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Original Article

Phytochemical Analysis and *in vitro* Antioxidant Potential of Aqueous and Ethanol Extracts of *Irvingia gabonensis* Stem Bark

Catherine Otitolaiye[1, 2], Akhere Omonkhua[2,*], Kelly Oriakhi[2], Edward Okello[3], Iyere Onoagbe [4], Friday Okonofua[5]

[1]Department of Biochemistry, Sokoto State University, Sokoto, NIGERIA.
[2]Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Edo, NIGERIA.
[3]Human Nutrition Research Centre, Population Health Sciences Institute, Faculty of Medical Sciences, Newcastle University, Tyne, UNITED KINGDOM.
[4]Department of Biochemistry, University of Benin, Edo-State, NIGERIA.
[5]Department of Obstetrics and Gynaecology, School of Medicine, University of Benin, Benin, NIGERIA.

ABSTRACT

Background: *Irvingia gabonensis* extracts are documented to possess antidiabetic, hypocholesterolemic, and antioxidant activities. The phytochemical components of medicinal plants are responsible for their therapeutic properties, particularly those with antioxidant properties. **Objectives:** The quantitative phytochemicals and *in vitro* antioxidant properties of the aqueous and ethanol extracts of *I. gabonensis* stem bark were assessed in this study. **Materials and Methods:** Standard techniques were used to measure the concentrations of phenols, flavonoids, tannins, alkaloids, steroids, and saponins in water and alcohol extracts of this plant. Total Antioxidant Power (TAP) and the extract's capacity to scavenge free radicals including superoxide, nitric oxide, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis-(3-ethylbenzoth iazoline-6-sulfonic acid) ($ABTS^+$), β-carotene, and hydrogen peroxide were also evaluated. The extracts' Ferric-Reducing Potential (FRAP) was also assessed. **Results:** The alcohol extract had significantly ($P < 0.05$) higher concentrations of all the phytochemicals measured as well as near significantly ($P < 0.066$) higher TAP in comparison to the aqueous extracts. In general, the *in vitro* antioxidant capacity of the ethanol extract surpassed that of the water extract. The ethanol extract had IC_{50} values that were comparable to or less than the reference standards for its ability to neutralize nitric oxide, H_2O_2, superoxide, and DPPH radicals. **Conclusion:** The outcomes of the quantitative phytochemical analysis and *in vitro* antioxidant effects of aqueous and ethanol extracts of *I. gabonensis* stem bark clearly show their potential as an excellent reservoir of bioactive compounds and scavengers of deleterious oxidants; properties that could be explored therapeutically.

Keywords: *Irvingia gabonensis*, Phytochemicals, Antioxidant, Free radicals, *In vitro*.

Correspondence:
Prof. Akhere A Omonkhua
Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Edo-State, NIGERIA.
Email id: akuekegbe.omonkhua@uniben.edu

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INTRODUCTION

Free radicals/oxidants are produced by the normal metabolic processes which occur daily in the body and these include peroxyl radical (RO_2^-), hydrogen peroxide (H_2O_2), nitric oxide (NO^-), superoxide ion (O_2^-), and hydroxyl radical ($OH^·$).[1,2] If not managed, the overproduction of these oxidants could overwhelm the endogenous antioxidants found in the body such as ascorbic acid, catalase, thiols, glutathione, and superoxide dismutase. This produces an imbalance that could lead to oxidative stress and subsequently, oxidative damage. This is because excess free radicals in the body can bind to biomolecules such as lipids, carbohydrates, nucleic acids, and proteins, to cause damage, which can induce and/or worsen various diseases.[3] Oxidative stress/damage has been associated with diseases like atherosclerosis, diabetes, chronic kidney disease, rheumatoid arthritis, cardiovascular disease, and neurological degenerative diseases.[4-6] Adequate levels of antioxidants in the body can scavenge these free radicals;[7,8] thus ameliorating their negative effects.

The treatment of oxidative stress-related illnesses with plant-derived antioxidants has gained increased interest as they are reported to be effective with very few or no negative effects.[9-11] Studies have shown that plants have natural antioxidants, such as flavonoids, phenols, tannins, saponins, and apigenin; which

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help boost the body's endogenous antioxidants and therefore ameliorate different diseases.[12-15]

Irvingia gabonensis, a medium-sized tree, can be found in the tropical rainforests of West Africa. It is a member of the Irvingiaceae family (order: Rutales). There are three species in the genus *Irvingia* (*Irvingia gabonensis, Irvingia wombolu*, and *Irvingia grandifolia*); and all are found in West and Central Africa.[16,17] Except for slight morphological variations in the trees and the palatability of the fruits, there are not many distinctions between the species. The soft fleshy part of *I. wombolu* is bitter and not edible whereas that of *I. gabonensis* is sweet and comestible. The *Irvingia* fruit is made up of a soft fleshy portion and a nut which encloses the seed or kernel. The seed has two white cotyledons and an outer brown testa.[18] In Nigeria, *I. gabonensis* is locally called by different names such as 'goron biri' in the Hausa language, 'Apon' or 'Oro' in Yoruba; and 'Ogbono' or 'Ugiri' in Igbo.[19,20] The sweet pulp is widely consumed by the Yorubas, while the seed is of interest to the Igbos as a soup thickener.

