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In vitro Antioxidant and Free Radical Activity of Some Nigerian Medicinal Plants: Bitter Leaf (*Vernonia amygdalina* L.) and Guava (*Psidium guajava* Del.)

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ABSTRACT

This study was aimed at assessing antioxidant and free radical scavenging properties of bitter leaf (*Vernonia amygdalina*) and guava (*Psidium guajava*) leaf in different extracting solvents (absolute ethanol, 70% ethanol and 50% ethanol) via *in vitro* assays including; Total Phenolic Content (TPC), Total Flavonoid Content (TFC), DPPH radical scavenging, metal chelating activity, reducing power and total antioxidant capacity. The results obtained indicated that both medicinal plants are antioxidant reservoir. The TPC and TFC of *P. guajava* was more in all the extracting solvents compared to *V. amygdalina*. The Inhibitory Concentration (IC_{50}) of *P. guajava* for DPPH radical scavenging were; absolute ethanol (1.564 μg mL^{-1}), 70% ethanol (1.723 μg mL^{-1}), 50% ethanol (4.102 μg mL^{-1}) while *V. amygdalina* were; absolute ethanol (33.18 μg mL^{-1}), 70% ethanol (56.21 μg mL^{-1}), 50% ethanol (73.46 μg mL^{-1}). Both DPPH radical scavenging and metal chelating activities of the two medicinal plants were dependent on the amount of phenols and flavonoids in the extract. The absorbance values for both reducing power and total antioxidant capacity of the two extracts also indicated their antioxidant potentials. Thus, the results obtained from the current study are indications of the possible use of the two plants in combating free radical related diseases often orchestrated by oxidative stress conditions, especially *P. guajava* since it showed more antioxidant activities than *V. amygdalina* following the *in vitro* results.

Key words: Free radicals, antioxidant, phenols, flavonoids, DPPH, *Vernonia amygdalina* and *Psidium guajava*

INTRODUCTION

In the past decades, there has been a growing interest in the screening of medicinal plants for their therapeutic properties and their possible use in combating ailments. These plants are specifically screened for the presence of bioactive compounds which are known to possess antioxidant and other therapeutic properties (Uyoh *et al*., 2013; Ikpeme *et al*., 2014). Parts of these plants like the leaves, bark and roots are very rich in phytochemicals such as phenols, alkaloids, flavonoids, terpenes, glycogens with the inherent capacity to mitigate diseases and attenuate toxicities (Ekaluo *et al*., 2013, 2014). The process of utilization of the bioactive compounds involve identification, isolation and characterization for use in cosmetics, perfumery, pharmaceutical and food industries (Farombi and Owoeye, 2011; Njan, 2012; Oriakhi *et al*., 2014). This new field of research has over years given rise to the screening and identification of many plants with inherent properties to combat diseases and this has been a great addition not only to traditional medicine but also to the pharmaceutical and food industries. Thus, it will not be an exaggeration to posit that

the search and possible prevention of aetiology of many pathological conditions in modern medicine today is more effective because it in tandem with traditional medicine.

Medicinal plants are of much interest due to their antioxidant and free radical scavenging properties. These antioxidants are electron sufficient compounds with the readiness to donate electron to electron-deficient compounds such as free radicals to stabilize their actions. Free radicals are electron-deficient compounds because they possess unpaired valence electrons (Kadam *et al*., 2010; Aluko *et al*., 2013) and in their quest to finding electrons, they can easily attack cells and biomolecules in the body resulting in generation of diseases. Several studies has linked the generation of reactive oxygen species (free radicals) such as hydroxyl (OH^-) radical, superoxide (O_2^-), nitric oxide ($NO\cdot$), nitrogen dioxide (NO_2^-), peroxyl (ROO^-), hydrogen peroxide (H_2O_2) to the development of pathological conditions including protein oxidation, lipid peroxidation, DNA damage and cellular degeneration and these conditions have been implicated in the aetiology of diseases such as diabetes, cancer, Alzheimer and Parkinson disease, cardiovascular disease, aging process, arthritis and inflammation (Knekt *et al*., 2002; Aruoma, 2003; Ismail *et al*., 2004; Sahlin *et al*., 2004; Tripathy *et al*., 2010).

