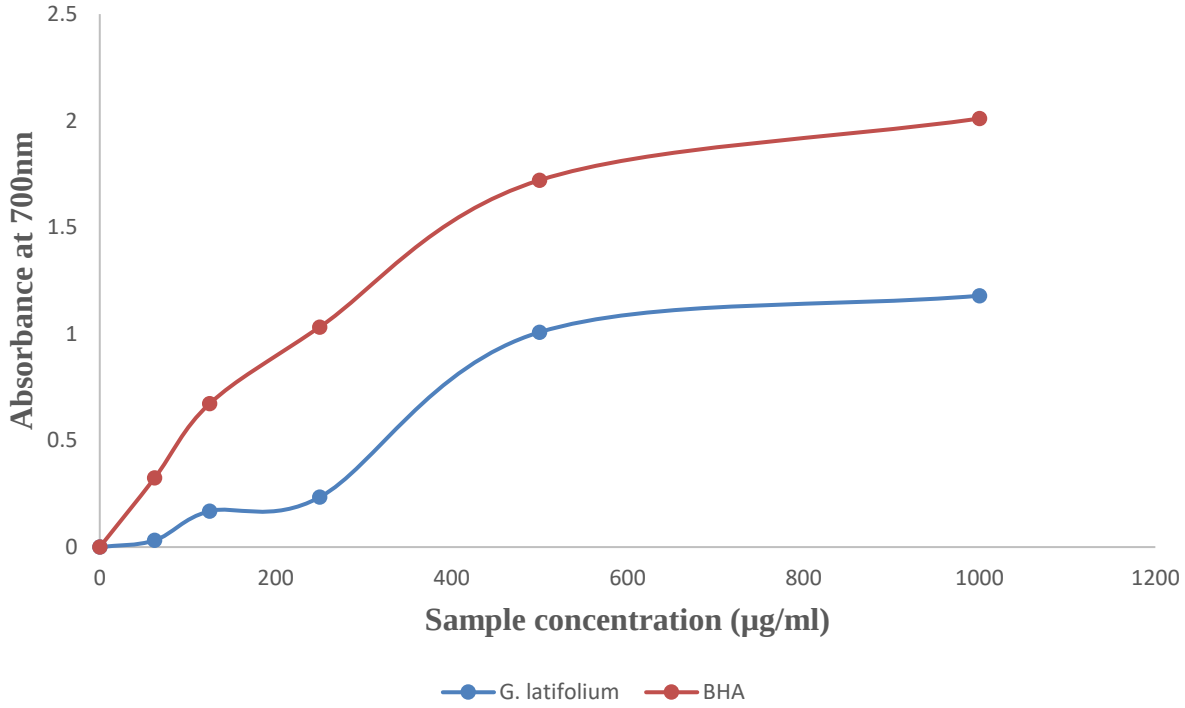

Figure 2: Reducing power of the fruit extract.

The reducing power in the fruit extract increased with an increase in concentration when compared to BHA standard. In **Figure 2**, the reducing power of the fruit extract was compared with BHA standard. The increased in the reducing power could be due to the presence of bioactive chemicals capable of donating electrons and causing reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) (Ebrahimzadeh *et al.*, 2010). The higher the reducing power the greater the antioxidant activity (Pakade *et al*., 2013). In comparison, a report by Adekanle and Omozokpia (2015) recorded high reducing power in the leaf extract of *G. latifolium*.

