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Article

Antioxidant and Phytochemical Profile of Crude Ethanol Extract and Fractions of *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill Seed

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Abstract

The study investigated the phytochemical constituents and antioxidant capacity of *Irvingia gabonensis* seeds, a plant known as African mango, renowned for its rich phytochemical content and potential health benefits. Phytochemicals, bioactive compounds in plants with therapeutic properties, and antioxidants, vital for combating oxidative stress caused by free radicals, were the focus of assessment. Standard phytochemistry procedures and antioxidant assays were employed for this purpose. The study aimed to determine the phytochemical constituents present in the seeds of *Irvingia gabonensis*; and evaluate the antioxidant capacity and free radical scavenging activity of the crude ethanol extract and its ethyl acetate fraction of *Irvingia gabonensis* seeds. Seeds of *Irvingia gabonensis* were processed to obtain a crude ethanol extract and an ethyl acetate fraction. Standard phytochemistry procedures identified Saponins, Tannins, Flavonoids, Glycosides, Anthraquinones, Steroids, Terpenoids, Alkaloids, and Phenols. Antioxidant potential was assessed through various assays, including DPPH, TPC, FRAP, and Total antioxidant capacity. Results revealed the presence of significant amounts of various active phytochemicals, including Saponins, Tannins, Flavonoids, Glycosides, Anthraquinones, Steroids, Terpenoids, Alkaloids, and Phenols. These compounds contribute to its medicinal properties. Additionally, the research findings indicated that *Irvingia gabonensis* exhibited considerable antioxidant capacity, as evidenced by the results of the antioxidant assays. This study demonstrated that *Irvingia gabonensis* seeds are a rich source of diverse phytochemicals and possess notable antioxidant capacity. The presence of these active compounds suggests the potential health benefits of *Irvingia gabonensis* and its possible use in natural antioxidant-based therapies..

Keywords: *Irvingia gabonensis*; vacuum liquid chromatography; total antioxidant capacity; total phenolic content; antioxidant; total phenolic content; free radical scavenger; phytochemicals.

1. Introduction

The use of plants for medicinal purposes is still very much adopted especially in the developing worlds. Many of such plants have been acclaimed to treat various diseases through unknown mechanisms. One major plant of such interest is *Irvingia gabonensis*. It is a species of African Trees, native to West Africa [1]. It belongs to the genus Irvingia and commonly referred to as Dika, "Wild Mango" or "Bush Mango". In Nigeria, the Igbos call it "Ogbonno" [2], "Goronor" by the Hausas while the Yorubas call it "Òòro" or "Àpòn". Dika is usually a large tree with a dense evergreen crown and large buttresses (Figure 1). The leaves are simple and arranged asymmetrically, they are glossy on the upper surface. The flowers are bisexual, yellow to pale greenish in colour and are borne in small clusters. The fruit is

a drupe, large, edible with thick fibrous flesh. The fruit is almost spherical, green when ripe with a bright orange pulp. The endocarp is woody and contains a single seed only [1].

Figure 1*:* *Irvingia gabonensis* Plant. *Picture taken at Ilora, Afijio Local Government, Oyo State, Nigeria (7°48´49.8´´N3°53´16.4´´E)*

The use of the mesocarp in traditional medicine has been documented for the treatment of gastrointestinal or hepatic disorders, diarrhea, infections, and as a purgative [3]. It is used as a soup (ogbono soup) and stew thickener, flavouring agent when roasted and as a spread on yam/plantain dishes when ground and fermented [4]. The stem bark is used to treat hunch back, infections and used as analgesic, antibacterial and antifungal activities gonorrhea, hepatic and gastrointestinal disorders. Furthermore, the leaf is widely used in traditional medicine for the treatment of several illnesses [5].

Many ailments and diseases have been closely linked with oxidative stress midwifed by reactive oxygen species generated in the body. ROS cause tissue damage and may result in systemic breakdown and ultimately cause diseases [6-8]. Owing to the facts that IG is widely consumed and its seed forms a major recipe for food and medicine and believed to alleviate many diseased conditions, it is of utmost importance to investigate the phytochemicals present and the antioxidant efficacy of its extracts, hence this study.