Interestingly, antioxidants (synthetic and natural antioxidants) have been used to ameliorate the actions of free radicals in biological systems. Several *in vitro* and *in vivo* studies has further indicated a positive correlation in the use of antioxidants to mitigate free radical related pathologies (Uyoh *et al*., 2013; Ikpeme *et al*., 2013, 2014; Ekaluo *et al*., 2015). However, the continuous use of synthetic antioxidants from pharmaceutical stores has over the years post high state of panic following scientific assertions on their side effects (Kukic *et al*., 2006; Vijayakumar *et al*., 2012; Ikpeme *et al*., 2013). This is not to completely assert that natural antioxidants are entirely risk free but since they are natural, there is a general believe of their safety over the synthetic ones (Calixto, 2000; Ikpeme *et al*., 2011, 2012). Also, the accessibility and affordability of natural antioxidants is another strong reason for increased utilization of natural antioxidants over the synthetics. As a result of this, medicinal plants reported with high antioxidants potentials have continuously been utilized both in traditional and contemporary medicine for management of free radical related diseases and other disease conditions.

Bitter leaf (*Vernonia amygdalina*, Del.) belongs to the Compositae family. It is one of such plants reported with high medicinal properties. The name bitter leaf originated from the bitter taste impacted by the leaves and stem. It is a major vegetable and occupies significant position in the diet of several Nigerian ethnic groups (Oriakhi *et al*., 2014). They are widely distributed in tropical Africa and Asia and are most common in natural forest. It has long history in traditional medicine and parts including the leaves, stem and roots have been exploited in the treatment of various ailments. Particularly, the leaves are utilized in the treatment of malaria, diabetes mellitus, venereal disease, wounds, hepatitis and cancer (Kambizi and Afolayan, 2001; Hamill *et al*., 2003; Erasto *et al*., 2007). Amongst the diverse uses of the plant in combating diseases, the antihelminthic properties of the plants have also been reported (Danquah *et al*., 2012). *in vitro* antioxidant studies of the ethanolic extract of the leaves were shown to inhibit diphenyl picryl hydrazyl radical (Ayoola *et al*., 2008). Similarly, *in vivo* antioxidant of *V. amygdalina* has been shown to ameliorate oxidation of linoleic acid and lipid peroxidation induced by Fe^{2+}/ascorbate in rat (Mbang *et al*., 2008).

Guava (*Psidium guajava*, L.) found in the Myrtaceae family is another plant with diverse medicinal values. Guava is reported to be rich in tannins and is widely cultivated in the tropics and subtropics for its edible fruits (Chuanoi *et al*., 2009). The leaves contain considerable amount of

phenols and have been used as health tea. The phenolic content in the leaves which indicates it antioxidant properties are utilized in the prevention of chronic diseases such as diabetes, cancer, heart diseases (Kimura *et al*., 1985). According to Chuanoi *et al*. (2009), guava leaves contain high amount of phenols and these bioactive compounds play major role in free radical scavenging, thus could combat free radical induced conditions such as oxidative stress.

Vernonia amygdalina and *P. guajava* have been reported with nutritional and therapeutic properties and are used in the treatment of free radical related pathologies such as diabetes. Although, these properties have been linked to the polyphenolic content of the medicinal plants, however, the amount of these phenols and other antioxidant compounds in the two medicinal plants may likely vary. Generally, the medicinal plant with more amounts of these antioxidant compounds could be more potent in scavenging free radical and ameliorating their pathological effects in the body. Thus, the aim of this research was to assess and compare the antioxidant and free radical scavenging properties of these medicinal plants in three extracting solvents (absolute ethanol, 70 and 50% ethanol) using *in vitro* assays.

