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Evaluation of Phytochemical Compositions, *in Vitro* Antioxidant and Cytotoxicity Potentials of *Irvingia gabonensis* Fruit Pulp for Possible Anticancer Activity

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Abstract

Background: Cancer continues to be a growing public health burden of the 21st century in both developed and developing countries with new incidence and mortality cases recorded annually. Several studies have implicated oxidative stress in the pathogenesis and initiation of cancer, which is why novel phytochemicals are sought to address this gap nutritionally and through drug discovery initiatives. This study was therefore carried out to investigate the phytochemical compositions, *in vitro* antioxidant and cytotoxicity potentials of *Irvingia gabonensis* with the aim of assessing its role in providing phytocompounds with possible anticancer relevance. **Methods:** Pulp of fresh fruits of *I. gabonensis* was collected, prepared and extracted using ethanol by Soxhlet extraction method. Standard phytochemical methods were employed to quantify phytochemicals whereas antioxidant assays were conducted using spectrophotometric determination of lipid peroxidation inhibition and glutathione activity, with butylated hydroxytoluene and ascorbic acid as standards and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Brine shrimp lethality assay was performed to assess its cytotoxic potential as an index of anticancer activity. **Results:** Findings showed alkaloids were the most abundant phytochemicals while phenols, tannins, flavonoids, and saponins were present in relatively low concentrations. The extract demonstrated moderate inhibition of lipid peroxidation with maximum inhibition of 84.50% at 100 µg/ml and an IC_{50} of 10.50 µg/ml compared to 9.23 µg/ml for ascorbic acid and 1.51 µg/ml for BHT. Reduced glutathione concentrations did not show a consistent dose-dependent increase, indicating limited ability of the extract to stimulate endogenous antioxidant defenses. *In vitro* DPPH results showed the highest scavenging activity of 80.68% at 80 µg/ml and the lowest at 10 µg/ml

of 48.03% with an IC_{50} of 11.17 mg/mL representing a dose-dependent scavenging activity with increasing plant extract concentration though values were observed to be lower than those of the reference standard (BHT) but comparable in activity. The brine shrimp lethality assay showed a moderate lethality of 53.33% at 1000 µg/mL and LC_{50} of 1077.71 µg/mL, compared with 66.67% mortality at 1000 µg/mL and LC_{50} of 511.30 µg/mL for the positive control. **Conclusion:** The study demonstrated *I. gabonensis* possesses moderate antioxidant and cytotoxic potentials specifically linked to scavenging free radicals, which is the hallmark biochemical event in cancer initiation and progression with the concomitant promise for optimization in novel cancer drug identification and development.

Keywords

Irvingia gabonensis, Cancer Therapy, Oxidative Stress, Antioxidant, Phytochemicals, Drug Discovery

1. Introduction

Cancer is the second most common cause of death globally and a major public health challenge of the twenty-first century, particularly in Nigeria, where over 120,000 new cases and more than 78,000 cancer-related deaths are reported annually [1]. In Nigeria, breast cancer, cervical cancer, prostate cancer, and liver cancer are the most prevalent among the various cancer types [2] [3]. Cancer treatment requires novel strategies to target the abnormal cells at the molecular level, both therapeutically and nutritionally. Though cancer therapy and care have made significant progress, more effective and safer therapeutic alternatives are urgently needed given the fact that conventional treatment strategies such as chemotherapy, radiotherapy, immunotherapy among others, have limited positive prognosis outcomes [4]. Amidst this huge treatment disparity, however, recent research has increasingly focused on the potential of tropical plants as sources of novel anticancer agents, given that many effective anticancer drugs have historically been derived from plant sources [5] [6].

Oxidative stress, characterized by an overproduction of reactive oxygen species (ROS) in excess of endogenous antioxidant levels resulting from aberrant redox signaling mechanisms [7], has been linked to the pathology of many debilitating diseases including cardiovascular diseases, diabetes mellitus and cancers [8]. In cancer, it is associated with dysregulation of redox cellular signaling pathways [9]. According to Jomova *et al.* [10], oxidative stress can induce a cellular redox imbalance that results in progressive damage to the cell, shutting off immune functions while leading to development of oxidative DNA damage. Shukla *et al.* [11] noted that mechanisms responsible for the ROS mediated injury to cells and tissues mainly include lipid peroxidation, protein oxidation, and oxidative DNA damage as a series of events cascading to tumor evolution and accompanied by

*h*TRT overexpression, ultimately implicating oxidative stress as a basic factor in carcinogenesis and tumorigenesis.

Tropical plants are rich in diverse bioactive compounds, including alkaloids, flavonoids, terpenoids, and phenolic compounds, which exhibit promising anticancer properties [12]. These compounds, also called phytochemicals, are naturally occurring and play crucial roles in the plant's defense mechanisms and interactions with the environment [13]. These phytochemicals work through various mechanisms by inducing apoptosis, inhibiting cell proliferation, modulating signaling pathways, and reducing oxidative stress and inflammation [14]. Despite the extensive studies on phytochemical constituents and antioxidant activity of plants [15], there remains paucity of data on the plant of interest, *Irvingia gabonensis*, in possessing anticancer bioactive compounds.

Irvingia gabonensis, commonly known as African bush mango, is one of the commonly consumed tropical plants in Africa particularly Nigeria. It is a large tree with a dense compact crown and evergreen large leaves with edible seeds and sweet edible fruit pulp found in rainforests. It is highly demanded for its high nutritional and medicinal benefits. It is commonly referred to as bush mango, dikanut, wild mango, and African mango [16] [17]. The seeds are called "Ogbono" in most Southern parts of Nigeria and are used widely to make a traditional delicacy known as Ogbono soup. Its biological activities include antidiabetic, antiulcer, antioxidant, analgesic, anti-obesity and antimicrobial. In some parts of Africa, its decoction is used for the treatment of gonorrhea, gastrointestinal, and hepatic disorders. According to some studies, the seed extracts improve blood sugar levels by increasing high-density lipoprotein cholesterol levels while reducing low-density lipoprotein (LDL), total cholesterol and triglycerides thereby acting as antidiabetic and anti-obesity agents [18].

