PRELIMINARY PHYTOCHEMICAL AND *IN VITRO* ANTIOXIDANT STUDIES ON METHANOL EXTRACT OF *VERNONIA CALVOANA* LEAVES AND ITS POLAR FRACTIONS

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Abstract

This study examines the possible in vitro antioxidant activities of the methanol extract of Vernonia calvoana leaves and its phytochemical constituents. Fresh leaves were collected, dried at room temperature and ground into powder with a laboratory mill. The powdered material was then de-fatted with petroleum ether and the dry marc, extracted in 80 % methanol for 72 h. The filtrate was dried in a rotary evaporator at 40°C. Fractionation of the crude methanol extract using gradedconcentrations (30, 40 and 50 %) of aqueous MeOH yielded F_1, F_2, F_3 and F_4 fractions. 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) and Ferric reducing/antioxidant power (FRAP) spectrophotometric methods were used to evaluate the in vitro antioxidant potentials of the crude and the polar fractions. In the DPPH assay, Fraction F_1 (F1) did not only exhibit the highest antioxidant potential compared to the crude and the rest of the fractions, its antioxidant activities were also consistently concentration dependent. FRAP assay was also revealed the high antioxidant capacity of F_1 with the highest value of 2.234 µM at 500 µg/ml relative to the crude and other fractions at this same concentration (crude=1.957; F_2 1.731; F_3 =1.245 and F_4 =1.025 µM). Flavonoids and saponins were detected in the crude extract; their derivatives may also be present as part of the readily soluble constituents of Vernonia leaf extract. Some of the components especially flavonoids, are known to possess high antioxidant potentials. This may be the reason behind the observed use of the plant leaves in different traditional therapies in Southern Nigeria.

Keywords:*Vernonia calvoana* leaves,antioxidant, phytochemical, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), IC_{50}

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Introduction

So many physiological defects, imbalances and pathologies are attributable to oxidative stress in living systems. Oxidative stress has been shown to be caused by free radicals, including the superoxide radical, hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), and lipid peroxides (Demidchik, 2015). These reactive oxygen species (ROS) are produced as a consequence of normal biochemical processes in the body but may be exacerbated under conditions of increased exposure to xenobiotics. There is a compelling need for novel antioxidants with high potencies to counter the hazards of ROS and to complement specific medical therapies. Fortunately, the plant kingdom offer valuable prospect in 'lead-finding' as a source of natural products and

new chemotherapeutic agents. The search for plant-derived medication has accelerated in recent years as ethnopharmacologists, botanists, microbiologists, and natural products chemists are greatly involved in exploring the universe for phytochemicals and ‘‘leads’’ which could be developed for treatment of numerous diseases (Nwaehujor *et al.*, 2013).

The genus, Vernonia has about one thousand species of forbs and shrubs in the family Asteraceae which include *V. calvoana, V. amygdalina* and *V. colorata.* These species are the most widely consumed leaf vegetables in West Africa. In Nigeria, *V. calvoana* is popular in Southern riverine (Niger delta) areas where the leaves, adorned for the sweet taste, are used as fresh vegetables in delicacies. The leaf is also component of traditional concoction used as an anthelmintic, antidiabetic and antidote to food poisoning. It was also employed as remedy for navel aches and constipation (Sobrinho *et al 2015*).
The present study evaluated the antioxidant properties of the methanol extract and the polar fractions of *V. calvoana* leaves.

Materials and methods

Source of Plant Material and identification

The leaves of *V. calvoana* were freshly harvested from a farm in Calabar municipal, Cross River State, Nigeria and were air-dried at room temperature. They were identified by Department of Botany, University of Calabar, Nigeria. A voucher specimen (UNICAL/BT/0125) was deposited in the herbarium of the department of Biochemistry, University of Calabar.

Extraction and fractionation of crude extract

The dried leaves were finely ground using a laboratory mill. A 300 g of the ground material was first de-fatted with petroleum ether and then macerated in 80 % aqueous methanol for 72 h. The filtrate was evaporated with a rotary evaporator at 40°C. The extract was loaded in a 3 cm x 50 cm column pre-loaded with silica gel Silica Gel 70-30 mesh, 60A (Sigma Aldrich, Germany)and pre-conditioned with methanol. The column was then successively eluted with 450 ml of 100 % MeOH (F_1), 300 ml of 70 % MeOH (F_2), 250 ml of 50% MeOH (F_3) and 250 ml 0f 20 % MeOH (F_4). The eluted fractions (F_1, F_2, F_3 and F_4) with different Rf values of 0.51, 0.43, 0.36 and 0.28 respectively in actone, chloroform, methanol (1:4:2) showing one spot on thin layer chromatography (TLC) were dried at 40 ^oC, weighed and used for further studies.

