



## ***In vitro* antioxidant activities of extracts of *Vernonia amygdalina* and *Ocimum gratissimum* leaves**

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### **Abstract**

*Vernonia amygdalina* and *Ocimum gratissimum* occupy prominent positions in the diets of several Nigerian ethnic groups. This study therefore compared the phenolic content and some antioxidative activities of the ethanolic leaf extracts of these two plants. Ethanolic extracts of freshly plucked leaves were obtained using standard procedures. The phenolic content and antioxidant capacity of the vegetables were quantified *in vitro*. The results show that *O. gratissimum* contains higher amounts of total phenolics ( $168.5 \pm 5.50$  and  $97.0 \pm 0.01$  mg gallic acid equivalents/g extract respectively) and flavonoids ( $126.7 \pm 1.00$  and  $65.4 \pm 4.42$  mg quercetin equivalents/g extract respectively) than *V. amygdalina*. A dose-dependent increase in flavonol content was observed for both extracts with higher contents obtained in the *O. gratissimum* extract. *O. gratissimum* also has higher DPPH radical scavenging activity (IC<sub>50</sub> values of 105.7 and 111.8 µg/ml respectively) and ferric reducing antioxidant potential (FRAP) ( $110.0 \pm 0.02$  and  $85.0 \pm 0.01$  mg Fe<sup>2+</sup>/g extract). *O. gratissimum* also had higher reducing power than *V. amygdalina* at the concentrations studied. Standard ascorbic acid however had higher antioxidant activity values for the DPPH, FRAP and reducing power assays. Both plant extracts reasonably inhibit lipid peroxidation at  $31.4 \pm 0.15\%$  for *V. amygdalina* and  $42.8 \pm 1.00\%$  for *O. gratissimum*. The results further substantiate the medicinal value of these vegetables. Consumption of these vegetables is encouraged to help militate against deleterious effects of reactive species.

**Keywords:** Phenolic; Flavonoids; DPPH; Ferric Reducing Antioxidant Potential (FRAP); Lipid peroxidation

### **INTRODUCTION**

The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytochemicals. There is currently, widespread screening of plants with potential bioactive properties and concomitant isolation and characterization of these bioactive principles for prevention and combating a wide array of diseases (Farombi and Owoeye, 2011; Njan, 2012). Different plant parts used in various treatment regimens

include leaves, barks, tubers and root that secrete phytochemicals such as alkaloids, terpenes and phenolic compounds. Their utilization may be in decoction, emulsion, apozems, liniments and powdered forms. Medicinal plants are also exploited in cosmetics, perfumery, pharmaceutical and food industries. Their successful exploitation depends on the identification, isolation and purification of desired phytochemicals (Belewu *et al.*, 2009).

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*Vernonia amygdalina* Del. (Compositae), commonly known as “bitter leaf” in Nigeria because of the bitter taste imparted by the leaves and the stem, is a major vegetable of the celebrated “bitter leaf soup” among the Nigerian people. Vernacular names include “ewuro” among the Yorubas and “onugbu” among the Ibos. It has a long history of use in folk medicine particularly among the people of sub-Saharan Africa. Previous studies have reported that it possess antimicrobial, antidiabetic, antimalarial, antiparasitic, insecticidal, anticancer, anti-inflammatory, antipyretic, analgesic, antihelminthic hepatoprotective, antioxidative and hypolipidaemic effects among others (Atangwho *et al.*, 2010; Yeap *et al.*, 2010; Danquah *et al.*, 2012; Ho *et al.*, 2012).

*Ocimum gratissimum*, popularly known as “scent leaf”, is an aromatic medicinal plant belonging to the family Lamiaceae. It is widely distributed in the tropics of Africa and warm temperature regions. In Nigeria, *O. gratissimum* is called “Efinrin” in Yoruba; “Nchoanwu” or “Ahuji” in Igbo and “Aramogbo” in Edo. Scent leaf is used to enhance food flavour (spice) and in the production of dental care products (Awah and Verla, 2010; Akinjogunla *et al.*, 2011).

Foods of plant origin contain a variety of important bioactive compounds such as vitamins, carotenoids and phenolics. Vegetables are widely consumed by humans for their bioactive compounds in addition to their rich nutrients. Many vegetables have been reported to possess antioxidative capacities which enable them to scavenge reactive oxygen species, chelate metal ions, inhibit nitrosation and modulate certain enzymatic actions (Ola *et al.*, 2009). *V. Amygdalina* and *O. gratissimum* occupy reasonably prominent positions in the diets of several Nigerian communities. This study therefore set out to compare the phenolic content and antioxidative capacities of these two widely consumed vegetables.

## EXPERIMENTAL

**Collection and identification of plant materials.** Fresh leaves of *O. gratissimum* and *V. amygdalina* were collected from a farmland in Benin City. They were then identified and authenticated by a Botanist in the Department of Plant Biology and Biotechnology of the University of Benin.