To evaluate the anticancer relevance of the plant fruit extract, the brine shrimp lethality assay (BSLA) offers a viable preliminary test to investigate the bioactivity of phytoconstituents. BSLA is a robust first step for testing the toxicity of an extract or compound and can be employed to pre-screen bioactive compounds in plants as well as natural products [19] [20]. The assay is simple, quick, cost-effective, has close correlation with conventional cytotoxicity tests such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Trypan Blue and Alamar Blue assays that employ cancer cell lines and is easy to perform with a high confidence level for estimating pharmacological activity or toxicity of any natural product [20] [21]. This assay uses *A. salina* larvae as experimental animals and expresses toxicity levels of samples in percent mortality of the animals converted into half-maximal lethal concentration (LC_{50}) values, which specify the concentration of compounds required to cause death in 50% of the brine larvae population [22]. Thus, to investigate the anticancer potential of *I. gabonensis* fruit pulp extract, the brine shrimp lethality assay was conducted on *prima facie* basis to estimate the plant's cytotoxic capacity.

Because of its varied phytochemical and proximate compositions, antioxidant

qualities and vast pharmacological attributes, *I. gabonensis* has attracted a lot of interest from both researchers and health experts [23]. *I. gabonensis* may exhibit promising anticancer properties due to its diverse phytochemical composition, ranging from flavonoids, tannins, saponins, to phenols and terpenoids [24]. This study therefore aimed to profile the phytochemicals and evaluate antioxidant and cytotoxic abilities of *I. gabonensis* fruit pulp for its potential in offering novel phytotherapeutics for possible anticancer relevance.

2. Materials and Methods

2.1. Source of Plant Material and Identification

Fresh *I. gabonensis* fruits were sourced from a local market, Eke-Awka, in Awka, Anambra state, Nigeria. They were identified and deposited at the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra state with the herbarium number NAUH-071[B].

2.2. Experimental Site

The experimental analyses were carried out at the laboratory of Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka.

2.3. Sample Preparation

The fruits were cleaned, shade-dried, and peeled to reveal the pulp, which was further dried and ground into fine powder using a laboratory grinder. The powdered samples were stored in airtight containers until analysis.

2.4. Sample Extraction

For extraction, the Soxhlet method was used as described by Ibrahim *et al.* [25]. Prior to extraction, 500 mL clean round-bottom boiling flasks were dried in an oven at 105˚C - 110˚C for 30 minutes to remove any moisture, after which they were transferred into a desiccator and allowed to cool to room temperature to prevent contamination and ensure accurate weighing. After drying the flasks, exactly 15 g of the powdered sample was weighed into a Soxhlet extraction thimble and lightly plugged with cotton wool to prevent loss of sample during extraction. Approximately 300 mL of ethanol was placed in a clean, dry boiling flask in the Soxhlet apparatus. Extraction was performed by refluxing the solvent at approximately 60˚C for 4 hours. After extraction, the thimble was carefully removed and the extract allowed to cool. The ethanolic extract obtained was concentrated at 70˚C for 8 hours using a rotary evaporator to remove the solvent, thus yielding the crude extract. The concentrated extract was weighed (1.2 g) and then transferred into a clean sample bottle and stored under appropriate conditions until required for further analyses. The concentrated extract percentage yield of the sample was determined to be 8% calculated from the below formula:

$$\%\ \text{yield} = \text{weight of extract} / \text{weight of sample} \times 100$$

2.5. Quantitative Phytochemical Analysis

2.5.1. Alkaloid Determination

Alkaloid content was determined following the method of Harborne [26]. Exactly five grams of the sample were weighed into a 250 ml beaker, and 200 ml of 20% acetic acid in ethanol was added. The mixture was covered and allowed to stand for 4 hours at 25˚C. It was filtered using Whatman's No. 42 filter paper and concentrated to one-quarter of the original volume on a water bath. Concentrated ammonium hydroxide (NH_4OH) was added dropwise until complete precipitation. The precipitate was filtered using a pre-weighed filter paper, washed with 1% NH_4OH, dried in the oven at 80˚C, and weighed. Alkaloid content was expressed as a percentage of the sample weight.

2.5.2. Total Flavonoid Content

Flavonoid content was determined using a colorimetric method described by Barros *et al.* [27]. A 0.5 ml aliquot of diluted sample solution (2 mg/2ml) was mixed with 2 ml distilled water and 0.15 ml of 5% $NaNO_2$. After 6 minutes, 0.15 ml of 10% $AlCl_3$ was added and allowed to stand for another 6 minutes, followed by the addition of 2 ml of 4% NaOH. The mixture was diluted to 5 ml with distilled water, mixed thoroughly, and allowed to stand for 15 minutes. Absorbance was measured at 510 nm using catechin as standard. Results were expressed as mg catechin equivalents (mg CE) per 100 g of sample.

2.5.3. Tannin Content

Tannin concentration was determined using the method of Association of Analytical Chemists (AOAC) [28]. One gram (1 g) of the sample was extracted with 10 ml of 70% ethanol and centrifuged at 2500 rpm for 5 minutes. The supernatant (0.5 ml) was diluted with 4.5 ml distilled water, followed by 0.5 ml of 0.1 M $FeCl_3$ and 0.3 ml of 0.1 M potassium ferrocyanide [$K_4Fe(CN)_6$]. Six milliliters (6 ml) of distilled water were added, and the absorbance was measured at 720 nm. Tannic acid was used as standard, and results were expressed as mg tannic acid equivalents (mg TAE) per gram of sample.

2.5.4. Total Phenol Content

Total phenols were determined using the method of Barros *et al.* [27]. Extract solution (1 ml) was mixed with 1 ml of Folin-Ciocalteu reagent. After 3 minutes, 1 ml of saturated sodium carbonate solution was added, and the volume was made up to 10 ml with distilled water. The mixture was incubated in the dark for 90 minutes, and absorbance was measured at 725 nm. Gallic acid was used as standard, and results were expressed as mg gallic acid equivalents (mg GAE) per gram of extract.