Acute toxicity test

Thirty (30) mature albino mice of both sexes (21-28 g) were randomly separated into 6 groups (1–6) of 5 mice per group. Groups 1–5 were dosed orally with varying doses (250, 500, 1000, and 2000 mg/kg) of the crude methanol leaf extract of *V. calvoana* respectively while group 6 was given an equivalent volume of distilled water (0.03 ml/10 g). The mice were allowed access to feed and water *ad libitum* for 72 h and observed for signs of toxicity and death.

Preliminary qualitative phytochemical analysis

The crude extract was subjected to phytochemical analysis as described by Bargah (2015). A 2 g of the crude extract was dissolved in distilled water to form 20 ml solution; this was screened for the presence of alkaloids, flavonoids, tannins, polyuronoids, saponins, terpenes, anthraquinones, carbohydrates and glycosides using standard methods.

Test for alkaloids

Mayer's test: 2 ml of the filtrate and control solution were pipetted into two separate test tubes. To the test tubes were added 3 drops of Mayer's reagent. The solutions were mixed and allowed to stand for 5 min and then observed for presence of precipitate and color change. Wagner's test: 2 ml of the filtrate and control solutions were pipetted into two separate test tubes. To the test tubes were added 3 drops of Wagner's reagent. The solutions were mixed and allowed to stand for 5 min and then observed for presence of precipitate and color change. Dragendorrf's test: 2 ml of the filtrate and control solutions were pipetted into two separate test tubes. To the test tubes were added 3 drops of Dragendorff's reagent. The solutions were mixed and allowed to stand for 5 min and then observed for presence of precipitate and color change.

Test for flavonoids

I. 2 ml of the filtrate and control solutions were pipetted into two separate test tubes. To the test tubes were added 3 drops of NaOH. The mixtures were allowed to stand for 2 min and then observed for presence of precipitate and color change.

II. 2 ml of the filtrate and control solutions were pipetted into two separate test tubes. To the test tubes were added 3 drops of NaOH and 3 drops of 0.5 N HCl. The mixtures were observed for presence of precipitate and color change.

Test for tannins

i. 2 ml of the filtrate and control solution were pipetted into two separate test tubes. To the test tubes were added 3 drops of 10 % ferric chloride. The mixtures were observed for presence of precipitate and color change.

ii. 2 ml of the filtrate and control solution were pipetted into two separate test tubes. To the test tubes were added 3 drops of 10 % lead acetate. The mixtures were observed for presence of precipitate and color change.

Test for polyuronoids

5 ml of ethanol and control solutions were pipetted into separate test tubes. 1 ml of filtrate was added drop-wise into the test tubes. The mixtures were observed for presence of precipitate and color change

Test for saponins

Emulsifying test: 2 ml of the filtrate and control solution were pipetted into two separate test tubes. To the test tubes were added 3 drops olive oil and the mixture shaken vigorously. The mixtures were observed for presence of brown emulsion. Frothing test: 1 ml of the filtrate and control solution were pipetted into two separate test tubes. To the test tubes were added 4 ml distilled water. The mixture was shaken vigorously and then observed for presence of frothing.

Test for terpenes

A given mass (0.1 g) of the crude extract was dissolved in 10 ml concentrated chloroform. The solution was filtered and used for this test.

i. To 1 ml of filtrate and control solutions in separate test tubes were added 1 ml acetic anhydride. The solutions were mixed thoroughly with a glass rod. The test tubes were then placed in a slanting positions and 1 ml H_2SO_4 was added by the side of each test tube into the mixture. The junction of the two liquid layers was observed for presence of color change.

Test for anthraquinone

A given mass (0.1 g) of the crude extract was dissolved in 10 ml concentrated chloroform. The solution was filtered and used for this test.

i. To 5 ml of filtrate and control solution in separate test tubes was added 5 ml ammonia solution. The mixtures were shaken vigorously. The mixtures were observed for presence of precipitate and color change.