2. Methods

2.1 Plant collection, authentication, extraction and fractionation

Fresh fruits and leaves of *I. gabonensis* were plucked at Ilora, Afijio Local Government, Oyo State, Nigeria and identified by Mr. Ademoriyo, a taxonomist in the Department of Botany, Obafemi Awolowo University, Ile-Ife where a voucher number (IFE-17976) was obtained, and specimens deposited in the herbarium for reference. The skin of the fresh fruits was peeled off and the edible part of the fruits was removed to expose the seed. The endocarp of the seed was broken. The seeds were

dried at room temperature and ground using an electric blender. 3000 g (3 Kg) of the dried blended seeds was transferred into a glass container where 10 L of pure 100% ethanol was added, stirred at 2 hrs interval using an electric shaker to allow the extraction process to take place. This was done for 72 hrs. The solvent (now containing the extract) was collected using muslin bag and the filtrate was further filtered using Whatman filter paper 1 mm. Filtrate was concentrated with the aid of rotary evaporator (Heidolph Laborota 400 Efficient, made in Germany, Model 517-01002-002) set at 40°C. Concentrate was further concentrated using a vacuum oven set at 40°C with a pressure of 700 mmHg. Percentage yield was calculated using the following formula:

$$\%\ Yield = \frac{EEIG\ (g)}{Powdered\ IG\ (g)} \times 100$$

Three fractions were isolated using three different solvents based on ascending order of polarity (n-hexane, ethyl acetate and ethanol). 91 g of the concentrated crude ethanol extract was poured into a clean beaker of 500 ml capacity, 100 ml ethanol was added, made into solution and pre-absorbed with 400 g of silica gel. The dried pre-absorbed extract was packed into a vacuum liquid chromatography (VLC) set up which has been initially packed with 200 g of silica gel in the presence of vacuum. n-Hexane, ethyl acetate and ethanol were respectively added after a clear solution of the preceding solvent was gotten. The respective fractions were concentrated with rotary evaporator at 40°C and a vacuum oven at 40°C with a pressure of 700 mmHg. They were then stored in a refrigerator.

2.2. Phytochemical screening

Qualitative photochemical analysis followed the procedures of Sofowora [9] while the method described by Krishnaiah *et al.* [10] was used for quantitative phytochemistry.

2.3 Total phenolic content

Total phenolic compound contents were determined by the Folin-Ciocalteau method. Different dilutions were mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na_2CO_3 (4 ml, 1 M) were then added. The mixture was allowed to stand for 15 min and the phenols were determined by colorimetric method at 765 nm. The standard curve was prepared in different concentration in ug/ ml solutions of Gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of Gallic acid equivalent (ug/ g of dry mass), which is a common reference compound.

2.4 Antioxidant Assay

2.4.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity

In order to evaluate the antioxidant potential of the extracts through free radical scavenging, the change in optical density of DPPH radicals was monitored according to the method of [11]. The different fractions were prepared with methanol into five different concentrations ranging from 200ug/ml-1000ug/ml. 1ml of the different concentrations were transferred into test tube and 1mL of DPPH solution (0.3 mM) was added. After 30 min, the absorbance was measured at 517 nm. The percentage of the DPPH radical scavenging was calculated using the equation as given below:

$$\%\ \text{Inhibition of DPPH radical} = \frac{\text{Abr} - \text{Aar}}{\text{Abr}} X\ 100$$

Where Abr is the absorbance before reaction (blank) and Aar is the absorbance after reaction has taken place.

2.4.2 Ferric ion reducing antioxidant power assay (FRAP)

Ferric ions reducing power was measured according to the method of Oyaizu [12] modified by Rohan and Anup [13]. The different fractions in different concentrations were mixed with 2.5ml of 20 mM

phosphate buffer and 2.5 ml 1%, w/v potassium ferricyanide, and then the mixture was incubated at 50 °C for 30min. Afterwards, 2.5 ml of 10%, w/v trichloroacetic acid and 0.5ml 0.1%, w/v ferric chloride were added to the mixture, which was kept aside for 10 min. Finally, the absorbance was measured at 700 nm. Ascorbic acid was used as positive reference standard. All assays were run in triplicate and averaged.

2.4.3 Total antioxidant determination

Total antioxidant activity was estimated by phosphomolybdenum assay. 1ml each of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate were added in 20 ml of distilled water and made-up volume to 50 ml by adding distilled water. Sample in different concentrations ranging from 200 ug/ml to 1000 ug/ml were added to each test tube individually containing 3 ml of distilled water and 1 ml of Molybdate reagent solution. These tubes were kept incubated at 95°C for 90 min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695 nm. Mean values from three independent samples were calculated for each extract. Ascorbic acid was used as positive reference standard.