I. gabonensis seeds have been reported to possess body weight reduction and blood anticoagulant effects.[21] Traditionally, the stem bark is given to women to decrease their breastfeeding period as well as to relieve pain.[22] It can also be used in the treatment of dysentery, colic, hernias, yellow fever, and as an anti-poison.[22,23] Our prior studies showed that *I. gabonensis* aqueous stem bark extract had a beneficial effect against oxidative damage, resulting in prolonged anti-obesity and hypoglycaemic benefits in apparently healthy rabbits as well as antidiabetic and antioxidant effects in streptozotocin-induced diabetes in rats.[24-26]

The presence of phytochemicals in medicinal plants, especially those that also have antioxidant activities, is attributed to their positive therapeutic effects. Therefore, this work was designed to quantitatively identify the natural compounds in the aqueous and ethanol extracts of *I. gabonensis* stem bark and assess the *in vitro* antioxidant capacity of these extracts as a means of ascertaining their potential to protect against oxidative stress.

MATERIALS AND METHODS

Extract Preparation

Irvingia gabonensis fresh stem bark was obtained from a farm in Akungba-Akoko, Ondo-State, Nigeria. A specimen, with herbarium ID No of FHI 112492, was deposited at the Forest Research Institute Herbarium, Ibadan, Oyo State; after authentication. The bark samples were washed, shade-dried, and pulverised using a grinding machine.

The air-dried powdered sample was divided into 2 portions. The first part was soaked (macerated) in distilled water for 48 hr and subsequently filtered and the marc (residue) was again macerated in distilled water for 24 hr. The procedure was replicated four times and the extract obtained was concentrated and freeze-dried to obtain a powdered aqueous extract. The second portion of pulverized stem bark was also soaked (macerated) in ethanol for 72 hr, filtered, and macerated again, this process was done four times for exhaustive extraction. Most of the solvent was removed via rotary evaporation and the residue was freeze-dried.

Quantitative Determination of Phytochemicals

Phenol Estimation

As described by Roy *et al.*,[27] the phenolic content of the extracts was ascertained using the Folin-Ciocalteu reagent (FCR) method. The FCR assay's principle is that phenolics in the extracts reduce FCR, which causes the development of a blue colour that gets darker as phenolic concentration increases. One and a half (1.5) mL of 10% FCR reagent was put to a test tube already holding 200µL of extract/standard. The solution was covered and put in a dark cupboard at ambient temperature for 5 min. After that, 1.5mL of 5% Na_2CO_3 was introduced and thoroughly mixed. This was again covered and left to stand in a dark place for 2 hr at room temperature. The absorbance was later read at 750nm. Gallic acid (1mg/mL) was prepared at different concentrations of 5, 10, 25, 50, 75, 100, 150, and 200µg/mL, to plot the standard curve. The equivalent amount of gallic acid, in milligrams (mgGAE/g), was used to determine the amount of phenol in the extracts. Three replicates of the assay were run.

Flavonoid Estimation

The flavonoid concentration in this assay was estimated using the aluminium chloride colourimetric method[27] but with some modifications. The reference, quercetin (1mg/mL), was prepared in a range of concentrations, such as 10, 50, 75, 100, 150, 200, 250, and 300 µg/mL. To 1 mL of either the aqueous or ethanol extract, or quercetin was added 0.3 mL of sodium nitrite (5%). After covering, this solution was incubated for 5 min at ambient temperature. Thereafter, 10% aluminium chloride (0.3 mL) was introduced, stirred, and the solution was once more allowed to stand for 5 min at ambient temperature. The solution was then combined with 2mL of 1M NaOH and left to stand at room temperature for an additional 10 min. The flavonoid concentration was quantified as milligrams of quercetin equivalent per gram of extract (mgQE/g) after the absorbance was measured at 510 nm. Triplicates of the assay were done.

Tannin Estimation

The estimation of tannin was done using the Folin-Ciocalteu Reagent (FCR)[27] but with little modifications. With this method, the presence of tannins in the extract reduces FCR from molybdate (VI) ions to molybdate (V) ions and a blue colour is produced which increases in intensity as the concentration of the tannins increases. For the reference standard, various quantities of 1mg/mL gallic acid were generated, including 10, 50, 100, 200, 300, 400, and 600µg/mL. A test tube containing 100 µL of extract or standard was then filled with 7.5 mL of distilled water, 0.5 mL of 10% FCR, and 1 mL of a 35% Na_2CO_3 solution. The

mixture was incubated at ambient temperature for 10 min. The extracts and the gallic acid standard absorbance were measured at 725nm. The corresponding amount of gallic acid per gram of extract, expressed in milligrams (mgGAE/g) was used to quantify the tannin concentration. The assay was carried out three times.

Alkaloid Estimation

This test was carried out using bromocresol green (BCG) solution[28] although with a small modification. To freshly prepare Bromocresol Green (BCG) solution; BCG (69.8mg), 2N NaOH (3mL), and distilled water (5mL), were heated together to fully dissolve. After that, distilled water was used to dilute the resulting solution and make it up to 1000mL. Separately, a sodium phosphate buffer (2N) with a pH of 4.7 was prepared. Additionally, distilled water was utilized in making the atropine reference solution (0.5 mg/mL) that was diluted to varying concentrations of 10, 50, 100, 200, 300, 400, and 500μg/mL. In order to recover the alkaloids, 100 mg of plant sample was combined with 10 mL of 2N HCl and filtered. After which 1mL of the filtrate and 1mL of standard (at the different concentrations) were separately added to 5mls of the phosphate buffer and 5mL of the bromocresol green solution in a separating funnel. This was well mixed and 4mL of chloroform was later added and shaken vigorously. As such, a BCG-Chloroform complex was formed and the chloroform fraction was collected into a test tube. The absorbance was then read at 470nm. Milligrams of atropine equivalent per gram of extract (mgAE/g) served as the unit of measurement for the overall alkaloid content. All determinations were in triplicates.