2.5.5. Saponin Content

Saponin content was estimated spectrophotometrically using the method of Hiai *et al.* [29]. One gram (1 g) of sample was dissolved in 10 ml of 80% methanol and allowed to stand for 2 - 3 hours. After centrifugation, 0.25 ml of the supernatant was mixed with 0.25 ml of 5% vanillin in methanol and 2.5 ml of 72% sulphuric acid.

The mixture was incubated in a water bath at 60˚C for 10 minutes, cooled in an ice bath, and the absorbance read at 544 - 550 nm against methanol blank. Diosgenin was used as standard, and saponin content was calculated in mg/g using the formula:

$$\text{Saponin}\left(\text{mg/g}\right)=\frac{C\times V}{M}$$

where:

C = concentration from standard curve (mg/ml);

V = volume of extract (ml);

M = mass of plant material (g).

2.5.6. Phytate Content

Phytate content was determined using the method of Young and Greaves [30]. Two grams (2 g) of sample were soaked in 100 ml of 2% HCl for 3 hours and filtered. Twenty-five milliliters (25 ml) of filtrate were diluted with 50 ml distilled water, followed by the addition of 5 ml of 0.3% ammonium thiocyanate. The mixture was titrated against $FeCl_3$ solution (0.00195 g Fe/ml). Phytate content was calculated using:

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

2.5.7. Terpenoid Content

Terpenoids were determined following the methods as described by Indumathi *et al.* [31]. One hundred milligrams (100 mg) of dried extract were soaked in 9 ml ethanol for 24 hours. The filtrate was extracted with 10 ml petroleum ether using a separating funnel. The ether layer was evaporated to dryness in pre-weighed vials, and the percentage yield of terpenoids was calculated using:

$$\text{Terpenoid yield}\left(\%\right)=\left[\left(W_i-W_f\right)/W_i\right]\times 100$$

where:

W_i = initial weight of extract (mg);

W_f = final weight after extraction.

2.6. Antioxidant Assays

2.6.1. Malondialdehyde (MDA) Assay

Lipid peroxidation was determined using the Thiobarbituric Acid Reactive Substance (TBARS) method described by Buege and Aust [32], Ohkawa *et al.* [33] and Halliwell and Gutteridge [34]. Exactly one gram of goat liver tissue obtained from a known abattoir in Awka according to appropriate ethical procedures as stipulated by recommended authorities, was homogenized in 10 ml cold phosphate buffer (0.1 M, pH 7.4) and centrifuged at 3500 rpm for 10 minutes. The supernatant (0.5 ml) was mixed with 0.5 ml of test sample/buffer/control and 0.1 ml of 0.1 mM $FeCl_3$. The volume was made up to 2 ml with phosphate buffer and incubated at 37˚C for 1 hour. After incubation, 1 ml each of 15% trichloroacetic acid (TCA) and 0.67% Thiobarbituric acid (TBA) were added, mixed, and boiled for 15

minutes. After centrifugation, the absorbance of the supernatant was read at 532 nm. Percentage inhibition (%) was calculated using the given formula below:

$$\%\ \text{Inhibition} = \left[\left(A_{\text{control}} - A_{\text{sample}}\right) / A_{\text{control}}\right] \times 100$$

where:

A_{sample} is absorbance of sample at 532 nm;

A_{control} is absorbance of standard.

2.6.2. Reduced Glutathione (GSH) Assay

Reduced glutathione was measured by the method of Ellman [35]. The principle of this assay is based on the reaction of sulfhydryl group of glutathione with 5,5'-dithiobis-(2-nitrobenzoic acid) [DTNB] producing a yellow colored 5-thiol-2-nitrobenzoic acid, whose absorbance is measured at 412 nm, directly proportional to the concentration of reduced GSH in the sample. The plant powder weighing 1 g per 5 mL was macerated in 5% trichloroacetic acid (TCA) solution in 0.2 M phosphate buffer, pH 8.0. and continuously stirred for 2 hours using a stirrer. The extract was centrifuged subsequently for 10 minutes at 67 g and supernatant collected. This was serially diluted with 0.2 M phosphate buffer, pH 8.0 into five different concentrations at extract-to-buffer ratios of 1:5, 1:6, 1:7, 1:8 and 1:9. For the blank, phosphate buffer was used while DTNB (2 mL) and phosphate buffer (1 mL) served as the control. The samples were then prepared by mixing 0.6 mM DNTB in 0.2 M of 2 mL phosphate buffer, 0.9 mL buffer and respective concentrations of supernatant and further incubated for 10 minutes at room temperature in the dark. Serial dilutions of reduced glutathione (0.1, 0.25, 0.5, 0.75 and 1 µM) solutions were prepared from 1 µM GSH stock solution of 15.4 mg of GSH in 50 mL of 0.1 N HCl. All mixtures were shaken well and incubated for 5 minutes at room temperature after which absorbance of each sample mixture was recorded at 412 nm and a standard curve plotted for the GSH concentrations.