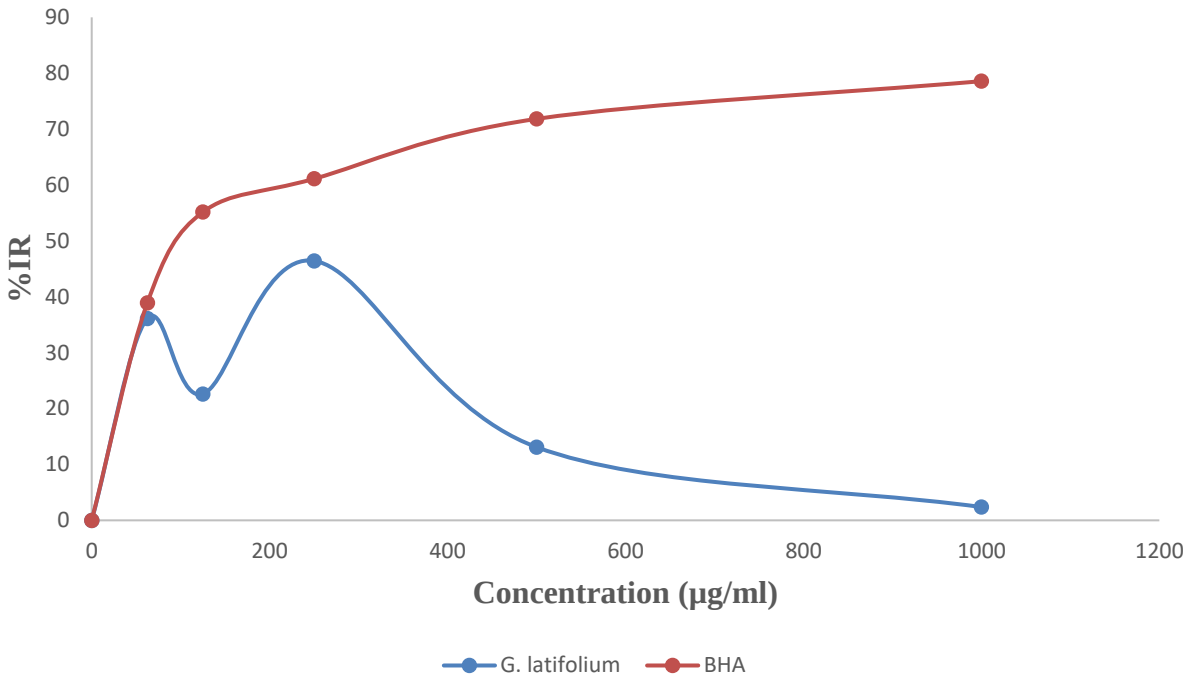

Figure 3: Lipid peroxidation of the extract and BHA standard

The inhibition of lipid peroxidation in the fruit extract of *G. latifolium* competed favorably with the BHA standard as shown in **Figure 3**. Phenolic compound in plant has the capacities to quench lipid peroxidation, prevent oxidative damage and scavenging of reactive oxygen species (Yoo *et al*., 2008). The fruit extract of *G. latifolium* could have antioxidant properties by reducing lipid peroxidation due to the high phenolic compound or other active phytochemicals.

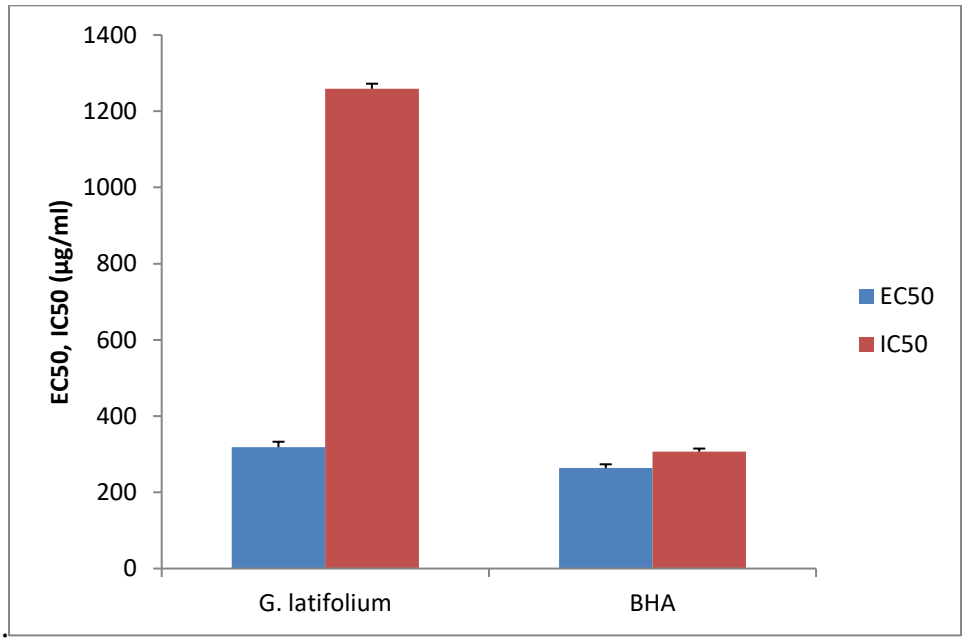

Figure 4: EC_{50} and IC_{50} of the fruit extract and standard BHA

The fruit extract has antioxidant effect with EC_{50}318.65µg/ml when compared to the standard BHA (263.56µg/ml). Also, the fruit extract showed inhibition of radicals with IC_{50}1259µg/ml when compared with the standard (306.84µg/ml). In **Figure 4,** the BHA standard has higher activity to obtain/inhibit 50% antioxidant effect/lipid peroxidation than the fruit extract. The lower the EC_{50} or IC_{50} the more potent the fruit extract (Meyer *et al*., 2019). The result showed that the fruit extract may exert potency near that of the standard BHA due to high active antioxidant phytochemicals. In line with Osuagwu *et al*. (2013), it was reported that the fruit of *G. latifolium* is more potent than the leaves.

