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Research Article

Phytochemicals, Antioxidant Activity and Acute Toxicity of Ethanol Stem Bark Extract of Irvingia gabonensis O'Rorke Baill

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Abstract

Irvingia gabonensis O'Rorke Baill (IG) stem bark has been utilized as medicine by herbal medicine practitioners and some locals. This study was therefore conducted to investigate the phytochemical composition, *in vitro* antioxidant activity and acute toxicity of *Irvingia gabonensis* O'Rorke Baill (IG) ethanol stem bark extract. Standard procedures were used for phytochemical screening, while ferric reducing power, as well as 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, nitric oxide ($NO^{\cdot}$) and hydrogen peroxide (H_2O_2) scavenging activities were used to assess the antioxidant properties of the extract at concentrations of 10, 20, 40, 80 and 160 µg/ml. Acute toxicity of the extract was determined by Lorke's method. The results obtained revealed the presence of flavonoids, alkaloids, saponins, tannins, anthraquinones, carbohydrates and cardiac glycosides in the stem bark extract. IG ethanol stem bark extract exhibited potent DPPH, $NO^{\cdot}$ and H_2O_2 scavenging activities, and reducing power in concentration-dependent manner when compared with ascorbic acid standard. Moreover, the LD_{50} of the ethanolic stem bark extract was above 5000 mg/kg body weight. The results indicate that ethanol stem bark extract of *Irvingia gabonensis* O'Rorke Baill contains vital phytochemicals and possesses high antioxidant activity. The stem bark extract may also be said to be relatively non-toxic and safe up to a dose of 5000 mg/kg body weight in Wistar albino rats.

Keywords: *Irvingia gabonensis O'Rorke Baill; Phytochemical; Antioxidant; Acute toxicity; Free Radical*

Abbreviations

DPPH: 1,1-Diphenyl-2-Picrylhydrazyl; NO.: Nitric Oxide; H_2O_2: Hydrogen Peroxide; IG: *Irvingia gabonensis*; ROS: Reactive Oxygen Species; RNS: Reactive Nitrogen Species; mg/kg b.w.: Milligram per Kilogram Body Weight; SD: Standard Deviation

Introduction

Several plants have been in utilized by herbal medicine practitioners and most locals as medicines owing to their acclaimed medicinal properties. One of such plants is *Irvingia gabonensis* O' Rorke Baill. *Irvingia gabonensis*, O'Rorke Baill of the Irvingiaceae family, is a tropical forest tree commonly found in Southern and Eastern Nigeria, Sierra Leone and Equatorial Africa. It has an edible, sweet fruit pulp with a turpentine-like flavor [1]. In Cameroon, where the plant is locally called "kaka", the stem bark is used to treat hunch back and infections [2]. The analgesic, antibacterial and antifungal activities of the stem bark of this plant have also been reported [3,4]. It is also known that the seed extract and the methanol stem bark extract have antioxidant activities [5,6]. In Senegal, the decoction of the stem bark is used in the treatment of gonorrhea, hepatic and gastrointestinal disorders [6]. Furthermore, the leaf is widely used in traditional medicine for the treatment of several illnesses [7].

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are an integral part of normal physiology [8]. For example, superoxide anion, hydrogen peroxide and hydroxyl radical are natural by-products of body metabolism [9]. However, excess free radicals can attack biological molecules such as proteins, lipids, enzymes, DNA and RNA, leading to cell or tissue injury associated with degenerative diseases [9]. Epidemiological studies have shown that many phytonutrients of fruits and vegetables might protect the human body against damage by ROS. The consumption of natural antioxidant phytochemicals was reported to have potential health benefits [10-12].

It is also very pertinent that in the selection of herbal medicines for health use, the safety of such herbs must be ascertained. The screening of plant extracts for their activities against diseases should be combined with evaluation of the toxicities of such plant extracts with traditionally acclaimed therapeutic properties. In view of the fact that the etiologies of many diseases involve oxidative stress, the present study was conducted to determine the phytochemical composition, *in vitro* antioxidant activity and acute toxicity of ethanol stem bark extract of *Irvingia gabonensis* O'Rorke Baill. The acclaimed medicinal properties of the stem bark, medicinal values of phytochemicals and the relevance of drug safety informed the selection of this plant for the current study.