2.6.3. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Assay

The free radical scavenging activity of *I. gabonensis* fruit pulp extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described by Blois [36]. The percentage inhibition of DPPH radicals by varying concentrations of the extract was determined and compared with that of the standard antioxidant. The samples (20 µl) were added to 0.5 ml of 0.1 mM methanolic solution of DPPH and 0.48 ml of methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank while DPPH in methanol without the samples was used as the positive control with butylated hydroxytoluene (BHT) as reference. After 30 minutes of incubation, the discoloration of the purple color was measured at 518 nm in a spectrophotometer (Genesys 10-S, USA). The radical scavenging activity was calculated as follows:

$$\text{Scavenging activity}\,(\%) = \frac{A_{518(\text{control})} - A_{518(\text{sample})}}{A_{518(\text{control})}} \times 100$$

2.7. Brine Shrimp Lethality Assay

The method according to Meyer *et al.* [37] was employed for this assay. In this assay, brine shrimp (*Artemia salina*) larvae were used. In 1 L of distilled water, 33.3 g of sea salt was dissolved to prepare the artificial sea water for cultivating the larvae. The shrimp eggs were then spread in the salt solution, exposed to light and aerated for 48 hours. After incubation, larvae that hatched into stages instar II and III were harvested for the lethality assay. Stock solutions of *I. gabonensis* fruit pulp extracts were then prepared by dissolving in artificial sea water and 1% DMSO at different concentrations of 10, 100, 500 and 1000 µg/mL for the test. For the positive and negative controls respectively, $K_2Cr_2O_7$ and 1% DMSO in artificial sea water were used. To test for lethality, ten brine shrimps were placed in 10 ml of sample solutions in a test tube at room temperature for 24 hours in triplicates. The percentage mortality was determined using the formula below:

$$\%\ \text{Mortality} = \left[\text{Died larvae}/\text{Total number of larvae}\right] \times 100$$

LC_{50} was thus calculated as the concentration of sample required to kill 50% of brine shrimps using Probit analysis while for any natural deaths in control groups, mortality rate was corrected using the Abbott's formula given below:

$$\text{Corrected Mortality}\left(\%\right) = \frac{\text{Test mortality}\left(\%\right) - \text{Control mortality}\left(\%\right)}{100 - \text{Control mortality}\left(\%\right)} \times 100$$

2.8. Data Analysis

Statistical analysis was performed using IBM Statistical Package for Social Sciences (SPSS) version 25. Results were presented in tables as Mean ± Standard Deviation of triplicate measurements while statistical significance between groups were established using One-way Analysis of Variance at 95% confidence interval ($p < 0.05$). Tukey's-HSD test was used to show exact points of significance between mean values where significant differences ($p < 0.05$) were obtained. Probit regression analysis was used to determine LC_{50} of log-transformed concentrations of extract and control.

3. Results

3.1. Quantitative Phytochemical Composition

The present study identified seven (7) phytochemicals in the sample. The result revealed that alkaloids were the most abundant phytochemicals in the extract (10.00% ± 0.01%), followed by phytates (0.81% ± 0.20%), terpenoids (0.50% ± 0.01%), and phenols (0.38 ± 0.13 mg GAE/g). Flavonoids and saponins were present in trace amounts, 0.004 ± 0.001 mg CE/100g and 0.005 ± 0.001 mg/g respectively. These results suggest that the plant possesses bioactive components, but relatively low concentrations of classical antioxidant compounds such as flavonoids, phenols, and saponins. The quantitative phytochemical composition of the bush mango extract is presented in **Table 1**.

Table 1. Quantitative phytochemical composition of *I. gabonensis* fruit pulp extract.

Phytochemicals	Composition
Alkaloids	10.00% ± 0.01%
Flavonoids	0.004 ± 0.001 mg CE/100g
Phytates	0.81% ± 0.20%
Saponins	0.005 ± 0.001 mg/g
Tannins	0.20 ± 0.02 mg TAE/g
Terpenoids	0.50% ± 0.01%
Total phenols	0.38 ± 0.13 mg GAE/g

Values are presented as Mean ± Standard deviation of triplicate determinations.

3.2. Inhibition of Malondialdehyde (MDA) and Reduced Glutathione (GSH) Concentration

The results of the antioxidant activity of *I. gabonensis* on inhibition of malondialdehyde formation and reduced glutathione concentration are presented in Table 2. In a dose-dependent manner, the % inhibition of MDA by the plant extract was reported to be highest at 100 µg/mL (84.50 ± 0.75) with the lowest documented at 10 µg/mL (48.54 ± 2.91). When compared to the standards, maximal inhibition of MDA was found to be at 100 µg/mL (94.88 ± 7.84 and 88.39 ± 4.29 for BHT and ascorbic acid respectively). Similarly, half-maximal inhibitory concentration (IC_{50}) of *I. gabonensis* fruit pulp extract was observed to be 10.5 µg/mL suggesting a moderate potent inhibitory activity of the plant though lower than those of the standards (1.51 µg/mL for BHT and 9.23 µg/mL for ascorbic acid) but comparable in activity. On the other hand, in a non-dose dependent manner, highest GSH concentrations were noted at 25 µg/mL (16.92 ± 0.50) while the lowest was reported for 10 µg/mL (14.61 ± 0.64). These values were found to be statistically non-significant ($p > 0.05$). Figure 1 and Figure 2 further illustrate the percentage inhibition of MDA by *I. gabonensis*, BHT and ascorbic acid and standard curve of GSH concentration at absorbance 412 nm respectively.

Table 2. Percentage inhibition of MDA and GSH concentration.

Concentration (µg/mL)	% Inhibition of MDA by *I. gabonensis*	% Inhibition by BHT	% Inhibition by Ascorbic Acid	GSH concentration of *I. gabonensis* (µg/mL)
10	48.54 ± 2.91^{a}	52.08 ± 5.57^{a}	50.11 ± 0.07^{a}	14.61 ± 0.64^{a}
25	58.25 ± 5.95^{b}	61.04 ± 4.11^{b}	55.34 ± 1.68^{b}	16.92 ± 0.50^{b}
50	63.21 ± 2.03^{c}	$76.67 \pm 7.84^{a, c}$	70.21 ± 4.29^{c}	16.84 ± 0.89^{c}
75	$75.33 \pm 2.84^{a, d}$	$88.23 \pm 2.48^{a, d}$	79.58 ± 2.11^{d}	14.70 ± 1.50^{d}
100	$84.50 \pm 0.75^{a, b, e}$	$94.88 \pm 3.40^{a, b, c, e}$	$88.39 \pm 2.56^{a,b,c,e}$	14.63 ± 1.19^{e}

Values are expressed as Mean ± Standard deviation of triplicate determinations. Superscripts of same letter indicate significant difference between mean values of the extract concentration.