MATERIALS AND METHODS

Collection of plant materials and extraction: Fresh leaves of *V. amygdalina* and *P. guajava* were obtained from Staff Quarters, University of Calabar, Calabar and authenticated in the Herbarium Unit of the Department of Botany, University of Calabar. The fresh leaves were freed from dirt, air dried at room temperature for one week and then finely milled separately using a blender (Model: 5KSB655CCSO). Ten grams of the milled sample was soaked in 100 mL of the three different solvents (absolute ethanol, 70 and 50% ethanol) for 72 h at room temperature. The soaked samples were shaken intermittently during the extraction period and subsequently filtered using Whatman No. 1 filter paper. The resulting extracts were concentrated under vacuum in a rotary evaporator at 45°C for complete solvent removal. A stock solution of each crude extract was prepared and desired working concentrations were made by appropriate dilutions.

Determination of extract yield (%): The percentage yield of each extract was obtained by dividing the weight of the concentrated crude extract by the initial weight (10 g) of dry milled starting material and multiplying the ratio by 100.

Determination of Total Phenolic Content (TPC): The total phenolic contents of the extracts were determined by the Folin-Ciocalteau method according to Duarte-Almeida *et al*. (2006). About 100 mL of Folin-Ciocalteau reagent was added to 500 mL of the different extract solutions containing 1000 mg mL^{-1}+6 mL of distilled water and shaken for one minute. Thereafter, 2 mL of 15% sodium carbonate was added to the mixture and shaken again for 30 sec. Finally, distilled water was added to the solution to make it up to 10 mL, then left to incubate for 1.5 h at room temperature. Thereafter, the absorbance at 750 nm was evaluated using a spectrophotometer (LABTECH UV/VIS Spectrophotometer, India-Single beam 295). Gallic acid monohydrate, a standard phenol, in the range of 5-150 μg mL^{-1} was used to prepare standard reference curve. The Total Phenol Contents (TPC) of the extracts were expressed as Gallic Acid Equivalents (GAE) from the linear regression curve of gallic acid.

Determination of Total Flavonoid Content (TFC): The total flavonoid contents of each extract concentration were determined using the aluminum chloride colorimetric method, according to

Dewanto *et al*. (2002). The different extract solutions (1 mL containing 1000 μg mL^{-1}) were diluted with 4 mL of distilled water in a 10 mL volumetric flask. Thereafter, 0.3 mL of 5% sodium nitrite ($NaNO_2$) solution was added to each extract solution. Five minutes later, 0.3 mL of 10% aluminium chloride ($AlCl_3$) was added; 1 min later, 2 mL of 1.0 M sodium hydroxide (NaOH) was added and finally, 2.4 mL of distilled water was added and mixed properly. Absorbance of the reaction mixture was read at 510 nm. Rutin, a standard flavonoid in the range of 10-150 μg mL^{-1} was used to prepare the standard reference curve. Total Flavonoid Content (TFC) of the extracts were expressed as Rutin Equivalents (RE) from the linear regression curve of Rutin.

DPPH radical scavenging activity: The ability of *V. amygdalina* and *P. guajava* leaf extracts to scavenge stable DPPH radical was measured using the method of Mensor *et al*. (2001). Five different concentrations of each test extracts were prepared in methanol (20, 40, 60, 80, 100 mg mL^{-1}). One milliliter of 0.3 mM of freshly prepared DPPH solution in methanol was added to 2.5 mL solution of each extract concentration and allowed to react in the dark at room temperature for 30 min. Absorbance of the resulting solution was measured at 518 nm. Methanol (1 mL) was added to 2.5 mL of each extract concentration was used as blank, while 1 mL of 0.3 mM DPPH solution added to 2.5 mL of methanol served as a negative control. Ascorbic acid and gallic acid were used as standard reference compounds (positive controls) for comparison. Percentage DPPH scavenging activities of the extracts and standards were determined using the equation:

$$\text{Scavenging activity } (\%) = 100 - \left| \frac{A_s - A_b}{A_c} \times 100 \right|$$

Where:
A_s = Absorbance of sample (extracts or reference standard)
A_b = Absorbance of blank
A_c = Absorbance of negative control

Results were expressed as inhibitory concentration, IC_{50} (concentration of extract or standard required to scavenge 50% of DPPH radicals), which were determined from a linear regression curve of concentration versus % scavenging activity.