Test for carbohydrates

0.1 g of the crude extract was mixed with 4 ml distilled water. The mixture was boiled for 3 min in a water bath and filtered. The filtrate was used for the following tests

i. Test for reducing sugar: To 2 ml of filtrate and control solutions in separate test tubes were added 3 drops of Molisch reagent. The mixtures were observed for presence of precipitate and color change.

ii. Test for starch: To 2 ml of filtrate and control solutions in separate test tubes were added 3 drops of 2 % iodine solution. The solutions were mixed thoroughly and boiled in a water bath for 2 min. The mixtures were observed for presence of precipitate and color change.

Test for glycoside

To 2 ml of filtrate and control solutions in separate test tubes were added 2 ml of Fehlings I and Fehlings II solutions. The solutions were mixed thoroughly and boiled in a water bath for 2 min. The mixture was observed for presence of precipitate and color change.

In vitro antioxidant analysis

The crude methanol extract of *V. calvoana*, the fractions (F_1, F_2, F_3 and F_4) fractions and reference antioxidants (ascorbic acid, butylated hydroxytoluene, catechin and gallic acid) were evaluated with different *in vitro* anti-oxidant assays. Test Samples (800 µg) were individually dissolved in 1 ml methanol and further reduced by serial dilution to 400 µg/ml, 200 µg/ml, 100 µg/ml and 50 µg/ml. Each test was performed in triplicates.

Evaluation of antioxidant capacity using the 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) spectrophotometric assay

The free radical scavenging activity of extracts was analyzed by the DPPH assay following a standard method (Boligon *et al., 2014*). A given volume (2 ml) of the extract at varying concentrations (10-500 µg/ml) 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 percentage antioxidant activity was calculated as follows:

% Antioxidant Activity [AA] = 100 – [{(Abs sample – Abs blank) X 100}/Abs control].

Methanol (1.0 ml) plus 2.0 ml of the extract was 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 employed as reference standard.

Ferric reducing/antioxidant power (FRAP) assay

The total antioxidant potential of sample was determined using a ferric reducing ability of plasma (FRAP) assay (Kaushik *et al 2012*) 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 $FeSO_4 \times 7H_2O$. All solutions were used on the day of preparation. In the FRAP assay, the antioxidant efficiency of the extracts 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 sample to be analyzed was first adequately diluted to fit within the linearity range. All determinations were performed in triplicates.

Calculations were made by a calibration curve. FRAP value of sample (µM) =
changes in absorbance from 0-4 min x FRAP value of std (1000 µM)
changes in absorbance of std 0 min-4 min

Hydrogen peroxide scavenging assay

The method described by Bokhari *et al. 2013* was followed to investigate hydrogen peroxide scavenging capacity of the test samples. Hydrogen peroxide (2 mM) solution was prepared in phosphate buffer (50 mM, pH 7.4). Samples (100 µl) were pipetted into flasks and their volumes 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 % scavenging activity = (1- absorbance of sample) x 100
absorbance of control

Ascorbic acid was used as the standard antioxidant.

Hydroxyl radical scavenging assay

This was performed on test samples according to the method adopted by Saumya and Mahaboob (2010). 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 sample. 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.

Hydroxyl scavenging activity (%) =1-(Absorbance of sample ×100)
Absorbance of control

ABTS radical cation scavenging activity

The method (Zampini *et al 2010*) was carried out with slight modification 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 sample of different dilution 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:
% ABTS scavenging effect = (control absorbance - sample absorbance) X 100
control absorbance

β-Carotene bleaching assay

Mueller and Boehm (2011) 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 sample (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:

$$\% \text{ bleaching inhibition} = \frac{(A_{ot} - A_{120t})}{A_{oc} - A_{120}} \times 100$$

Superoxide anion radical scavenging assay

Riboflavin light NBT system assay was followed for superoxide radical scavenging activity as described by Masuoka *et al* (*2016*). 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 sample 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:

% Percent scavenging activity = (1- Absorbance of sample /Absorbance of control) ×100

Phosphomolybdate (Anti-lipid) assay for Total antioxidant capacity

The total antioxidant potency of test compounds was investigated by phosphomolybdate method (Shabbir *et al 2013*). An aliquot of 0.1 ml of different concentrations (800, 400, 200, 100 and 50 µg/ml) of each sample was added to 1 ml of reagent (0.6 M H_2SO_4, 0.028 M sodium phosphate, 0.004 M ammonium molybdate) and incubated for 90 min at 95°C in a water bath. Absorbance was recorded at 765 nm after the samples cooled to room temperature. Ascorbic acid served as standard.

Statistical analysis

All data were expressed as mean±SEM and subjected to one-way analysis of variance followed by post-hoc multiple-comparison Dunnett's test using the SPSS software to determine the level of significance between "test" and "control" group data means. Values of $p<0.05$ was considered significant.

Results

Acute toxicity studies

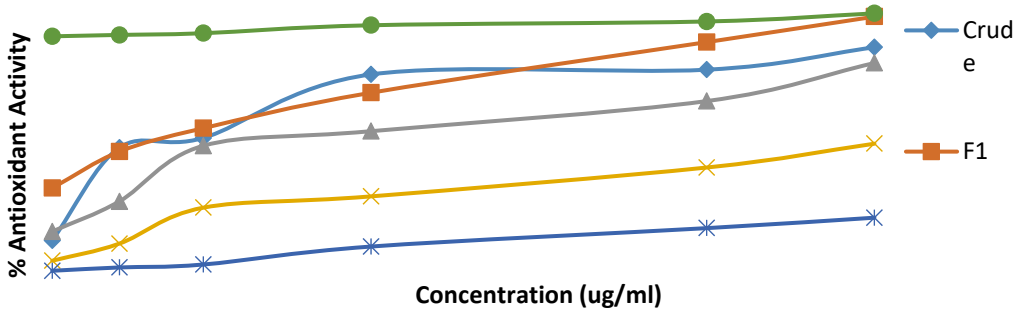
No mortality or overt clinical manifestation detected in mice within 72 h observation period following oral administration of the crude extract up to a dose of 2000 mg/kg.

Phytochemical analysis

Phytochemical analysis of the crude methanol extract showed the presence of alkaloids ++, flavonoids ++++, tannins +, phenols +++, steroids +, saponins +++, terpenes +, anthraquinones ++, carbohydrates++ and glycosides ++.

Antioxidant capacity using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical spectrophotometric analysis

Fraction F_1 and the crude displayed an almost equipotency with ascorbic acid at the maximal test concentration of 500 µg/ml. F_1 had 81.40 % relative to 72.37% for the crude while ascorbic acid produced 82.37 % antioxidant activity. The rest of the fractions however had comparatively reduced antioxidant values with 67.73 % for F_2, 44% for F_3 and 22.17% for F_4 at the same concentration (500 µg/ml) (Fig. 1).

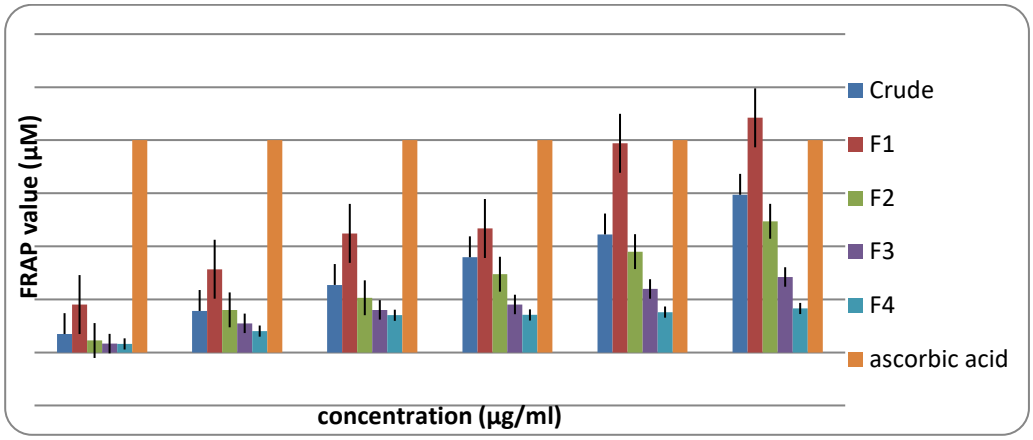


* F < 0.05 significantly different from reference compound (Ascorbic acid)

Figure 1: Antioxidant activities of the crude methanol extract of *Vernonia calvaona* leaves and polar fractions with DPPH assay

Ferric Reducing/Antioxidant Power Assay (FRAP)

In the FRAP assay, F_1 was consistent in producing the highest antioxidant value of 2.234 µM at 500 µg/ml. The crude and the rest of the fractions exhibited appreciable but reduced antioxidant power compared to ascorbic acid at the maximal test concentration of 500 µg/ml. The crude extract had 1.957 µM but F_2, F_3 and F_4 gave 1.731, 1.245 and 1.025 µM values in antioxidant power respectively. Ascorbic acid is known to have a FRAP value of 2 at varying concentrations (Fig. 2).



* P < 0.05 significantly different from reference compound (Ascorbic acid). FRAP value of ascorbic acid between 10 – 1000 µg/ml = 2.000

Figure 2: Antioxidant activities of methanol leaf extract of *V. calvoana* and its fractions using the FRAP method

Hydrogen peroxide scavenging assay

F_1 was most effective among the crude and other polar fractions from *V. calvoana* leaves in scavenging hydrogen peroxide radicals but its potency to do this was halved that of ascorbic acid. F_1 had a value of 119.00IC_{50} µg/ml compared to 53.00 IC_{50} (µg/ml) for ascorbic acid. F_2 displayed a minimal H_2O_2 scavenging activity, less than double the effect of ascorbic acid with IC_{50} µg/ml of 146.67±1.45. The crude extract, F3 and F4 had no significant ($p<0.05$) scavenging effect on hydrogen peroxide (Table 1).

Hydroxyl radical scavenging assay

F_1 and F_2 were also the most effective at neutralizing hydroxyl radicals among the crude and the rest of the fractions but their efficacies were comparatively lower than that of ascorbic acid. The IC_{50} (µg/ml) for F_1, F_2 and ascorbic acid was 133.67±0.67, 173.00±1.00 and 105.00 respectively (Table 1).

ABTS radical cation scavenging activity

In ABTS assay, it was only F_1, that exhibited significant ($p<0.05$) radical cations scavenging capacity among the test samples but it demonstrated with a twice reduced potency compared to ascorbic acid as revealed in the inhibitory concentrations (IC_{50} F_1 =111.00±2.31 while IC_{50} ascorbic acid=51.00) (Table 1).

Anti-lipid assay

F_1 was the only test sample that inhibited lipid peroxidation but with a value less than twice that of ascorbic acid. The crude extract and the rest of the polar fractions had no significant ($p>0.05$) effect (Table 1).

β-Carotene bleaching assay

The crude extract and all the isolated fractions did not induce inhibition of β-carotene bleaching activity.

Superoxide anion radical scavenging assay

Ascorbic acid had more than double effect compared to F_1, but more than triple effect relative to F_2 in superoxide radical scavenging activity IC_{50} F_1 =96.67±3.76; IC_{50} F_2 = 123.67±1.33 while IC_{50} ascorbic acid=37.00) (Table 1).

Table 1: IC_{50} values of the crude extract and polar fractions of *V. calvoana* leaves different antioxidant assays

Activity	IC_{50} (µg/ml) Crude	F_1	F_2	F_3	F_4	Ascorbic acid
H_2O_2	320.33±2.33*	119.00±0.00	146.67±1.45	178.00±0.00*	489.00±1.53*	53.00±0.00
Hydroxyl	254.33±2.96*	133.67±0.67	173.00±1.00	211.67±2.03*	479.00±1.00*	105.00±0.00
ABTS	240.67±1.33*	111.00±2.31	181.33±1.20*	223.00±1.73*	258.67±2.03*	51.00±0.00
Anti-lipid	212.00±1.53*	98.33±1.76	142.00±1.15*	201.67±2.40*	278.33±8.19*	34.00±0.00
B-carotene	239.00±1.00*	118.33±1.33*	156.00±1.53*	182.33±1.45*	281.67±2.91*	30.00±0.00
Superoxide	152.33±1.33*	96.67±3.76	123.67±1.33	178.33±1.33*	207.00±3.21*	37.00±0.00

Source: Laboratory Analysis

Values are expressed as mean±SD (N=3); Mean denoted*in a row vary significantly ($p<0.05$) from that of ascorbic acid.

Discussion

Oxidative damage at cellular level denatures proteins affecting their functions as biological catalysts and signaling components, carbohydrates by changing their structural conformation and lipids via lipid peroxidation. Cell membranes are made up of a high proportion of phospholipids which are initial targets for invading micro-organisms, chemical toxicants, and radiation which are harmful to tissues under lethal exposure. Potentially harmful reactive oxygen species (ROS) are also produced as a consequence of normal aerobic respiration (Aristidis *et al 2012*). These free radicals are usually removed or inactivated *in vivo* by a team of antioxidants (Gabriele *et al 2017*). Individual members of the antioxidant defense team are mobilized to prevent generation of ROS, to destroy potential oxidants, and to scavenge ROS. Thus oxidative stress-induced tissue damage is minimized. However, an absolute or relative deficiency of antioxidant defense may lead to a situation of oxidative stress, and this may give rise to or complicate a variety of pathologies including heart ailments and cancer (Gutteridge, 1994). Thus, there is increasing interest in antioxidants, particularly in

those intended to prevent the deleterious effects of free radicals in the body, and to prevent the deterioration of fats and other constituents of foodstuffs.

Antioxidants are known to be 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 block processes of oxidation by neutralizing free radicals thereby becoming oxidized themselves (Nwaehujor *et al.*, 2013). Endogenous antioxidants prevent oxidation by reducing the rate of chain initiation. Antioxidants are considered useful in preservation of foods, beverages, raw materials and parts of formulations for different drugs. Antioxidants consist of vitamins, polyphenols, flavonoids, minerals and endogenous enzymes such as superoxide dismutase, catalase and glutathione peroxidase that have the capability to neutralize unstable molecules (Trouillas *et al.*, 2003).

The results of DPPH assay showed that the crude extract of *V. calvoana* leaves had a high antioxidant activity of 72.37% at 500 µg/ml but fraction (F_1) exerted an almost comparable antioxidant activity to that of the standard, ascorbic acid at 500 µg/ml; F_1 had 81.40 % while ascorbic acid produced 82.37 % (Fig.1). The high percentage inhibition of free radicals by the crude extract and the component polar fractions was indicated in the significant reduction of colour of DPPH reagent from purple to yellow in the assay. A high antioxidant activity is significant in free radical scavenging activity of extracts. FRAP assay also collaborated the high antioxidant potential of F_1 in having the highest value of 2.34 µM at 500 µg/mlrelative to other fractions and ascorbic acid (Fig. 2). Ascorbic has a known FRAP value of 2.0 at different concentrations (Henkler *et al.*, *2010*). F_1,among other fractions was most effective in scavenging hydrogenperoxide radicals and radical cations but at a reduced (half) potency compared to ascorbic acid. The fraction (F_1) also inhibited lipid peroxidation with half efficacy of ascorbic acid. Fraction F_2 (F2) on the other hand, was only able to show appreciable antioxidant activity in scavenging free hydroxyl radicals, hydrogen peroxides and superoxide radicals but at reduced potencies compared to either F_1 or ascorbic acid. It was ineffective against lipid peroxidation and radical cations scavenging. The rest of the fractions (F_3 and F_4) exhibited negligible antioxidant activities as seen in Figures 1 and 2 as well as in Table 1. The test extract and its polar fractions did not confer inhibition of β-carotene bleaching activity.

Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, phenols, saponins, terpenes, anthraquinones and carbohydrates in the crude methanol extract of *V. calvoana* leaves. Flavonoids are valuable dietary supplements duehigh antioxidant properties (Igile *et al.*, 2013). Luteolin, a flavonoid is reported to possess high antioxidant activity, measured in Trolox test, is twice stronger than vitamin E (*Lakovleva et al.*, *2015*). Further studies are presently on-going to purify F_1 and to elucidate its molecular properties.

Conclusion

The results of the different *in vitro* antioxidant assays demonstrated that the crude extract of *V. calvoana* leaves contained potent antioxidant principles. The polar bioactive fraction responsible for the observed antioxidant effects was identified as F_1 with Rf value of 0.51 in acetone, chloroform, methanol (1:4:2) showing one spot on TLC. This finding supports the use of the plant leaves in various traditional medicine therapies in Niger delta areas of Nigeria.

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