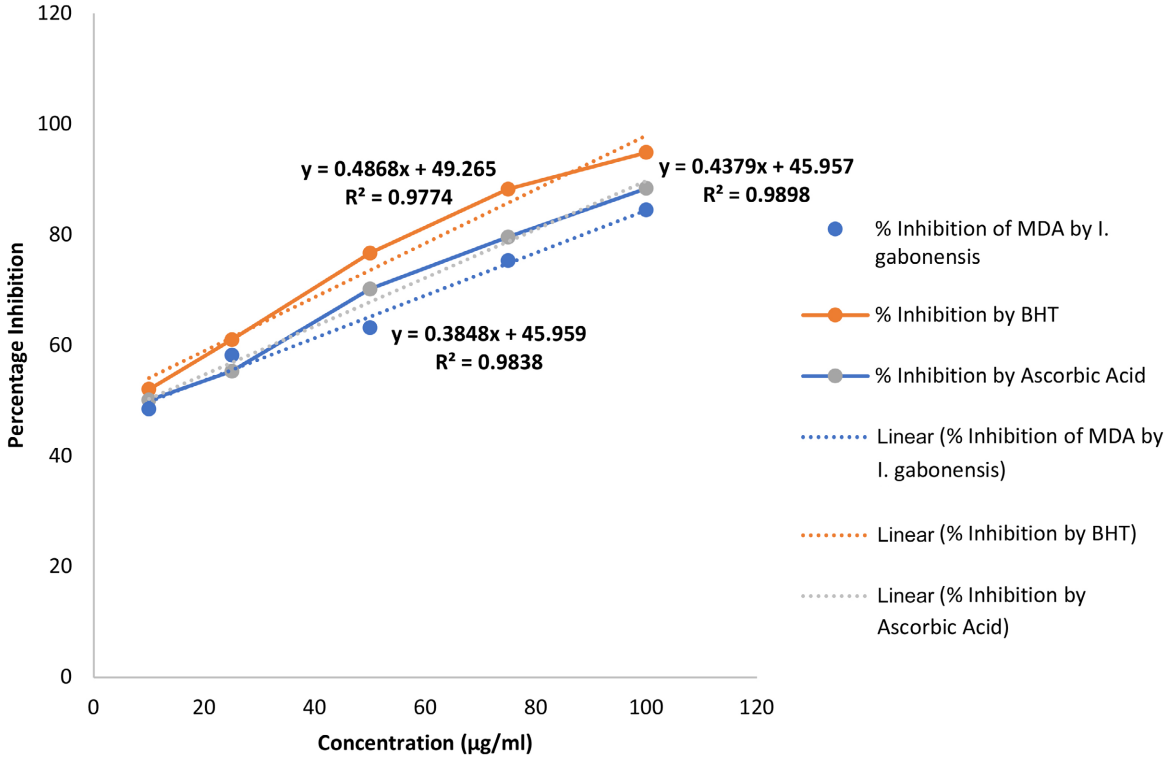

Figure 1. Scatter plot showing percentage inhibition of MDA by *I. gabonensis* fruit pulp extract against standards, BHT and ascorbic acid.

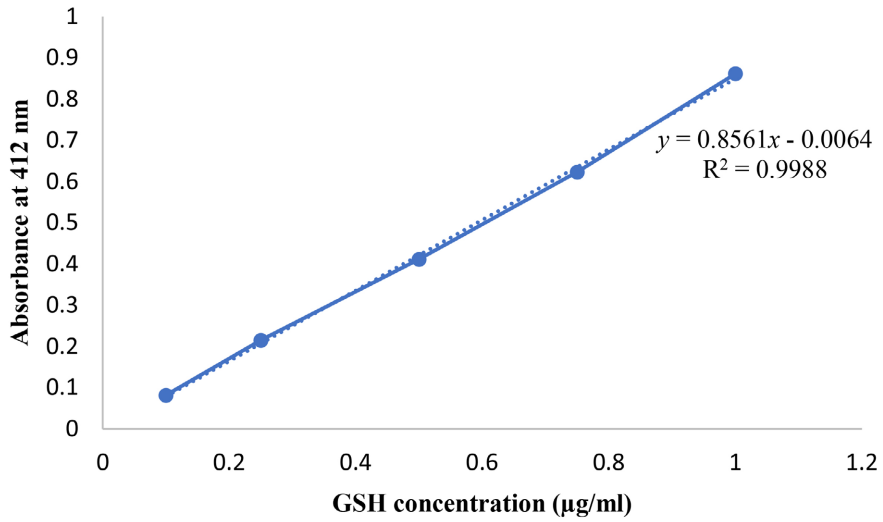

Figure 2. Standard calibration curve for GSH concentrations at 412 nm.

3.3. DPPH *in Vitro* Antioxidant Assay

Table 3 shows the DPPH radical scavenging activity of *I. gabonensis* fruit pulp extract at different concentrations. The percentage scavenging activity increased from 48.03% at 10 mg/mL to a maximum of 80.68% at 80 mg/mL, indicating increased antioxidant activity with increasing extract concentrations. The antioxidant effect of the plant extract was noted to be comparable with the reference

standard though BHT showed higher scavenging activity than the plant extract. The analysis also showed that there was a significant difference ($p < 0.05$) between the respective concentrations of sample extract and reference standard (BHT). As shown in Figure 3, the IC_{50} of the plant extract was determined to be 11.17 mg/mL indicating that the plant extract may be potent at scavenging half of the free radicals at lower concentrations.

Table 3. DPPH antioxidant activity of *I. gabonensis* fruit pulp extract.

Concentration of Extract	Absorbance	% Scavenging Activity (Mean ± Standard deviation)
10 mg/mL	1.005	48.03 ± 0.02[a]
20 mg/mL	0.863	55.49 ± 0.01[a]
40 mg/mL	0.673	63.25 ± 0.01[a]
80 mg/mL	0.374	80.68 ± 0.03[b]
Blank (methanol)	0.087	
Blank (positive control DPPH in methanol)	1.934	
Reference (BHT)	0.116	98.50 ± 0.10[c]

Results are expressed in Mean ± Standard Deviation of triplicate determinations. Superscripts of different letters indicate significant difference between concentration of sample and reference standard.

