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Isolation of pentacyclic triterpene, phenolic content and antioxidant activity of root bark of *Irvingia gabonesis* Baill. (Irvingiaceae)

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Abstract
The aim of this study was to investigate the phytochemical constituents and antioxidant potentials of the ethanol extract and fractions of *Irvingia gabonensis* root bark in order to establish the scientific basis and rationale for its folkloric use. The powdered root bark of *I. gabonensis* was macerated with 70% ethanol and then partitioned with different solvents based on their polarity to afford 4 fractions. The extract and fractions were subjected to phytochemical analysis. The *in vitro* antioxidant activity was evaluated using DPPH assay. The quantitative phytochemical determination showed that ethyl acetate fraction of *I. gabonensis* root bark (EFIGR) had the highest content of tannins (154.44 mg/GAE g), phenol (106.26 mg/GAE g and flavonoid (76.07 mg/QE g). The *in vitro* antioxidant activity of the extract and the fractions had IC_{50} values of 76.0±0.03, 27.0± 0.05, 39.0±0.07 and 28.0±0.10 µg/mL for Ethanol extract, EFIGR, Butanol fraction and Aqueous fraction respectively while the standard had 29.0±0.03 µg/mL.Further fractionation of EFIGR led to the isolation of hederagenin which was characterized using 1D-NMR and mass spectrometric data. The present study revealed that root bark of *I. gabonensis* is a potential source of natural antioxidant which justified its uses in the traditional medicine.

Keywords: *Irvingia gabonensis;* Qualitative; Quantitative; Hederagenin; Natural antioxidant

INTRODUCTION

The use of plants in the management and treatment of diseases started with life. In more recent years, with considerable research, it has been found that many plants do indeed have medicinal value [1]. Medicinal plants contain phytochemicals that are mainly primary and secondary metabolites which can be utilized as drug [2]. These phytochemicals are naturally occurring and biologically active plant compounds that have potential disease inhibiting properties [3]. Plant phytochemicals are potent antioxidants against reactive oxygen species and have several health benefits [4]. Free radicals are chemically unstable species containing unpaired electrons that can cause damage to lipid cells, proteins and DNA as a result of imbalance between the generation of

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reactive oxygen species (ROS) and the antioxidant enzymes [5]. They are known to be the underlying cause of oxidative stress which is implicated in the pathogenesis of many diseases such as cancer, diabetes, cardiovascular diseases, aging and metabolic syndrome [6,7]. These radicals can be scavenged by the protective role of natural and synthetic antioxidant agents [8]. Natural antioxidants are broad-spectrum and effective in regulating destructive processes triggered by oxidative stress, induced by free radicals overproduction [9]. Recently, the use of natural antioxidant has gained much attention from consumers because they are considered safer than synthetic antioxidants [8].

Irvingia gabonesis is a genus of African and Asian tree which belongs to Irvingiaceae family and common names are wild mango, African mango or bush mango. It is locally known as "Pekpeara" in Nupe, "Ogwi" in Bini, "Ogbono/Ugiri" depending on the variety in Igbo, "Uyo" in Efik and "Oro" (tree) "Apon" (Kernel) in Yoruba and ''goronbiri" in Hausa language of Nigeria [10]. Ethno-medicinally, it has been reported to be useful in treating diseases such as hernia, yellow fever, gastrointestinal and liver conditions, sterility and urethral discharge, and it is also considered to be an antidote to poisoning [11,12]. Bark shavings are used to relieve pain [13] and stop diarrhoea and dysentery, and powdered chocolate prepared from the kernels is applied to burns [14]. In a recent study carried out in Ilorin, Nigeria, it was found that the root bark is also used as a fertility enhancer among males [15]. The antioxidant activity of the kernel and stem bark has also been reported [16,17]. The kernels are used for weight reduction and for the management of type 2 diabetes [18]. Donfack and co-workers [19] reported the isolation of seven compounds from the methanolic extract of *I. gabonensis* stem bark; identified as: 3-friedelanone; betulinic acid; oleanolic acid; 3,3',4'-tri-*O*-trimethylellagic acid; methyl gallate; hardwickiic acid and 3-β-acetoxyursolic acid. Therefore, this study was undertaken to investigate the phytochemical properties and antioxidant potential of the ethanol extract and fractions of *I. gabonensis* root bark in order to explore the rationale for its folkloric use.

EXPERIMENTAL METHODS

Plant collection and authentication. The plant *I. gabonensis* was collected at "Oke Egbo" village, Ondo East Local government area of Ondo state, Nigeria, in December, 2017. It was identified by Mr. Bolu Ajayi (Plant Taxonomist) of the Herbarium Unit, Department of Plant Biology, University of Ilorin, Ilorin, where a herbarium specimen with specimen voucher number (UILH/001/1364) was deposited for future reference.