Materials and Methods

Collection, Authentication and Preparation of Plant Extract

Fresh and mature stem bark of *Irvingia gabonensis* O'Rorke Baill were harvested from Itak Ikot Akap village in Ikono local government area of Akwa Ibom State, Nigeria. The samples were identified and authenticated by Mr. Daniel Etefia of the Department of Pharmacognosy and Herbal Medicine, University of Uyo, Akwa Ibom State, Nigeria. They were washed and then pulverized using mortar and pestle (due to their hardness) and then air-dried at room temperature for about seven days in the Biochemistry laboratory of University of Uyo. The pulverized stem bark (2000g) was macerated in absolute ethanol (JHD, China) and allowed to stay for 72h with intermittent stirring to ensure proper extraction. The sample was sieved through muslin cloth and the filtrate was concentrated in a stainless steel bowl using a water bath (Preciseterm) at 45°C. The paste-like gel extract obtained after continuous concentration was then transferred into preweighed transparent containers, weighed and stored in the refrigerator prior to use.

Phytochemical Screening

Qualitative phytochemical screening was carried out on IG ethanol stem bark extract. The presence of saponins, alkaloids, cardiac glycosides and anthraquinones, were determined according to the method of Sofowora [13]. Determination of flavonoids and terpenoids were done according to the method of Trease and Evans [14], while the method of Antherden [15] was employed in the determination of tannins, Molisch test was used for detection of carbohydrates.

In vitro Antioxidant Assays

1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay

1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of the extract was investigated as described by Gyamfi [16]. About 450 µl of 50 mmol/l Tris-HCl buffer (pH 7.4) was mixed with solutions of the extract and ascorbic acid (standard) at different concentrations (10, 20, 40, 80 and 160 µg/ml), followed by addition of 10 ml of 0.1 mmol/L DPPH-methanol solution. The mixture was swirled and kept in the dark for 30 min incubation time. Absorbance was measured at 517 nm using a mixture of distilled water, buffer and methanol as blank.

The DPPH radical scavenging activity was calculated as follows:

$$\% \text{ DPPH inhibition} = \frac{\text{Absorbance of control-Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

Nitro oxide (NO) Radical Scavenging Assay

Nitric oxide radical scavenging activity was investigated as described by Green [17]. About 3.0 ml of 10 mM sodium nitroprusside in phosphate buffer was added to 2.0 ml of the stem bark extract and ascorbic acid (standard) at different concentrations (10, 20, 40, 80, and 160 µg/ml). The resulting solutions were incubated at 25^0C for 60 minutes and 5 ml of Griess reagent was added. The absorbance of the chromophore formed was measured at 540 nm.

The percentage of nitric oxide scavenged was calculated using the formula:

$$\% \text{ NO inhibition} = \frac{\text{Absorbance of control-Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

Hydrogen Peroxide (H_2O_2) Scavenging Assay

The H_2O_2 scavenging activity was estimated according to the method of Ruch [18]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4). Stem bark extract and ascorbic acid (standard) solutions at different concentrations (10, 20, 40, 80, and 160 µg/ml) were added to hydrogen peroxide solution and absorbance was read after 10 minutes at 230 nm.

The percentage of hydrogen peroxide scavenging was calculated as follows:

$$\%\ H2O2\ \text{inhibition} \quad \frac{\text{Absorbance of control-Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

Reducing Power Assay

The reducing power of the ethanolic stem bark extract was determined by the method of Oyaizu [19]. This assay method is based on the principle of increase in the absorbance of the reaction mixtures. Increases in the absorbance of the reaction mixture indicates increased reducing power and hence, antioxidant activity. About 1.0ml the stem bark extract and ascorbic acid (standard) at different concentrations (10, 20, 40, 80, and 160 µg/ml) were mixed with 2.5 ml of 0.2M phosphate buffer and 2.5 ml of 1% potassium ferricyanide and incubated for 20 minutes at 50^0C. 2.5 ml of trichloroacetic acid was thereafter added to the reaction mixture and centrifuged at 3000 rpm for 10 minutes. The supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$ and absorbance was measured at 700 nm.