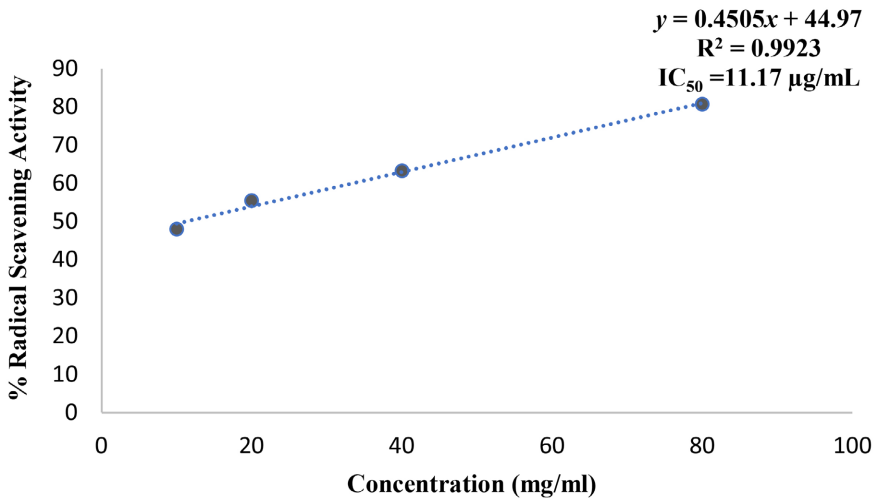

Figure 3. DPPH scavenging activity of *I. gabonensis* fruit pulp extract.

3.4. Brine Shrimp Lethality of *I. gabonensis* Fruit Pulp Extract

The results of the brine shrimp lethality assay presented in Table 4 revealed that *I. gabonensis* fruit pulp extract had a moderate toxicity of 53.33% at 1000 µg/ml and at 500, 100 and 10 µg/mL, lethality were 33.33%, 26.67% and 6.67% respectively. Consequently, the LC_{50} was determined at 1077.71 µg/mL. Similarly, the

positive control showed the highest mortality rate at 1000 µg/mL of 66.67% while at 500, 100 and 10 µg/ml concentrations, lethality were 40%, 30% and 10% respectively with an LC_{50} value of 511.30 µg/mL. The percent mortalities of the positive control were however higher than those of the plant extract at the different concentrations tested and similarly showed a more potent cytotoxic effect than the *I. gabonensis* plant extract. The negative control showed no lethality indicating a no dose-response on the brine shrimps. Figure 4 further illustrates the percent mortality of brine shrimp lethality assay by the plant extract and controls.

Table 4. Percent mortality of brine shrimp larvae of *I. gabonensis* fruit pulp and corresponding LC_{50} values.

Concentration (µg/mL)	10	100	500	1000	LC_{50}
I. gabonensis fruit pulp extract	6.67 (3.50, 2)	26.67 (4,38, 8)	33.33 (4.57, 10)	53.33 (5.08, 16)	1077.71
$K_2Cr_2O_7$ (positive control)	10 (3.72, 3)	30 (4.48, 9)	40 (4.79, 12)	66.67 (5.43, 20)	511.30
1% DMSO (negative control)	0	0	0	0	Non-lethal

Values are presented as percent mortality (probit, number of died larvae) of triplicate determinations.

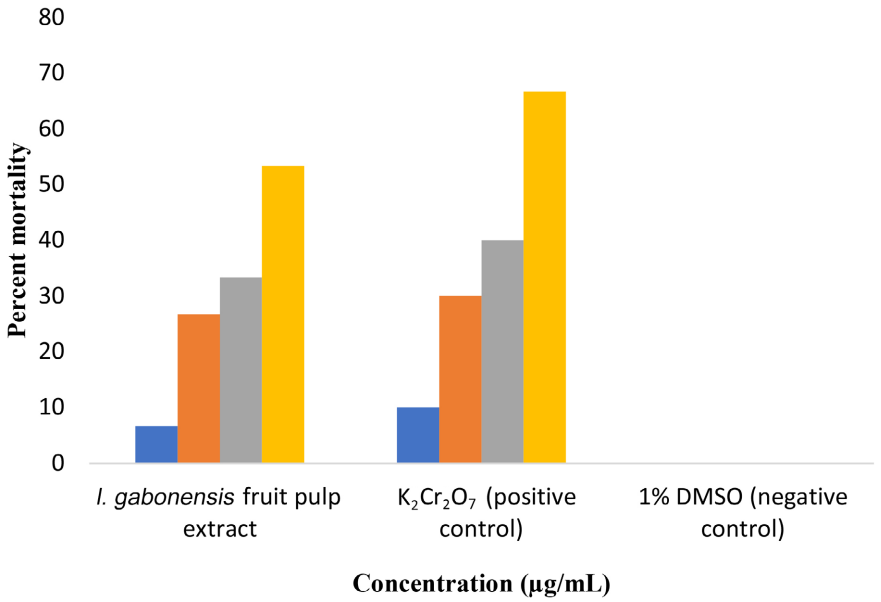

Figure 4. Percent mortality of brine shrimp larvae by *I. gabonensis* pulp extract and controls.