***In vivo* Antioxidant Enzyme Activity**

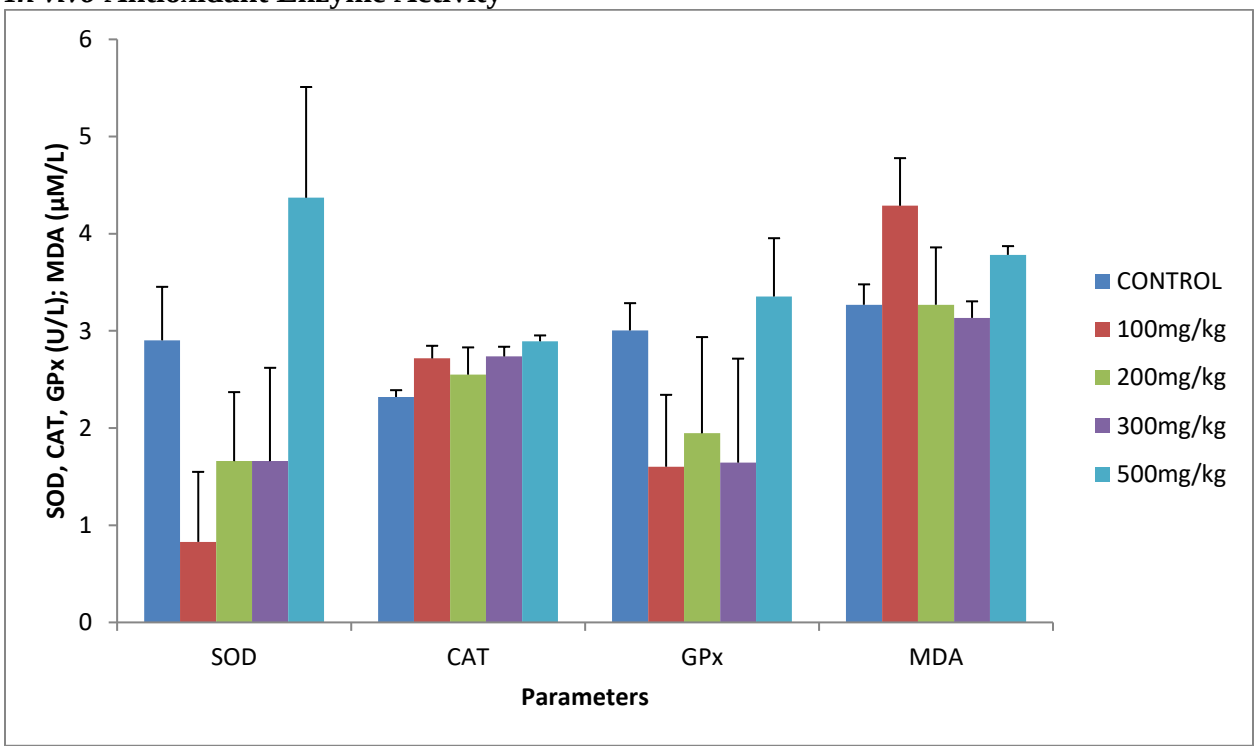

Figure 5: Effect of *G. latifolium* fruit extract on activities of superoxide dismutase, catalase, and glutathione peroxidase and malondialdehyde concentration in the treated groups.

There was a marked decrease in the SOD and GPx activity of groups administered 100, 200, and 300mg/kg compared to control. However, groups administered 500mg/kg displayed elevated SOD activity and slight elevation in GPx levels when compared to control respectively. There were varying increases in the catalase activity levels across the treatment groups when compared to control. The MDA showed marked increases in groups administered 100mg/kg and 500mg/kg when compared to control. However, groups administered 200mg/kg and 300mg/kg showed little difference in the MDA levels when compared to control. The result in **Figure 5**therefore shows that at doses of ≤300mg/kg, the fruit extract reduced the activity of antioxidant enzymes when compared to control, whereas at doses of ≥500mg/kg, the activity of antioxidant enzyme where markedly higher than control. This could be attributed to the presence of high phytochemicals like phenols with its capacity to quench lipid peroxidation,

prevent oxidative damage and increase the scavenging of reactive oxygen species (Yoo *et al.*, 2008). This result agrees with Ighodaro and Akinloye (2018) and Nnodim and Emejulu (2011) who reported same marked increased activities of catalase, superoxide dismutase, glutathione peroxidase at higher dose.