Extraction and fractionation of the ethanol extract of the root bark of *I. gabonensis*. The powdered root bark (2 kg) of *I. gabonensis* was extracted using cold maceration technique with 10 L of 70% ethanol in a glass jar for 3 days (72 hours) at room temperature. The extract was filtered, the filtrate was further concentrated by using a rotary evaporator and finally evaporated to dryness using a water bath set at a temperature of 50°C. Hundred grams (100 g) of crude ethanol extract (EEIGR) was suspended in 500 ml of water and filtered. The filtrate was partitioned thrice with 300 ml of hexane, ethyl acetate and butanol saturated with water to afford the hexane fraction (HFIGR), ethyl acetate fraction (EFIGR), butanol fraction (BFIGR) and the aqueous fraction (AFIGR) respectively. The crude ethanol extract, partitioned fractions and the residual aqueous fraction were placed over a water bath labelled appropriately and stored for further use [20].

Qualitative phytochemical screening. The crude ethanol extract and fractions (ethyl acetate, butanol and aqueous) of *I. gabonensis*

root bark were subjected to phytochemical screening using standard procedure in order to identify the phytochemical constituents of the plant [21,22,23].

Quantitative phytochemical evaluation. The phytochemical constituents of the crude ethanol extract and fractions of *I. gabonensis*root bark were further evaluated quantitatively by spectrophotometric methods using standard procedures as mention below.

Determination of total phenolic content (TPC). Total phenol content in *I. gabonensis* ethanol root bark extract was measured spectrophotometrically by Folin–Ciocalteu colorimetric method, using gallic acid as the standard and expressing results as Gallic Acid Equivalent (GAE) per gram of sample. Different concentrations (0.01 - 0.1 mg/ml) of Gallic acid were prepared in methanol. Aliquots of 0.5 ml of the test sample and each sample of the standard solution were taken, mixed with 2 ml of Folin–Ciocalteu reagent (1:10 in deionised water) and 4 ml of saturated solution of sodium carbonate (7.5% w/v). The tubes were covered with silver foils and incubate at room temperature for 30 minutes with intermittent shaking. The absorbance was taken at 765 nm using methanol as blank. All the samples were analysed in three replications. The total phenol was determined with the help of standard cure prepared from pure phenolic standard (Gallic acid) [24].

Determination of total flavonoid content (TFC). The TFC of *I. gabonensis* ethanol root bark extract was determined by aluminium chloride colorimetric assay [25]. Briefly, 0.5 ml aliquots of the samples (ethanol extract and fractions) and standard solution (0.01 – 0.1 mg/ml) of Quercetin were mixed with 2 ml of distilled water; and subsequently with 0.15 ml of sodium nitrite (5% $NaNO_2$, w/v in methanol). After 6 minutes, 0.15 ml of (10% $AlCl_3$, w/v in methanol) solution was added. The solution was allowed to stand for 6 min and after that 2 ml of sodium hydroxide (4% NaOH, w/v in methanol) solution was added to the mixture. The final volume was adjusted to 5 ml with immediate addition of distilled water, mixed thoroughly and allowed to stand for another 15 min. The absorbance of each mixture was determined at 510 nm. TFC was determined as mg Quercetin equivalent per gram of sample with the help of calibration curve of Quercetin. All determinations were performed in triplicate.

Determination of tannin content. The tannins were determined by Folin-Ciocalteu method. 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu-phenol reagent, 1 ml of 35 % Na_2CO_3 solution and diluted to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of Gallic acid (0.01 – 0.1 mg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract [26].

Determination of saponin content. Total saponins were determined according to the method described by [27]. One gram (1 g) of freeze-dried extract was dissolved in 50% aqueous methanol and an aliquot (5 mg/ml) was taken. Vanillin reagent (0.25 ml; 8%) was added followed by sulphuric acid (2.5 ml; 72% v/v). The reaction mixtures was mixed well and incubated at 60°C on a water bath for 10 min. After incubation, the reaction mixtures were cooled on ice and absorbance at 544 nm (UV visible spectrophotometer) was read against a blank that does not contain extract. The standard calibration curve was obtained from aliquots of diosgenin (0.01 – 0.1 mg/ml in 50% aqueous methanol). The total saponin concentration was expressed as mg diosgenin equivalents (DE) per g dry weight (DW).