4. Discussion

The present study investigated the phytochemical components of *I. gabonensis* as well as its antioxidant and cytotoxic potentials with the aim of evaluating the plant's potential in offering novel phytocompounds for cancer management and

treatment. Findings showed phytochemical screening of *I. gabonensis* indicated the presence of alkaloids, phytates, terpenoids, phenols, saponins, tannins and flavonoids. Phytochemicals are secondary metabolites of plant origin that have diverse therapeutic and pharmacological impact when administered *in vivo* for specific disease ailments. Alkaloids were reported in this study as the most abundant phytochemical with pharmacological application as anesthetics and central nerve system simulant [38]. Its clinical use includes as analgesics, morphine and codeine, the muscles relaxants and as anti-cancer agent [39]. Alkaloids have been reported to inhibit cancer cells proliferation through the initiation of cell apoptosis and by stimulating the termination of cancer cell cycle at G1-phase or G2/M-phase [40]. Jie *et al.* [41] and Hamsa and Kuttan [42] reported the ability of alkaloids to inhibit tumor angiogenesis by a possible pathway of modulating the cellular redox signaling pathway. Additionally, the study showed appreciable levels of phytate in the plant. Dietary phytates have been claimed to prevent kidney stone formation, protect against diabetes mellitus, caries, atherosclerosis and coronary heart disease, as well as to fight the progression of a wide range of cancers [43]-[45]. Terpenoids play an important role in health by providing provitamin A activity for vision, influencing the human immune system function and gap conjunctional communication. Flavonoids and saponins were shown to be the least occurring phytochemicals by concentration in *I. gabonensis*. The flavonoids have been reported to be toxic to cancer cells and exhibited high free radical scavenging activity [46]. The low concentration of flavonoids aligns with previous studies that reported high anticancer efficacy at low concentrations [47]. The flavonoids inhibited expression of NF-κB, a protein complex required for angiogenesis, proliferation and survival of cancer cells [48]. In previous studies, flavonoids and its variants have been shown to suppress breast cancer cells' progression by interfering with the G0/G1 cell cycle phases [49], inhibit the resistance of cancer cells to many drugs [50] and promote the AMPK/Nrf2 pathway thereby enhancing acetaminophen-induced injury hepatoprotective effect [51]. Triterpene saponins triggered apoptosis and inhibited the development of cancer cells as documented by Park *et al.* [52]. By aggregating in the S-phase, obstructing the G2/M-phase, inhibiting p21 expression, and inhibiting cyclin-dependent kinase activity, saponins demonstrated antiproliferative effects. The moderate amounts of saponins as reported in the study could establish a strong point for scientifically investigating the plant for its potential in cancer therapy. However, antioxidant activity is often linked to flavonoids, phenols, and saponins [53], which were comparatively lower in the bush mango sample. This low abundance could explain the moderate antioxidant activity observed.

The inhibitory effect on lipid peroxidation (measured by MDA formation) showed that bush mango extract exhibited dose-dependent activity, with maximum inhibition (84.50%) at 100 μg/mL. However, its half-maximal inhibitory concentration (IC_{50}) was 10.50 μg/mL, which is higher (less potent) compared to BHT (1.51 μg/mL) and ascorbic acid (9.23 μg/mL). This indicates that bush mango has moderate antioxidant potential but not as effective as the standard an-

tioxidants. Similar moderate activity was reported in other plant extracts with low phenolic/flavonoid content [54]. The reduced glutathione (GSH) concentrations did not show significant elevation with increasing extract concentration, unlike standard antioxidants. For instance, GSH levels ranged between 14.40 - 16.92 μg/mL across the extract concentrations, suggesting a plateau effect. The lack of significant increase implies that the extract may not strongly induce endogenous antioxidant defenses. However, it may be alluded that the plant extract exhibits a biphasic, hormetic dose-response curve where at lower concentrations, increases in GSH concentrations are observed peaking at 25 - 50 μg/mL and then declining at 75 - 100 μg/mL. This may be explained by the pro-oxidant shift of the plant extract at overwhelming concentrations of the plant's constituents resulting in a surge of reactive oxygen species that deplete endogenous GSH pool. This observed effect in decreased GSH concentration may also be attributed to its limited phenolic and flavonoid contents, as phenolics have been reported to synergistically repair GSH and synthesis and subsequently enhance antioxidant activity [55]. The findings suggest that while bush mango possesses bioactive compounds with some ability to suppress lipid peroxidation, its antioxidant strength is relatively lower compared to ascorbic acid and BHT. The extract may therefore function better as a supportive antioxidant agent rather than a primary therapeutic compound or serve in complementary medicine strategies to combat inflammatory pathways linked to oxidative stress in cancer pathology.

To further investigate the antioxidant potential of the plant extract, DPPH assay was used to determine the scavenging ability of *I. gabonensis* pulp extract by estimating its total antioxidant capacity. Antioxidant studies represent models for determining whether a natural plant product can inhibit formation of free radicals or scavenge already formed free radicals [56] [57]. In the present study, results showed a comparable potent antioxidant effect of the plant to the standard reference with an IC_{50} (11.17 mg/mL) almost closer to the lowest concentration (10 mg/mL) against which the plant extract was tested, suggesting a role as a natural plant source for providing compounds that can scavenge free radicals, which have been largely implicated in the onset of debilitating chronic illnesses such as cancer. In comparison with other studies, Atanu *et al.* [53] reported IC_{50} values ranging from 21.42 to 36.42 μg/mL for different solvent extracts of the plant leaves using n-hexane, chloroform, ethanol and aqueous. For our study with an IC_{50} of 11.17 μg/mL, it may suggest that the edible fruit pulp performs better at scavenging radicals than the leaves. The findings of this present study also align with previous studies that investigated the antioxidant potentials of the different plant parts including the leaves [53] [58] and seeds [59]-[61] by DPPH assay indicating a very potent antioxidant and efficacious plant at mopping up free radicals and inhibiting their formation. However, Okolieuwa *et al.* [13] who studied the antioxidant effect of the pulp extract investigated the effect on glutathione and superoxide dismutase concentrations of CCl_4-treated rats and reported compelling evidence of the fruit pulp effectiveness at increasing the levels of these endogenous antiox-

idant enzymes while concluding that the fruits could be valuable dietary additions to promoting antioxidant defense mechanisms against oxidative stress-related conditions. The study did not include DPPH scavenging activity assay of the fruit pulp but however indicated potential antioxidant activity using other approaches. The scavenging ability of the fruit pulp observed in this study could be attributed to the presence of phenols and polyphenols in the plant, which have been linked to their redox properties and thus play roles in absorbing and neutralizing free radicals [62]-[64]. This finding can be further substantiated by the study of Ni *et al.* [65] who reported the correlation of antioxidant activity to presence of polyphenols. Phenols and polyphenols have been known to possess anticancer properties and consequently were demonstrated in this study. The study also showed an increasing antioxidant activity with increasing sample concentration indicating a dose-dependent response suggesting a plant source of natural antioxidants that can be optimized in cancer treatment and management.