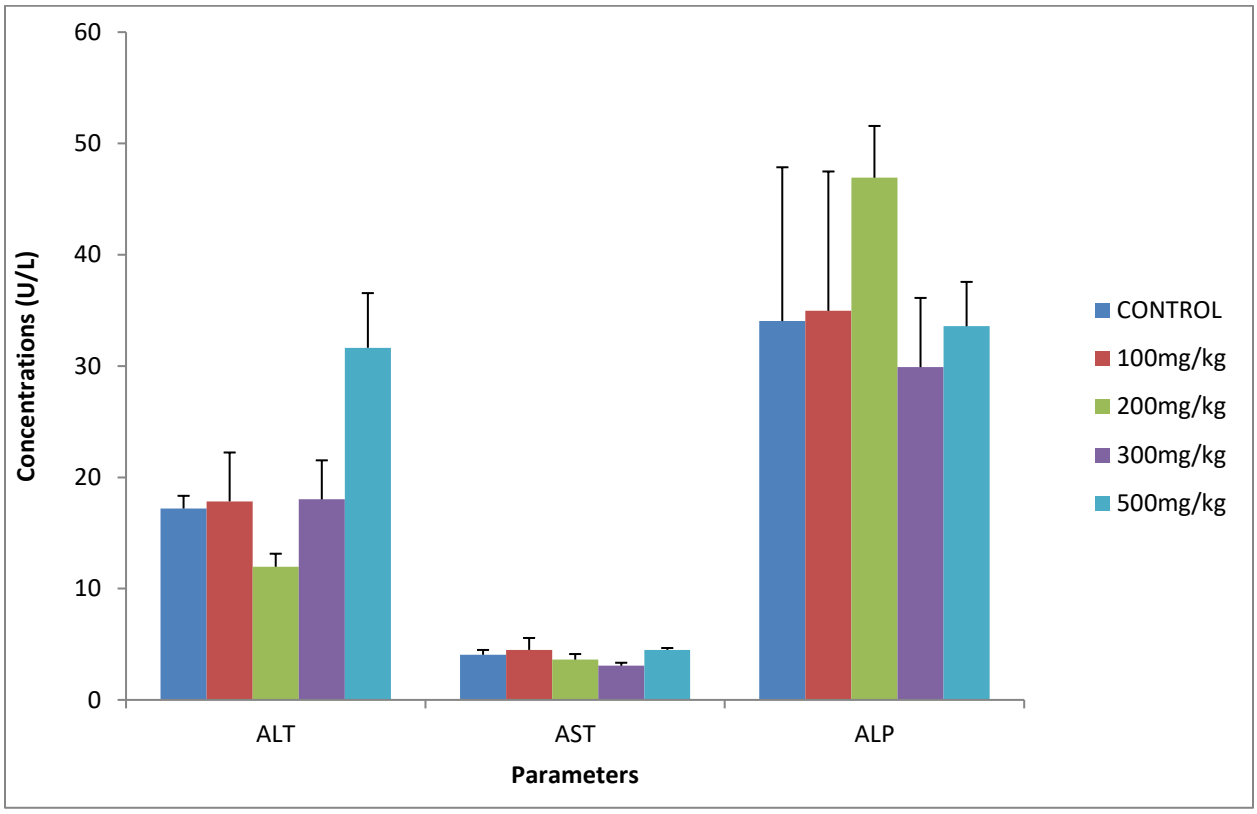

Figure 6: Effect of *G. latifolium* fruit on concentrations of ALT, AST, and ALP in the treated groups.

There were slight increases recorded in the ALT activity in groups administered 100 and 300mg/kg, but a slight decrease was recorded in the group administered 200mg/kg when compared to control. However, groups administered 500mg/kg showed marked increase in the AST activity levels when compared to control. The AST activity levels showed increases in groups administered 100mg/kg and 500mg/kg, while groups administered 200mg/kg and 300mg/kg showed decreases when compared to control. The ALP activity levels of the animals were markedly elevated in groups administered 200mg/kg when compared to control while decrease in the ALP activity levels was recorded in the group administered 300mg/kg. This result is in line with Balogun *et al*. (2016) and Adegbenro *et al.* (2021). The increase in the concentration on ALT at higher dose could be due to the presence of high bioactive bitter substances (phytochemicals) in the fruit extract. Ijah and Ejike (2011) also attributed to the bitter taste in the leaves of *G. latifolium* to be the presence of flavonoids, alkaloids, glycosides, saponins, tannins. This bitter substance in the fruit extract especially phenols or other phytochemicals may have protective effect in prevention of oxidative damage and increase the scavenging of reactive oxygen species (Yoo *et al.*, 2008).

