International Journal of Science Academic Research
Vol. 04, Issue 11, pp.6585-6595, November, 2023
Available online at http://www.scienceijsar.com

ISSN: 2582-6425

Research Article

INVITRO COMPOSITION AND THE EFFECTS OF ETHANOL EXTRACT OF *GONGRONEMA LATIFOLIUM* (BENTH) FRUIT ON SOME BIOCHEMICAL PARAMETERS IN ALBINO RATS

[1,*]Onwukeme, Chigozie A., [1]Udedi, Stanley C., [2]Ubaezuonu Augustine N., [1]Asogwa, Kingsley K., and [1]Nwodo,Nnanna M.

[1]Department of Applied Biochemistry, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Anambra state, Nigeria
[2]Department of Biomedical Science, Edinburgh Napier University, Sighthill Court, Edinburgh, Scotland

Received 18th September 2023; **Accepted** 24th October 2023; **Published online** 28th November 2023

Abstract

The purpose of the study was to evaluate the *invitro* composition and the effects of ethanol extract of *G. latifolium* fruit on some biochemical parameters in albino rats. Acute toxicity study was carried out with eighteen (18) albino rats using lorke's method. Thirty-five (35) albino rats weighing between 90-105grams were acclimatized for 7days, divided into 5 groups of 7 rats per group. Group A is the normal control while Groups B, C, D, and E received 100, 200, 300 and 500mg/kg bodyweight extract respectively. 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: *Gongronemalatifolium*fruit, Chemical constituents, Nutritional composition, Antioxidant enzyme activities.

INTRODUCTION

The plant, *Gongronemalatifolium*, is of huge importance in food and medicine, it's nutritional and ethno medicinal uses are practiced in the southern part of Nigeria [1]. 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 [2]. *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 [3]. 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 [3]. 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 [4]. 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 [2]. 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

Materials

The equipment used were Electronic Weighing Balance (Model: Adam AFP 800L), Spectrophotometer (KJ -72IG China), Refrigerated centrifuge (PEC – MEDICAL, USA), Randox test kits, 3015 Single Chamber Water-jacketed Laboratory Incubator. All reagents/chemicals were of analytical grade. All the analytical chemicals were purchased from British Drug House (BDH) products. Glass distilled water was used for all preparations of solutions.

Methods

Study Site

The fruit of *G. latifolium* was collected from Osete village in Umuchu, Aguata L.G.A of Anambra State, Nigeria, located at latitude 5° 56' 00"N and longitude 7° 08' 00"E.

Duration of Study

The study was completed within the period of seventy (70) days equivalent to Ten (10) weeks, 14days for air-drying, 5days for pulverization and extraction, 7days for acclimatization, 30days for animal studies, and 14days for *in vitro* assays, collection of results and data analyses.

Identification and Authentication of G. latifolium Specimen

The plant sample collected was taken to the Department of Botany Herbarium Nnamdi Azikiwe University Awka, where it was identified and authenticated by Mr. IrokaChisom, 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 in order 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.

Corresponding Author:** ***Onwukeme, Chigozie A.,
Department of Applied Biochemistry, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Anambra state, Nigeria.

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°C using water bath (Memmert WTB). The crude extract of *G. latifolium* fruit was stored at 4°C in refrigerator and used for further analyses. Percentage Extract

$$\text{Yield} = \frac{Weight\ of\ extract\ of\ dried\ homogenised\ sample}{Weight\ of\ dried\ homogenised\ sample} \text{x } 100 \text{ [5].}$$

Quantitative Analyses of the Crude Extract of *G. latifolium* Fruit

Determination of Alkaloids

The alkaloid content was determined according to the method of Harborne (1998) [6]. Five grams (5g) of the sample was weighed into a 250ml beaker, which was added to 200ml of 20% acetic acid in ethanol, covered and allowed to stand for 4 hours at 25°C. The resulting solution was filtered with Whatmann filter paper No.42 and the filtrate was concentrated using a water bath. Concentrated ammonium hydroxide (NH_4OH) was added drop wise to the extract until the precipitate was collected, washed with dilute NH_4OH (1% ammonia solution) and then filtered with pre-weighed Whatmann filter paper No.42. The residue on the Whatmann filter paper No.42 was the alkaloid which was dried in an oven (precision electrothermal model BNP 9052 England) at 60°C. The alkaloid content was calculated and expressed as percentage of the weight of the sample.

$$\text{Alkaloid (\%)} = \frac{\text{Weight of filter paper + Residue − Empty filter paper}}{\text{Weight of sample}} \text{ x } 100$$

Determination of Total Flavonoid

The flavonoid content was determined according to the method described by Barros *et al.* (2008) [5]. Aliquot of appropriately (0.5ml) diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5% sodium nitrite ($NaNO_2$) solution. After 6 minutes, 0.15 ml of 10% aluminium chloride ($AlCl_3$) solution was added and allowed to stand for 6 minutes, and 2 ml of 4% sodium hydroxide (NaOH) solution was added to the mixture. Immediately, water was added that brought the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for 15 minutes. Absorbance of the mixture was read at 510 nm against water blank with reference standard prepared with catechin concentrations. This analysis was performed in triplicate and the result was expressed as mg Catechin Equivalents per gram of sample (mg CE/100 grams).

Determination of Tannins

The tannin content of the fruit extract was determined according to the AOAC method (1998) [7]. One gram (1g) of the sample was extracted with 10ml of 70% ethanol and centrifuged at 2500rpm for 5 minutes to remove the residue. The resulting supernatant (0.5ml) was diluted with 4.5ml of distilled water. A volume of 0.5ml of 0.1M ferric chloride ($FeCl_3$) and 0.3ml of 0.1M potassium ferrocyanate was added to 6ml of distilled water to dilute the whole mixture. The absorbance was read at 720nm using a UV-visible spectrophotometer (KJ-72IG China). Tannic acid was used as standard and the result recorded as mg Tannic Acid Equivalent (mg TAE) per gram of the sample.

Evaluation of Total Phenolic Content

The total phenol content of the sample was determined according to the method of Barros *et al.* (2008) [5]. The extract solution (1ml) was mixed with Folin and Ciocalteu's phenol reagent (1ml). After 3minutes, 1ml saturated sodium carbonate (Na_2CO_3) solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 minutes. The absorbance was read at 725 nm (UV-Visible spectrophotometer (KJ-72IG China)). Gallic acid was used as standard and the results were expressed as mg of Gallic Acid Equivalents (GAEs) per gram of extract.