The brine shrimp lethality assay revealed an LC_{50} of 1077.17 µg/mL higher than that of the reference standard (511.30 µg/mL), indicating a less potent or cytotoxic plant material. According to Meyer's toxicity classification, LC_{50} of lower than 1000 µg/mL are considered toxic while LC_{50} above 1000 µg/mL are non-toxic. Further, Clarkson *et al.* [66] specifies that bioactive substances are toxic when their LC_{50} are higher than 1000 µg/mL, weakly toxic when in the range between 500 and 1000 µg/mL, moderately toxic when between 100 and 500 µg/mL and highly toxic if below 100 µg/mL. Thus, according to these classifications, the fruit pulp extract was found to be non-toxic, which explains its edibility and possible dietary contribution to the fare of the locales. Previous studies [13] investigating the toxicity profile of *I. gabonensis* fruit pulp using rat models found similar results of non-toxicity of the plant material. In relation to assessing the cytotoxic potential of *I. gabonensis* fruit pulp extract, the brine shrimp lethality assay suggested a concentration-dependent response of the plant extract, which may be linked to the presence of secondary metabolites [67], specifically tannins and flavonoids, also demonstrated in this study. Flavonoids are known to possess antioxidant and anti-inflammatory properties exhibited by modulating certain enzyme activities thereby impacting key biochemical events in cancer initiation and progression [68]. By impacting certain enzymes crucial to cell division and growth, flavonoids can initiate apoptotic pathways and subsequently cell death. For example, naringenin, a member of a flavonoid subclass, flavanones, was reported to suppress breast cancer progression by interfering with the G0/G1 cell cycle [49] and exerted antiproliferative action in HT29 colon cancer cell lines [69].

Tannins, similarly, exhibit antioxidative effects by targeting important cellular signaling pathways and molecular agents involved in cancer development thereby offering chemo-preventive and therapeutic benefits [70]. They modulate EGFR/Jak2/STAT1/3 and P38/STAT1/p21Waf1/Cip1 pathways thereby inducing cell cycle G1-arrest and apoptosis in breast cancer [71], hence preventing carcinogenesis. Overall, the brine shrimp lethality assay findings of this study may

be linked to an underlying factor, which is the presence of secondary phytochemicals that have been reported in studies, known to induce cytotoxicity and these phytochemicals can, therefore, be subsequently optimized in drug development strategies for cancer treatment and management.

5. Conclusions

This study is a preliminary work to support global initiatives on the drive to identify novel therapeutics in the management and treatment of cancer leveraging on the scientific knowledge gap of the potential of *I. gabonensis* to offer novel phytocompounds in cancer therapy. The work therefore demonstrated that *I. gabonensis* extract contains appreciable amounts of phytochemicals, with alkaloids being the most abundant and phenols, flavonoids, and saponins present in very low concentrations. The extract showed moderate inhibition of lipid peroxidation as indicated by its effect on MDA formation comparable to standard antioxidants. The extract did not significantly elevate glutathione levels, suggesting that its antioxidant activity may be mainly due to direct radical scavenging activity rather than modulation or regulation of endogenous antioxidant defense systems. The DPPH and cytotoxicity assays suggested translatable relevance and options in anticancer treatment. Overall, *I. gabonensis* possesses moderate antioxidant potential which may contribute to its traditional use, but its lower content of key antioxidant phytochemicals limits its effectiveness compared to standard antioxidants.

Our study, however, presented some limitations. The study fundamentally comprised crude phytochemical screening. Going forward, in a holistic attempt within the pipeline of studies to profile the plant, future research will focus on employing advanced quantitation techniques such as GC-MS, HPLC and FTIR to identify novel phytocompounds of profound bioactivities with functional groups of anticancer potential while computational molecular docking and molecular dynamics simulation studies among others will be used to investigate pharmacokinetic and drug-likeness properties of identified compounds. Similarly, next steps will include biosynthesizing hit compounds of anticancer impact and conducting preclinical and clinical studies to validate findings.

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Data Availability

Data generated will be shared upon request.

Ethics Approval

Ethical approval was not required for this study as no live animals were used,

grown, fed or euthanized specifically for the purpose of this study. The liver tissues used for this experiment were obtained post-mortem from goat slaughtered at a commercial abattoir after regular slaughter procedures for meat production. All procedures were followed according to local veterinary and livestock humane handling regulations.

Plant Authentication

The fruit plant was identified and authenticated, according to local, national and international guidelines regarding studies involving plants, by a botanist in the Department of Botany, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria after which the plant was later deposited in the herbarium of the department with voucher number NAUH-071[B].

Author Contributions

UCO conceptualized, designed and supervised the study. EPN performed the experimental aspects of the study and wrote the first draft of manuscript. UCO analyzed and interpreted the data and wrote the final draft of manuscript for submission. All authors read and approved final draft of manuscript.

Conflicts of Interest

The authors declare that they have no competing interests.

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List of Abbreviations

AOAC	Association of Official Analytical Chemists
BHT	Butylated Hydroxytoluene
BSLA	Brine Shrimp Lethality Assay
DTNB	5,5'-dithiobis-(2-nitrobenzoic acid)
IC_{50}	Half Maximal Inhibitory Concentration
FTIR	Fourier Transform Infrared Spectroscopy
GC-MS	Gas Chromatography-Mass Spectrometry
GSH	Reduced Glutathione
HPLC	High Performance Liquid Chromatography
LC_{50}	Half Maximal Lethal Concentration
MDA	Malondialdehyde
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid