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Deciphering the Antioxidant and Free Radical-Scavenging Capacity of *Anthocleista vogelii*

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Abstract
The empirical survey comparatively explores the antioxidant activity and the free radical-scavenging capacity of various fractions of *Anthocleista vogelii* leaves. The dried and pulverized leaves of *A. vogelii* were subjected to cold maceration for 4, 8 and 12 days using 70 % methanol and filtered. The filtrate was concentrated to a slurry crude extract by means of a rotary evaporator at 40ºC with a salvaged yield of 34.10 %. Exactly 0.23 kg of crude extract was defatted and fractionated using two chromatographic techniques in chloroform-methanol-hexane solvent system (3:21 v/v). The fractions were screened qualitatively for polyphenolic compounds. The fractions containing these bioactive compounds were quantified and further subjected to free radical-scavenging activity: DPPH assay, ferric reducing power (FRP) assay, hydrogen peroxide scavenging activity (HPA) and total antioxidant capacity (TAC) respectively. There was marked significant difference ($p<0.05$) in the total phenolic and flavonoid contents (TPC and TFC) among the fractions with ample amount found in n-hexane fraction. There were marked significant differences ($p<0.05$) in the IC_{50} values among fractions for DPPH, FRP, HPA and TAC assays. Thus, the ample antioxidant activity of n-hexane fraction among other fractions was further validated by the extrapolated IC_{50} values. The study inferred that *A. vogelii* leaves are possible sources of antioxidants with health benefits, especially for disease conditions characterized by oxidative stress.
Keywords: *Anthocleista vogelii*, Antioxidant activity, Free radical-scavenging capacity, IC_{50} value, Oxidative stress
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1. Introduction

The undue generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) triggers the compromise of antioxidant defence system as a result of over-exposure to environmental stressors, and its degree of reactivity necessitates the development of deleterious effects, comprising the onset of pathologies and loss of cellular functions. The over reactivity of these species termed "Free Radicals" were characterized by lone pair of electrons and were observed to be associated with normal metabolic processes (Tiwari 2001; Sasikumar & Kalaisezhiyen 2014). Superoxide anion, peroxyl radical, hydroxyl radical, nitric oxide and many more were notable free radicals reported to cause aberration of cellular functions characterized by oxidative stress (Halliwell & Gutteridge 1990; Sudha *et al*. 2011). The onset of oxidative stress was observed to be associated with the development of diverse pathologies like diabetes, neurodegenerative disorders, cardiovascular disorders and a host of others linked to excessive synthesis of oxygen and nitrogen-derived species (Tiwari 2001; Olugbami *et al.* 2014). Despite the affirmative role played by free radicals in the stimulation of signalling pathways, enhancement of phargocytosis and initiation of detoxification pathways for stressors (Costa & Magalhães 2009), the overwhelming harmful effects of oxidative stress, which centred on the onset of cellular injury, diverse pathologies and programmed cell death, cut the interest of toxicologists to finding a lasting solution on how to curb the detrimental effects of free radical-related diseases (Riley 1994; Olugbami et al. 2014). Therefore, scientific reports revealed that the boost of intrinsic antioxidant defence system through dietary supplementation rich in bioactive compounds with high antioxidant potential was suggested to reducing the risk of associated diseases (Willcox et al. 2004; Sasikumar & Kalaisezhiyen 2014).

The capability of antioxidants to switch-off hazardous effects of ROS associated with the development of various diseases at a non-overwhelming rate was indicated in epidemiological reports (Valko et al. 2007; Godic et al. 2014). To this end, Duarte and Lunec (2005) emphasized that the reducing function of antioxidants, involving their

hydrogen-donating ability, was fundamental in the cessation of every trace of oxidative stress initiated by ROS and a host of related pathologies in biological systems on exposure to a stressor. Intrinsic antioxidant capacity of medicinal plants was generally reported to fight or mop-up free radicals by offering protection against free radical-related diseases, which were observed in the significant reduction of lipid peroxidation and cellular injury (Osawa 1990; Saeed et al. 2012; Sasikumar & Kalaisezhiyen 2014). The increasing potency of free radical scavenging ability of natural antioxidants inherent in *Anthocleista vogelii* was taken into cognisance in this scientific investigation, hence the need for dietary ingestion of these compounds of plant origin may not be exaggerated in the enhancement of *in vivo* antioxidant defence system of complex multicellular organisms predisposed to environmental stressors.