Antioxidant studies. 2, 2-Diphenyl-1-picryl-hydraxyl (DPPH) radical scavenging assay method as described [28] was used to evaluate the extract and fractions for antioxidant activity. A 0.1 mM solution of DPPH in methanol was prepared by dissolving 39.4 mg of DPPH in 1 L of methanol and it served as the blank (control). Five serial dilutions (0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml) of the ethanol extract and ascorbic acid (AA) as standard antioxidant was prepared using methanol as the diluent. 1 ml of the prepared DPPH was added to 3 ml of the various serially diluted concentrations of ethanol extract and ascorbic acid and kept in the dark for 30 minutes. The absorbance of the various samples was measured at 517 nm using UV spectrophotometer. All tests were performed in triplicate. The radical scavenging activity was calculated as follows:

Radical scavenging activity (%) =

$$\frac{absorbance\ of\ control - absorbance\ of\ test\ sample}{absorbance\ of\ control} X\ 100$$

The obtained percentages were plotted against the various concentrations for each sample and IC_{50} values were estimated by non-linear regression analysis. The same procedure was repeated using ethyl acetate, butanol and residual aqueous fractions.

Isolation and characterization of bioactive compounds from EFIGR. Column chromatographic separation of the EFIGR was performed using silica gel (60 - 120 µm in size) stationary phase and hexane and ethyl acetate mobile phase in a gradient elution method after carried out series of thin layer chromatographic (TLC) profile of the sample. Eight gram of EFIGR was pre-absorbed in silica gel (dry method) and loaded into the glass column (3.0 x 60 cm). The polarity of the solvent system was increased by increasing the proportion of the more polar solvent (ethyl acetate) gradually by 5%. The profiles of collections made were monitored using TLC plate (Silica gel-60 F_{254}aluminiumpre-coated plates from Merck, Germany)and collections having similar profiles from the column were pooled together. Furthermore, combined fractions 92 - 94 (200 mg) was purified on 50 mL burette packed with silica gel stationary phase and eluted with 100% hexane, hexane : ethyl acetate (9:1 and 8:2) to afford a pure compound coded EA1, which was monitored on TLC plate using hexane: ethyl acetate (4:1; 3:1). Physiochemical studies conducted on the isolated compound include; colour, solubility in different solvents, melting point and TLC using sprayed detecting reagents. The compound EA1 was subjected to 1D Nuclear magnetic resonance (NMR) spectroscopic and mass spectrometric analysis.

Data analysis. Data obtained from the study was analysed using Statistical Package for Social Sciences (SPSS), IBM version 20. The results was expressed as mean ± standard error of mean (SEM) of three replicate.

RESULTS AND DISCUSSION

Qualitative phytochemical constituents. Qualitative phytochemical investigation of *I. gabonensis* root bark extract and fractions revealed the presence of carbohydrates, saponin, cardiac glycoside, flavonoids, tannins, alkaloids and steriods/ triterpenes but absences of anthraquinones glycoside (Table 1). The result of this study is supported by the finding of [29] who reported the presence of alkaloids, tannin, phlobatannins, saponin, flvonoids, anthraquinones, phenol and cardiac glycosides on the stem bark of *I. gabonensis*. Preliminary phytochemical screening gives a brief idea about the qualitative nature of active phytochemical constituents present in plant extracts, which will help the future investigators regarding the selection of the particular extract for further investigation or isolation of the active principle [30]. The information on the presence or absence and the type of phytochemical constituents especially the secondary metabolites are useful taxonomic keys in identifying a particular species and distinguishing it from a related

species, thus helping in the delimitation of taxa [31]. The therapeutic or biological activities of any medicinal plant are usually a direct function of the chemical constituents present in the plant and phytochemical constituents often vary with plant parts, method of extraction and solvent for the extraction [32].

Quantitative phytochemical determination. The results for the quantitative phytochemical content of crude ethanol extract and fractions of *I. gabonensis* root bark are presented in Figures 1 – 4. BFIGR had the highest content of saponins 372.67 mg diosgenin equivalent/g and followed by EEIGR with 172.33 mg diosgenin equivalent/g. EFIGR had the highest content of tannins 154.44 mg Gallic acid equivalent/g, phenol 106.26 mg Gallic acid equivalent/g and flavonoid 76.07 mg quercetin equivalent /g. AFIGR had the lowest tannin content 7.78 mg Gallic acid equivalent/g. This analysis is concerned with the determination of the amount of chemical substances.

Table 1: Qualitative phytochemical analysis of extract and fractions of *Irvingia gabonensis* root bark

Constituents	Tests	EEIGR	EFIGR	BFIGR	AFIGR
Carbohydrate	Molisch	+	+	+	+
	Fehling	+	+	+	+
Saponin	Frothing	+	+	+	+
Anthraquinones	Borntrager	-	-	-	-
	Modified Borntrager	-	-	-	-
Cardiac glycosides	Keller- Kiliani	+	+	+	+
	Baljet	+	+	+	+
Flavonoids	Shinoda	+	+	+	+
	Sodium Hydroxide	+	+	+	+
Tannins	Ferric chloride	+	+	+	+
	Lead sub acetate	+	+	+	+
Alkaloids	Dragendorff	+	+	+	+
	Mayer	+	+	+	+
	Wagner's Test	+	+	+	+
Steroids/Triterpenes	Salkowski	+	+	-	-
	Lieberman-Burchard	+	+	-	-

EEIGR: Ethanol Extract of *Irvingia gabonensis* Root Bark; EFIGR: Ethyl acetate Fraction of *Irvingia gabonensis* Root Bark; BFIGR: Butanol Fraction of *Irvingia gabonensis* Root Bark; AFIGR: Aqueous Fraction of *Irvingia gabonensis* Root Bark; +: Present; -: Absent

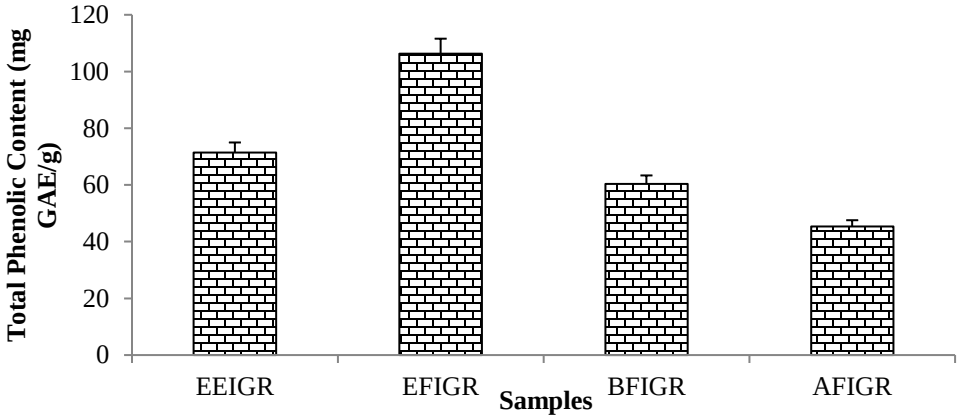

Figure 1: Total Phenolic Content of the Extract and Fractions of *Irvingia gabonensis* Root Bark
Values are presented as Mean ± SEM (three determination) in mg GAE/g. GAE :Gallic acid equivalent; EEIGR: Ethanol Extract of *Irvingia gabonensis* Root Bark; EFIGR: Ethyl acetate Fraction of *Irvingia gabonensis* Root Bark; BFIGR: Butanol Fraction of *Irvingia gabonensis* Root Bark; AFIGR: Aqueous Fraction of *Irvingia gabonensis* Root Bark

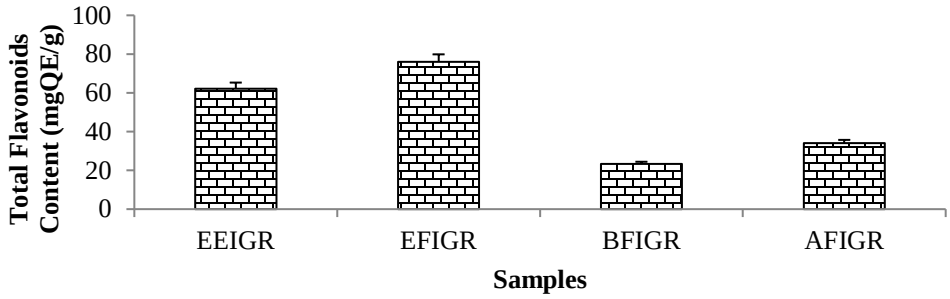

Figure 2: Total Flavonoid Content of the Extract and Fractions of *Irvingia gabonensis* Root Bark
Values are presented as Mean ± SEM (three determination) in mg QE/g. QE: Quercetin equivalent; EEIGR: Ethanol Extract of *Irvingia gabonensis* Root Bark; EFIGR: Ethyl acetate Fraction of *Irvingia gabonensis* Root Bark; BFIGR: Butanol Fraction of *Irvingia gabonensis* Root Bark; AFIGR: Aqueous Fraction of *Irvingia gabonensis* Root Bark

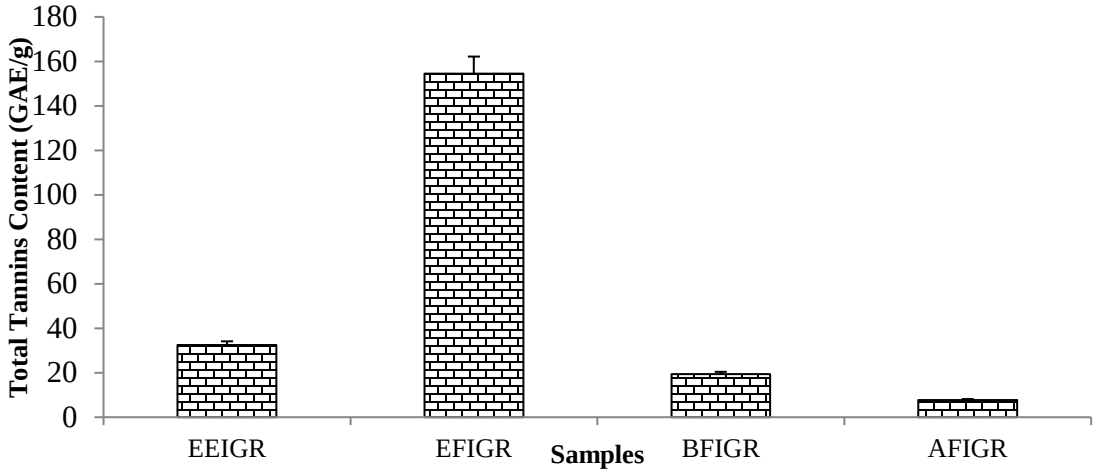

Figure 3: Total Tannin Content of the Extract and Fractions of *Irvingia gabonensis* Root Bark
Values are presented as Mean ± SEM (three determination) in mg GAE/g. GAE: Gallic acid equivalent; EEIGR: Ethanol Extract of *Irvingia gabonensis* Root Bark; EFIGR: Ethyl acetate Fraction of *Irvingia gabonensis* Root Bark; BFIGR: Butanol Fraction of *Irvingia gabonensis* Root Bark; AFIGR: Aqueous Fraction of *Irvingia gabonensis* Root Bark

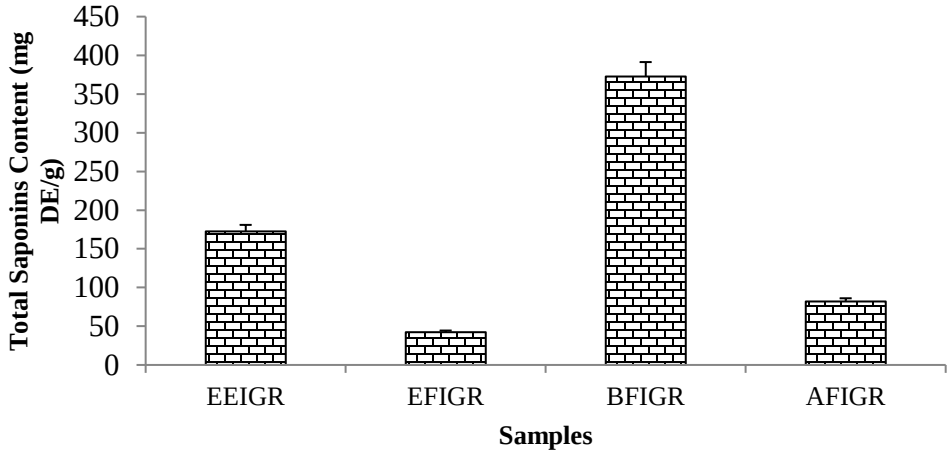

Figure 4: Total Saponin Content of the Extract and Fractions of *Irvingia gabonensis* Root Bark
Values are presented as Mean ± SEM (three determination) in mg DE/g.DE: Diosgenin equivalent; EEIGR: Ethanol Extract of *Irvingia gabonensis* Root Bark; EFIGR: Ethyl acetate Fraction of *Irvingia gabonensis* Root Bark; BFIGR: Butanol Fraction of *Irvingia gabonensis* Root Bark; AFIGR: Aqueous Fraction of *Irvingia gabonensis* Root Bark

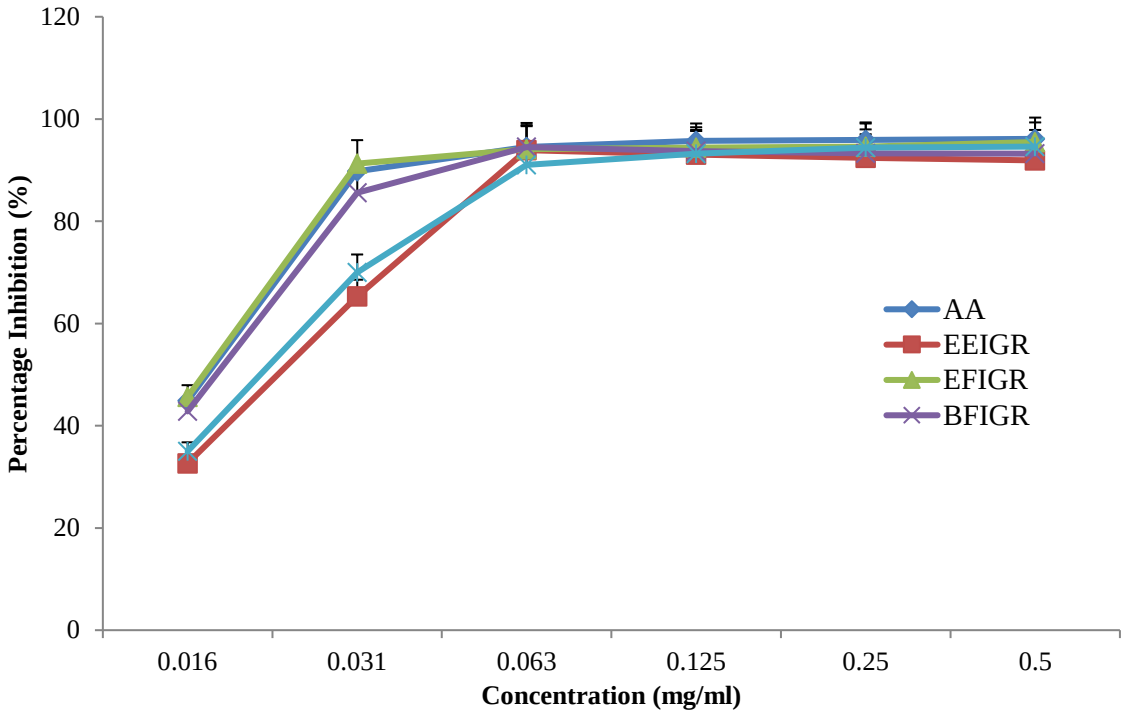

Figure 5:% Inhibition of Ethanol Extract and Fractions of *Irvingia gabonensis* in DPPH Scavenging Activity Assay AA: Ascorbic Acid (Standard); EEIGR: Ethanol Extract of *Irvingia gabonensis*Root Bark; EFIGR: Ethyl acetate Fraction of *Irvingia gabonensis* Root Bark; BFIGR: Butanol Fraction of *Irvingia gabonensis* Root Bark; AFIGR: Aqueous Fraction of *Irvingia gabonensis* Root Bark; DPPH: 2, 2-Diphenyl-1-picryl-hydraxyl

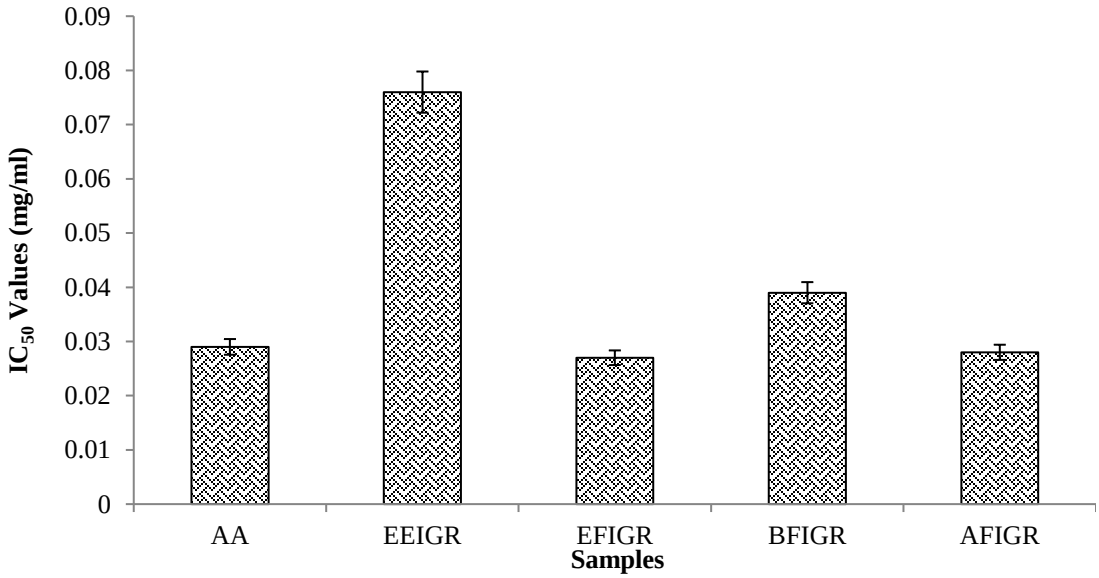

Figure 6: IC_{50} values of Extract and Fractions of *Irvingia gabonensis* root bark in DPPH Scavenging Activity Assay AA: Ascorbic Acid (Standard); EEIGR: Ethanol Extract of *Irvingia gabonensis* Root Bark; EFIGR: Ethyl acetate Fraction of *Irvingia gabonensis* Root Bark; BFIGR: Butanol Fraction of *Irvingia gabonensis* Root Bark; AFIGR: Aqueous Fraction of *Irvingia gabonensis* Root Bark; IC_{50}: Median Inhibitory Concentration.

Table 2: Comparison between chemical shift data of hederagenin and compound EA1 as obtained from literature

	Hederagenin*		Compound EA1	
Position	δ* ^{1}H (J Values in Hz)	δ* ^{13}C	δ ^{1}H (J Values in Hz)	δ ^{13}C
1	1.07 m, 1.59 m	38.8	1.03 m, 1.58 m	36.89
2	1.89 m	27.7	1.89 m	26.63
3	4.23 dd (11.2, 5.1)	73.4	4.29 t, (8.0)	75.80
4	-	42.9	-	
5	1.55 m	48.6	1.56 m	
6	1.48 m, 1.69 m	18.6	1.44 m, 1.69 m	18.57
7	1.32 m, 1.67 m	33.0	1.29 m, 1.67 m	32.57
8	-	39.8	-	
9	1.80 m	48.2	1.87 m	
10	-	37.3	-	36.64
11	1.95 m	23.9	1.96 m	23.81
12	5.51 t (2.9)	122.6	5.33 t (2.9)	123.96
13	-	144.9	-	145.19
14	-	42.4	-	42.07
15	1.18 m, 2.13 m	28.4	1.20 m, 2.09 m	29.77
16	1.99 m, 2.13 m	23.7	1.99 m, 2.09 m	23.81
17	-	46.7	-	46.67
18	3.32 dd (13.8, 4.0)	42.0	3.20 dd (8.0, 4.0)	
19	1.33 m, 1.81 m	46.5	1.29 m, 1.86 m	46.67
20	-	31.0	-	32.57
21	1.22 m, 1.45 m	34.3	1.24 m, 1.44 m	35.94
22	1.80 m, 2.03 m	33.1	1.74 m, 2.04 m	32.57
23	3.74 d, 4.21 d (10.3, 10.3)	68.0	3.65 s, 4.28 s	65.08
24	1.07 s	13.2	1.03 s	13.79
25	0.99 s	16.0	0.97 s	
26	1.07 s	17.5	1.04 s	18.09
27	1.26 s	26.2	1.27 s	26.63
28	-	180.2	-	184.73
29	0.95 s	33.3	0.95 s	32.57
30	1.02 s	23.8	0.93 s	23.81

*****[39]; Hz: Hertz; S: Singlet; D: Doublet; T: Triplet; M: Multiplet

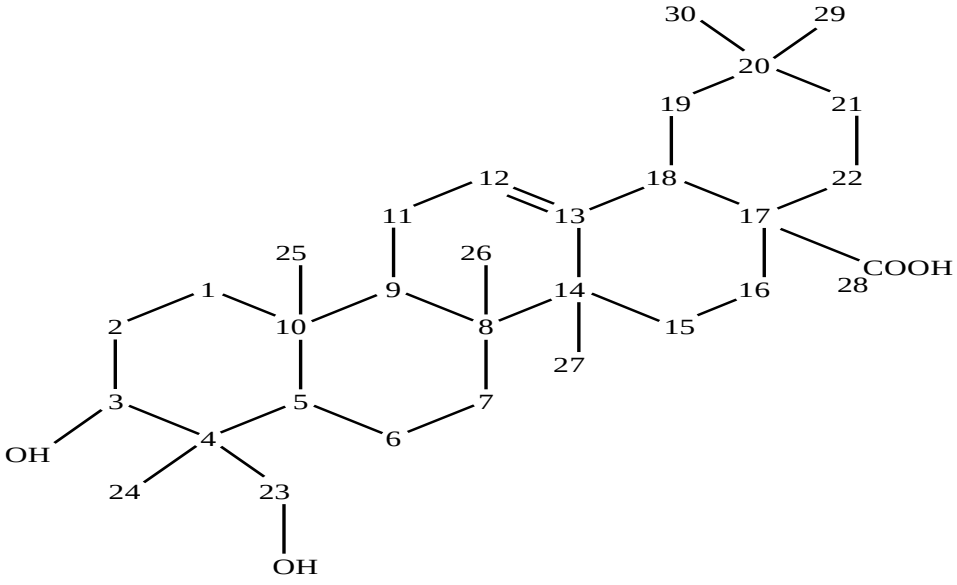

Figure 7: Structure of Compound EA1 (Hederagenin) from root bark of *I. gabonensis*

Phenolic compounds of plant origin exhibit anticarcinogenic effect which is attributed to their antioxidant properties, as well as their capability to modulate the activity of enzymes, block hormone receptors, and lower the activity of mutagens [33]. Polyphenols also protect blood vessels, reduce the aggregation of blood platelets and lower the LDL-cholesterol level in the blood [34]. Alkaloids have a wide range of pharmacological activities including antimalarial (e.g., quinine), anticancer (e.g. homoharringtonine), antibacterial (e.g. chelerythrine), and antihyperglycemic activities (e.g. piperine) [35]. Saponins are another type of bioactive chemical constituents which are involved in plant disease resistance because of their antimicrobial activity [36]. Saponins help reduce congestive heart failure by inhibiting sodium efflux via the blocking of the entrance of the sodium ions into the cell [37].

Antioxidant studies. The radical scavenging activity of ethanol extract and fractions of *I. gabonensis* root bark using 2, 2-Diphenyl-1-picryl-hydraxyl (DPPH) radical assay showed that the ethanol extract and fractions were capable of scavenging the DPPH free radical to various extents. The radical scavenging effects produced were in the following order: AA > EFIGR > AFIGR > BFIGR > EEIGR (Figure 5).The Median Inhibitory Concentration (IC_{50}) values of EEIGR, EFIGR, BFIGR and AFIGR were 76.0, 27.0, 39.0 and 28.0 µg/mL respectively while the IC_{50} of ascorbic acid (standard) was 29.0 ± 0.03 µg/mL as shown in Figure 6. From this findings, crude ethanol extract and fractions of *I. gabonensis* root bark exhibited significant free radical scavenging activity in the DPPH assay, with increase in concentration i.e. they are concentration dependent. Also, the EFIGR had the highest IC_{50} of 27.0 ± 0.05 µg/mL; follow by AFIGR (28.0 ± 0.10 µg/mL), BFIGR (39.0 ± 0.07 µg/mL) and lastly EEIGR (76.0 ± 0.03 µg/mL). These suggest that they possess significant antioxidant activities and it's an indicative of genuine antioxidants. This is because they are effective at lower concentration. This assertion is supported by [38] who reported that the IC_{50}values less than 100 µg/mL is indicative of a very good free radical scavenging potential. Phenolic compounds with known antioxidant activity are flavonoids, tannins, chalcones, coumarins and phenolic acids [39]. However, it was reported that phenolic compounds are essentially representing varieties of natural antioxidants, which are used as nutraceuticals and also in control of human pathogenic diseases [40]. Therefore, the antioxidant activity observed in this study may be related to the presence of those phytochemicals reported.

Isolation and characterization of bioactive compounds from EFIGR. The ethyl acetate fraction of *I. gabonensis* root bark(EFIGR) was observed to have the highest antioxidant activity and was chromatographed to isolate some of the bioactive compounds. The compound EA1 isolated was obtained as white crystalline powder, readily soluble in chloroform. Its chromatogram (developed using hexane: ethyl acetate 7.5:2.5) gave R_f value of 0.48 when sprayed with 10% sulphuric acid in methanol. It showed a positive reaction with Liebermann-Burchard spraying reagent (pink coloured spot) suggesting the presence of a steroidal or a triterpenoid nucleus. It has a melting point of 330 – 334^{0}C, which was relatively close to melting point value 331-333^{0}C reported for hederagenin by [41].

The ESI-MS (electrospray ionization mass spectrometry) spectrum of compound EA1 revealed a molecular ion (*m/z*) peak at 471.53 in the negative mode $[M-H]^-$ with molecular formula of $C_{30}H_{48}O_4$, which corresponds with reported literature [42]. The diagnostic signals observed in the ^{1}H NMR spectrum of compound EA1 include; δ 5.33

(1H, t, H-12), δ 4.29 (1H, m, H-3), δ 3.65 (2H, s, H-23), δ 3.20 (1H, dd, H-18), , δ 1.03 (3H, s, H-24), δ 0.97 (3H, s, H-25), δ 1.04 (3H, s, H-26), δ 1.27 (3H, s, H-27), δ 0.95 (3H, s, H-29) and δ 0.93 (3H, s, H-30) ppm according to their chemical shift. The ^{13}C NMR spectrum diagnostic signals include; a carboxylic acid carbon resonating at δ_c 184.73 ppm (C-28) and olefinic carbons resonating at δ_c 123.96 ppm (C-12) and 145.19 ppm (C-13) [43]. A signal resonating at 75.80 ppm (C-3) and 65.08 ppm (C-23) indicated the presence of oxymethine and oxymethylene carbon [43]. Compound EA1 was proposed to be hederagenin (3β, 23-dihydroxy-12-oleanan-28-oic acid); oleanane-type pentacyclic triterpene by analysis of the spectra data and comparison with reported data as in Table 2 [43]. Wide range of pharmacological activities of hederagenin has been reported including anti-tumor, anti-inflammatory, anti-diabetic, anti-depressant and anti-neurogenerative activity [41], this may be due to its antioxidant properties.

Conclusion. The finding of this study revealed that root bark of *I. gabonensis* is a great source of phytochemicals that could be exploited in therapeutic purposes and exhibited high antioxidant capacity which justified the folkloric uses of the plant.

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