Metal (Ferrous ion) chelating activity: The ferrous ion chelating activity of *V. amygdalina* and *P. guajava* leaf in different extracting solvents (Absolute ethanol, 70 and 50% ethanol) were determined by the method of Ebrahimzadeh *et al*. (2009). Here, the ability of the extracts to chelate ferrous ion (Fe^{2+}) was estimated. Different concentrations (20-100 mg mL^{-1}) of each extract were prepared and 1 mL of each concentration were mixed with 1 mL of $FeSO_4$ (0.125 M) and 1 mL of ferrozine (0.3125 mM) and shaken vigorously. After incubating for 10 min at room temperature, the mixture solution was measured using a spectrophotometer at 562 nm against a blank containing the same components as stated above but the extracts were replaced with distilled water (1 mL of distilled water). The blank was incubated under the same conditions as the test samples. Sodium EDTA (Na_2EDTA) was used as control. The percentage inhibitions of ferrozine (Fe^{2+}) by the extracts were determined using the equation:

$$\text{Inhibition of ferrozine (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

Where:
A_c = Absorbance of control and A_s = Absorbance of sample

Results were expressed as IC_{50} (concentration of extract or standard required to chelate 50% of ferrous ions), which were determined from a linear regression curve of concentration versus % chelating activity.

Total Antioxidant Capacity (TAC) assay: The Total Antioxidant Capacity (TAC) of *V. amygdalina* and *P. guajava* leaf extract in different extracting solvents (absolute ethanol, 70% and 50% ethanol) were determined by the phosphomolybdate method according to Jayaprakasha *et al*. (2002). An aliquot (30 mL) of different concentrations (20, 40, 60, 80 and 100 mg mL^{-1}) of the test extracts were mixed with 3 mL of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) taken in test tubes. The tubes were capped with aluminium foil and incubated in a boiling water bath at 95°C for 90 min. The reaction mixture was allowed to cool to room temperature and the absorbance of the solution was measured at 695 nm against a blank containing 3 mL of reagent solution and the appropriate volume of the dissolving solvents. The blank was incubated under the same conditions as the test samples. Ascorbic acid and gallic acid were used as standard reference compounds to compare the activities of the extracts.

Reducing power assay: Antioxidant activity of the leaf extract of *V. amygdalina* and *P. guajava* in different extracting solvents (absolute ethanol, 70 and 50% ethanol) were determined to assess their ferric ion (Fe^{3+}) reducing ability according to the method of Anandjiwala *et al*. (2007). Different concentrations (20, 40, 60, 800, 100 mg mL^{-1}) of each extract were prepared and 1 mL of each concentration was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.8) and 2.5 mL of potassium ferricyanide. The mixture was incubated in a water bath at 50°C for 20 min. To this mixture, 2.5 mL of 10% trichloroacetic acid was added and then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride was added. Absorbance of the Pert Prussian blue solution formed was measured at 700 nm. Ascorbic acid and gallic acid were used as standard reference compounds for comparison and prepared in same concentrations as the extracts.

Statistical analysis: Analysis of variance (ANOVA) was used to analyze absorbance values for total phenolic content, total flavonoid content, reducing power and total antioxidant capacity of the two medicinal plants against the reference standards. Mean separation was done using the Least Significant Difference (LSD) test at $p<0.05$.

RESULTS

Extract yield, Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and inhibitory concentration of *Vernonia amygdalina* and *Psidium guajava*: From Fig. 1a, the extract yield in the different extracting solvent (absolute ethanol, 70% ethanol and 50% ethanol) increased as the concentration of the extracting solvent decreased. *Vernonia amygdalina* had more