Although the plant, "*A. vogelii*" formerly belongs to the Longniaceae family, but it is presently accepted into the Gentaniaceae family in view of its morphology and phytochemically rich history with diversification in Africa, most especially Nigeria, where it is typically found in swampy and marsh terrestrial habitats (Osadebe et al. 2014; Anyanwu et al. 2015; Apiamu et al. 2017)). The broad spectrum of therapeutic applications of the aerial parts of *A. vogelii* for various pathologies was scientifically proven to show antimicrobial, antiplasmodial, antihypertensive and antihypotensive, spasmolytic and spasmogenic, anti-obesity, antitrypanosomal, antihelmintic, diuretic and laxative, and antioxidant properties (Lawal et al. 2011; Sunday et al. 2011; Anyanwu et al. 2013; Ateufack et al. 2014; Christophe et al. 2015) respectively. The pharmacological competence of the plant was scientifically validated by bioactive compounds such as alkaloids, flavonoids, phenolics, saponins, steroids, anthraquinones and tannins reported to be inherent in its root and stem (Jegede et al. 2011;Gboeloh et al. 2015; Christophe et al. 2015; Apiamu et al. 2017). Therefore, the comparative investigation of *in vitro* antioxidant activity as well as free radical scavenging potential of leaf extracts of *A. vogelii* was novel and fundamental in the present study to identifying its potential antioxidant efficacy for the improvement of intrinsic antioxidant defence system during exposure of biological systems to environmental stressors.

2. Materials and Methods

2.1 Chemicals/reagents

All chemicals employed in the present study were of analytical grade.

2.2 Plant sampling and preparation

The plant, "*A. vogelii*", was authenticated by a taxonomist, Dr. H.A. Akinnibosun, at the Department of Botany, University of Benin, Benin City, Nigeria., with herbarium specimen marked as UBHa0258. A large chunk of the plant was harvested from the vicinity of Western Delta University, Oghara, Nigeria, and dried for three weeks at room temperature. The dried plant leaves were milled by means of a mechanical blender into a powdery paste, weighing 1.955 kg and then sieved for uniformity. The leaf paste was subjected to cold maceration technique using 70 % methanol for 4, 8 and 12 d, and the filtrate was concentrated to dryness under pressure to a slurry crude leaf extract at 40^{o}C by means of a rotary evaporator with a salvage yield of 34.10 %. This was refrigerated for further use in an air-tight container. To this end, 0.23 kg of crude leaf extract was defatted using 40-60^{o}C petroleum ether and then fractionated using Thin-layer Chromatography (TLC) with pre-coated silica gel plate and Vacuum Liquid Chromatography (VLC) techniques respectively. The insoluble defatted mass was further extracted using chloroform-methanol-hexane solvent system (3:2:1 v/v) (Figure 1). The respective fractions were obtained using rotary evaporator and subjected to *in vitro* antioxidant screening and free radical scavenging activities.

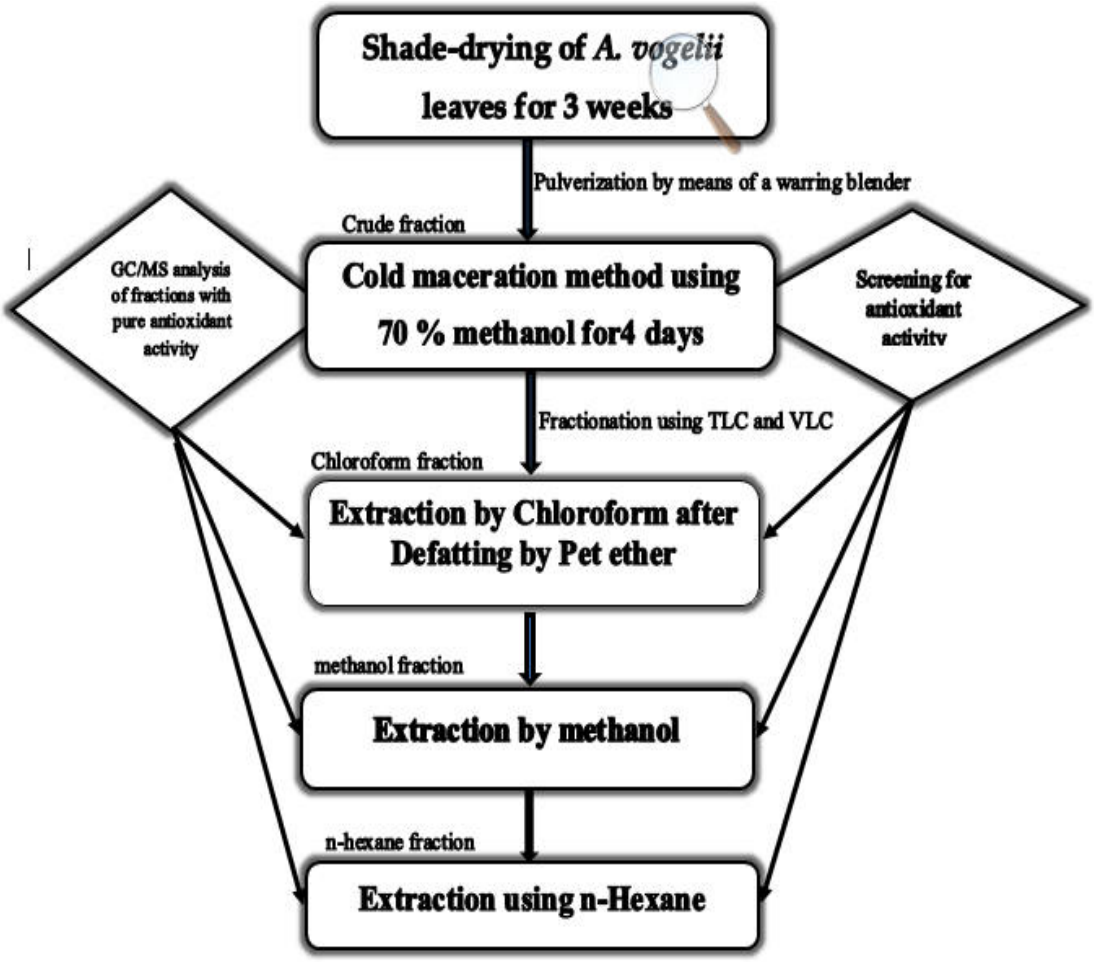

Figure1. Extraction scheme for *A. vogelii*

2.3 Preliminary phytochemical screening for phenols and flavonoids

The qualitative assessment of the fractions was accomplished using the method described by Jegede and his colleagues (Jegede et al. 20111).

Test for phenols: (a) To 2 ml of test sample in a test tube, 5 ml of 5 % iron (iii) chloride, ($FeCl_3$), was added. The development of a deep blue-black colour indicated the presence of phenolics. (b) To 2 ml of test sample in a test tube, 5 ml of 10 % lead (ii) Trioxonitrate (v), $Pb(NO_3)_2$, were added. The formation of white precipitate confirmed the presence of phenolic compounds in the plant.

Test for flavonoids: Five millilitres (5 ml) of methanolic leaf samples was pipetted into a test tube containing 10 ml of ethylacetate, which was subjected to heating in a hot water bath for 5 min and filtered after cooling. Methanolic filtrate (1.4 ml) was then added to 3 ml of 10% aqueous ammonia and shaken intensely for colour development. The formation of yellow coloration indicated the presence of flavonoids.

2.4 Assessment of total phenolic content (TPC) in samples

The TPC of *A. vogelii* was estimated spectrophotometrically in the respective fractions by assay technique described by Kim and colleagues (Kim et al. 2003). A millilitre of each test fraction at varying concentrations, (100-1000 µg/ml), was pipetted into a test tube containing one millilitre of Folin-Ciocalteu reagent, (previously diluted with H_2O in 1:10 v/v) and incubated at room temperature for 5.00 min. Ten millilitres of saturated sodium

carbonate, (7.00 w/v), was vortexed with thirteen millilitres of double distilled H_2O for few minutes, and allowed to stand in the dark for another 90.00 min at 23°C for colour development. The absorbance of the mixture was read and recorded at 765 nm against a reagent blank using UV-VIS spectrophotometer double beam (Labtech 2802). Following the above assay procedure, a calibration curve was developed using standard gallic acid at similar varying concentrations with the test fractions, and the TPC was estimated as milligram gallic acid equivalent (milligram GAE) per gram of plant sample.

2.5 Determination of total flavonoid content (TFC)

The spectrophotometric evaluation of TFC of *A. vogelii* in the respective fractions employed the assay technique described by Park and his colleagues (Park et al. 2008). The assay mixture containing one thousand microliter, (1000 µl), of test fraction with varying concentrations of 100-1000 µg/ml and one hundred and fifty microliter, (150 µl), of 300 mM hydrated aluminium chloride, ($AlCl_3.6H_2O$), was allowed to stand for 5min at room temperature. One thousand microliter, (1000 µl), of 1000 mM sodium hydroxide, (NaOH), was pipetted into the mixture and the absorbance was read and recorded instantaneously at 506 nm against a reagent blank using UV-VIS spectrophotometer double beam (Labtech 2802). Following the above assay procedure, a calibration curve was developed using standard rutin at similar varying concentrations with the test fractions, and the TFC was estimated as milligram rutin equivalent (mg RE)) per gram of plant sample.

2.6 Determination of DPPH scavenging capacity of samples

The capacity to scavenge the "unwavering" free radical, 2, 2- diphenyl-1-picrylhydrazyl, (DPPH), by *A. vogelii* was estimated spectrophotometrically in the respective fractions by assay technique described by Hasan et al. (2006) and Alam et al. (2008). Four milligram percent of DPPH-methanol, which served as positive control, was prepared and kept in the dark for 10.00 min at an ambient temperature. One hundred microliters, (100 µl), of test fraction at graded concentrations of 200-1000 µg/ml was pipetted into a test tube containing three thousand microliters, (3000 µl), of DPPH-methanol solution, vortexed and allowed to stand for 15 min at room temperature. The absorbance of the mixture was read and recorded instantaneously after incubation at 517 nm against a reagent blank using UV-VIS spectrophotometer double beam (Labtech 2802). Correspondingly, the absorbances of assay mixture containing ascorbic acid as the standard antioxidant in place of the test fractions was also read and recorded. Following the assay procedure, a control test tube devoid of standard or test fraction was prepared and its absorbance was measured. The percentage inhibition (Equation 1) was evaluated using the model hereunder, and this was employed in the extrapolation of the respective IC_{50} values.

$$\% \text{ DPPH Inhibition} = \frac{A_{control} - A_{test}}{A_{control}} \times \frac{100}{1} \quad (1)$$

Such that: A_{test} = absorbance of test fraction or standard at 517 nm; $A_{control}$ = absorbance of methanol-DPPH devoid of test sample or standard at 517nm

2.7 Ferric reducing power (FRP) assay of samples

The spectrophotometric antioxidant capacity of *A. vogelii* was monitored through its ferric reducing power following the assay procedure authored in literature (Yildirim et al. 2001). Here, the assay mixture at first contained two millilitres each of test fractions at graded concentrations, (200-1000 µg/ml), potassium ferricyanide (1.0 % w/v) and phosphate buffer (0.2 M, pH 6.6), which was incubated at 50°C for 20 min. Two millilitres of Trichloroacetic acid (10.0 % w/v) was added to the assay mixture, centrifuged for the supernatant at 3000 g for 20.0 min. Two millilitres of supernatant was collected and added to two millilitres of ddH_2O and four hundred microliters of freshly prepared ferric chloride (0.1 % w/v) in a test tube, and allowed to stand for 10.0 min at room temperature. The absorbance was measured at 700 nm using UV-VIS spectrophotometer double beam (Labtech 2802). Following the above assay procedure, the absorbances of the standard antioxidant, (ascorbic acid), and control, (devoid of standard or test fraction), were also measured. The percentage inhibition (Equation 2) was evaluated using the model hereunder, and this was employed in the extrapolation of the respective IC_{50} values for the respective fractions.

$$\text{FRP (\% Inhibition)} = \frac{A_{control} - A_{test}}{A_{control}} \times \frac{100}{1} \quad (2)$$

Such that: $A_{control}$ designated absorbance for control at 700nm; A_{test} designated absorbance for test fraction or standard at 700nm.

2.8 Hydrogen peroxide (H_2O_2) scavenging activity (HPA) in samples

The capacity of *A. vogelii* to scavenge H_2O_2 was evaluated following the assay procedure established in literature (Yildirim et al. 2001). Here, 4000 µl of test fraction or standard ascorbic acid at graded concentrations of 0-1000 µg/ml, and 600 µl of 0.004 M hydrogen peroxide (H_2O_2) were added to test tubes, allowed to stand for 10.0 min and absorbances were measured at 230 nm using UV-VIS spectrophotometer double beam (Labtech 2802). Thus, the percentage H_2O_2-scavenging activities (Equation 3) of the test fractions were computed, following the equation hereunder, and extrapolated in terms of their IC_{50} values.

$$H_2O_2 \text{ scavenging activity } (\%) = \left[\frac{(A_{control} - A_{test})}{A_{control}} \times \frac{100}{1} \right] \quad (3)$$

2.9 Total antioxidant capacity (TAC: phosphomolybdate assay) of samples

The application of ascorbic acid as standard antioxidant was anchored in the assessment of free radical scavenging capacity of *A. vogelii* following phosphomolybdate method on the basis of its reducing power on Mo^{6+} to Mo^{5+}, which resulted in the formation of phosphate-Mo^{5+} complex at a pH less than 7 (Umamaheswari & Chatterjee 2008). The assay mixture in a test tube contained 1000 µl of test fraction or ascorbic acid at graded concentrations of 100-1000 µg/ml and 1000 µl of reagent A (0.60 M H_2SO_4, 28.0 mM Na_3PO_4 and 4.0 mM $(NH_4)_2MoO_4$). The mixture was incubated at 95°C for 90 min in a water bath and allowed to cool. In each case, the absorbance was measured immediately at 765 nm using UV-VIS spectrophotometer double beam (Labtech 2802). Therefore, the total antioxidant capacity (TAC) was computed and extrapolated in terms of percentage inhibition and IC_{50} values for the respective fractions (Equation 4).

$$\text{Total antioxidant effect } (\%) = \left[\frac{(A_{control} - A_{test})}{A_{control}} \times \frac{100}{1} \right] \quad (4)$$

Such that: $A_{control}$ designated absorbance for control at 765 nm; A_{test} designated absorbance for test fraction or standard at 765 nm.

2.10 Statistical analysis

Experimental results are expressed as mean ± SEM (= standard error of mean) of triplicate determinations. One-way analysis of variance (ANOVA) was employed using Duncan's Multiple Range Tests (DMRT) to establishing the degree of significance among test fractions, and mean differences whose $p < 0.05$ were considered to differ significantly. GraphPad Prism 6.0 software was also employed in the extrapolation of IC_{50} value as an inverse function of antioxidant activity of *A. vogelii*.

3. Results

The evaluation of TPC and TFC in the respective fractions, as indicated in Table 1, was based on their characterized antioxidant potentials in samples of interest. Following the assay procedures highlighted above, there was marked significant difference ($p<0.05$) among various fractions for TPC and TFC respectively. Comparatively, the n-hexane fraction showed the highest amount of these polyphenolic compounds, where TPC and TFC were numerically expressed as (263.30 ± 0.60) mgGAE and (1159.67 ± 8.82) mgRE per gram of dried plant sample among other fractions. Therefore, n-hexane fraction > methanol fraction > crude fraction in terms of their TPC and TFC respectively.

Table 1 Quantifications of bioactive compounds with antioxidant properties from various fractions of *A. vogelii*

Samples	% Yield	Assay Type	Quantification
Crude fraction	34.09	TPC	2.81 ± 0.50*
		TFC	503.00 ± 15.28*
n-Hexane fraction	22.61	TPC	263.30 ± 0.60**
		TFC	1159.67 ± 8.82**
Methanol fraction	15.40	TPC	241.67 ± 8.00***
		TFC	1076.33 ± 8.82**

NB: S.I units for phenolic and flavonoid contents were determined in mg Eq of gallic acid and rutin per gram of dried sample. Results were expressed as mean ± SEM of triplicate determinations. Phenolic fractions marked with different asterisks differ significantly ($p<0.05$) and flavonoid fractions marked with the same asterisks showed no significant difference ($p>0.05$). The petroleum ether, chloroform and the combined fractions were devoid of polyphenolic compounds.

Table 2 reveals the amount of *A. vogelii* leaves in various fractions responsible for 50 % inhibition of DPPH, FRP, HPA and TAC. In view of the various assays, the results showed marked significant difference ($p<0.05$) among the fractions with the exception of crude and methanol fractions for DPPH and TAC assays. The IC_{50} was highest in crude extract followed by methanol fraction, n-hexane fraction and lowest in standards used, in each case, for the respective free radical-scavenging assays. Therefore, this clearly shows that the antioxidant activity among the fractions increases in this fashion: standard > n-hexane fraction > methanol fraction > crude extract.

Table 2 IC_{50} values of various fractions of *A. vogelii* for different free radical scavenging assays.

Samples	IC_{50} values expressed in µg/ml			
	DPPH	FRP	HPA	TAC
n-Hexane fraction	317.33 ± 19.22^{a}	270.17 ± 5.68^{a}	490.43 ± 0.33^{a}	439.40 ± 1.11^{a}
Methanol fraction	556.00 ± 3.21^{b}	442.87 ± 14.99^{b}	645.30 ± 2.55^{b}	519.40 ± 3.95^{b}
Crude Extract	613.20 ± 32.75^{b}	504.97 ± 1.46^{c}	762.33 ± 23.74^{c}	531.03 ± 10.16^{b}
Standard	16.71 ± 0.31^{c}	4.03 ± 0.06^{d}	2.01 ± 0.06^{d}	8.86 ± 0.08^{c}

*Mean IC_{50} values designated by different alphabets down each column differ significantly at $p < 0.05$. **IC_{50} (µg/ml) represents the effective inhibition concentration at which 50 % of the respective radicals are scavenged.

Figure 2gives a progressive inhibition of DPPH radical in a dose-dependent manner for various fractions from 0-1000 µg/ml. This reflects a significant decrease ($p<0.05$) in the amount of DPPH radical due to the free radical-scavenging capacity of the plant sample inherent in the respective fractions. The figure clearly shows optimal inhibition of DPPH radical at 1000 µg/m. Here, the positions of the sample fractions correspond to their IC_{50}s, as indicated in Table 2.

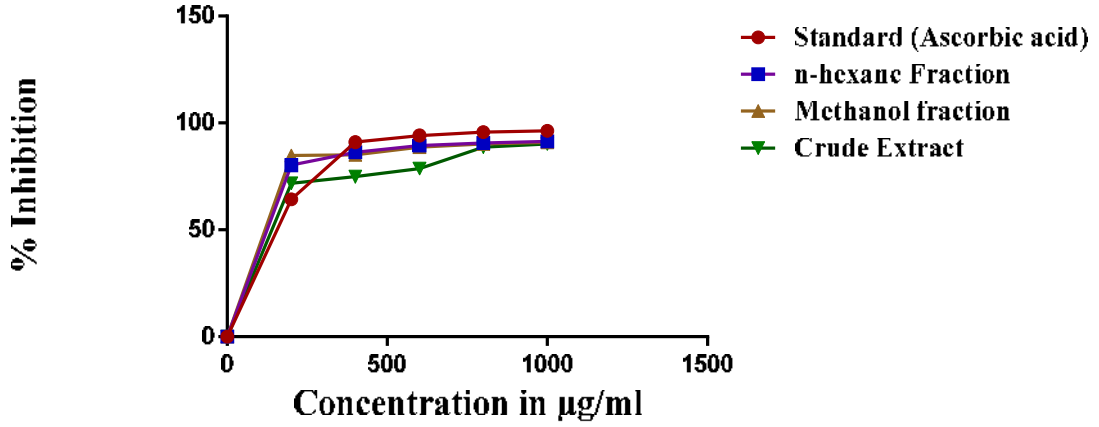

Figure 2. DPPH radical scavenging activity for different fractions of *A. vogelii*

Figure 3 explains that a dose-dependent increase from 0-1000 µg/ml for various fractions (*A. vogelii* leaves) in relation to standard ascorbic acid significantly caused a progressive inhibition of the oxidant (ferric chloride), which marks the reducing power of the plant to change the oxidation state of ferric chloride from +3 to +2. Therefore, the reducing power among the fractions was best in standard ascorbic acid, followed by n-hexane,, methanol and then crude extract with maximum inhibition observed at 1000 µg/ml in each case.

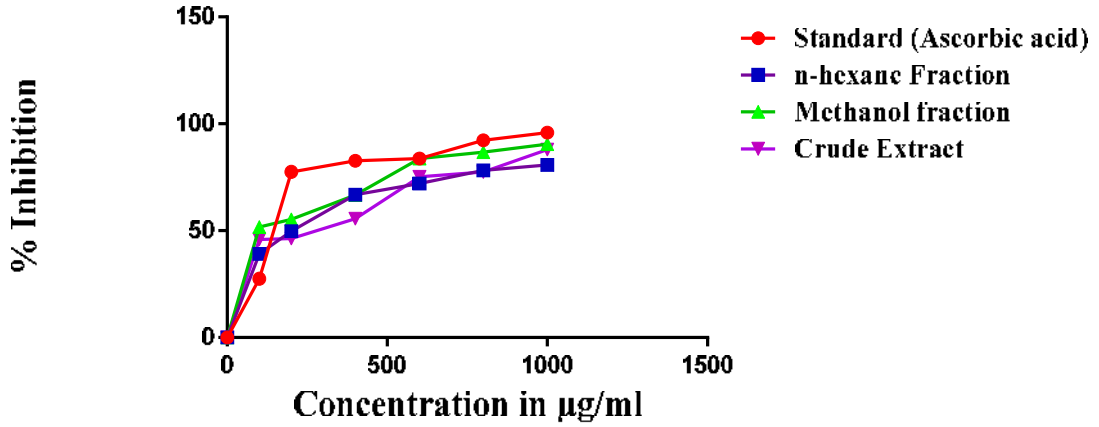

Figure3. Reducing power of various fractions from *A. vogelii*

The H_2O_2-scavenging activity of the various fractions of *A. vogelii* leaves is illustrated in Figure 4. The significant inhibition at $p<0.05$ in an increasing dose reflects the ability of the plant to scavenge H_2O_2 with maximum inhibition obtained at 1000 µg/ml. The figure then suggest n-hexane fraction as the fraction with the best free radical-scavenging capacity in relation to standard ascorbic acid for H_2O_2.

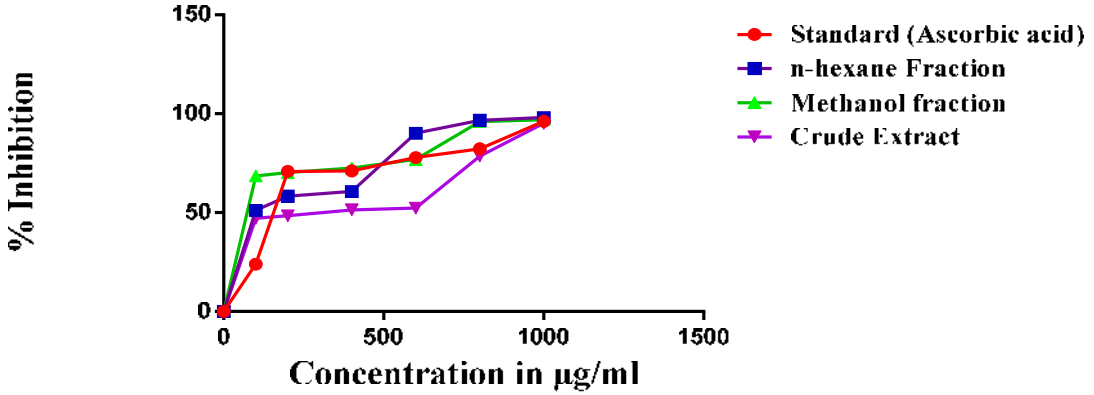

Figure 4. H_2O_2 scavenging activity of various fractions of *A. vogelii*

The totality of antioxidant activity in the leaves of *A. vogelii* is shown in Figure 5 following phosphomolybdate method. There was marked significant inhibition ($p<0.05$) of phosphomolybdate, which corresponds to the reducing power of the designated samples on Mo^{6+} to Mo^{5+} at increasing concentrations. Here, the reducing power on phosphomolybdate was significantly highest in n-hexane fraction and least in crude extract in relation to standard ascorbic acid.

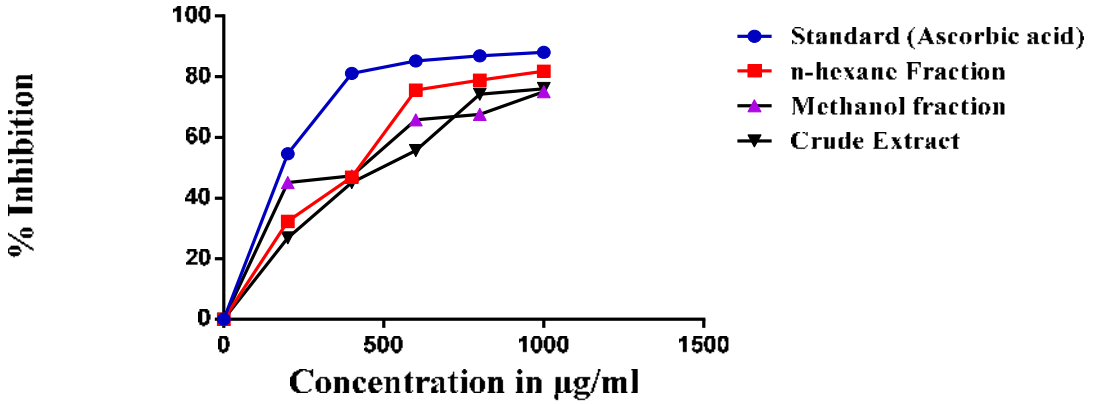

Figure 5. Total antioxidant capacity of various fractions of *A. vogelii*.

4. Discussion

Although the chloroform fraction and the three solvent combinations were devoid of phenolics and flavonoids, but the TPC and TFC of the respective fractions revealed the comparative antioxidant potential of the bioactive compounds present in *A. vogelii* (Table 1). The table also indicated the percentage yields of crude, n-hexane and methanol fractions. Therefore, the order of antioxidant capacity of these fractions encompasses; n-hexane fraction > methanol fraction > crude fraction, on the basis of their TPC and TFC respectively. Regardless of the fractions involved, the intrinsic phenolic and flavonoid contents of *A. vogelii* validated earlier reports that these

bioactive compounds exhibits antioxidant potential independently, but with greater efficacy when they act synergistically (Pereira et al. 2009; Yadav et al. 2016).

The deleterious effects initiated by undue synthesis of ROS, especially during induction of various diseases was reported to be circumvented by natural antioxidants, which are chiefly known to sequester these free radicals with concomitant enhancement of antioxidant defence system (Umamaheswari & Chatterjee 2008; Saeed et al. 2012). Consequently, this development necessitated the evaluation of antioxidant activity of *A. vogelii* by various in vitro assay methods like DPPH, FRP, HPA and TAC respectively. The results of the DPPH scavenging capacity by *A. vogelii* relative to standard ascorbic acid showed substantial DPPH inhibitory effect at increasing concentrations with n-hexane fraction taking the lead, followed by methanol fraction and then least by crude fraction (Figure 1). In a dose-dependent fashion, the efficacy of the respective fractions to significantly scavenge DPPH radical was clearly observed with maximum scavenging activity observed at 1000 µg/ml (Figure 1). Scientific reports showed the usefulness of DPPH radical to form a stable diamagnetic molecule in view of its role as an electron acceptor in the presence of an antioxidant, which was an implication of its discoloration and scavenging activity (Mahdi-Pour et al. 2012; Yadav et al. 2016). This was scientifically validated through the extrapolation of IC_{50} values of n-hexane fraction, methanol fraction, crude fraction and standard ascorbic acid, which differed significantly ($p < 0.05$) (Table 2). Therefore, the IC_{50}, which depicts the concentration of *A. vogelii* fractions that caused 50 % inhibition of DPPH radical via its proton-donating ability, is a function of scavenging effect occurring in the following order: n-hexane fraction < methanol fraction < crude extract relative to the reference antioxidant (ascorbic acid), with the least IC_{50} value. This plausibly suggested that n-hexane fraction exhibited the best antioxidant potential with significantly low IC_{50} value comparable to other fractions. Thus, the above inferential statement correlates with literature that the antioxidant activity of a substance is inversely related to its free radical scavenging capacity (Mahdi-Pour et al. 2012; Olugbami et al. 2014).

