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In vitro Free Radical Scavenging Activities of the Saponin-rich Fractions from *Vernonia amygdalina* Del. (Compositae)

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Abstract *Vernonia amygdalina* leaves is consumed fresh or dried after removal of bitter taste by the peoples of the South-Eastern Nigeria, especially the Igbos. It is also widely used in cooking and serves as a vegetable and ethno-medicinal herb against many ailments. Not much is known of its *in vitro* antioxidant potentials, especially the major saponins and those of its fractions. In this study, the 80 % methanol extract of the leaves was fractionated by analytical TLC-guided column chromatography, preparative TLC and further purified with HPLC using gradient concentrations of solvent mixtures of chloroform, n-butanol, methanol and distilled water yielding VASC, VASB, VASM70, VASM65 and VASM50. Quantitative phytochemical analysis gave their % total saponin content as 55.8, 64.6, 21.4, 78.8 and 33.8 respectively. The 5 saponin-rich fractions (VASC, VASB, VASM70, VASM65 and VASM50) showed no significant signs of toxicity in mice at oral doses of 1500 mg/kg although VASM50 at 1500 mg/kg caused increased urination and watery fecal deposits which disappeared after 18hrs. The saponin-rich fractions of *Vernonia amygdalina* were evaluated for *in vitro* free radical scavenging properties using 2, 2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$), ferric reducing antioxidant power (FRAP), hydrogen peroxide scavenging assay, hydroxyl radical scavenging assay, ABTS radical cation scavenging activity assay, anti-lipid peroxidation assay, B-carotene bleaching assay and superoxide anion using ascorbic acid, butylated hydroxytoluene (BHT), catechin and gallic acid as reference standards. The results showed that the saponin-rich fractions from *Vernonia amygdalina* leaves had a potent DPPH radical scavenging and FRAP activities comparable to those of reference standards used. The IC50 values were also comparable to those of standards especially that of VASM65. Vernonia species are known to contain abundant saponins and flavonoids which are polar compounds and readily soluble in methanol. Most saponins and their derivatives of plant origin are known to possess great antioxidant potentials. This may explain the above observed antioxidant activities and thus, the use of the leaves in different traditional curative therapies in Eastern Nigeria, and as vegetable.

Keywords: *Vernonia amygdalina, Saponins, Antioxidants, free radicals, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP)*

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1. Introduction

Oxidants, commonly called free radicals, have been shown to be responsible for oxidative damage to blood [23], organs, tissues and cells, and may therefore be implicated in the etiology and progression of many cellular and molecular diseases, including testicular and cervical cancers, hepato-toxicities, nephro-toxicities, cardio-toxicities, lymphomas and leukemia and may potentially induce diabetes and spontaneous aging via lipid peroxidation. Oxidants can initiate endocrine disruption, hemolysis, cell lysis, immunosuppression and mal-steroidogenesis.

Several physiological defects, imbalances and disease states have been attributed to these oxidants in living systems which induce oxidative stress. Oxidative stress has been shown to be caused by oxidants (including the superoxide radical, hydroxyl radical (OH•), hydrogen peroxide (H_2O_2) and lipid peroxide radicals, reactive oxygen species (ROS)) produced as a normal consequence of biochemical processes in the body due to increased exposure to xenobiotics and contaminated environments [23].

Several dietary and medicinal phytochemicals that inhibit, reverse or retard diseases caused by oxidative and inflammatory processes have been documented [24]. Many plant materials including vegetables and fruits are known to contain anti-oxidative phytochemicals such as saponins, flavonoids, phenolic and polyphenolic compounds. These plants include, *Vernonia amygdalina, Garcinia kola, Gongronema latifolia, Sida cordata, Curcumbita maxima, Eryngium foetidum, Bridelia micrantha, Allium cepa and Lasianthera africanum*. There is therefore need for novel antioxidants with better

pharmacological potencies and medicinal plants to readily provide sources for such new drugs discovery.

The genus Vernonia has about one thousand species, and members of the genus are widely used as food and medicine. In Nigeria, *Vernonia amygdalina* is domesticated and used as fresh vegetable for preparing soup and yam porridge and other dishes after several squeezing and washing with cold or hot water to reduce the excessive bitter taste to a desired level. It is very different from *Vernonia calvoana* which has a sweet taste and is only popular in the Southern riverine areas of Nigeria. It is used in traditional medicine as an anthelmintic and antidiabetic. It is also used for curing naval aches and constipation [8]. There is also a folkloric claim that it is used to quench food poisoning.

Saponins are naturally occurring tri-terpenoids found in many food materials derived from plants. They are secondary plant metabolites containing a steroid or tri-terpenoid aglycone with a number of different carbohydrate moieties which are linked through either an ether or an ester linkage at one or more glycosylation sites.

Saponins are known to have important biological activities in humans, including hypocholes- terolaemic, haemolytic, immunostimulatory and anti-tumourigenic activities [14], as well as chemoprotective activities [3]. Despite a great deal of analytical research over the past 30 years, detailed studies of the biological activities have been hampered owing to the lack of availability of large quantities of purified saponins, as well as the lack of efficient procedures for the detection and quantification of these compounds in natural products, foods and food products. A number of acceptable HPLC protocols exist [5,19,20,31,32,33], but the preparation of pure analytical standards is laborious and difficult owing to the large numbers of very similar saponin glycosides.

Searches for plant-derived medications have accelerated in recent years as ethno-pharmacologists, botanists, microbiologists, and natural products chemists are greatly involved in exploring the universe for phytochemicals and ‘‘leads’’ which could be developed for the treatment of numerous diseases [24].

The present study was carried out to investigate the possible antioxidant properties of the saponin-rich fractions from *Vernonia amygdalina* leaves using series of *in vitro* models.

2. Materials and Methods

2.1. Source of Plant Material and Identification

The leaves of *V. amygdalina* were harvested from Owerri, in Imo State, Nigeria. The plant was identified and authenticated by Mr. A. Ozioko, a taxonomist with the Biodiversity Development Centre Program (BDCP) Nsukka, Enugu State, Nigeria.

2.2. Extraction and fractionation of Crude Extract

The air-dried leaves were pulverized using a laboratory mill. 800 g of the pulverized material was first de-fatted with petroleum ether using a soxhlet extractor (Büchi, Switzerland) at 40°C. It was then extracted by cold percolation with a mixture of Methanol: Distilled water (80:20) for 72 h at room temperature. The solvent was evaporated from the extract using a rotary evaporator at 40°C. The alcohol-free extract was centrifuged at 3000 rpm for 8 minutes to sediment gummy impurities. The supernatant was loaded into a 4 cm x 50 cm glass column pre-loaded with silica gel (Silica Gel 60-120 mesh, 60A, Oxford, India) pre-conditioned with 30% methanol. The column was then successively eluted with 500 ml of chloroform (VASC), 500 ml of n-butanol (VASB), 500 ml of 70 % MeOH (VASM70), 450 ml of 65 % MeOH (VASM65), 450 ml of 50 % MeOH (VASM50) and 300 ml of 30% MeOH (VASM30) respectively.

2.3. Purification of the Saponin-rich Fractions from the Methanol Leaf Extract of *V. amygdalina*

5 mg of each fraction was centrifuged separately, filtered and was loaded onto a C-18 preparative column (C18 Extract-Clean™, Alltech Associates Inc, Deerfield, IL, USA). Inorganic impurities were further eluted with 600 ml/L methanol:water. The eluted fractions were diluted with water (final methanol concentration of 400 ml/L) and were further purified using HPLC to isolate the saponins. The HPLC system (Thermo Separation Products, Riviera Beach, FL, USA) was equipped with a diode-array detector (UV6000) and a column oven (35°C). A reverse-phase C-18 column (250 × 4.6 mm, ‘Luna-2’ Phenomenex, Torrance, CA, USA) was employed. Elution was performed using water and methanol, acidified with 0.1 g/L formic acid, at a flow rate of 1 ml/min. A chromatography program was developed, starting with an isocratic step at 100 ml/L methanol for 3 min, then a linear gradient up to 950 ml/L methanol in 8 min, an isocratic step at 950 ml/L methanol for 4 min, and equilibrating at the starting conditions for an additional 4 min. The solution was then lyophilized (Heto Dry Winner, Heto-Holten, Gydevank, Denmark) and the saponins powder were kept in a desiccator. Saponins were quantified using a colorimetric reaction mixture thus: dry powder (0.2–1 mg) was dissolved in acetic acid (1.5 ml), to which sulfuric acid (1 ml) was added. The absorbance (530 nm) of the reaction mixture was determined after 15 min incubation at room temperature [10].

Each 100ml effluent collected was profiled using TLC with a mobile phase system of acetone, chloroform and methanol (1:4:2) [16]. Spots were located using saturated iodine chamber. Aliquots with similar profile were pooled together, concentrated over a water bath and allowed to evaporate to dryness at room temperature.

All fractions were concentrated with rotary evaporator at 40°C, weighed and used for the various anti-oxidative studies. VASM30 showed no presence of saponins in the preliminary phytochemical analysis and was not used further in the antioxidant assays

2.4. Acute Toxicity Test

Mature albino mice (22 – 33 g) of both sexes were weighed and randomly separated into 4 groups (1–11) of 5

mice per group. Groups 1–10 were given varying doses (250 and 1500 mg/kg b.w.) of the saponin-rich fractions from *V. amygdalina* respectively. Group 11 was given 10 ml/kg distilled water to serve as control. The mice were allowed access to feed and water *ad libitum* for 72 h and observed for signs of toxicity and death. Animals for the experiment were kept in accordance with ethics in the guidelines for animal care as contained in the animal ethics handbook of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria.

2.5. *In vitro* Antioxidant Analysis

Fractions VASC, VASB, VASM70, VASM65, VASM50 and positive standards (ascorbic acid, butylated hydroxytoluene, catechin and gallic acid) were assayed for different *in vitro* anti-oxidant capacities. Of each fraction, 800 µg was dissolved in 1 ml analytical grade methanol. These solutions were further serially diluted to 400, 200, 100, 50 and 25 µg/ml. In all the different antioxidant assays, same dilutions of sample and standards were used. Standards were altered as per assay requirement. The fractions at different concentrations were prepared in triplicates.

2.5.1. Evaluation of Antioxidant Capacity Using the 1, 1-diphenyl-2-picrylhydrazyl Radical (DPPH) Spectrophotometric Assay

The free radical scavenging activity of the saponin-rich fractions were analyzed by the DPPH assay following a standard method [21]. A given volume (2 ml) of each fraction at varying concentrations ranging from 25-800 µg/ml each was mixed with 1 ml of 0.5 mM DPPH (in methanol) in a cuvette. The absorbance at 517 nm was taken after 30 min of incubation in the dark at room temperature. The experiment was done in triplicate. The percentage antioxidant activity was calculated as follows:

$$\%\ \text{Antioxidant Activity}\ [\text{AA}] = 100 - \frac{(\text{Abs sample} - \text{Abs blank})\ \text{X}\ 100}{\text{Abs control}}.$$

Methanol (1.0 ml) plus 2.0 ml of the fractions were used as the blank while 1.0 ml of the 0.5 mM DPPH solution plus 2.0 ml of methanol was used as the negative control. Ascorbic acid was used as reference standard.

2.5.2. Ferric Reducing/Antioxidant Power (FRAP) Assay

The total antioxidant potential of fractions was determined using a ferric reducing ability of plasma (FRAP) assay of Benzie and Strain [2] as a measure of "antioxidant power". FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe^{II}-tripyridyltriazine compound from colorless oxidized Fe^{III} form by the action of electron donating antioxidants. Standard curve was prepared using different concentrations (100-1000 µmol/L) of $FeSO_4$ x $7H_2O$. All solutions were used on the day of preparation. In the FRAP assay, the antioxidant efficiency of the saponin-rich fractions under the test was calculated with reference to the reaction signal given by an Fe^{2+} solution of known concentration, this representing a one-electron exchange reaction. Ascorbic acid was measured within 1 h after preparation. The fractions to be analyzed were first adequately diluted to fit within the linearity range. All determinations were performed in triplicate. Calculations were made by a calibration curve.

$$\text{FRAP value of sample}\ (\mu\text{M}) = \frac{\text{changes in absorbance from 0 – 4 or 8 min}}{\text{changes in absorbance of std 0 min – 4 or 8 min}} \times \text{FRAP value of std}\ (1000\ \mu\text{M}).$$

2.5.3. Hydrogen Peroxide Scavenging Assay

The method of Bokhari et al. [4] was followed to investigate hydrogen peroxide scavenging capacity of fractions. Hydrogen peroxide (2 mM) solution was prepared in phosphate buffer (50 mM, pH 7.4). Fractions (100 µl) were pipetted into flasks and their volume made up to 400 µl with 50 mM phosphate buffer (pH 7.4). H_2O_2 solution (600 µl) was added and absorbance at 230 nm was taken 10 min after vortexing the flasks. Percent scavenging activity was determined by following formula;

$$H_2O_2\%\ \text{scavenging activity} = (1 - \frac{\text{absorbance of fraction}}{\text{absorbance of control}})\text{x}\ 100$$

Ascorbic acid served as standard.

2.5.4. Hydroxyl Radical Scavenging Assay

The antioxidant activity was evaluated by method reported by Halliwell et al [12]. The reaction mixture comprised of 2-deoxyribose (2.8 mM, 500 µl) in 50 mM of phosphate buffer, 100 µl of 0.2 M hydrogen peroxide solution, 200 µl of 0.1M ferric chloride, 0.1M EDTA and 100 µl of test fraction. The reaction was initiated by the addition of 100 µl of ascorbate (0.3M). The mixture was incubated at 37°C for 60 min. TCA (2.8% w/v, 1 ml) and 1 ml of thiobarbituric acid (TBA) solution in 50 mM of sodium hydroxide (1%; w/v) was added. This reaction mixture was heated for 15 min in boiling water bath and then allowed to cool. Absorbance was recorded at 532 nm.

$$\text{Hydroxyl scavenging activity}\ (\%) = \left(1 - \frac{\text{Absorbance of fraction}}{\text{Absorbance of control}}\right) \times 100.$$

2.5.5. ABTS Radical Cation Scavenging Activity

Re et al. [29] methodology was slightly modified as followed for ABTS (2, 2 azobis, 3 ethylbenzothiozoline-6-sulphonic acid) radical cation scavenging activity. ABTS (7 mM) solution was reacted with 2.45 mM potassium persulfate and kept overnight in dark for generation of dark colored ABTS radicals. For the assay, the solution was diluted with 50 % ethanol for an initial absorbance of 0.7 at 745 nm. Activity was determined by adding 100 µl fractions of different dilutions with 1 ml of ABTS solution in glass cuvette. Decrease in absorbance was measured after one min and 6 min of mixing. The difference was calculated and compared with control. Percent inhibition was calculated by formula:

$$\% \text{ ABTS scavenging effect} = \left(\frac{\text{control absorbance} - \text{fraction absorbance}}{\text{control absorbance}}\right) X100.$$

2.5.6. Anti-lipid Peroxidation Assay

This assay was performed as illustrated by Dorman et al. [6]. An aliquot of egg yolk (10%, w/v) was prepared in KCl (1.15 %, w/v). The yolk was homogenized for 30 sec and subsequently subjected to centrifugation for 15 min. Each fraction (100 µl) at varying concentrations (800, 400, 200, 100, 50 and 25 µg/ml in methanol) and 500 µl of yolk homogenate were pipetted into flasks and volume was made up to 1 ml with distilled water. It was mixed with 1.5 ml of acetic acid (20 %, pH 3.5) and TBA (0.8 %, w/v) in sodium dodecyl sulphate (1.1 %, w/v). The reaction mixture was vortexed and incubated for 60 min in water bath. n-Butanol was added after cooling at room temperature, stirred and then centrifuged for 10 min at 3000 rpm. Butylated hydroxytoluene served as standard. The absorbance at 532 nm of supernatant was recorded.

The percent anti lipid peroxidation was determined by the formula (1-S /C) ×100

Where, C = Absorbance of control and, S= Absorbance of test fraction.

2.5.7. β -Carotene Bleaching Assay

Elzaawely et al. [7] modified method was used for β-carotene bleaching assay. β-carotene (2 mg) was dissolved in 10 ml of chloroform and blended with 20 mg of linoleic acid and 200 mg of Tween 20 followed by removal of chloroform under nitrogen with subsequent addition of 50 ml of distilled water with vigorous shacking to prepare β-carotene linoleate emulsion. An aliquot of each fraction (50 µl) was mixed with 1ml of the emulsion, vortexed and absorbance was determined at 470 nm immediately against the blank solution. Capped tube was then kept in a water bath at 45°C for 2 h and the difference between the initial readings is calculated by measuring the reading after 2 h. β-Carotene bleaching inhibition was estimated by the following equation:

$$\% \text{ bleaching inhibition} = \left(\frac{A_{ot} - A_{120t}}{A_{oc} - A_{120}}\right) x100.$$

2.5.8. Superoxide Anion Radical Scavenging Assay

Riboflavin light NBT system assay was followed for superoxide radical scavenging activity as described by Nishikimi [22]. The reaction mixture containing 0.5 ml of phosphate buffer (50 mM, pH 7.6), 0.3 ml riboflavin (50 mM), 0.25 ml PMS (20 mM), and 0.1 ml NBT (0.5 mM), prior to the addition of 1 ml fraction in methanol. Florescent lamp was used for starting the reaction. Absorbance was recorded at 560 nm after incubation for 20 min under light. The percent inhibition of superoxide anion generation was calculated using the following formula:

$$\% \text{ Percent scavenging activity} = \left(1 - \frac{\text{Absorbance of fraction}}{\text{Absorbance of control}}\right) \times 100.$$

2.6. Statistical Analysis

All data were expressed as Mean ± SD or % mean. Data were analyzed using One-way analysis of variance (ANOVA) at 5% level of significance.

3. Results

3.1. Quantitative Phytochemical Analysis

A major saponin band was observed at R_f = 0.25. The identity of this band as a saponin was primarily confirmed by anisaldehyde staining showing a violet-blue band at the same R_f. This major saponin was clearly observed in the butanol:water and methanol:water extracts. A second violet-blue band was observed at R_f = 0.5 and 0.75 suggesting the presence of other saponins in *Vernonia amygdalina* leaves.

Phytochemical analysis of the fractions showed the presence of saponins. The individual weight of the saponin content in each fraction were as follows:

Table 1.

Fractions	VASC	VASB	VASM70	VASM65	VASM50
Saponin content in %	55.8	64.6	21.4	78.8	33.8

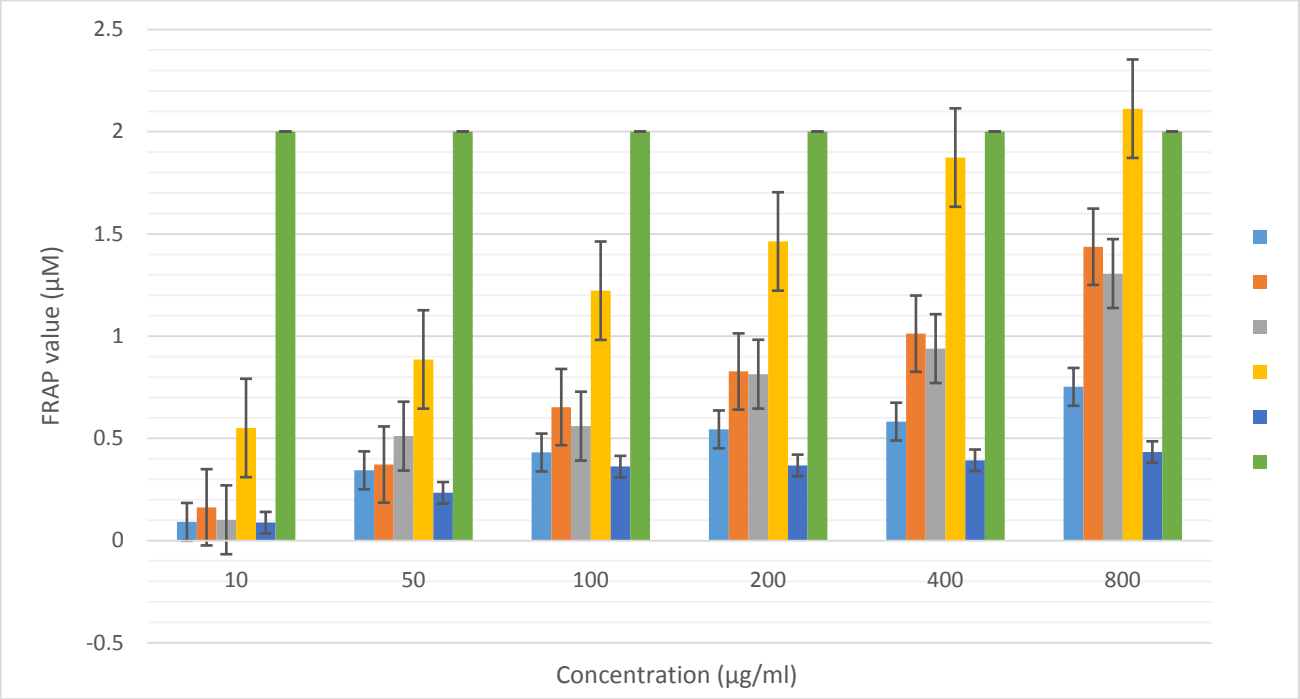
3.2. Acute Toxicity Studies

No mortality or adverse reaction was detected in mice during the 72 h observation period following oral administration of the fractions up to a dose of 1500 mg/kg although fraction VAM50 at 1500 mg/kg caused increased urination and watery fecal deposits which disappeared after 18 h.

Table 2. Antioxidant activities of saponin-rich fractions from methanol leaf extract of *V. amygdalina* using the DPPH assay method

Concentration (µg/ml)	% anti-oxidant activity					
	VASC	VASB	VASM70	VASM65	VASM50	Ascorbic acid
25	8.21*	12.32*	11.44*	33.14	9.74*	75.61
50	8.92*	38.14	18.29*	44.27	12.36*	76.02
100	15.36*	41.37	33.78	51.32	28.12*	76.52
200	29.42	48.44	41.26	66.18	35.66	78.87
400	44.66	55.27	44.78	77.18	39.82	79.98
800	51.36	63.45	56.29	79.34	49.21	82.37

* F < 0.05 significantly different from reference compound (Ascorbic acid).



* P < 0.05 significantly different from reference compound (Ascorbic acid).

Figure 1. *In vitro* anti-oxidant performance of saponin-rich fractions from *Vernonia amygdalina* leaves using the Ferric reducing antioxidant power (FRAP) spectrophotometric method and compared to ascorbic acid.

Table 3. IC_{50} values for different antioxidant assays of saponin-rich fractions of *Vernonia amygdalina* methanol leaf extract

	IC_{50} (µg/ml)					
Activity	VASC	VASB	VASM70	VASM65	VASM50	Standard
H_2O_2	303.33±1.44*	133.48±1.33	151.22±0.18*	98.12±0.77	289.29±1.29*	53.00±0.00
Hydroxyl	271.00±1.00*	147.12±1.19	156.41±2.10*	119.21±0.52	188.12±2.51*	105.00±0.12
ABTS	244.52±1.81*	101.41±11.34	217.00±1.61*	88.00±2.12	180.51±1.51*	51.01±0.22
Anti-lipid	171.41±1.17*	97.18±1.21*	138.41±2.13*	69.31±1.57	211.24±1.21*	34.12±0.13
B-carotene	177.41±2.51*	101.16±1.33*	127.33±1.51*	91.14±1.53	217.82±1.12*	30.11±0.22
Superoxide	188.72±2.78*	101.21±1.18	144.21±1.32*	81.67±2.61	131.27±1.28*	37.13±0.11

Values are expressed as mean±SD (n=3). * Means in rows significantly (P<0.05) different from the standard.

3.2.1. Antioxidant Capacity Using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) Radical Spectrophotometric Analysis

The result showed that the crude, VASM65 and VASB had comparable percentage activities at high concentrations (800 µg/ml) when compared with Ascorbic acid. VASM65 showed 79.34 % activity at 800 µg/ml while VASB gave 63.45 % at the same concentration compare to 82.37 % given by ascorbic acid (Table 2 and Figure 1). VASM50 on the other hand showed activities different from ascorbic acid even at high doses of 800 µg/ml (49.21 %), though not significant.

3.2.2. Ferric Reducing/Antioxidant Power Assay (FRAP)

The FRAP results were similar to the DPPH with VASM65 at 800 µg/ml giving a FRAP value of 2.112 ± 0.08 which is slightly higher than that of ascorbic acid even at 1000 µg/ml (FRAP value of ascorbic acid between 10 and 1000 µg/ml is 2) (Figure 1). It was also observed that the FRAP values increased with time between 4 and 8 min of measurement.

3.2.3. IC_{50} Values for Different Antioxidant Assays of saponin-rich Fractions of Vernonia Amygdalina Methanol Leaf Extract

The IC_{50} values show a remarkable correspondence in effects to those observed from values seen in DPPH and FRAP assays above with VASM65 having the lowest IC_{50} across the assays. Its values were not significantly different (P<0.05) from the different standards used for each assay (Table 3).

4. Discussion

Free radical scavenging agents popularly known as antioxidants are intermediates between chemical reactions and biological activities. They do not completely get rid of free radicals in the body but retard or minimize the damage caused and also inhibit processes of oxidation by neutralizing free radicals thereby becoming oxidized themselves [24]. Endogenous antioxidants prevent oxidation by reducing the rate of chain initiation. Antioxidants have been implicated as being useful

compounds in preservation of foods, beverages, raw materials and parts of formulations for different drugs used in treating several ailments.

Antioxidants consist of vitamins, polyphenols, flavonoids, saponins, minerals and endogenous enzymes such as superoxide dismutase, catalase and glutathione peroxidase that have the capability to neutralize unstable molecules [35].

Saponins, constitutively produced in many plant species, both in the wild and in domesticated crops exert a wide range of biological activities [18]. Some saponins have been shown to exhibit antibacterial, antifungal and insecticidal activities and, as such, have been suggested to constitute part of some plant defense systems. In cultivated plants, including many vegetables such as *Vernonia amygdalina* and *Gongronema latifolia*, triterpenoid saponins are generally predominant. Steroidal saponins are common in plants used as herbs or for their health-promoting properties [25,27]. The unique chemical nature of saponins demands tedious and sophisticated techniques for their isolation, structure elucidation and analysis [9,26]. The task of isolating saponins from plant material is complicated even more by the occurrence of many closely related substances in plant tissues, and by the fact that most saponins lack a chromophore. Thus, for many years, the complete characterization of saponins from even well-known saponin-containing plants was limited. However, recent renewed interest in medicinal plants and foods, along with the dramatic evolution of analytical tools, has resulted in a burst of publications presenting numerous novel saponins.

Purification of saponins traditionally consists of a liquid–liquid extraction step following the extraction procedure. The amphipathic nature of these compounds make this extraction tedious and inefficient. In this work, the 80 % methanol extract was used for further purification and identification of the major saponin in *Vernonia amygdalina* leaves were made. Chloroform, n-butanol and aqueous fractions, that form separate layers, were used in a single extraction step. Including the liquid–liquid extraction in the isolation process thus enhanced the efficiency and selectivity. The saponins were resolved using a preparative C-18 column and then an analytical C-18 column. The choice of solvents used in the isolation may serve as a separation tool.

DPPH and to a lesser extent, FRAP are fast, reliable and reproducible methods widely used to measure the *in vitro* antioxidant activities of pure compounds as well as plant extracts [1]. Substances that increase percentage antioxidant activity in DPPH spectrophotometric assay and FRAP value as seen in the concentration dependent increase in DPPH and FRAP by the fractions in this study (Table 2 and Figure 1), are assumed to have antioxidant activity [28]. These were confirmed by the IC_{50} values observed in the subsequent anti-oxidant tests (Table 3). These antioxidant activities may help in slowing or terminating the production of free radicals especially reactive oxygen species and/or other radicals produced in the process of generating them.

There have been several works on potential antioxidant compounds which will replace the suspected cancer-causing synthetic analogues like butylated hydroxytoluene (BHT) (Ito *et al.*, 1985). These results may lead to another avenue for novel sources of antioxidants and again be useful especially to those in rural areas. Saponins are valuable dietary supplement partly because they have high antioxidant potentials [17].

5. Conclusion

The results of different *in vitro* antioxidant activity assays indicate that the saponin fractions of *Vernonia amygdalina* possess appreciable DPPH, ABTS scavenging effects and metal ion chelating activity in a concentration-dependent manner. The antioxidant activities of the fractions may be attributed to their phenolic and saponin contents. This study helps in promoting increased consumption of *Vernonia amygdalina* leaves by the general public and offers opportunity to develop valued addition in the Nigerian vegetable supply chain. More works are on-going on the plant extracts to isolate, purify and characterize the biologically active antioxidant compounds.

Conflict of Interest

The authors declare no conflict of interest

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