3. Results and Discussion

This study aimed to determine the phytochemical constituents and antioxidant capacity of *Irvingia gabonensis* (IG) seed. The results indicated that crude ethanol extract of the seed is rich in various phytochemicals including saponins, tannins, flavonoids, glycosides, anthraquinones, steroids, terpenoids, alkaloids, and phenol (Table 1). The crude ethanol extract of I. gabonnensis was subjected to fractionation using N-hexnae, ethyl acetate and ethanol respectively. Figure 2 shows the percentage yield of different fractions from crude ethanol extract of *I. gabonenesis*.

Table 1: Phytochemical contents of ethanol extract and fractions of *I. Gabonensis*.

Parameters	n-Hexane fraction	Ethyl acetae fraction	Ethanol fraction	Crude extract
Saponins	-ve	+ve	++ve	++ve
Tannins	-ve	+ve	++ve	++ve
Flavonoids	-ve	++ve	+ve	++ve
Glycosides	-ve	-ve	++ve	+ve
Anthraquinones	+ve	++ve	-ve	+ve
Steroids	+ve	++ve	-ve	+ve
Terpenoids	+ve	++ve	+ve	++ve
Alkaloids	-ve	+ve	+ve	+ve
Phenol	-ve	+ve	+ve	+ve

(+) = Weak positive test. (++) = Strong Positive test -ve = Absent

The presence of various phytochemicals in IG seeds indicates its potential use as a therapeutic agent. Saponins, for instance, are known to possess anti-inflammatory, hypocholesterolemic, and anti-tumor properties [14]. Tannins have antidiabetic, anti-inflammatory, and antioxidant properties [15]. Flavonoids have potent antioxidant activity and have been reported to possess antiviral, anti-inflammatory, and anticancer properties. Glycosides have been found to have antifungal and antitumor activity. Anthraquinones possess antibacterial and antifungal activity and are used in the treatment of gastrointestinal disorders. Steroids and terpenoids have been reported to possess anticancer and anti-inflammatory properties [16]. Alkaloids have been found to possess analgesic, anti-inflammatory, and anti-tumor properties.

Phenolic compounds possess potent antioxidant activity and are used in the treatment of several diseases including cancer, diabetes, and cardiovascular disorders and also play a vital role in balancing the excessive production of free radicals or ROS over oxidative stress mechanisms of

enhancing redox homeostasis for protection against oxidative stress [17]. Phytochemicals have been reported to mediate the medicinal activity of various plant extracts [18]. Tannins have been reported to possess free-radical scavenging activity, antimicrobial, antiviral and anti-inflammatory properties [19].

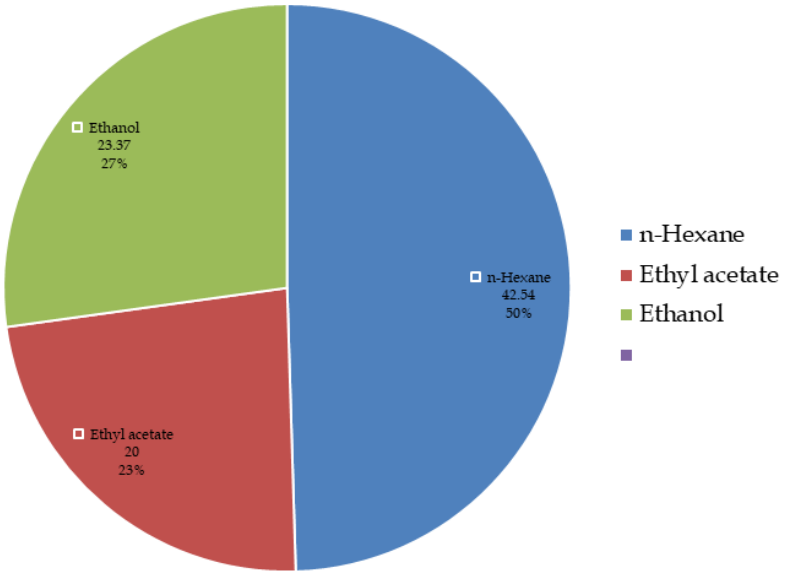

Figure 2: Phytochemical composition of different fractions of *Irvingia gabonensis. crude (ethanol) extract of IG, upon subjection to fractionation, yielded 50% (42.5g) of n-hexane fraction, 27% (23.4g) ethanol fraction and 23% (20.0g) of ethyl acetate fraction*

Saponins are common in most plants and have been postulated to have a wide range of biological activity including antioxidant, anticarcinogenic as well as having immunostimulant properties thereby exhibiting the potential to cure a number of diseases [120.

Furthermore, phytochemicals such as flavonoids and tannins possess potent antioxidant and anti-inflammatory properties, making them useful in medicinal and pharmaceutical applications. For instance, flavonoids have been reported to possess antidiabetic, anticancer, and neuroprotective properties. Some flavonoids have been shown to inhibit the growth of cancer cells and reduce the risk of cardiovascular diseases. Tannins, on the other hand, have been reported to exhibit antimicrobial, antiviral, and antiparasitic activities. Some tannins, have been shown to possess anticancer and antidiabetic properties [21].

The study also revealed that the crude ethanol extract and ethyl acetate fraction of IG possess significant antioxidant capacity (Figure 3). The antioxidant activity of the plant material may be attributed to the presence of various phytochemicals such as flavonoids, phenolics, and tannins, which possess potent antioxidant activity [22]. The antioxidant potential of the plant material is important in the prevention and treatment of oxidative stress-related diseases such as cancer, cardiovascular disorders, and diabetes. The findings of the study are in agreement with previous studies that have reported the presence of various phytochemicals and antioxidant activity in IG seeds [23- 25]. The study, however, extends the previous findings by fractionating the crude ethanol extract of IG seeds and evaluating the antioxidant activity of the fractions. The study also provides important information on the phytochemical constituents of IG seeds, which may be useful in the development of new drugs and formulations.

Ferric ion reducing capacity (FRAP) and scavenging capacity are important parameters for evaluating the antioxidant potential of plant extracts. Ferric ion reducing capacity is the ability of a substance to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), which are less reactive and can chelate with free radicals. This study shows that all the fractions of IG have significantly high reducing power as evidenced by high absorbances (Fig. 4). Higher absorbance indicates higher reducing potency [26].

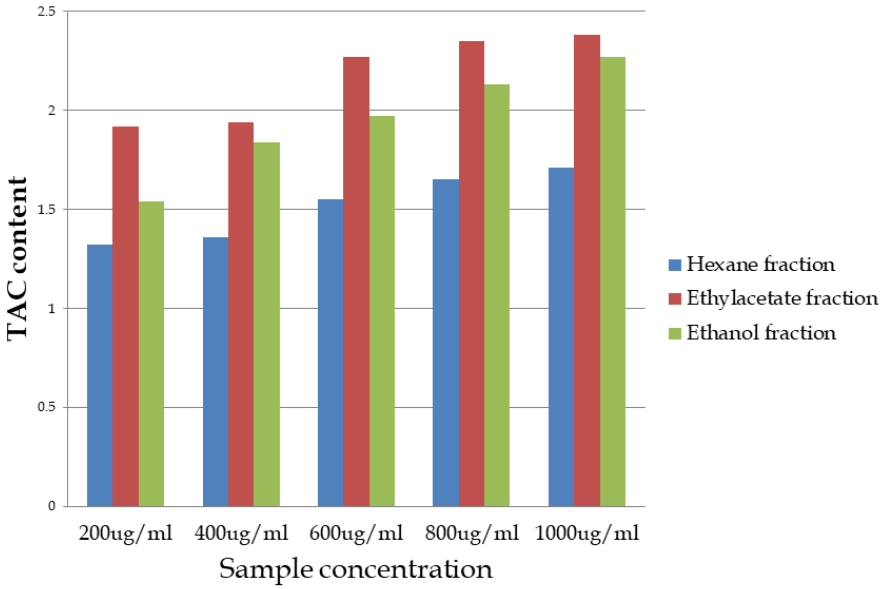

Figure 3: Total Antioxidant Capacity of different fraction of *Irvingia gabonensis. the total antioxidant capacity of ethyl acetate fraction is the highest among the three fractions, then, closely followed by ethanol fraction and then, n-hexane fraction at 200 to 1000 µg/ml concentrations.*

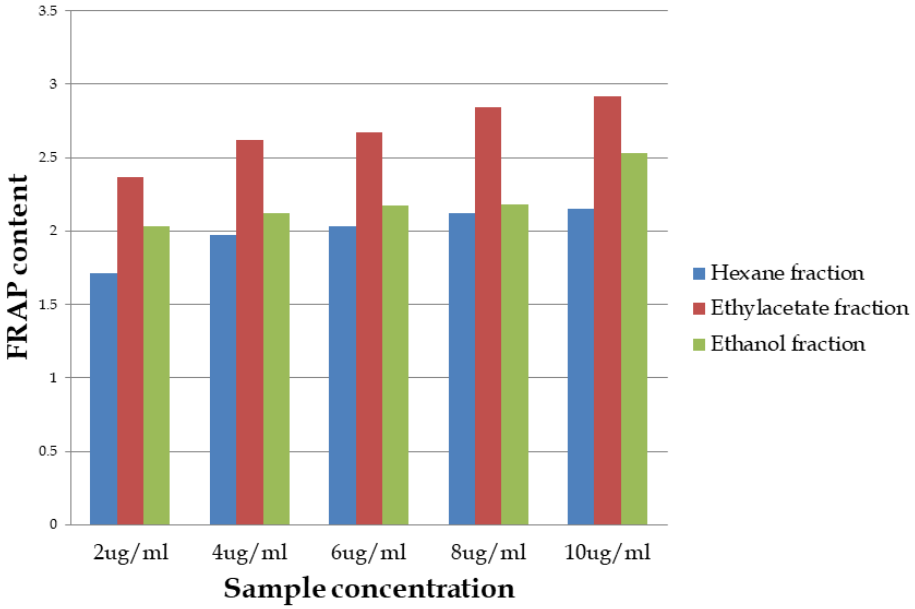

Figure 4: FRAP of different fraction of *Irvingia gabonensis. The ferric ion reducing power of ethyl acetate fraction was higher compered to ethanol and n-hexane fractions at 200 to 1000 µg/ml concentrations.*

In this system, electron from oxidized antioxidant transfers to the substrate by inhibiting oxidation of oxidant [27]. This study shows that IG has potent reducing capacity and reduction capacity of free oxidative metallic ions. This renders oxidant less potent and reduces or completely eliminates stress incurred by the oxidant [28]. FRAP assay is one of the methods involving the mechanism of single electron transfer system (SET). The reducing capacity of any phytochemical depends largely on the phenolic contents [27]. This study shows that the fractions of IG have appreciable phenol contents (Figure 5). Phenols are effective free radical scavengers. Nixwell and co had previously alluded the presence of phenol in extract of IG and the free radical scavenging capacity [28].

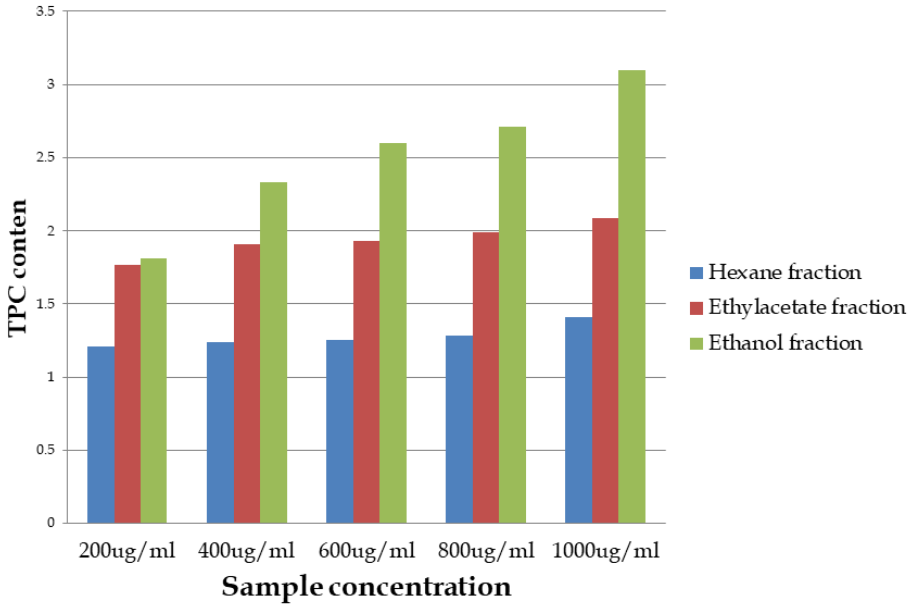

Figure 5: Total Phenolic Content (TPC) of different fraction of *Irvingia gabonensis. The ethanol fraction has the highest total phenols compared to the n-hexane fraction closely followed by ethyl acetate fraction at 200 to 1000 µg/ml concentrations.*

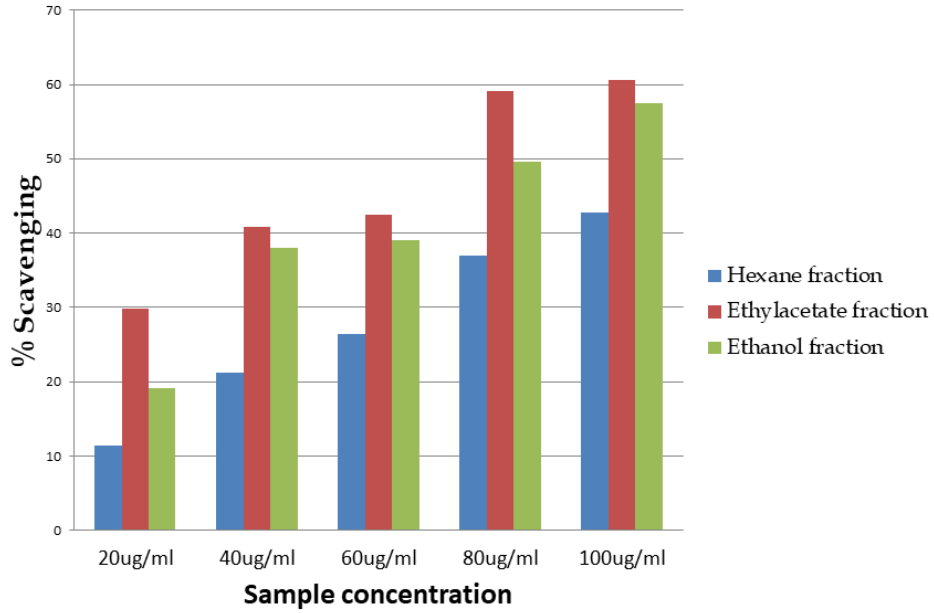

Figure 6: DPPH scavenging activity of different fraction of *Irvingia gabonensis. Ethyl acetate fraction showed higher scavenging activity compared with ethanol and n-hexane fractions at 200 to 1000 µg/ml concentrations.*

Phytochemicals such as phenolic compounds, flavonoids, and terpenoids have been shown to have strong ferric ion reducing capacity and scavenging capacity. For example, flavonoids are known to scavenge reactive oxygen species (ROS) and nitrogen species, chelate metal ions, and inhibit lipid peroxidation. These antioxidants play a role in donating electrons to oxidants and forming compounds that could not oxidize other compounds [29]. Phenolic compounds also have strong antioxidant potential, with the ability to scavenge free radicals and prevent oxidative damage to biomolecules. Terpenoids also have antioxidant properties and can protect against oxidative stress.

Scavenging capacity refers to the ability of a substance to neutralize free radicals by donating an electron and thereby terminating the chain reaction of oxidative stress. The free radical scavenging

ability of IG was assessed using 2,2-diphenylpicrylhydrazyl (DPPH) assay which is widely used in plant biochemistry to evaluate the properties of plant constituents for scavenging free radicals. This study showed that extracts of IG exhibit remarkable free radical scavenging capacity (Figure 6).

DPPH and total antioxidant capacity (TAC) are widely used as parameters to characterize different substances and food samples with the ability of scavenging or neutralizing free radicals. This capacity is related to the presence of compounds capable of protecting the biological system against harmful oxidation [30, 31]. The medicinal and pharmaceutical potential of plants rich in phytochemicals with strong antioxidant capacity is vast. These compounds have been shown to have anti-inflammatory, anticancer, antimicrobial, and cardioprotective properties. For example, flavonoids such as quercetin and catechins have been shown to have anti-inflammatory effects, while phenolic compounds have been shown to have anticancer properties. Terpenoids protective against cardiovascular diseases and diabetes [31].

5. Conclusions

In conclusion, the study demonstrated that extract of *Irvingia gabonensis* seed (especially its ethyl acetate fraction) is rich in various phytochemicals and possess significant antioxidant capacity. The findings of the study suggest that IG seed has the potential to be used as a therapeutic agent in the treatment of various diseases. The study provides a basis for further investigation into the therapeutic potential of IG seeds and its bioactive compounds.

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Conflict of Interest
The authors declared no conflict of interest in the manuscript.

Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them

Author Contributions
Concept – O.P.A.; Design – O.S.A., O.P.A.; Supervision – O.S.A; Resources – O.P.A, A.O.A.; Materials – O.P.A.; Data Collection and/or Processing – O.P.A, O.S.A, A.O.A; Analysis and/or Interpretation – O.P.A, O.S.A, A.O.A; Literature Search – O.P.A; Writing – O.P.A, O.S.A, A.O.A; Critical Reviews – O.S.A, A.O.A

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