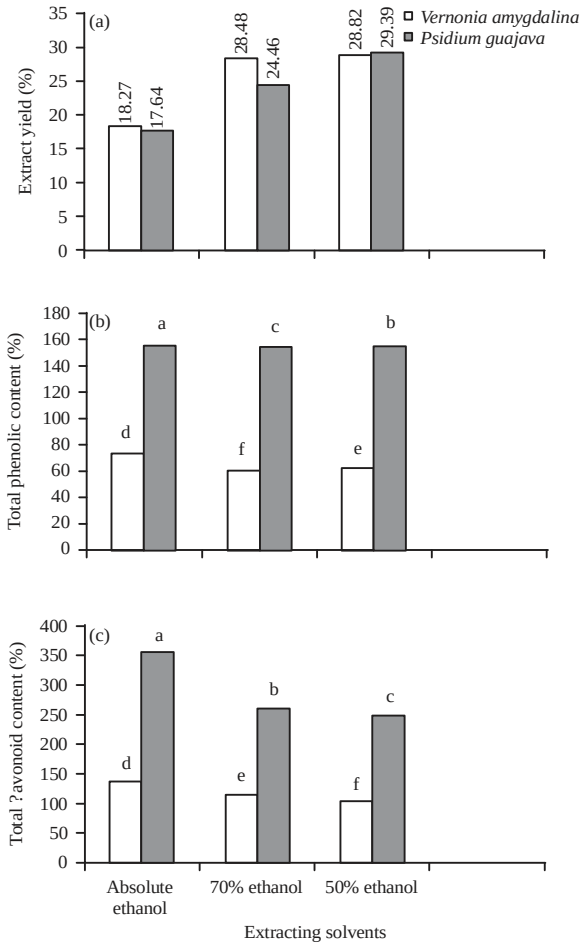

Fig. 1(a-c): (a) Extract yield, (b) Total phenolic content and (c) Total flavonoid content of *Vernonia amygdalina* and *Psidium guajava* in different solvents, bars with different alphabet indicate significant difference ($p<0.05$)

yield in all the concentrations; absolute ethanol (18.27%), 70% ethanol (28.48%), 50% ethanol (28.82%) than *P. guajava*; absolute ethanol (17.64%), 70% ethanol (24.46%) and 50% ethanol (29.39%). Figure 1b shows TPC of the two medicinal plants showed significant differences ($p<0.05$) in all the concentrations of the extracting solvent. The highest TPC was obtained in absolute ethanol of *P. guajava* extract (156.49 µgGAE/mg), which was significantly higher than absolute ethanol of *V. amygdalina* (73.68 µgGAE/mg). Similarly, from Fig. 1c, TFC was more in *P. guajava* leaf extract than *V. amygdalina*. TFC obtained in the different extracting solvent were as follows: absolute ethanol (134.18 µgRE/mg), 70% ethanol (112.55 µgRE/mg), 50% ethanol (101.17 µgRE/mg) for *V. amygdalina* and absolute ethanol (354.22 µgRE/mg), 70% ethanol (259.50 µgRE/mg) and 50% ethanol (247 µgRE/mg) for *P. guajava* (Fig. 1c). The Inhibitory Concentration (IC_{50}) of *P. guajava* was also better than *V. amygdalina* ($p<0.05$) in all the extracting solvent for DPPH radical scavenging (Fig. 2). *Psidium guajava* was a better metal chelator in absolute ethanol since it required a lower concentration (445.61 µg mL^{-1}) to chelate 50% of ferrous ion (Fe^{2+}) than *V. amygdalina* (896. 98 µg mL^{-1}) at the same concentration of extracting solvent (Fig. 3).

Effect of concentration on the reducing power and total antioxidant capacity of *Vernonia amygdalina*, *Psidium guajava* and standards: Our results revealed that the reducing power and antioxidant capacity of *V. amygdalina* and *P. guajava* were increased as the concentrations of the extracts increased (Table 1). Ascorbic acid, a standard antioxidant showed more reducing power than extracts of the two plants. The reducing power of *P. guajava* extract was generally higher ($p<0.05$) than *V. amygdalina* in all the extracting solvents. At 20 μg mL^{-1}, 70% of *P. guajava* reduced more of the ferrous ion with absorbance value of 0.978 while, *V. amygdalina* reduced the metal with absorbance value of 0.720 at the same concentration. At 40, 60, 80 and 100 μg mL^{-1}, the highest reducing power was observed in 50% ethanol of *P. guajava*. Similarly, ascorbic and gallic acid standards used to compare the total antioxidant capacity of the two plant extracts showed more potency than the test extracts although 50% ethanolic extract of *P. guajava* compared favourably with the standards. There were significant differences ($p<0.05$) in the total antioxidant capacity of the two extracts in the different extracting solvent. At the least concentration of 20 μg mL^{-1}, the highest antioxidant capacity was recorded in 70% ethanol of *P. guajava* (0.172) while the least absorbance was in absolute ethanol of *V. amygdalina* (0.057). At the highest concentration of 100 μg mL^{-1}, 70% of *P. guajava* extract also recorded the highest antioxidant activity (0.304) while, the least was observed in absolute ethanol of *V. amygdalina* (0.107), as shown in Table 1.

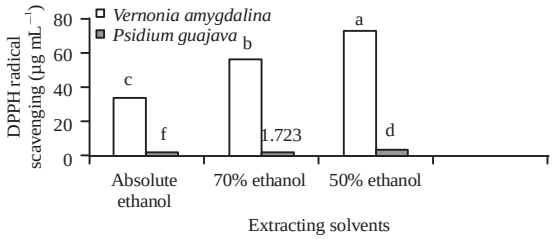

Fig. 2: DPPH (IC_{50}) of *Vernonia amygdalina* and *Psidium guajava* in different extracting solvents, Bars with different alphabet indicate significant difference ($p<0.05$), IC_{50} for ascorbic acid standard control = 5.950 μg mL^{-1}, IC_{50} for gallic acid standard control = 0.505 μg mL^{-1}

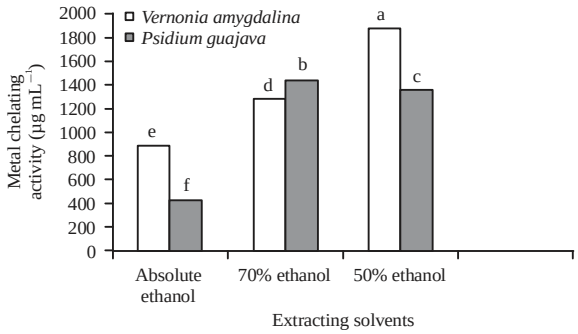

Fig. 3: Metal chelating activity of *Vernonia amygdalina* and *Psidium guajava* in different extracting solvents, Bars with different alphabet indicate significant difference ($p<0.05$), IC_{50} for NA_2EDTA standard control = 0.024 μg mL

Table 1: Effect of concentration on the reducing power and total antioxidant capacity of *Vernonia amygdalina, Psidium guajava* and standards

	Vernonia amygdalina			*Psidium guajava*			Standards	
Concentration of extracts (μg mL^{-1})	Absolute ethanol	70% ethanol	50% ethanol	Absolute ethanol	70% ethanol	50% ethanol	Ascorbic acid	Gallic acid
Reducing power								
20	0.720±0.01^{g}	0.728±0.01^{f}	0.768±0.01^{e}	0.858±0.01^{d}	0.978±0.01^{b}	0.963±0.01^{c}	1.909±0.01^{a}	-
40	0.740±0.01^{g}	0.833±0.03^{e}	0.772±0.02^{f}	1.032±0.03^{d}	1.163±0.02^{c}	1.308±0.01^{b}	2.149±0.13^{a}	-
60	0.787±0.01^{f}	0.850±0.02^{e}	0.774±0.03^{g}	1.188±0.03^{d}	1.268±0.03^{c}	1.422±0.03^{b}	2.239±0.11^{a}	-
80	0.812±0.02^{f}	0.932±0.01^{e}	0.777±0.04^{g}	1.364±0.02^{c}	1.360±0.04^{d}	1.530±0.04^{b}	2.459±0.13^{a}	-
100	0.850±0.02^{f}	0.941±0.02^{e}	0.815±0.04^{g}	1.540±0.04^{d}	1.451±0.04^{d}	1.557±0.04^{b}	2.464±0.13^{a}	-
Antioxidant capacity								
20	0.057±0.02^{h}	0.074±0.01^{f}	0.065±0.01^{g}	0.133±0.02^{e}	0.172±0.01^{c}	0.137±0.03^{d}	0.651±0.03^{a}	0.400±0.02^{b}
40	0.069±0.01^{h}	0.076±0.03^{g}	0.090±0.03^{f}	0.162±0.02^{d}	0.214±0.01^{c}	0.159±0.02^{e}	0.778±0.03^{a}	0.431±0.03^{b}
60	0.082±0.02^{h}	0.100±0.01^{f}	0.095±0.03^{g}	0.177±0.03^{e}	0.247±0.02^{c}	0.194±0.01^{d}	0.849±0.02^{a}	0.434±0.04^{b}
80	0.093±0.03^{h}	0.102±0.04^{g}	0.105±0.04^{f}	0.212±0.03^{e}	0.287±0.02^{c}	0.219±0.03^{d}	1.011±0.03^{a}	0.459±0.04^{b}
100	0.107±0.02^{h}	0.115±0.03^{f}	0.111±0.04^{g}	0.240±0.04^{e}	0.304±0.02^{c}	0.245±0.03^{d}	1.050±0.03^{a}	0.460±0.03^{b}

Means with different superscript along the same horizontal array differ significantly ($p<0.05$) from each other, -: Particular standard was not used

DISCUSSION

The growing search for plants with high medicinal values has so far brought about better understanding of the invaluable medicinal properties of plants and their subsequent exploitation in the management of diseases. These properties are linked to the phytochemical constituent of plants and since plants resources and products are reported to be safer upon consumption, accessible and affordable their usage are more recommended over synthetic products (Ikpeme *et al*., 2013, 2014, 2015; Ekaluo *et al*., 2015). The therapeutic properties of these plants are attributed to antioxidant compounds (Padmanabhan and Jangle, 2012), phytochemicals found abundantly in medicinal plants. These antioxidants are known to scavenge free radicals, ameliorate free radical related diseases and conditions such as oxidative stress. Thus, plants identified with antioxidant properties are a great addition to traditional medicine and could also serve as additives in products from food/pharmaceutical industries.

From Fig. 1b-c, the quantitative analysis of the polyphenolic constituent in the leaf extract of *Vernonia amygdalina* and *Psidium guajava* revealed the presence of phenols and flavonoids in extract of the two medicinal plants. Phenols play a major role as primary antioxidants or free radical terminators (Oriakhi *et al*., 2014), while flavonoids are reported as the most widespread group of natural compounds and probably the most important natural phenolic compounds because of the health benefits it confers to the body (Gil *et al*., 2002; Padmanabhan and Jangle, 2012). Phenols and flavonoids are primary antioxidants due to their hydroxyl group (OH^{-}), directly bonded to the benzene ring, thus allowing them to easily donate electrons to electron-deficient free radicals in order to reduce their menace in biological systems (Uyoh *et al*., 2013). Thus, plants quantified with appreciable amount of polyphenolic compounds could serve as reliable free radical scavengers. Relating this to our findings, it thus suggest that the amount of phenol and flavonoid estimated in *V. amygdalina* and *P. guajava* in the different extracting solvents are indications of the antioxidant and free radical scavenging potentials of the two plants. The more the amount of these compounds in a plant, the more likely the plant's antioxidant potentials. Thus, *P. guajava* may be a better antioxidant source since the amount of phenols and flavonoids were generally higher in all its extracting solvent than *V. amygdalina* (Fig. 1b-c). The inhibitory concentration (IC_{50}), which is the concentration of the extracts required to inhibit 50% of DPPH radical obtained in this study may not be unconnected with these polyphenols. The lower the amount of IC_{50} of the extract, the more the ability of the extract to scavenge free radicals. As clearly shown

in Fig. 1b-c, the amount of phenols and flavonoids was more in the extract of *P. guajava* than *V. amygdalina* which could be why it required lower concentration of *P. guajava* extract to inhibit 50% of DPPH radical compared to *V. amygdalina*. Furthermore, the IC_{50} of *P. guajava* for DPPH in absolute ethanol (1.564 μg mL^{-1}), 70% ethanol (1.723 μg mL^{-1}) and 50% ethanol (4.102 μg mL^{-1}) were even lower than ascorbic acid standard control (5.950 μg mL^{-1}). Considering the fact that *P. guajava* is more readily available in our locality, our results clearly suggest that the leaves of this medicinal plant could be more utilized in mitigating oxidative stress and other free radical related conditions rather than complete reliability on synthetic antioxidants including ascorbic acids.

Transition metals are reported to play part in the generation of free radicals (Ghimeray *et al*., 2009). Transition elements such as iron and copper are capable of fronting free radical generation because they are catalyst due to lone pair of electrons on their outermost shell (Ekaluo *et al*., 2015). As a result of the lone pair of electrons, they become structurally unstable and often orchestrate the conversion of H_2O_2 to OH^- in the Fenton reaction and in decomposition of alkyl peroxide to heavy reactive alkyl and hydrogen radical (Hsu *et al*., 2006). Antioxidant compounds have the capacity to chelate and bond free radicals into forms that can easily be removed from the body. This property of antioxidant compounds are due to their redox properties which allow them to act as reducing agents, electron donors, oxygen quenchers or metal chelators (Demiray *et al*., 2009; Premanath and Lakshmidevi, 2010). From the results of the present study, the IC_{50} of the extract for metal chelating may be attributed to the amount of phenols in the extract. Flavonoids have been reported to act as a barrier to enzymes responsible for oxidative stress generation, quenching free radicals, chelating transition metals and rendering them inactive in Fenton reaction (Wong *et al*., 2006; Aiyegoro and Okoh, 2009). Thus, the metal chelating property of the two plants under review may not only be attributed to phenols but also to the synergistic interaction between phenols, flavonoids and other bioactive compounds which may also be present in the extracts. Although, the IC_{50} for metal chelating activity was generally low for the two extracts, however, the DPPH scavenging of the plants particularly *P. guajava* is highly recommended.

There is a strong relationship between phenols and total antioxidant capacity of some fruits, vegetables and grain products (Demiray *et al*., 2009). It could therefore suggest that the absorbance values for total antioxidant capacity of *V. amygdalina* and *P. guajava* are positive indications of their antioxidant/free radical scavenging. The reducing power of a compound is an indication of its possible antioxidant properties due to the presence of reductants (Duh *et al*., 1999; Chanda *et al*., 2011). These reductants facilitates the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) with high absorbance values on spectrophotometer (Ekaluo *et al*., 2015). The more the concentration of Fe^{2+}, the more the absorbance value, which indicates the extract electron donating capacity. Since, free radicals are electron-deficient and structurally unstable compounds with dire need of electrons to stabilize them, it thus suggests that reducing agents such as antioxidant compounds can easily donate electrons to these radicals in order to quench their deleterious effects. Therefore, the reducing power of *V. amygdalina* and *P. guajava* indicated by the absorbance values are a good indication of their antioxidant potentials and this may be a good addition to pharmaceutical industries in the search to replace synthetic antioxidants with natural antioxidants from plant origin considering the side effects pose by the former.

CONCLUSION

Plant products are currently occupying vital position in food and pharmaceutical industries and will continue to be in front line for the management of diseases. Countless efforts are put in place

to explore, discover and exploit more plants with medicinal values. From the findings of our study, it could be concluded that the leaf extract of *Vernonia amygdalina* and *Psidium guajava* are good antioxidant sources. However, comparing the two plant extracts, *Psidium guajava* was better than *V. amygdalina* in eliciting antioxidant activity and free radical scavenging. Thus, we recommend the use of these plants in combating free radical menace such as oxidative stress and other related conditions. It will be of significant importance to further test the potentials of these two extracts in eliciting antioxidant/free radical scavenging properties *in vivo* for a more categorical assertion.

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