Acute toxicity study

Lorke's method [20] was adopted in the determination of the LD50 of the stem bark extract. The study was divided into two phases using a total of fifteen (15) male Wistar albino rats of weights between 100 - 168g. They were allowed to acclimatize for two weeks in the animal house of Faculty of Basic Medical Sciences, University of Uyo, Nigeria and fasted overnight just before the commencement of the experiment for body weight determination. In phase one, nine (9) rats were randomized into three groups of three rats each and were administered the stem bark extract at 10, 100, and 1000 mg/kg body weight (b.w) respectively in order to possibly establish the range of doses that would produce any toxic effect. Additionally, a fourth group of three rats was set up as control group fed with normal feed and water only. In phase two, 1600, 2900 and 5000 mg/kg b.w. of the extract were administered to three rats of one rat per dose to further determine the correct LD_{50} value. Extract administration was done by oral intubation. The experimental animals were observed for the first 30 minutes of administration for signs of acute toxicity, then 24rs and 14 days for signs of acute toxicity or mortality.

Statistical Analysis

The experiments were done in triplicates and data expressed as mean ± standard deviation (SD) using Microsoft Excel. Data were analysed using one way analysis of variance (ANOVA) where applicable with the aid of SPSS statistical software. P values at < 0.05 were considered statistically significant for difference between means.

Results

Results of the phytochemical screening of ethanol stem bark extract of Irvingia gabonensis O'Rorke baill

Preliminary phytochemical screening of *Irvingia gabonensis* (O'Rorke) baill ethanol stem bark extract revealed the presence of saponins, alkaloids, tannins, flavonoids, anthraquinones, cardiac glycosides, and carbohydrates as seen in table 1 below.

S/N	Phytochemical Constituent	Inference
1	Saponins	++
2	Alkaloids	+
3	Flavonoids	++
4	Tannins	+++
5	Anthraquinones	+
6	Cardiac Glycosides	++
7	Carbohydrate	++
8	Terpenoids	-

Table 1: *Phytochemical Composition of Irvingia gabonensis O'Rorke Baill Ethanol Stem Bark Extract*

Keys: *+: Present in a trace concentration; ++: Present in a medium concentration; +++: Present in a high concentration; -: Absent or in negligible amount*

1, 1-diphenyl -2- picrylhydrazyl (DPPH) Free Radical Scavenging Activity of Irvingia gabonensis O'Rorke Baill Ethanolic Stem bark extract

The figure 1 below shows the DPPH free radical scavenging activity of IG ethanol stem bark extract and ascorbic acid (standard). At different concentrations of 10, 20, 40, 80 and 160 µg/ml, percentage inhibitions of the stem bark extract were 20.89 ± 0.13%, 25.41 ± 0.06%, 30.05 ± 0.19%, 35.54 ± 0.13% and 53.53 ± 0.19%. Those of ascorbic acid (standard) were 34.55 ± 0.12%, 40.04 ± 0.18%, 55.18 ± 0.12%, 72.28 ± 0.07%, 91.43 ± 0.00%. The IC_{50} of the stem bark and ascorbic acid as extrapolated from the line graph were 143.95 µg/ml and 38.76 µg/ml respectively.

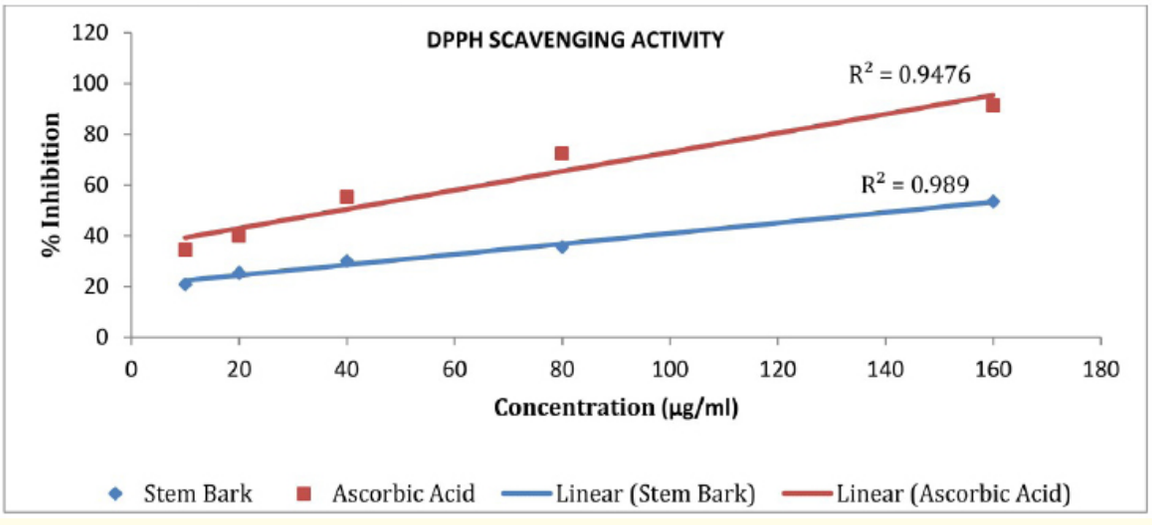

Figure 1: *1, 1-diphenyl -2- picrylhydrazyl (DPPH) Free Radical Scavenging Activity of Irvingia gabonensis O'Rorke Baill Ethanol stem bark extract in comparison with ascorbic acid standard.*

Nitric Oxide (NO.) Scavenging Activity of Irvingia gabonensis O'Rorke Baill Ethanol Stem bark extract

The figure 2 below shows the NO. free radical scavenging activity of IG ethanol stem bark extract and ascorbic acid (standard). At different concentrations of 10, 20, 40, 80 and 160 µg/ml, percentage inhibitions of the stem bark extract were 32.81 ± 0.01%, 33.97 ± 0.00%, 36.41 ± 0.00%, 40.56 ± 0.00%, 48.64 ± 0.00%. Those of ascorbic acid (standard) were 33.76 ± 0.00%, 35.19 ± 0.00%, 44.43 ± 0.00%, 46.7 ± 0.05%, 57.04 ± 0.00%. The IC_{50} of the stem bark and ascorbic acid as extrapolated from the line graph were 171.75 µg/ml and 106.05 µg/ml respectively.

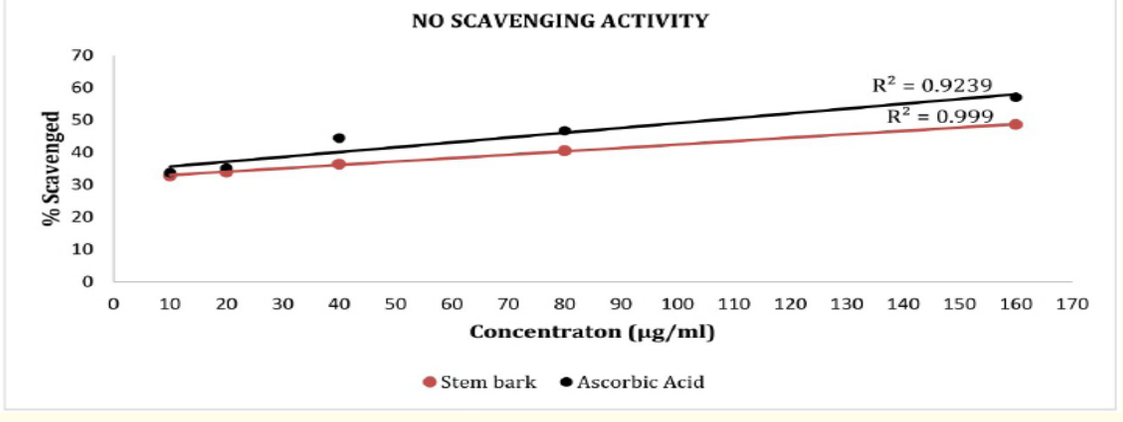

Figure 2: *Nitrogen Oxide (NO) Scavenging Activity of Irvingia gabonensis O'Rorke Baill Ethanolic stem bark extracts in comparison with ascorbic acid standard.*

Hydrogen Peroxide (H2O2) Scavenging Activity of Irvingia gabonensis O'Rorke Baill Ethanol Stem Bark Extract

The figure 3 below shows the H_2O_2 free radical scavenging activities of IG ethanol stem bark extract and ascorbic acid (standard). At different concentrations of 10, 20, 40, 80 and 160 µg/ml, percentage inhibitions of the stem bark extract were 17.27 ± 0.00%, 22.51 ± 0.00%, 29.32 ± 0.00%, 29.84 ± 0.00%, 38.22 ± 0.00%. Those of ascorbic acid (standard) were 37.17 ± 0.00%, 38.22 ± 0.00%, 42.93 ± 0.00%, 50.26 ± 0.01%, 59.16 ± 0.01%. The IC_{50} of the stem bark and ascorbic acid as extrapolated from the line graph were 249.12 µg/ml and 91.83 µg/ml respectively.

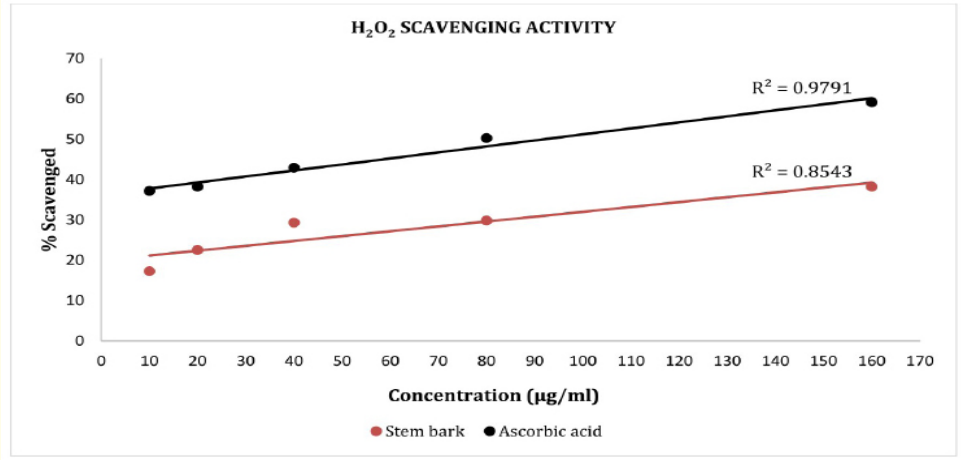

Figure 3: *Hydrogen peroxide scavenging activity of Irvingia gabonensis O'Rorke Baill Ethanol stem bark extract in comparison with ascorbic acid standard.*

Reducing Power of Irvingia gabonensis O'Rorke Baill Ethanolic Stem Bark Extract

The figure 4 below shows the reducing power of IG ethanol stem bark extract and ascorbic acid (standard). At different concentrations of 10, 20, 40, 80 and 160 µg/ml, the reducing power of the stem bark extract were 0.414 ± 0.00, 0.425 ± 0.00, 0.498 ± 0.00, 0.568 ± 0.00 and 0.638 ± 0.00. Those of ascorbic acid (standard) were 0.487 ± 0.01, 0.528 ± 0.01, 0.643 ± 0.00, 0.775 ± 0.01 and 1.235 ± 0.00.

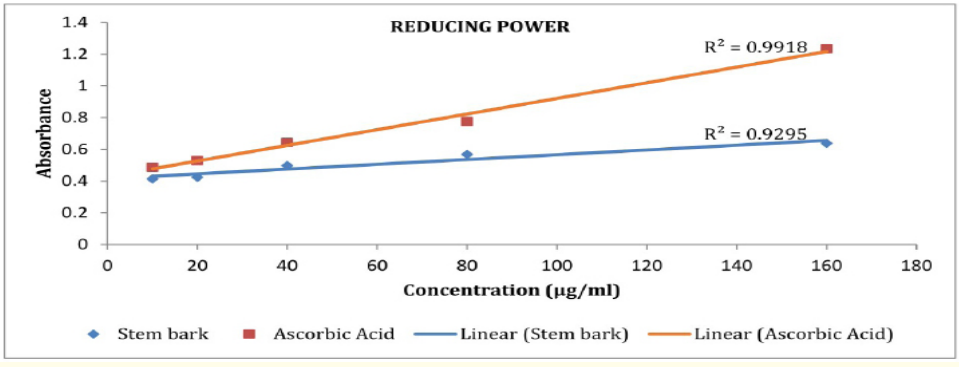

Figure 4: *Reducing Power of Irvingia gabonensis O'Rorke Baill Ethanol stem bark extract in comparison with ascorbic acid standard.*

Acute lethal effect of ethanol stem bark extract of Irvingia gabonensis O'Rorke baill on male wistar albino rats

No death was recorded for any of the experimental rats even at the highest dose of 5000 mg/kgbw after 24 hours of treatment and after 14 days period of observation and no signs of toxicity was observed (Table 2). The extract also had little or no effect on the body weights of the experimental animals. There were no significant differences ($P > 0.05$) in the percentage change in body weights between the control and experimental groups as shown in table 3 below. The oral LD_{50} was therefore found to be higher than 5000 mg/kgbw.

Experiment/Groups	Treatment	No of deaths after 24 hours
Phase1 1 2 3	 10 mg/kg/bw IG extract 100 mg/kg/bw IG extract 1000 mg/kgbw IG extract	 0/3 0/3 0/3
Control	0	0/3
Phase2 1 2 3	 1600 mg/kgbw IG extract 2900 mg/kgbw IG extract 5000 mg/kgbw IG extract	 0/1 0/1 0/1

Table 2: *Acute lethal effect of ethanol stem bark extract of Irvingia gabonensis O'Rorke baill on male Wistar albino rats.*

mg/kgbw: Milligram Per Kilogram Body Weight; IG: Irvingia gabonensis

Groups	Initial body weight (g)	Final body weight (g)	% change in body weight
1 (10 mg/kgbw)	138.7 ± 8.96	157.67 ± 16.26	11.82 ± 3.53
2 (100 mg/kgbw)	148.33 ± 4.73	173.33 ± 10.21	14.32 ± 3.32
3 (1000 mg/kgbw)	163.33 ± 3.21	189.67 ± 23.03	13.01 ± 10.83
Control	126.5 ± 9.19	151 ± 1.41	16.19 ± 4.86
Phase 2			
1 (1600 mg/kgbw)	100.00	97.00	3.00
2 (2900 mg/kgbw)	114.00	135.00	15.56
3 (5000 mg/kgbw)	134.00	133.00	0.75

Table 3: *Effect of Irvingia gabonensis O'Rorke Baill Ethanol Stem Bark Extract on the Body Weights of Experimental Animals.*

Data expressed as mean ± standard deviation; mg/kgbw: Milligram Per Kilogram Body Weight

Discussion

Over the last decade, there has been massive scientific research into the therapeutic utilization of different plants owing to the use of different plant parts as foods and as medicine. Herbal medicine practitioners and most locals use plant parts for therapeutic purposes [21]. It is a common knowledge that most therapeutic effects of medicinal plants are a function of their phytochemical constituents and as such in recent years, secondary plant metabolites (phytochemicals) have been investigated extensively as sources of medicinal agents [22]. Furthermore, oxidative stress, caused by an accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and counteracted by antioxidants, has been implicated in the etiology of different diseases [23,24].

In this study, qualitative phytochemical screening of the ethanol stem bark extract of *Irvingia gabonensis* O'Rorke baill revealed the presence of saponins, tannins, flavonoids, alkaloids, anthraquinones, cardiac glycosides and carbohydrates. Deleterious effects of free

radicals are known to be combated by tannins, phenols and polyphenols including flavonoids [25]. Flavonoids have been reported to possess antioxidant, anti-carcinogenic, antimicrobial and antitumor properties [26] and reduce heart diseases [27]. Flavonoids have also been suggested to function by scavenging free radicals, acting as reducing agents, acting as singlet oxygen quenchers, donation of hydrogen atom, chelating metal ions and sparing of other antioxidants [28]. Saponins help in the control of blood cholesterol levels, bone health, building of the immune system and cancer [29]. Tannins have also been reported to possess antioxidant, antimicrobial and anti-inflammatory properties [30]. Anthraquinones are used as laxatives and alkaloids have been reported to possess analgesic, antispasmodic, antibacterial, anticancer and antioxidant effects. Cardiac glycosides possess anti-cancer activities [31-35] and help in the treatment of congestive heart failure and arrhythmia [36].

Phyto-antioxidants have been linked with the amelioration of chronic diseases since they have the capacity of terminating the propagation of free radicals in the body [37]. This is utilized as a parameter in characterizing medicinal potentials of plants.

1,1-diphenyl-2-picrylhydrazyl (DPPH) is characterized as a stable free radical due to the delocalization of the spare electron over the entire molecule, preventing the molecule from dimerizing. The delocalization of electron also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 517 nm. When DPPH solution is mixed with that of a substrate (antioxidant) that can donate a hydrogen atom, it this gives rise to the reduced form with the loss of this violet color [38]. In the present study, IG ethanol stem bark extract scavenged DPPH radical in a concentration dependent manner comparable to that of ascorbic acid (standard). This is indicative of the very high antioxidant activity of the extract which may not be unconnected to the presence of antioxidant phytochemicals as seen from the results obtained from the phytochemical screening of IG ethanol stem bark extract. This may be via the donation of hydrogen atom to it by hydrogen-donating antioxidant phytochemicals present in the extract thereby producing the non-radical form [38]. Ewere., *et al*. [39] had reported the DPPH free radical scavenging activity of *Irvingia gabonensis* (O'Rorke) baill ethanol leaf extract.

Nitric oxide (NO.) is a free radical that is produced in biological tissues by enzymes called nitric oxide synthases [38]. These enzymes catalyze the metabolism of arginine to citrulline with the formation of NO. through a five electron oxidative reaction [40-42]. Excess NO. causes some negative health effects like blurred vision, confusion, dizziness, fever, headache, unusual bleeding, rapid heart rate among others. In the present study, IG ethanol stem bark extract scavenged NO. in a concentration-dependent manner comparable to ascorbic acid (standard). This therefore also connotes the antioxidant activity of the extract.

Hydrogen peroxide (H_2O_2) is a reactive oxygen species that decomposes rapidly into oxygen and water and this may culminate in hydroxyl radical (OH.) production that can initiate lipid peroxidation and cause DNA damage in the body. Humans are indirectly exposed to H_2O_2 through the environment mostly from the consumption of leaf crops [38]. It can enter into the human body by inhalation of vapor or mist and through eye/skin contact [38]. In the present study, IG ethanol stem bark extract scavenged H_2O_2 in a concentration-dependent manner comparable to ascorbic acid. This is also reflective of the potent antioxidant activity of the extract.

Reducing power of a compound is a measure of its antioxidant activity [43]. Compounds that possess high reducing power are electron donors and can reduce oxidized intermediates of lipid peroxidation processes and therefore act as primary and secondary antioxidants [44]. In the present study, the reducing power of IG ethanol stem bark extract increased in a concentration-dependent manner comparable to that of ascorbic acid (standard). This also further reflects the potent antioxidant activity of the extract and may corroborate its medicinal use by locals.

Acute toxicity studies are necessary in understanding the toxicity profiles of plant extracts [45]. This study revealed the acute oral toxicity of ethanol stem bark extract of IG in Wistar albino rats to be beyond 5000 mg/kg body weight since the experimental rats tolerated the extract with no symptom of acute toxicity (no mortality, skin changes, aggressiveness, diarrhea, restiveness, seizures, dizziness, weakness, or withdrawal from either food or water) even at higher doses of the extract. Thus, IG ethanol stem bark extract is considered safe as extracts or chemicals with LD_{50} beyond 5000 mg/kg body weight are considered safe [20].

Conclusion

In conclusion, ethanol stem bark extract of *Irvingia gabonensis* O'Rorke baill contains very vital phytochemicals, has high antioxidant activity and can be considered to be non-toxic. Further *in vivo* studies are recommended to further justify its medicinal value.

Conflict of Interest

No conflict of interest exists.

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Volume 6 Issue 6 June 2018