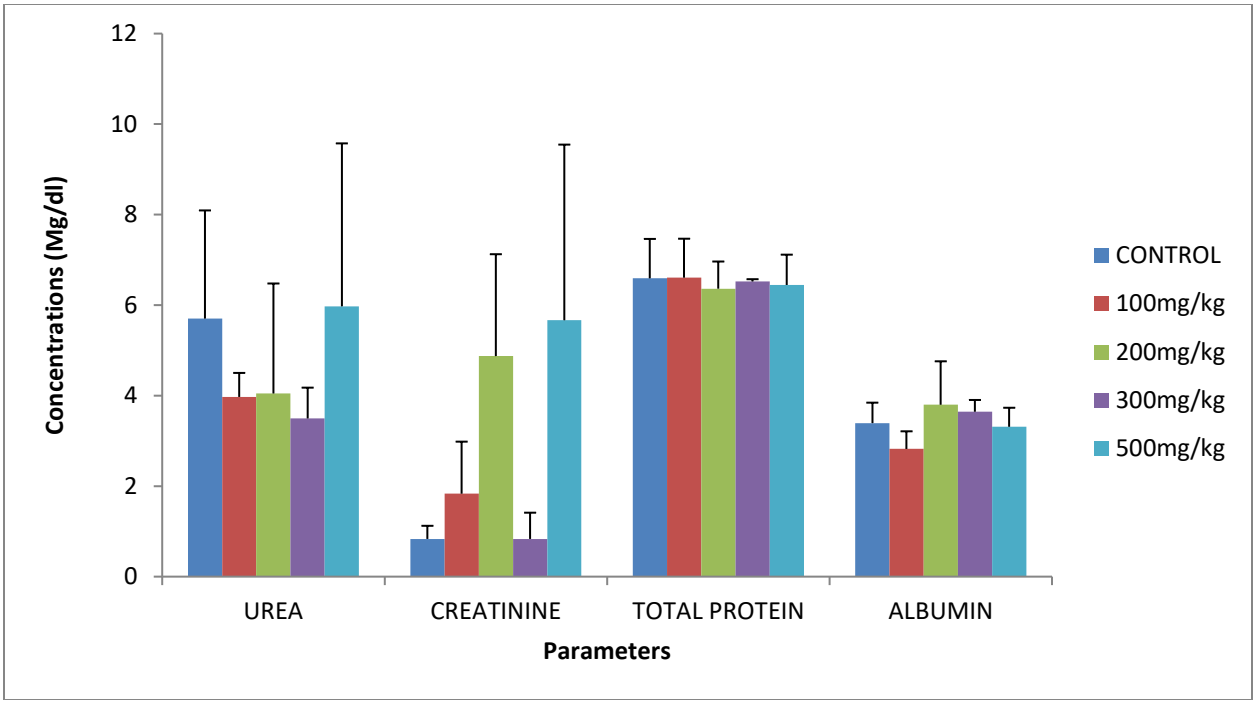

Figure 7: Effect of *G. latifolium* fruit on concentrations of urea, creatinine, total protein, and albumin in rat groups.

There was no significant ($p>0.05$) difference in serum urea, total protein, and albumin concentration in the experimental rats across the treatment groups when compared to control. The serum creatinine levels of the animals were markedly elevated in groups administered 200mg/kg and 500mg/kg when compared to control. The highest recorded creatinine level was in groups administered 500mg/kg of *G. latifolium* extract. This could be due to the presence of high bitter substances (bioactive phytochemicals) contained in the fruit extract. This is in line with Ijah and Ejike (2011) who also reported that the presence of flavonoids, alkaloids, glycosides, saponins, tannins contribute to the bitter taste in the leaves of *G. latifolium*. These bitter substances in the fruit extract at higher dose increase the serum creatinine levels (Kingsley *et al*.,2017).

The histopathology examination of the liver reported by Agwaramgbo *et al*. (2014) showed that *G. latifolium* fruit extract at high doses produced vacuolar degeneration at the 91st day which however showed convincing signs of reversibility after 28days post treatment. They insist that the vacuolar changes may be due to high concentration of the extract leading to glycogen accumulation. Osuagwu *et al*. (2013) and Agwaramgbo *et al*. (2014), in their separate research, advised that lesser quantities of the fruit should be consumed to avoid the effect of overdose.

CONCLUSION

This finding suggests that the fruit of *G. latifolium* (utazi) has active phytochemicals, good energy nutrients and antioxidant potentials at lower doses of ≤300mg/kg. Therefore, it can be included in our diet in moderate quantities to help remedy oxidative stress and other related diseases.

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