One of the significant mechanism of action employed by bioactive compounds with antioxidant potential reflects its electron-donating capacity to cause a drastic reduction of Fe^{3+}/ferricyanide complex to Fe^{2+} ions with consequent transformation of the yellow test solution to green or blue colour, which is dependent on the degree of reducing power of the sample (Mahdi-Pour et al. 2012; Olugbami et al. 2014). It was recapped in literature that the electron-donating capacity of the antioxidant causes a breakage in free radical synthesis thereby preventing the onset of lipid peroxidation (Mathew & Abraham 2006). Based on the results obtained in the present study, FRP assay for the respective fractions of *A. vogelii* showed a marked significant increase of percentage inhibition of Fe^{3+}/ferricyanide complex with increasing concentration such that the maximum reducing power was observed at 1000 µg/ml (Figure 3). Relatively besides the reference antioxidant, n-hexane fraction showed the best reducing power capacity. Thus, the reducing power capacity of the three fractions was observed to increase in the following order: crude extract < methanol fraction < n-hexane fraction. The ferric reducing power of *A. vogelii* leaves was expressed in terms of IC_{50} values, where it showed a marked significant difference ($p < 0.05$) among the respective fractions in relation to the reference antioxidant (Table 1). The findings from the present study indicated that the various fractions of *A. vogelii* exhibited antioxidant activities. However, FRP assay revealed that n-hexane fraction had the best antioxidant capacity, (with significantly lower IC_{50}), comparable with other fractions, and this conforms with previous reports (Olugbami et al. 2014; Sasikumar & Kalaisezhiyen 2014).] Therefore, the leaves of *A. vogelii* may be promising in view of its antioxidant profile for good health of humans.

The present study succinctly revealed the comparative efficacy of the various fractions to scavenge H_2O_2 and enhance its transformation to H_2O as a result of the intrinsic polyphenolic and flavonoid contents of *A. vogelii* (Figure 4). Studies have shown that the toxicity of H_2O_2 to tissues on passage of cell membrane was associated with the synthesis of hydroxyl radicals (Gulcin et al. 2010). The relevance of H_2O_2 scavenging capacity to modern toxicologists may not be over-emphasized as a result of its toxicity indications, and the in vitro assessment of H_2O_2-scavenging capacity was linked to the presence of polyphenolic compounds in plants with electron-donating ability to neutralize H_2O_2 to H_2O (Chanda & Dave 2009; Miaffo et al. 2016). The toxicity impact of H_2O_2 was observed in the inactivation of sulfhydryl groups of amino acid side chains at the active site of some enzymes and transversing of biological membranes to react with internal Fe^{2+} and Cu^{2+} ions with the formation of toxic hydroxyl radical in tissues (Miaffo et al. 2016). Therefore, the sequestration of H_2O_2 by bioactive compounds with antioxidant activities is significant in the enhancement of antioxidant defence system of biological systems. The IC_{50} values reflecting the concentration of the plant that caused 50 % inhibition of H_2O_2 toxic action showed marked significant differences ($p < 0.05$) among the various fractions in relation to standard rutin (Table 1). Although the various fractions elicit moderate antioxidant activity comparable to the reference antioxidant (rutin), but n-hexane fraction indicated the best antioxidant activity and greatest capacity to scavenge H_2O_2 with significantly lowest IC_{50}.

It was reiterated scientifically that the availability of polyphenolic compounds in plants may generally be a reason for the change of oxidative state of molybdenum from +6 to +5 during *in vitro* assessment of TAC where a stable phosphate-Mo^{5+} complex was formed (Umamaheswari & Chatterjee 2008; Sasikumar & Kalaisezhiyen 2014). At graded concentrations of 200-1000 µg/ml, all fractions from the leaves of *A. vogelii* showed marked antioxidant effect with increasing inhibition of phosphomolybdate (Figure 5). Although the total antioxidant effect exhibited by various fractions on phosphomolybdate was less than that of the reference antioxidant, but it was observed that the increasing order of inhibition among the fractions encompassed; crude extract < methanol fraction < n-Hexane fraction. This observation was validated through the extrapolation of IC_{50} values among the various fractions of *A. vogelii* leaves, with marked significant differences ($p < 0.05$) (Table 1). This trend of higher IC_{50} value with lower antioxidant efficacy comparatively agrees with earlier reports (Mahdi-Pour et al. 2012; Sasikumar & Kalaisezhiyen 2014). Also, the correlation between TAC and IC_{50} exhibited by *A. vogelii* leaves was a clear-cut indication that the intrinsic phenolic and flavonoid contents of the plant plays a synergistic role (Shahriar et al. 2013). Generally, the empirical survey carried out by Sunday and his research team on in vitro assessment of antioxidant capacity of *A. vogelii* roots using DPPH assay technique showed a similar correlation of moderate antioxidant potential with the present study (Sunday et al. 2016). To this end, the antioxidant potential of the plant may be suggestive of its usefulness in the effective management of diseases characterized by oxidative stress irrespective of the aerial parts of the plant employed.

5. Conclusion

The exhibition of antioxidant activity associated with intrinsic polyphenolic compounds suggested the usefulness of *A. vogelii* leaves for the development of antioxidant-based drugs in pharmaceutical industries. The data generated in the present study also suggested that *A. vogelii* leaves are natural sources of antioxidants for good health, and the isolation of these bioactive compounds may be of pharmacological priority in further studies.

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