Steroid Estimation

With few modifications, the estimation of the steroid content was done as Madhu *et al.*[29] had stated. Diosgenin was used as a standard steroid. Two mL of the extract or standard (2 mg/mL Diosgenin), 2 mL of 2 M H_2SO_4, and 2 mL of 0.5% $FeCl_3$ were placed in a test tube. After which, 0.5% potassium hexacyanoferrate (III) reagent was introduced. The final solution was placed in a water bath maintained at 70°C for 30 min. while being occasionally shaken. After allowing it to cool, the absorbance was measured at 780 nm. Milligram of diosgenin equivalent (mgDE/g) per gram of extract was used to express the steroid content. This test was run three times.

Total Saponins Estimation

The vanillin-sulphuric acid method[30] was used to estimate the amount of saponins present in *I. gabonensis* stem bark extracts. Seventy-two percent H_2SO_4 (2.5 ml), 0.25 mL of 8% vanillin (dissolved in ethanol) was introduced to a test tube holding 0.25 mL of plant extract or standard (1 mg/mL Diosgenin). This was left in a water bath for 15 min at 60°C. After allowing to cool, the extracts' and the standard's absorbance were measured at 560 nm. Following that, milligrams of diosgenin equivalent per gram of extract (mgDE/g) was used to determine the total saponin content. The test was run three times.

In vitro Antioxidant Assays

Total Antioxidant Power (TAP)

Here, the antioxidants present in the extracts reduce the phosphomolybdate (VI) ion to a green phosphomolybdate (V) ion. This assay is also called phosphomolybdate assay[31] and in this case, the reactions of sodium phosphate and ammonium molybdate in the presence of H_2SO_4 produce the free radical (phosphomolybdate ion). First, equal amounts of 4 mM ($(NH_4)_2MoO_4$), 28 mM Na_3PO_4, and 0.6 M H_2SO_4 were added to produce the standard phosphomolybdate reagent solution. The standard used for this assay was (gallic acid) and was prepared in concentrations of 10, 25, 50, 100, 200, 300, 400, and 500μg/mL while 500μg/mL of extracts were utilized. To 1mL of extract or gallic acid reference was added 2 mL of the standard phosphomolybdate solution. Then, it was placed in a water bath at 95°C for 90 min. A subtle green colour developed after it was allowed to cool to ambient temperature.

After determining the absorbance at 695 nm, the overall antioxidant strength was shown as the corresponding amount of gallic acid (mgGAE/g) in one gram of the extracts.

ABTS (2,2-azino-bis(3-ethylbenzothi azoline-6-sulfonic acid)) Oxidant Quenching Potential

The ABTS radical decolourisation assay was also used to assess the oxidant quenching capacity of the extracts of *I. gabonensis* stem bark,[32] but with little variation. Antioxidants found in plant extracts reduce the ABTS radical from blue to colourless; this change in absorbance is detected at 734 nm. By mixing distilled water with equal quantities of 8 mM ABTS and 3 mM $K_2S_2O_8$, ABTS oxidant ($ABTS^+$) was generated from ABTS. In the dark, at ambient temperature, this was allowed to sit and react for 16 hr. In a ratio of 1:10, the $ABTS^+$ reagent produced was diluted with methanol before it was used. The solution was protected from light rays by covering it with aluminium foil. The extracts and standard (gallic acid), 1mg/mL of each, at different concentrations of 10, 25, 50, 100, 200, 300, 400 and 500μg/mL were prepared. Then, in a test tube, 2mL of the diluted $ABTS^+$ solution was carefully poured into 200μL of the extract or standard. These were combined and then allowed to sit in the dark for 15 min, at ambient temperature. At 734 nm, the absorbance was then measured. The assay was

$$\%\,Inhibition\ of\ ABTS\ radical = \frac{Abs(control) - Abs(extract/standard)}{Abs(Control)}\ X\ 100$$

DPPH (2, 2-diphenyl-1-picrylhydrazyl) Oxidant Quenching Potential

The capability of antioxidants present in *I. gabonensis* stem bark extracts to decolourize DPPH radical from purple to yellow was measured as reported by Roy *et al*.[27] The extracts stock solutions (1mg/mL) were prepared into various amounts of 2.5, 5, 10, 20, 40, 80, 160, 320 and 640µg/mL. While the ethanol extract was dissolved in methanol, the aqueous extract was dissolved in distilled water. Gallic acid (1mg/mL) was the reference standard that was used. The 0.3mM DPPH radical solution was made by mixing DPPH powder with methanol. The solution was protected from light rays by covering it with aluminium foil. The extract or standard (1mL) was added at different concentrations to 2mL of 0.3mM DPPH solution. The tubes were vigorously shaken and then allowed to sit in the dark for 30 min. Then, at 517 nm, the absorbance was determined. The test was carried out three times.

$$\% \, Inhibition \, of \, DPPH \, radical = \frac{Abs(control) - Abs(extract/standard)}{Abs(Control)} X \, 100$$

Nitric Oxide Radical Scavenging Assay

The nitric oxide quenching potential of the aqueous and ethanol extracts of *I. gabonensis* stem bark was assessed by using Griess reagent. Nitric oxide is produced by sodium nitroprusside in an aqueous solution, and it can react with oxygen to produce nitrite ions (radicals). This in turn can be quenched by the antioxidants in the extracts which donate protons to the nitrite ions. The aqueous and ethanol extracts as well as standard gallic acid (1mg/mL) were prepared and serially diluted with distilled water into varied amounts of 2.5, 5, 10, 20, 40, 80, 160, 320, and 640 µg/mL. Griess reagent (0.1%) and 10mM sodium nitroprusside were also freshly prepared in distilled water.[33] In a test tube containing 1mL of the extracts or standard at different concentrations, 0.5 mL of a newly prepared sodium nitroprusside was added. After that, it was allowed to sit in the dark at ambient temperature for two hours. Newly made Griess reagent (1.5 mL) was included after the mixture had rested at ambient temperature for another 10 min. Later, at 546 nm, the absorbance was taken. There were three runs of the test.

$$\% \, Inhibition \, of \, nitric \, oxide \, radical = \frac{Abs(control) - Abs(extract/standard)}{Abs(control)} x \, 100$$

Superoxide radical quenching test

The reaction between NADH and PMS produce superoxide oxidants, which can cause the color of Nitroblue Tetrazolium (NBT) to change to purple. Plant antioxidants turn the purple color into a colorless tint.[31] Here, the aqueous and ethanol extracts and standard gallic acid (1mg/mL) were serially diluted to several amounts of 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100 µg/mL. To 1 mL of extracts or gallic acid at the different concentrations, was added 1 mL of 0.1 M phosphate buffer (pH 7.2), 1 mL NADH (2 mM), 1 mL NBT (0.5 mM), and 0.1 mL PMS (0.03mM). These were thoroughly combined and stood at the prevailing temperature for 5 min. At 562 nm, the absorbance was afterwards recorded. Triplicate tests were done.

$$\% \, Inhibition \, of \, superoxide \, radical = \frac{Abs(control) - Abs(extract/standard)}{Abs(Control)} X \, 100$$

Beta carotene bleaching assay

Linoleic acid when oxidized produces radicals which can react with β-carotene and change its colour from yellow to colourless. However, antioxidants decrease the acceleration of β-carotene bleaching.[34] In this test, the reference standard, butylated hydroxyanisole (BHA), and stock solutions of the aqueous and ethanol extract of *I. gabonensis* stem bark were prepared (1mg/mL). Using distilled water, these were divided into different quantities of 50, 100, 200, 300, 400, and 500 µg/mL. Next, 10mL of chloroform was used to dissolve 2mg of β-carotene.

Then, 1 mL of the β-carotene in chloroform was combined with 0.02 mL of linoleic acid and 0.20 mL of Tween 40. The chloroform was then vaporized in a steam bath at 40°C; after which, 50 mL of distilled water was carefully included. Finally, 4.8mL of the resultant linoleic acid/β-carotene water mixture was put into a test tube with 200µL of the extracts or standard solutions. The absorbance was read instantly (t = 0 min); the test tubes were immediately covered and lowered into a water bath that was heated to 50°C. Later, the absorbance was measured every 30 min for 120 min. The assay was carried out three times.

$$\% \, Inhibition = 1 - \frac{As(0min) - As(120min)}{Ac(0min) - Ac(120min)} X \, 100$$

Where As = Absorbance of sample and Ac = Absorbance of control

Hydrogen Peroxide Radical Scavenging Assay

The standards (gallic acid and ascorbic acid) and extracts (1mg/mL) were serially diluted to 12.5, 25, 50, 100, 200, 400, 800 and 1000µg/mL. To 350µL of the standards and plant extracts were added 350µL of 12mM phenol solution, 100µL of 0.5mM 4-aminoantipyrine, 160µL of 0.7mM hydrogen peroxide, and 350µL of 1U/mL horse radish peroxidase (HRP) made in sodium phosphate buffer (84mM, pH 7). These were thoroughly mixed and allowed to sit at 37°C for 30 min. The absorbance was determined at 504 nm, and the percentage inhibition was computed. [35] The assay was carried out three times.

$$\% \, Inhibition \, of \, hydrogen \, peroxide \, radical = \frac{Abs(control) - Abs(extract/standard)}{Abs(Control)} X100$$

Ferric Reducing Antioxidant Power (FRAP) Test

The ability of antioxidants in plant extracts to convert ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) is the basis for this test.[36] The extracts stock solutions, as well as the ascorbic acid standard, were serially diluted to 12.5, 25, 50, 100, 200, 400, 800, and 1000 µg/mL. In a ratio of 1:1:10, 20 mM $FeCl_3.6H_2O$, 10 mM tripyridyltriazine (TPTZ), and 300 mM sodium acetate buffer (pH 3.6) were

Table 1: Phytochemical Analysis and Total Antioxidant Power of *Irvingia gabonensis* Stem Bark Aqueous and Ethanol Extracts.

Sl. No.	Phytochemical Analysis	Aqueous Extract	Ethanol Extract
1	Phenolic Content (mgGAE/g of extract)	156.27 ± 4.72	253.39 ± 11.31*
2	Flavonoid Content (mgQE/g of extract)	185.33 ± 3.85	606.44 ± 21.11*
3	Tannin Content (mgGAE/g of extract)	199.33 ± 3.16	284.79 ± 13.51
4	Total Alkaloid Content (mgAE/g of extract)	61.11 ± 2.08	74.72 ± 1.28*
5	Total Steroid Content (mgDE/g of extract)	80.87 ± 10.26	162.67 ± 3.56*
6	Total Saponin Content (mgDE/g of extract)	223.44 ± 4.48	333.45 ± 15.43*
7	Total Antioxidant Power (mgGAE/g of extract)	319.89 ± 8.73	488.78 ± 47.31

QE-Quercetin Equivalent; GAE-Gallic Acid Equivalent; AE-Atropine Equivalent; DE-Diosgenin Equivalent.* Indicate values that are significantly different at $P < 0.05$.

combined to make the FRAP reagent. While $FeCl_3.6H_2O$ was dissolved in water, TPTZ was dissolved in methanol. The serially diluted extracts and standard (1 mL each) were combined with 3 mL of FRAP solution. These were thoroughly mixed and allowed to sit for 30 min at 37°C. Later, at 593 nm, the increase in absorbance was recorded. The assay was carried out three times.

Data Analysis

Each test was done three times and results were presented as mean ± SEM. Using IBM SPSS version 26, the variations between the aqueous and ethanol stem bark extracts of *I. gabonensis* for the quantitative phytochemicals and total antioxidant power assays were analysed by the Independent Samples *t*-test. $P<0.05$ was accepted as significant.

RESULTS

The ethanol and aqueous extracts of the powdered stem bark of *I. gabonensis* gave respective yields of 2.77% and 1.78%. The quantitative phytochemicals measured in this plant showed significantly greater values for the alcohol extract when referenced against the water extract ($p<0.05$) (Table 1).

The Total Antioxidant Power (TAP) of the aqueous and ethanol extracts of *I. gabonensis* stem bark was measured via a gallic acid standard graph. The ethanol extract's TAP (488.78 ± 47.31 mgGAE/g) was found to be nearly significantly ($P<0.066$) greater than the aqueous extract (319.89± 8.73 mgGAE/g) at 500 g/mL.

From Figure 1A, the standard (gallic acid) and the ethanol extract of *I. gabonensis* had higher inhibitory activity against the ABTS radical compared to the aqueous extract. The IC_{50} value for gallic acid was 3.36 µg/mL, and that of the ethanol extract was 17.58µg/mL while the water extract had an IC_{50} value of 283.19µg/mL (Table 2).

Likewise, the inhibitory activity of the extracts and standard against DPPH radical (Figure 1B) showed the alcohol extract and the standard (gallic acid) had stronger inhibitory activities against the radical than the aqueous extract. Gallic acid had the least IC_{50} value of 0.002 µg/mL (highest inhibitory potential), followed by the ethanol extract s with an IC_{50} of 1.17 µg/mL when compared to water extract with IC_{50} of 35.37 µg/mL.

When compared to the standard (gallic acid), the percentage inhibition against the nitric oxide radical (Figure 1C) showed that the aqueous and ethanol extracts exhibited stronger inhibition. The water and ethanol extracts had IC_{50} values of 0.83 g/mL and 6.42 g/mL, respectively, while the standard had an IC_{50} value of 427.09 g/mL (Table 2).

The ability of the aqueous and ethanol extracts of *I. gabonensis* stem bark to inhibit superoxide radicals was compared to gallic acid, the reference standard. From Figure 1D, the ethanol extract showed a higher percentage inhibition (IC_{50} of 18.17µg/mL) than the aqueous extract (38.24 µg/mL) while the standard (9.21 µg/mL) had the highest inhibitory activity.

The β-carotene bleaching test showed the ability of the extracts and standard (BHA) to inhibit β-carotene oxidation in the presence of linoleic acid (Figure 2A). The IC_{50} of the water (195.49 µg/mL) and ethanol (90.20 µg/mL) extract were higher than the standard BHA (0.025µg/mL). With reference to the water extract, the alcohol extract had better inhibitory potential.

The extracts' capacity to quench hydrogen peroxide oxidants was also assessed. As shown in Figure 2B, it was observed that at

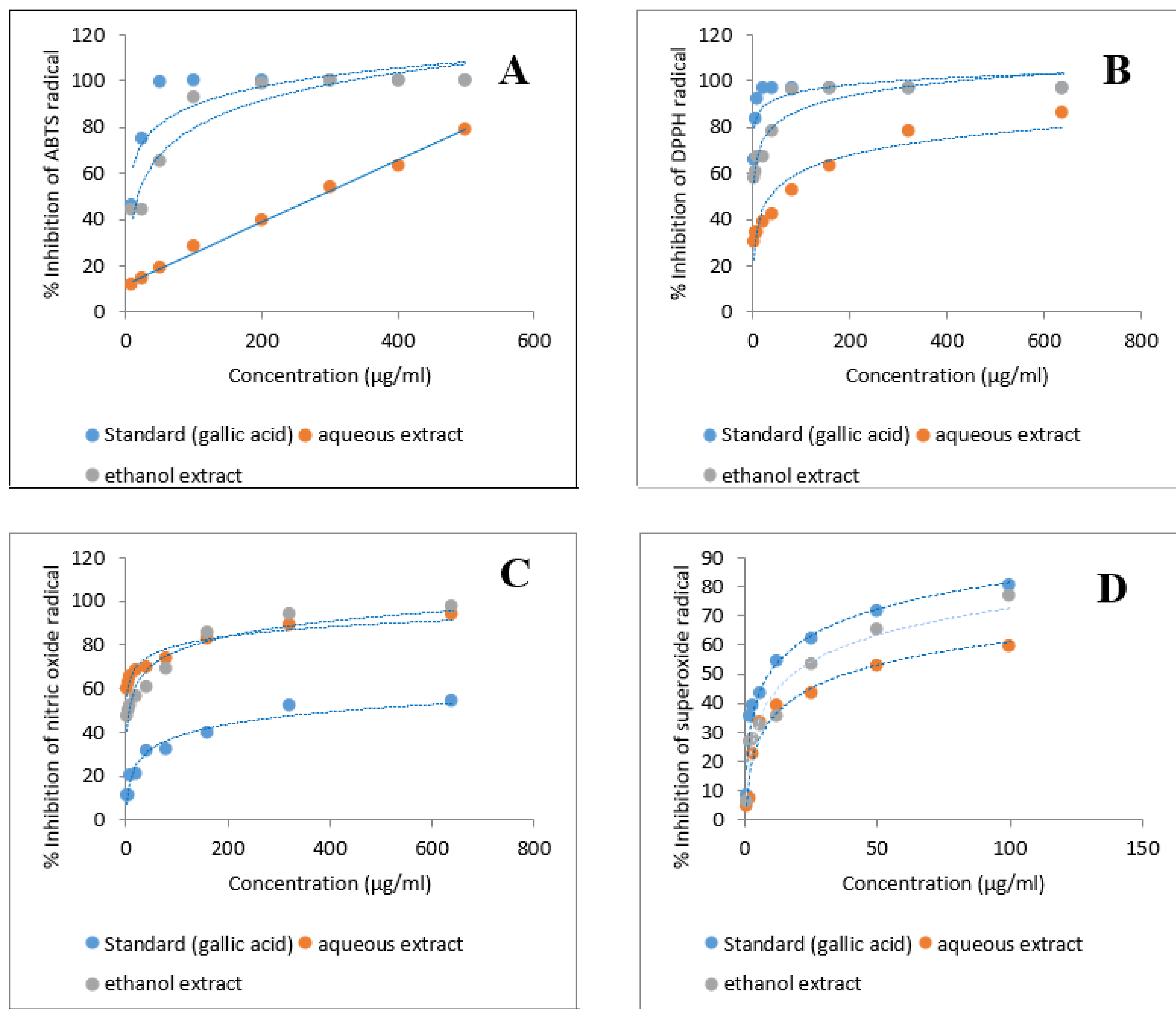

Figure 1: 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) [A], 2, 2-diphenyl-1-picrylhydrazyl (DPPH) [B], nitric oxide [C], and superoxide [D] radical scavenging activities of different concentrations of standard gallic acid, and aqueous and ethanol extracts of *Irvingia gabonensis* stem bark. All assays were done in triplicates.

lower concentrations, the percentage inhibition increased as the concentration increased but decreased drastically from 200 µg/mL to 1000 µg/mL. Ascorbic acid and gallic acid were employed as benchmarks. The results showed that the ethanol extract performed better than the aqueous extract.

The FRAP assay estimates the ability of extracts to act as antioxidants capable of reducing ferric ions to ferrous ions. It was observed the absorbance increased with increasing concentrations of extracts (Figure 2C). However, the ethanol extract competes favourably with the standard (ascorbic acid).

DISCUSSION

It is widely known that disruptions in oxidative status contribute to the emergence and progression of a number of diseases.[2,37] Increased production of free radicals can overwhelm endogenous antioxidant mechanisms and cause damage to cells. Studies have shown that endogenous and exogenous antioxidants can quench radicals by either transferring a hydrogen ions or donating electrons to the oxidants.[38] The antioxidant contents of a medicinal plant have been postulated to account for the ability of such plants to ameliorate several disease conditions.[39-41]

This investigation has shown that large amounts of phytochemicals are present in both the water and ethanol extracts of *I. gabonensis* stem bark namely: phenols, flavonoids, tannins, alkaloids,

Table 2: The IC_{50} values (µg/mL) of aqueous and ethanol extracts of *I. gabonensis* stem bark compared with the respective standards.

Antioxidant Activity	Ascorbic acid (µg/mL)	Gallic acid (µg/mL)	BHA (µg/mL)	Aqueous extract (µg/mL)	Ethanol extract (µg/mL)
$ABTS^+$	-	3.36	-	283.19	17.58
DPPH	-	0.002	-	35.37	1.17
Nitric oxide	-	427.09	-	0.83	6.42
Superoxide	-	9.21	-	38.24	18.17
β-carotene	-	-	0.025	195.49	90.20
H_2O_2	<12.5	1641.01	-	202.27	7.11

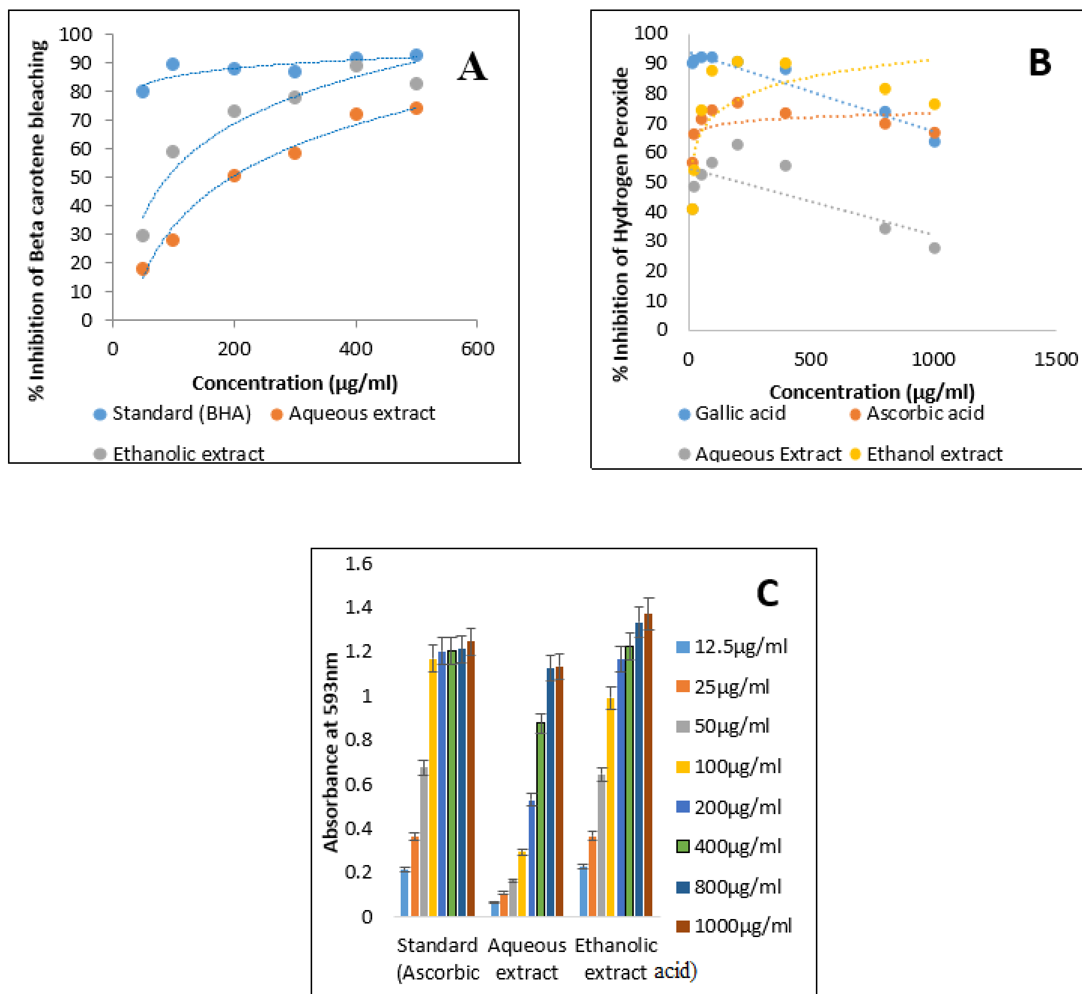

Figure 2: β-carotene bleaching inhibition [A], hydrogen peroxide inhibition [B], and ferric reducing antioxidant potential (FRAP) [C] of respective standards, aqueous, and ethanol extracts of *Irvingia gabonensis* stem bark. All assays were done in triplicates.

steroids, and saponins (Table 1). In this study, the total phenolics, flavonoids, and tannins found in both water and alcohol extracts of *I. gabonensis* stem bark were far higher than the total phenolics (1.15 mgGAE/g), flavonoids (0.77 mgQE/g) and tannins (1.25mgCE/g) reported for *I. gabonensis* kernels.[42,43] This shows the stem bark has a higher antioxidant content than the kernels. In addition, it was found that the ethanol extract of *I. gabonensis* stem bark contained more phenolic (253.39 mgGAE/g) and flavonoid contents (606.44 mgQE/g) than *Moringa oleifera* leaves which have phenolic content (12.33mgGAE/g) and flavonoid content (4.826 mgQE/g) as reported by Mwamatope *et al.*[44] Other prior studies have revealed that these phytochemicals have powerful antioxidant, anti-diabetic, anti-microbial,

anti-hypercholesterolemic, anti-inflammatory, and anti-tumour activities.[45-48]

This current study has revealed that the ethanol extract had greater total antioxidant power (488.78 mgGAE/g) when compared to the aqueous extract (319.89 mgGAE/g) and the difference was nearly statistically significant (Table 1). This study also found out that the high antioxidant power of the ethanol extract might have been influenced by the total phenolic and flavonoid compounds which have been well-established as antioxidants that inhibit oxidants such as superoxide anion, lipid peroxyl, and hydroxyl radicals.[49-52] This shows that *I. gabonensis* stem bark is rich in antioxidants and can be explored for its ability to eliminate free radicals.

The ethanol extract showed a greater antioxidant inhibitory effect against the ABTS radical in this investigation compared to the water extract (Table 2). This study also showed the extracts were able to eradicate the DPPH radical by giving out hydrogen ions or electrons to the free radical as seen in the transformation from a purple DPPH radical to a yellow reduced DPPH. The higher the concentration of the extract, the more powerful the ability of the extract to give out a hydrogen atom to the radical (Figure 1B). The IC_{50} value of the ethanol extract was lower (1.17 µg/mL) than the aqueous extract (35.37 µg/mL); implying a superior ability of the ethanol extract to eradicate free radicals and so defend against oxidative stress.

The extracts were discovered to be far more effective than the reference standard (gallic acid) in inhibiting nitric oxide radicals (Figure 1C and Table 2). Nitric oxide is very important in both cell signaling and oxidative/nitrosative stress and can create the extremely reactive peroxynitrite anion ($ONOO^-$) when reacted with superoxide radical.[37,53,54] Furthermore, nitric oxide generation has been linked to an increase in the production of proinflammatory mediators including cytokines and reactive oxygen species, which can worsen inflammatory diseases like ulcerative colitis, diabetes, multiple sclerosis, and arthritis.[55,56] As such, the stronger potential of *I. gabonensis* extracts to inhibit the production of nitric oxide radicals could have positive implications for the ability of this plant to ameliorate oxidative stress and inflammation in diseases.

In addition, the ethanol extract of *I. gabonensis* had higher percentage inhibition against superoxide radical compared to the aqueous extract (Figure 1D). Excess superoxide radicals can be transformed into more reactive and damaging hydroxyl radicals in the Haber-Weiss reaction.[57] The ability of the ethanol extract of *I. gabonensis* stem bark to have higher inhibitory activity against the superoxide ion implies that the extract has more effective scavenging power against superoxide radicals which may lead to better protection against oxidative damage.

The findings of this investigation also demonstrated that the inhibition efficiency of ABTS+, DPPH, nitric oxide, and superoxide radicals increased as the extracts' amount increased. This supports the finding by Adebiyi *et al.*[58] that extract concentration affects the extracts' capacity to transfer electrons and hydrogen ions to oxidants.

With an IC_{50} value of 90.20 g/mL compared to the aqueous extract's 195.49 g/mL, the β-carotene bleaching assay result showed that the ethanol extract had better inhibitory efficacy. The extracts were found to be less effective than the standard butylated hydroxyanisole (BHA) which had an IC_{50} value of 0.025µg/mL. This corroborates the report of Farooq *et al.*,[9] that BHA has high inhibitory potential against the bleaching of β-carotene which may not be matched by many extracts; however, the ethanol extract of *I. gabonensis* showed a relatively good ability to inhibit β-carotene bleaching.

In this study, we used a recent method[35] to test the inhibitory capacity of aqueous and ethanol extracts of *I. gabonensis* against H_2O_2. The results showed that the reference standard (ascorbic acid) and the ethanol extract had strong inhibitory activities (<12.5µg/mL) against H_2O_2 compared to the aqueous extract and gallic acid (another standard) with IC_{50} values of 202.27 µg/mL and 1641.01 µg/mL respectively (Table 2). As a metabolic byproduct, hydrogen peroxide is not particularly reactive on its own but can produce an extremely reactive hydroxyl radical ($OH^{\cdot}$) when it accumulates in the presence of metal ions, a process called Fenton's reaction.[59] Likewise, hydroxyl radicals can be produced when hydrogen peroxide accumulates in the presence of superoxide radicals, a process referred to as the Haber-Weiss reaction.[57] These hydroxyl radicals are responsible for lipid oxidation and oxidative damage in cells. The high inhibitory effects of these extracts (especially the ethanol extract) against H_2O_2 could therefore provide protection from reactive oxygen species' harmful effects.

The results for FRAP demonstrated that the ethanol extract competes favourably with the reference standard (ascorbic acid) and showed higher reducing power as the concentration increases when compared to that of the aqueous extract. This clearly demonstrates the extracts' ability to act as antioxidants by donating electrons to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). This study agrees with previous studies that when there is an increase in absorbance, the reducing capability of the plant extracts also increases.[58,60,61]

CONCLUSION

I. gabonensis stem bark extracts' ability to function as superior exogenous scavengers of both hydrophilic and lipophilic oxidant systems is demonstrated more comprehensively by the use of multiple tests to determine the *in vitro* antioxidant capacity of these extracts. Both the aqueous and ethanol extracts of *I. gabonensis* stem bark contain numerous natural compounds with a variety of biological effects. The ethanol extract from the stem bark of *I. gabonensis* exhibited higher phytochemical contents and

also had significant *in vitro* antioxidant potential (DPPH, nitric oxide, superoxide, and hydrogen peroxide scavenging assays), often comparable or superior to that of standard antioxidants. The results of the quantitative phytochemical analysis and *in vitro* antioxidant effects of aqueous and ethanol extracts of *I. gabonensis* stem bark clearly show their potential as excellent reservoirs of bioactive compounds and scavengers of deleterious oxidants; properties that could be explored therapeutically.

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CONFLICT OF INTEREST

The authors declare there are no conflicts of interest.

ABBREVIATIONS

ABTS: 2,2-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid); **BCG:** Bromocresol green; **BHA:** Butylated hydroxyanisol; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **FCR:** Folin-Ciocalteu reagent; **FRAP:** Ferric-reducing potential; **mgAE/g:** Milligrams of atropine equivalent per gram of extract; **mgDE/g:** Milligram of diosgenin equivalent per gram of extract; **mgGAE/g:** Milligrams of gallic acid equivalent per gram of extract; **mgQE/g:** Milligrams of quercetin equivalent per gram of extract; **NADH:** Nicotinamide adenine dinucleotide; **NBT:** Nitroblue tetrazolium; **($(NH_4)2MoO_4$):** Ammonium molybdate; **NO:** Nitric oxide; **PMS:** Phenazine methosulfate; **SEM: S**tandard error of mean; **TAP:** Total antioxidant power; **TPTZ:** Tripyridyltriazine.

SUMMARY

Ethanol and aqueous extracts of *Irvingia gabonensis* stem bark contained high amounts of several phytochemicals. The phytochemical content was higher for the ethanol extract. The antioxidant capacity of the ethanol extract surpassed the aqueous extract. The ethanol extract scavenged NO•, O_2•, DPPH., and H_2O_2 better than the standard antioxidants. Both extracts were good scavengers of hydrophilic and lipophilic oxidant systems

ETHICAL APPROVAL

The Ethics Committee, Faculty of Pharmacy, University of Benin, Nigeria provided clearance for this study with the reference number EC/FP/019/19.

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