Oxalate Determination by Titration Method

This was determined according to the method described by Oke (1966) [8]. This determination involves three major steps: digestion, oxalate precipitation and permanganate titration. In digestion: Two gram (2g) of the sample was suspended in 190ml of distilled water in a 250ml volumetric flask; 10ml of 6M hydrochloric acid (HCl) was added and the suspension was digested at 100°C for 1 hour. The mixture was cooled and filled up to 250ml mark before filtration. In oxalate precipitation: A duplicate portion of 125ml of the filtrate was measured into beakers and four drops of methyl red indicator added. Ammonium hydroxide (NH_4OH) solution was added drop wise until the test solution changes from salmon pink colour to a faint yellow colour (pH4-4.5). Each portion was heated to 90°C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was heated again to 90°C and 10ml of 5% calcium chloride ($CaCl_2$) solution was added and stirred constantly. After heating, it was cooled and left overnight at 250°C. The solution was centrifuged at 2500rpm for 5 minutes. The supernatant was decanted, precipitated completely and dissolved in 10ml of 20% sulfuric acid (H_2SO_4) solution. In permanganate Titration:

The total filtrate resulting from digestion of two gram (2g) of sample was made up to 300ml. Aliquot of 125ml of the filtrate was heated until near boiling and then titrated against 0.05M standardized potassium permanganate ($KMNO_4$) solution to a faint pink color which persists for 30seconds. The calcium oxalate content was calculated using this formula: $T \times VMe \times Df \times 105\ (mg/100g) \times ME \times MF$

Where T = Tilter of $KMNO_4$ (ml)
Vme = Volume – mass equivalent (i.e. 1ml of 0.05m $KMNO_4$ solution is equivalent to 0.00225g anhydrous oxalic acid)
Dilution factor (Df) = Vt/A (where Vt = total volume of titrate (300ml) and A is the aliquot used (125ml)
ME = Molar Equivalent of $KMNO_4$ in oxalate (KMO_4 redox reaction)
Mf = Mass of sample used.

Determination of Saponins

This was determined according to the AOAC method (1990) [9]. Saponin extraction was carried out using two different solvents. The first solvent (acetone) was used to extract crude lipids from the sample while the second solvent (methanol) was used for the extraction of the saponin itself. Two grams (2g) of the sample was folded using Whatmann filter paper

No.42 into a thimble placed in a Soxhlet extractor and a reflux condenser fitted. Extraction was done with acetone in a 250cm^3 capacity round bottomed flask for 3 hours, after which the apparatus was dismantled and another 150cm^3 capacity round bottomed flask containing 100cm^3 of methanol fitted to the extractor and extraction was carried on for another 3 hours. The weight of the flask was taken before and after the second extraction in order to determine the change in weight. At the end of the second extraction, the methanol was recovered by distillation and the flask was oven-dried to remove any remaining solvent in the flask. The flask was allowed to cool and the weight of the flask taken.

$$\text{Saponin (\%)} = \frac{\text{Z}}{Sample\ Weight} \times 100$$

Where Z (weight of residue) = Y – X
X = Weight of empty beaker
Y = Weight of empty beaker + sample after drying

Determination of Phytates

The phytate content was determined according to the method of Young and Greaves (1999) [10]. Two grams (2g) aliquot of the ground sample was weighed into different 250ml conical flask. It was soaked with 100 ml of 2% concentrated hydrochloric acid (HCl) for 3hours. The sample was filtered using Whatmann filter paper No.4. A volume of 25ml of the filtrate was placed in 250ml beaker and 50ml distilled water was added too. A volume of 5ml of 0.3% ammonium thiocyanate (NH_4SCN) solution was added as indicator and titrated with standard iron (III) chloride ($FeCl_3$) solution which contained 0.00195gram iron per ml. The percentage phytic acid was calculated using the formula:

$$\text{Phytic acid (\%)} = \frac{\text{Titre value} \times 0.00195 \times 1.195}{2} \times 100$$

Proximate Analyses of the Crude Extract of *G. latifolium* Fruit

Determination of Moisture Content

This was determined according to the method of McDonald *et al.* (1996) [11]. A cleaned dried glass container was weighed with its lid to the nearest 1mg in a desiccator. Five grams (5g) of the sample was weighed and spread evenly onto the weighed container. The container was placed without its lid in the oven (precision electrothermal model BNP 9052 England) preheated to 103^{o}C. The container was introduced rapidly as possible and allowed to dry for 3 hours reckoned from the time when the oven temperature returns to 103^{o}C. The lid on the container was replaced as latter was removed from the oven, allow to cool in the desiccator and weighed to the nearest 1mg.

$$\text{Calculation of result: DM (\%)} = \frac{M3-M1}{M2-M1} \times 1000$$

M1= weight of glass container (g)
M2 = weight of sample and glass container before drying (g)
M3 = weight of sample glass container after drying (g)

Protein Determination by Kjeldahl

This was determined according to the method of Skoog *et al.* (1992) [12].The determination involves three major steps: digestion, distillation and titration. In digestion: An aliquot sample was weighed to the nearest 0.001gram and transferred to a kjeldahl flask of the digestion apparatus. Fifteen grams (15g) of potassium sulphate (K_2SO_4) and catalyst (copper (II) sulphatepentahydrate) mixture was added with a volume of 12ml of sulfuric acid (H_2SO_4). The kjeldahl flask was heated moderately at first swirled from time to time until the mass has carbonized and the foam has disappeared; heat more intensively until the liquid boils steadily. Heating was adequate if the boiling acid condenses on the wall of the flask. When the solution becomes clear and light green, continue to boil for another two hours and allow cooling. In distillation: A collecting flask containing 60 ml of boric acid (H_3BO_3) solution was placed under the outlet of the condenser in such a way that the delivery tube was below the surface of the excess boric acid solution. The distillate was collected in the boric acid receiving solution by addition of sodium hydroxide (NaOH). In titration: The contents of the collecting flask were titrated with sulfuric acid (H_2SO_4) using a burette and the amount of titrant was read. When colorimetric end-point detection was applied, the end-point was reached when color of the solution changes from green to red. The burette reading was estimated to the nearest 0.01 ml.

Calculation of result:

$$\text{Nitrogen content in the sample (\%)} = \frac{Va-Vb \times c\text{HCl} \times \text{mN}}{Mvz}$$

V_a = Volume of standard HCl solution when titrating sample (l)
V_b = volume of standard HCl solution when titrating blank (l)
cHCl = concentration of HCl (mol/l)
m_N = nitrogen molar mass (g/mol);
mvz = weight of sample (g)

Determination of Crude Fat

This was determined according to the method of Perry *et al.* (1999) [13]. The sample was extracted with petroleum ether. The solvent was distilled off and the residue was dried and weighed. Three grams (3g) of the sample was weighed to the nearest 1 mg transferred to an extraction thimble and covered with a fat free wad of cotton wool. A clean and dry extraction flask was weighed to the nearest 1 mg. The thimble containing the sample was placed in an extractor for 80 minutes with light petroleum. Extraction procedure was automated. The dried residue was placed in the drying oven for one (1) hour in the drying oven at 98^{o}C and left to cool in a desiccator and weighed.

$$\text{Calculation of result: CF (\%)} = \frac{(b-a) \times 1000}{\text{c}}$$

b = Weight of dried and cooled flask and extract after extraction (g)
a = Weight of dried and cooled flask (g)
c = Weight of sample (g).

Determination of Ash Content

This was determined according to the method of AOAC (1980) [14]. A clean dried crucible was weighed out to the nearest mg. Four grams (4g) of the sample was weighed into the crucible approximately and the weighed record was collected. The crucible was placed into the muffle furnace set at 550^{o}C until

white light grey or reddish ash colour was obtained which appears to be free from carbonaceous particles. The crucible was placed in a desiccator, left to cool and weighed immediately. Calculation of result:

$$CA\ (\%) = \frac{(Mc-Ma)}{(Mb-Ma)} \times 1000$$

m_a= weight of porcelain crucible (g)
m_b= weight of porcelain crucible and sample (g)
m_c= weight of porcelain crucible and ash (g)

Determination of Crude Fibre

The determination of crude fibre was determined according to the AOAC method (2000) [15]. Three grams (3g) of the prepared sample was weighed to the nearest 10 mg and placed in the glass beaker. A volume of 200ml Sulfuric acid (H_2SO_4) was added and the beaker was covered with a condenser. The liquid was boiled vigorously for 30minutes. The tap was opened to the discharge pipe and under the vacuum, the suspension of sample was filtered in 1.25% sulfuric acid through the fritted glass crucible (containing cca 1.5 cm of sea sand). The residue was washed with three (3) consecutive 30 ml portions of boiling water and the residue was filtered dry after each washing. The sample from fritted glass crucible was place back into the glass beaker (with minimal proportion of the sea sand) with a volume of 200ml of 1.25% potassium hydroxide solution well covered in a condenser. The liquid boiling point was within 5 ± 2 minutes which boiled vigorously for exactly 30 minutes. Filtration and the washing procedure used for the sulfuric acid step were repeated. After the final washing and liquid aspiration, the residue in the crucible was washed with acetone and water ensuring that the residues are filtered dry after each washing. The crucible was dried to constant weight in the oven at 103^{o}C. After drying, it was cooled in the desiccator and weighed rapidly. The crucible was placed in a muffle furnace and ash at 520-550^{o}C overnight. After heating, it was cooled in the furnace and placed in the desiccator before weighing.

$$\text{Calculation of results: CF } (\%) = \frac{(Ma-Mb)}{\text{Mvz}} \times 1000$$

m_a = weight of crucible and fibre after drying (g)
m_b = weight of crucible and fibre after ashing (g)
m_{vz}= weight of sample (g).

Determination of Total Carbohydrate

The total carbohydrate was determined according to the AOAC method (1990) [9]. The sum of the percentages of all other proximate components was subtracted from 100 using the relation below:

% Carbohydrate = 100 - (% protein + % fat + % fiber + % ash + % moisture).

***In Vitro* Antioxidant Activity Assays**

DPPH Free Radical Scavenging Assay

Principle: This was assayed according to the method of Manzocco *et al.* (1998) [16]. DPPH (2, 2-diphenyl-1-picrylhydrazyl-hydrate) free radical method is an antioxidant assay based on the transfer of electron that produces a deep violet color. In order to evaluate the antioxidant potential on the test sample through free radical scavenging, the change in optical density of DPPH radicals was monitored.

Procedure: The mixture of 0.5ml methanol containing the plant fruit extract at different concentrations (0-1000µg/ml) and 2.5 ml of methanol containing 75µM DPPH solution was at room temperature in dark maintained for 90 minutes. After 90 minutes, the free radical scavenging activity was tested via measuring the absorbance at 517 nm.

The percentage of the DPPH radical scavenging was calculated using the formula as given below:

$$\%\ \text{Inhibition f DPPH radical} = \frac{[\text{Abr}-\text{Aar}]}{\text{Abr}} \times 100$$

Where A_{br} is the absorbance before reaction and A_{ar} is the absorbance after reaction has taken place.

Reducing Power Capacity Assay

Principle: This was assayed according to the method described by Oyaizu (1986) [17]. Antioxidant compound forms a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride measured at 700nm. Increase in absorbance of the reaction mixture indicates the reducing power of the sample.

Procedure: A volume of 2.5ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide ($K_3[Fe(CN)_6]$) was added to 1ml of sample dissolved in distilled water. The resulting mixture was incubated at 50°C for 20 minutes, with the addition of 2.5 ml of Trichloro acetic acid (10% w/v). The mixture was centrifuged at 3000 revolution per minutes for 10 minutes. A volume of 2.5ml of the supernatant was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride ($FeCl_3$). The absorbance was measured at 700 nm against reagent blank.

Inhibition of Lipid Peroxidation Assay

Principle: This was determined to the method of Barros *et al.* (2008) [5]. Determination of the extent of inhibition of lipid peroxidation was carried out using brain homogenate of a goat.
Procedure: The brain of a goat weighing approximately 90kg was purchased from Kwata and slaughter at Awka. The brain was dissected and homogenized with pestle and mortar in an ice cold Tris-HCl buffer (pH 7.4,20mM) to produce 10% w/v brain homogenate which was centrifuged at 3000rpm from 10minutes. An aliquot (0.1ml) of the supernatant was incubated with 0.2ml of the sample extract at various concentrations (0-1000µg/ml) in the presence of 0.1ml of 10µM ferrosulphate and 0.1ml of 0.1mM ascorbic acid at 37^{o}C for 1hour; followed by addition of 0.5ml of 28% trichloroacetic acid (TCA) with 0.38ml of 2% thiobarbituric acid (TBA). The mixture was heated at 80^{o}C for 20minutes. After centrifugation at 3000rpm for 10 minutes to remove precipitated protein, the colour intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532nm. The inhibition ratio (%) was calculated using the following formula:

$$\text{Inhibition ratio } (\%) = [(A-B)/A] \times 100\%$$

Where A and B are the absorbance of the control and the compound solution respectively. The extract concentration

providing 50% lipid peroxidation inhibition (EC_{50}) was calculated from the graph of antioxidant activity percentage against the extract concentrations using butylatedhydroxy anisole (BHA) as the standard.

Animal Studies

Purchase of Experimental Animals (Albino rats), Acclimatization and Feeding A total of forty-eight (48) albino rats were purchased from Onyebuchi Farm, Ifite, Awka. These animals were acclimatized for 1week. They were fed daily and given water ad libitum.

Determination of LD_{50} of Extract

The acute toxicity study was conducted in accordance with Lorke's method (Lorke, 1983) [18]. This study was carried out for 2 (two) days using thirteen (13) male albino rats and the experiment have two phases.

In phase 1: Nine (9) rats were divided into 3 groups, that is; each group has 3 rats. Group 1, 2, and 3 animal received 10, 100, and 1000mg/kg body weight of the extract respectively to establish the range of doses producing any toxic effect. Each rat received a single dose after at least 5 days of adaption. The treated rats were monitored for twenty four (24) hours for mortality. In phase 2: After 24 hours, further specific doses (1600, 2900, and 5000mg/kg body weight) of the extract was administrated to three rats, one rat from each group (one rat per dose) to determine the correct LD_{50} value. The rats were monitored again for 24 hours for mortality.

$$LD_{50} = \sqrt{Highest\ None\ Lethal\ Dose \times Least\ Lethal\ Dose}$$

Grouping of the Animal

Thirty-five (35) male albino rats are needed for this study and the rats were divided into five (5) groups of seven (7) rats in each group. The study was carried out for 28 days. The groups are: Group A (Normal control): This group received only food and water. Group B (100 mg/100kg bodyweight extract): This group was treated with 100mg/kg dose of the plant fruit extract. Group C (200mg/100kg bodyweight extract): This group was treated with 200mg/kg of the plant fruit extract. Group D (300mg/100kg bodyweight extract): This group was treated with 300mg/kg of the plant fruit extract. Group E (500mg/100kg bodyweight extract): This group was treated with 500mg/kg of the plant fruit extract.

Measurement of Mean Body Weight Variation of the Experimental Rats

The initial weights of the animals were taken after acclimatization using electronic weighing balance (Model: Adam AFP 800L). The weights were taken four (4) more times in seven (7) days interval.

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 placed inside a rack via close cardiac puncture method and centrifuged for 10 minutes at 4000rpm. The serum obtained were used for further analyses.

***In Vivo* Antioxidant Activity Assays**

Superoxide Dismutase (SOD) Activity Assay

Principle: Superoxide dismutase (SOD) activity was determined according to the method of Sun and Zigma (1978) [19]. This is based on its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm.

Procedure: The reaction mixture (3 ml) contained 2.95 ml of 0.05M sodium carbonate buffer, pH 10.2, 0.02 ml of serum sample and 0.03 ml of 0.3mM adrenaline in 0.005N hydrochloric acid (HCl) was used to initiate the reaction. The reference cuvette contains 2.95 ml buffer, 0.03 ml of substrate (epinephrine) and 0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 3 minutes. $\sum = 4020M^{-1}\ cm^{-1}$.

Determination of Catalase Activity

Principle: The activity of catalase was determined according to the method of Sinha (1972)[20]. Dichromate in acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide (H_2O_2) with the formation of per chromic acid as an unstable intermediate. Catalase split H_2O_2 for different period of time. The reaction is stopped at different time interval by the addition of dichromate acetic acid mixture, and the remaining H_2O_2 is determined by measured chromic acetate colorimetrically at 620nm after heating the reaction mixture and is expressed as micromoles of H_2O_2 consumed/min/mg protein at 25°C.

Procedure: The reaction mixture (1.5ml) contained 0.1ml of serum sample, 1.0ml of 0.01 phosphate, buffer (PH 7.0) and 0.4ml of 2M H_2O_2. The reaction was stopped by the addition of 2.0ml of 5% potassium dichromate ($K_2Cr_2O_2$) acid reagent and glacial acetic acid (CH_3COOH) was mixed in the ratio of 1:3 respectively) $\sum = 40m^{-1}\ CM^{-1}$.

Determination of Glutathione Peroxidase Activity

Principle: This was carried out according to the method of Paglia and Valentine (1967) [21]. Glutathione peroxidase (GPx) catalyzes the oxidation of glutathione reductase and NADPH. The oxidized glutathione (GS-SG) is converted to the reduced form with a concomitant oxidation of NADPH and $NADP^+$. The decrease in absorbance at 340nm is measured.

Procedure: A volume of 2ml diluting reagent (Hydrogen peroxide) was mixed with 0.1ml of serum sample. The diluted sample (50ml) was mixed with 1ml of reagent 1(Phosphate buffer (50mM), Triton X-100 (0.1%) pH 7.0) and reagent 2 (glutathione (24µmol), phosphate reduced (NADPH) (4.8 µmol) respectively. The initial absorbance of both test and blank was read again after 1 minute and the timer starts simultaneously. Absorbance was read again after 1 minute and 2 minutes interval at 340nm.

Estimation of Extent of Lipid Peroxidation (Malondialdehyde)

Principle: Malondialdehyde (MDA) an index of lipid peroxide reacts with thiobarbituric acid (TBA) to give a complex pink

colour. The extent of lipid peroxidation was estimated using the method of Buege and Aust (1978)[22].

Procedure: An aliquot of the serum (0.1ml) was mixed with 0.9ml of normal saline. This was followed by the addition of 2.0ml of (1:1:1 ratio) TCA-TBA-HCL reagent (0.37% TBA, 0.24mM HCl and 15% TCA). The mixture was boiled at 100°C for 15 minutes and allowed to cool. Flocculent materials were removed by centrifuging at 3000rpm for 10 minutes. The supernatant was removed and the absorbance read at 532nm against the blank. Malondialdehyde (in µM) was calculated using the molar extinction coefficient for MDA – TBA complex of $1.56x105M^{-1}cm^{-1}$.

Biochemical Analyses

Liver Function Test

The blood was centrifuged at 4000rpm for 15minutes and the serum was used for this assay. Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin and total protein were determined according to the method of Limdi and Hyde (2003) [23].

Alanine aminotransferase (ALT) Test

Principle: The alanine aminotransferasewas determined by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine at 546nm.

Procedure: An aliquot of the serum (0.1ml) was mixed with 0.5ml of Randox ALT R1 buffer containing 100mmol phosphate buffer, 200mmol L-alanine and 2mmol α-oxoglutarate. It was allowed to stand for 30minutes at room temperature followed by the addition of 0.5ml of 2mmol 2,4-dinitrophenylhydrazine. After 20minutes, 5ml of 0.4M sodium hydroxide (NaOH) was added and the absorbance was taking at 546nm after 5minutes. The concentration of ALT in the serum was calculated from the standard values given by Randox.

Aspartate aminotransferase (AST) Test

Principle: The aspartate aminotransferase was determined by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine at 546nm.

Procedure: An aliquot of the serum (0.1ml) was mixed with 0.5ml of Randox AST R1 buffer containing 100mmol phosphate buffer, 100mmol L-aspartate and 2mmol α-oxoglutarate. It was allowed to stand for 30minutes at room temperature followed by the addition of 0.5ml of 2mmol 2,4-dinitrophenylhydrazine. After 20minutes, 5ml of 0.4M sodium hydroxide (NaOH) was added and the absorbance was taking at 546nm after 5minutes. The concentration of AST in the serum was calculated from the standard values given by Randox.

Alkaline Phosphatase (ALP) Test

Principle: Alkaline phosphatase (ALP) hydrolyses p-nitrophenylphosphate to produce phosphate and p-nitrophenol.
Procedure: In the assay, 20µL of the serum was mixed with 1ml of 10mmol/L p-nitrophenylphosphate in 1mol/l Diethanolamine buffer. The initial absorbance was read immediately with Axiom 752 UV-VIS Spectrophotometer at 405nm, and then the absorbance was taken again after 1minute, 2minutes and 3minutes. The ALP activity was calculated as follows:

ALP (U/L) = 2760ΔA405nm/min

Where ΔA405 = change in absorbance at 405nm.

Albumin Test

Principle: This is based on its quantitative binding to the indicator 3,3',5,5'-tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG). The albumin-BCG-complex absorbs maximally at 578nm, the absorbance being directly proportional to the concentration of albumin in the sample.
Procedure: An aliquot of the serum (10µL) was mixed with 3ml of BCG reagent. This was allowed to stand for 5minutes at 30°C. The absorbance was read at 578nm and the concentration of albumin was calculated using the standard.

Albumin (mg/dl) = $\frac{Absorbance\ of\ sample}{absorbance\ of\ standard}$x concentration of standard.

Determination of Total protein

Principle: The proteins, in an alkaline medium, bind with the cupric ions present in the biuret reagent to form a blue-violet coloured complex. The intensity of the colour formed is directly proportional to the amount of protein present in the sample. This was determined using Biuret method.

Procedure: The serum (0.1ml) was diluted in 0.9ml of normal saline. 5.0 ml of blank Biuret reagent was added to samples, mixed properly and allowed to stand for 20minutes at room temperature 25°C - 27°C. Absorbance was read for one test and standard against a blank at 540 nm. The concentration of protein was calculated using:

Protein (mg/dl) = $\frac{Absorbance\ of\ sample}{absorbance\ of\ standard}$x concentration of standard.

Renal Function Test

Creatinine and Urea were determined according to the method of Limdi and Hyde (2003)[23].

Serum Creatinine (CREAT) Test

Principle:Creatinine is based on its reaction with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration.

Procedure: An aliquot of the serum (50µL) was mixed with 0.5ml of randox reagent containing 35mmol/l picric acid and 0.32mol/l NaOH. This was read with auto analyzer and the concentration of creatinine displayed on the machine was recorded.

Urea (Blood Urea Nitrogen) Test

Principle: Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia liberated is measured photometrically by Berthelot's reaction.

Procedure: An aliquot of the serum (5µL) was mixed with 50µL of Randox reagent containing 116mmol/L sodium

nitroprusside and 6mmol/L urease. It was allowed to stand for 10minutes at 37°C after which 1.25ml of 120mmol/l phenol and 27mmol/l sodium hypochlorite was added and allowed to stand again for 15minutes at 37°C. The concentration of urea was recorded with auto analyzer.

Data Analysis

The data's obtained were analyzed using the Statistical Package for Social Science (SPSS) Version 25 for windows. Different groups were measured using one-way Analysis Of Variance (ANOVA) to compare the mean ± SD of the parameters between and within the treated groups to check for the level of significant differences and their values were considered significant at $p<0.05$.

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 are 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)[2] and Offor *et al.* (2015)[24]; 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 [2][25]. Tannins extracted from *G. latifolium* fruit extract could inhibit food deterioration through inhibition of oxidative enzymes such as lipoxygenase [26].

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

Phytochemicals	Compositions
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 are 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)[27] and Mgbeje *et al.* (2019)[28] 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 [28]. The amount of protein in the fruit may be used as a protein supplement for patients with protein deficiency diseases [28]. Vegetables with high moisture content are prone to spoilage [29]. The fruit extract indicates moderate moisture content which could minimize degradation by microorganisms during storage [30]. The fruit extract contain lesser quantity of fat, which could aid control of hypertension and prevent obesity [31]. 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)[28] 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. latifolim* 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

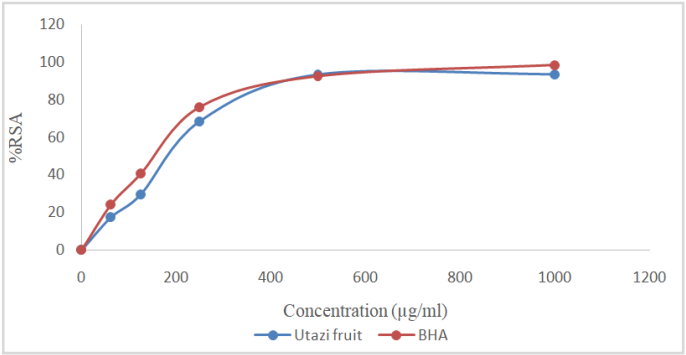

Figure 1. Free radical scavenging activities of *G. latifolium* fruit extract

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 [32].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 [33].This

result is in line with that reported by Emeka *et al.* (2015)[34], who observed that the *in vitro* free radical scavenging activity of *G. latifolium* leaves increased with increasing concentration against DPPH test.

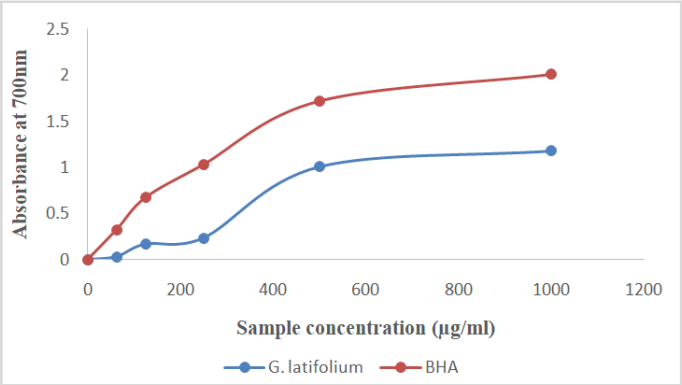

Figure 2. Reducing power of the fruit extract

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+}) [33]. The higher the reducing power the greater the antioxidant activity [35]. In comparison, a report by Adekanle and Omozokpia (2015) [36] recorded high reducing power in the leaf extract of *G. latifolium*.

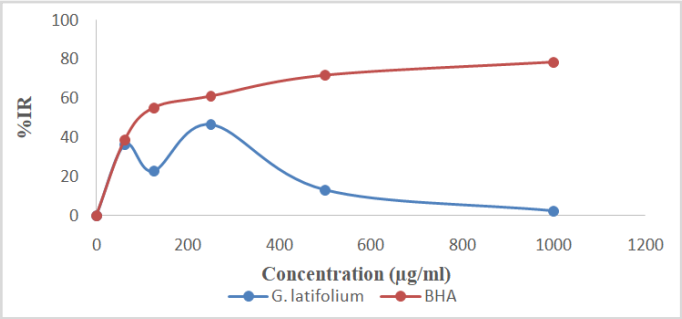

Figure 3. Lipid peroxidation of the extract and BHA standard

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 have the capacities to quench lipid peroxidation, prevent oxidative damage and scavenging of reactive oxygen species [37]. The fruit extract of *G. latifolium* could have antioxidant properties by reducing lipid peroxidation due to the high phenolic compound or other active phytochemicals.

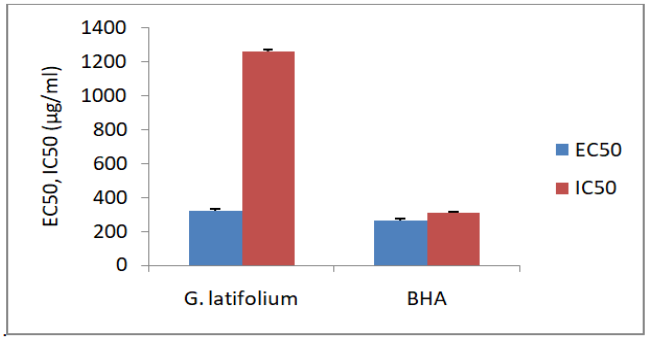

Figure 4. EC_{50} and IC_{50} of the fruit extract and standard BHA

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 [38]. 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) [2], it was reported that the fruit of *G. latifolium* is more potent than the leaves.

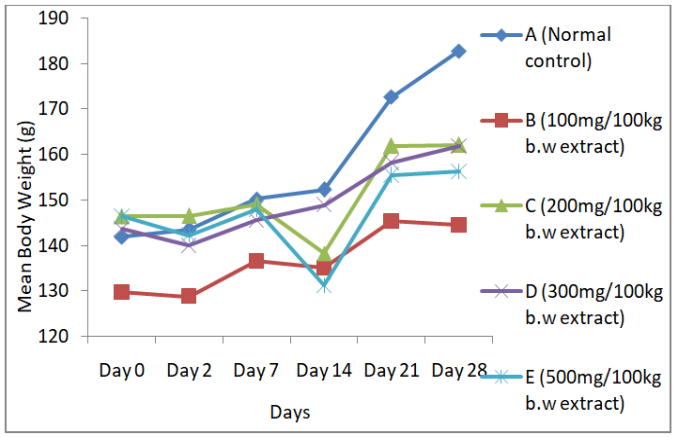

Figure 5. Mean body weight variation of experimental rats.

The mean body weights of experimental animals administered orally with *G. latifolium* fruit extract are shown in Figure 5. Data are mean of three replicates ± standard error of the mean (SEM). In Group A (Normal control) fed with feed and water, there was a significant increase in the mean body weight on day 21 and day 28 when compared to day 0. And their values are spread out over a wide range when compared to the values of other days. In Group C and D administered with 200 and 300mg/kg body weight of the extract, there was a significant increase on day 21 and day 28 when compared to day 14 and day 2 respectively and a significant decrease was recorded on day 14 and day 2 when compared to day 0, day 21 and day 28 respectively. In Group B and E administered with 100 and 500mg/kg body weight of the extract, there was no significant difference (no weight change). There mean body weight shows fluctuating increase and decrease during days of administration.

In vivo Antioxidant Enzyme Activity

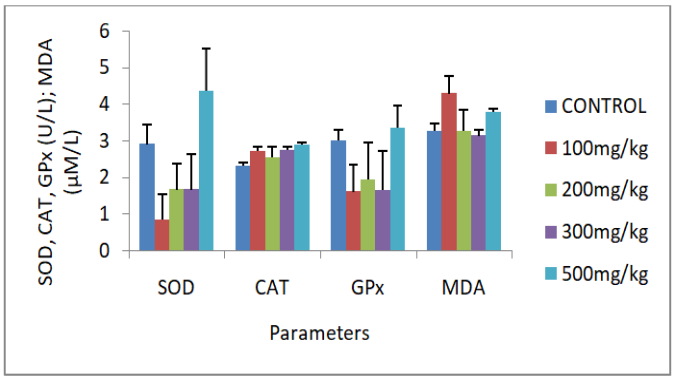

Figure 6. Effect of *G. latifolium* fruit extract on activities of superoxide dismutase, catalase and glutathione peroxidase and malondialdehyde concentration in the treated groups

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 was 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 6 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 [37]. This result agrees with Ighodaro and Akinloye (2018) [39] and Nnodim and Emejulu (2011) [40] who reported same marked increased activities of catalase, superoxide dismutase, glutathione peroxidase at higher dose.

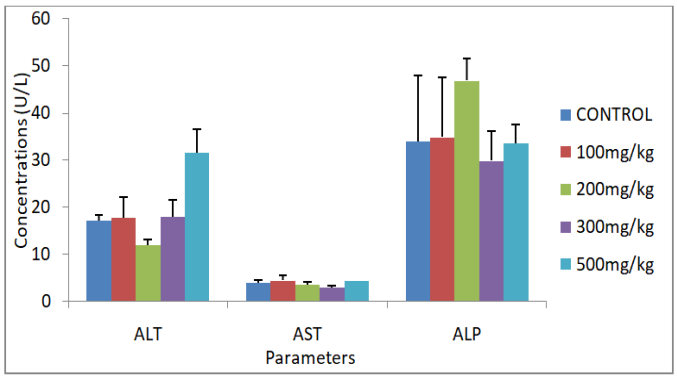

Figure 7. Effect of *G. latifolium* fruit on concentrations of ALT, AST, and ALP in the treated groups

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, in Figure 7, 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) [3] and Adegbenro *et al.* (2021)[41]. 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) [42] 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 [37]. 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 shown in Figure 8.

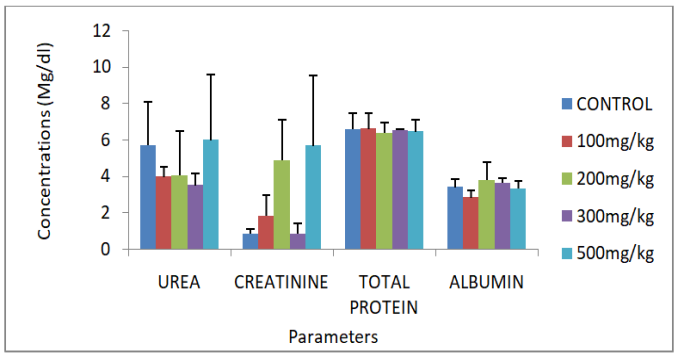

Figure 8. Effect of *G. latifolium* fruit on concentrations of urea, creatinine, total protein and albumin in rat groups

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. latifonium* 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) [42] who also reported that the presence of flavonoids, alkaloids, glycosides, saponins, tannins contribute to the bitter taste in the leaves of *G. latiolium*. These bitter substances in the fruit extract at higher dose increase the serum creatinine levels [43].

The histopathology examination of the liver reported by Agwaramgbo *et al.* (2014)[32] showed that *G. latifolioum* 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) [2] and Agwaramgbo *et al.* (2014)[32], in their separate research, advised that lesser quantities of the fruit should be consumed to avoid the effect of over dose.

Conclusion

This finding suggests that the fruit of *G. latifolium* 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. For the interest of the scientific community, there should be nutritional experiments on greater usage of *G. latifolium* fruit in preparation of meals, elixirs and snacks; and molecular studies is highly recommended; to study more of its effect, potency and bioinformatics application in solving some health issues.

Statement of competing interests

The authors have no competing interests

REFERENCES

1. Morebise, O., "A review on *Gongronemalatifolium*, an extremely useful plant with great prospects", *European Journal of Medicinal Plants*, 10(1):1–9. January 2015.
2. Osuagwu, A.W., Ekpo, I. A., Okpako, E.C., Otu, P., and Ottoho, E., "The biology, utilisation and phytochemical composition of the fruits and leaves of Gongronemalatifolium B", *Agrotechnology*, 2(3):1-15. January 2013.

3. Balogun, M. E., Besong, E. E., Obimma, J. N., Mbamalu, O.S., and Djobissie, S. F., "Gongronema Latifolium: A Phytochemical, Nutritional and Pharmacological Review", *Journal of Physiology and Pharmacology Advances*, 6(1): 811-824. January 2016.
4. Ciobanu, L., Olivier, R., Lynn, U., Bechir, J., and Denis, L. B.,"Effects of anesthetics agents on brain blood oxygenation level revealed with ultra–high field MRI.", *Asian Journal of Civilizational Studies*, 7(3):e32645. March 2012.
5. Barros, L., Falcão, S., Baptista, P., Freire, C., Vilas-Boas, M., and Ferreira, I., "Antioxidant activity of Agaricus sp. mushrooms by chemical, biochemical and electrochemical assays", *Food Chemistry*, 111(1):61–66. November 2008.
6. Harborne, A. J., *Phytochemical methods: A guide to modern techniques of plant analysis*, Springer Dordrecht, 1998, XIV, 302.
7. *Official Methods of Analysis of AOAC INTERNATIONAL* 13th Ed., AOAC International, Washington DC, Official Method 1998, pp. 1040.
8. Oke, O. L., "Chemical studies on some Nigerian Vegetables", *Cambridge University Press*,1(2):125-129. April 1965.
9. *Official Methods of Analysis of AOAC INTERNATIONAL* 15th Ed.,AOAC International, Washington DC, Official Method 1990, 2217-2280.
10. Young, S. M. and Greaves, J. S., "Influence of varieties and treatment of phytin contents of wheat", *Journal of Food Science*, 5(1):103-108.January 1940
11. McDonald, P., Edwards, R., Greenhalgh, D., and Morgan, C. A.,*Animal Nutrition*5th Ed., Longman Scientific & Technical, 1996, pp. 448.
12. Skoog, D. A., West, D. M., and Holler, F. J., *Fundamentals of analytical chemistry* 6th Ed., Saunders College Publishing/Harcourt Brace, 1992, pp. 802.
13. Perry, T. W., Cullison, A. E., and Lowrey, R. S., *Feeds and Feeding* 5th Ed., Prentice Hall, 1999, pp. 74.
14. *Official Methods of Analysis of AOAC INTERNATIONAL* 13th Ed.,AOAC International, Washington DC, Official Method 1980, pp. 1040.
15. *Official Methods of Analysis of AOAC INTERNATIONAL* 17th Ed., AOAC International, Gaithersburg, MD, USA, Official Method 2000, pp. 2176.
16. Manzocco, L., Anese, M., and Nicoli, M. C., "Antioxidant properties of tea extracts as affected by processing", *Food Science and Technology*, 31(7–8):694–698.November 1998.
17. Oyaizu, M., "Studies on products of browning reaction antioxidative activities of products of browning reactionprepared from glucosamine", *Japanese Journal of Nutrition and Dietetics*, 44(6):307–315.April 1986.
18. Lorke, D., "A new approach to practical acute toxicity testing", *Archives of Toxocity*, 53:275-287. December 1983.
19. Sun, M., and Zigma, S.,"An improved spectrophotometric assay of superoxide dismutase based on ephinephrine autoxidation", *Anaytical Biochemistry*,90(1):81-89. October 1978.
20. Sinha, K.A.,"Colorimetric assay of catalase",*Annals of Biochemistry*, 47: 389–394. June 1972.
21. Paglia, D. E., and Valentine, W. N.,"Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase", *Journal of Laboratory and Clinical Medicine,*70(1):158-69. July 1967.
22. Buege, J. A. and Aust, S. D., "Microsomal lipid peroxidation", *Methods in enzymology*, 52: 302-310. January 1978.
23. Limdi, J. K., and Hyde, G. M., "Evaluation of abnormal liver function tests", *Postgraduate medical journal*,79(932):307-312. June 2003.
24. Offor, C. and Uchenwoke, I., "Phytochemical analysis and proximate composition of the leaves of Gongronemalatifolium", *Global Journal of Pharmacology*, 9(2):159-162. July 2015.
25. Ujong, G. O., Beshel, J. A., Nkanu, E., Ubana, O. P., and Ofem, O. E., "Ethanolic extract of *Gongronemalatifolium* improves learning and memory in Swiss albino Mice", *Journal of Drug Delivery and Therapeutics*, 12(1): 45-50.January-February 2022.
26. Eze, S. O. and Nwanguma, B. C., "Effect of tannin extract from Gongronemalatifolium Leaves on Lipoxygenase Cucumeropsismanii Seeds", *Journal of Chemistry*, 2013:1-7. July 2012.
27. Asaolu, S. S., Adefemi, O. S., Oyakilome, I. G., Ajibulu, K. E., & Asaolu, M. F., "Proximate and mineral composition of Nigerian leafy vegetables", *Journal of Food Research*, 1(3):214-218. May 2012.
28. Mgbeje, B. I. A., Umoh, E. U., and Emmanuel-Ikpeme, C., "Comparative Analysis of Phytochemical Composition of Four Selected Tropical Medicinal Plants Namely: Ocimumgratissimum, Piper guineense, Gongronemalatifolium and Vernoniaamygdalina", *Journal of Complementary and Alternative Medical Research*, 7(3):1–11. June 2019.
29. Gomez, S., Kuruvila, B., Maneesha, P. K., and Joseph, M., "Variation in physico-chemical, organoleptic and microbial qualities of intermediate moisture pineapple (*Ananascomosus* (L.) Merr.) slices during storage", *Food Production, Processing and Nutrition*, 4(5): 1-11. February 2022.
30. Adeniran, A. A., Ntamanwuna, E. C., and Bassey, V. O., "Microscopical characterization and physicochemical standardization of leaves, stems and roots of *Spondiasmombin* L. (Anacardiaceae)", *Nigerian Journal of Pharmaceutical Research***,** 17(1):15-25. August 2021.
31. Uhegbu, F. O., Emeka, E. I., and Kanu, I., "Studies on the chemical and anti-nutritional content of some Nigerian spices", *International Journal of Nutrition and Metabolism*, 3(6):72–7. January 2011.
32. Agwaramgbo, A., Ilodigwe, E. E., Ajaghaku, D. L., Onuorah, M. U., and Mbagwu, S. I., "Evaluation of antioxidant, immunomodulatory activities, and safety of ethanol extract and fractions of Gongronemalatifolium fruit", *International Scholarly Research Noticess*, 2014:10. October 2014.
33. Ebrahimzadeh, M. A., Nabavi, S.M., Nabavi, S. F., Bahramian, F., and Bekhradnia, A. R., "Antioxidant and Free Radical Scavenging Activity of H. officinalis L. Var. angustifolius, V. odorata, B. hyrcanaand C. speciosum", *Pakistan Journal of Pharmaceutical Science*, 23(1):29–34. January 2010.
34. Emeka, J., Fang-Fang, L., Rong-Rong C., Yan, L., Asotie, O., and Ying-Jun, Z., "Anti-Cancer and Free Radical Scavenging Activity of Some Nigerian Food Plants in vitro", *International Journal of Cancer Research*, 11:41-51. January 2015.
35. Pakade, V., Cukrowska, E. and Chimuka, L., "Metal and flavonol contents of Moringaoleifera grown in South

Africa", *South African Journal of Sciences*, 109(3):835–837. January 2013.
36. Adekanle, E., and Omozokpia, M., "Antioxidant potentials of *Gongronemalatifolium* (utazi) leaf extracts", *Biokemistri*, 27(2):85-88. June 2015.
37. Yoo, K. M., Lee, H. C., Lee, H., Moon, B. and Lee, .Y. C., "Relative antioxidant and cytoprotective activities of common herbs", *Food Chemistry*, 106:929–936. February 2008.
38. Meyer, C. T., Wooten, D. J., Paudel, B. B., Bauer, J., Hardeman, K. N., Westover, D., Lovly, C. M., Harris, L. A., Tyson, D. R., and Quaranta, V., "Quantifying drug combination synergy along potency and efficacy axes", *Cell Systems*, 8(2):97-108. February 2019.
39. Ighodaro, O. M., and Akinloye, O. A., "First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid", *Alexandria Journal of Medicine*, 54(4):287–293. November 2017.
40. Nnodim, J., and Emejulu, A., "The protective role of Gongronemalatifolium in acetaminophen induced hepatic toxicity in wistar rats", *Asian Pacific Journal of Tropical Biomedicine*, 1(2):151–154. October 2011.
41. Adegbenro, A. A., Salawu, S. O., and Akindahunsi, A. A., "Antioxidant activities of Celosia argentea Linn and GongronemalatifoliumBenth and the antihyperlipidemic effect of the vegetable supplemented diets on fat induced hyperlipidemic rats", *Journal of Food Measurement & Characterization*, 15(5):1–12. February 2021.
42. Ijeh, I. I. and Ejike, C. E., "Current perspectives on the medicinal potentials of Vernoniaamygdalina Del", *Journal of Medicinal plant Research*, 5(7):1051-1061. April 2011.
43. Kingsley, C. K., Solomon, N. I., and Odudu, A., "Haematological, Biochemical and Antioxidant Changes in Wister Rats exposed to Dichlorvos based Insecticide Formulation used in Southeast Nigeria", *Toxics*, 4(4):1-28. November 2016.

****