**Preparation of plant extracts.** The leaves were washed, air-dried, macerated and then extracted with ethanol at room temperature for 48 hours with stirring at interval. The extracts obtained were concentrated to dryness at 40°C using a rotary evaporator under reduced pressure (Ayoola *et al.*, 2008). The dried extracts were weighed and then stored at 4°C for subsequent analysis.

**Determination of Total Phenolic Content.** Total phenolic content was determined according to Folin and Ciocalteu’s method (1927). Concentrations of 0.2, 0.4, 0.6, 0.8, and 1 mg/ml of gallic acid were prepared in methanol. Concentrations of the extracts were also prepared in methanol. 4.5 ml of distilled water was added to 0.5 ml of the extract and mixed with 0.5 ml of a ten-fold diluted Folin-Ciocalteu reagent. 5ml of 7% sodium carbonate was then added to the tubes and another 2 ml of distilled water was added. The mixture was allowed to stand for 90 min at room temperature then absorbance read at 760 nm. All determinations were performed in triplicates with gallic acid utilized as the positive control. The total phenolic content was expressed as gallic acid equivalent (GAE).

**Determination of Total Flavonoid Content.** Total flavonoid content was determined using the method of Miliauskas *et al* (2004). 2 ml of 2% AlCl<sub>3</sub> in ethanol was added to 2 ml of extracts (concentrations of extracts were 0.1 – 1.0 mg/ml), in methanol. The absorbance was measured at 420 nm after one hour incubation at room temperature. Similar concentrations

of quercetin, the positive control were used. The total flavonoid content was calculated as mg quercetin equivalent /g of extract.

#### **Determination of Total Flavonol Content.**

The flavonol content was determined by the Yermakov *et al* (1987). The quercetin calibration curve was prepared by mixing 2 ml of varying concentrations of standard quercetin (0.1 - 1.0 mg/ml) in methanolic solutions with 2 ml of 2 g% aluminium trichloride and 6 ml of 5 g% sodium acetate. The absorbance was read at 440 nm after 2.5 hour incubation at 20° C. The content of flavonols in quercetin equivalent was calculated.

#### **Estimation of Diphenyl-2-picryl-hydrazyl (DPPH) Radical Scavenging Activity.**

The free radical scavenging capacity of the plant extracts against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined by a slightly modified method of Brand-Williams *et al* (1995). Briefly, 0.5 ml of 0.3 mM DPPH solution in methanol was added to 2 ml of various concentrations (0.2 - 1.0 mg/ml) of the extracts. The reaction tubes were shaken and incubated for 15 min at room temperature in the dark, and the absorbance read at 517 nm. All tests were performed in triplicate. Vitamin C was used as standard control, with similar concentrations as the test samples prepared. A blank containing 0.5 ml of 0.3 mM DPPH and 2 ml methanol was prepared and treated as the test samples.

$$\text{DPPH radical scavenging activity (\%)} \\ = [(A_0 - A_1) / (A_0)] \times 100,$$

Where  $A_0$  was the absorbance of DPPH radical + methanol;  $A_1$  was the absorbance of DPPH radical + sample extract or standard.

The 50% inhibitory concentration value ( $IC_{50}$ ) was calculated as the effective concentration of the extract that is required to scavenge 50% of the DPPH free radicals.

#### **Ferric ion Reducing Antioxidant Power (FRAP) Assay.**

A modified method of Benzie

and Strain (1996) was adopted for the ferric reducing antioxidant power (FRAP) assay which depends on the ability of the sample to reduce the ferric tripyridyltriazine (Fe (III)-TPTZ) complex to ferrous tripyridyltriazine (Fe(II)- TPTZ) at low pH. Fe (II)-TPTZ has an intensive blue colour which can be read at 593 nm. 1.5 ml of freshly prepared FRAP solution (25 ml of 300 mM acetate buffer pH 3.6, 2.5 ml of 10 mM 2,4,6-tripyridyls-triazine (TPTZ) in 40 mM HCl, and 2.5 ml of 20 mM ferric chloride ( $FeCl_3 \cdot 6H_2O$ ) solution) was mixed with 1ml of the extracts at concentration of 1.0 mg/ml. The reaction mixtures were incubated at 37 °C for 30 min and increase in absorbance at 593 nm measured.  $FeSO_4$  was used for the calibration curve and ascorbic acid served as the positive control. FRAP values (expressed as mg Fe (II)/g of the extract) for the extracts were then extrapolated from the standard curve.

**Reducing power assay.** The reducing power of extract was determined according to the method described by Lai *et al* (2001). 1 ml of different concentrations of extracts (0.1-1.0 mg/ml) in water was mixed with 2.5 ml of 0.2 M phosphate buffer, pH 6.6 and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. Thereafter, 2.5 ml of trichloroacetic acid (10%) was added to the mixture to stop the reaction. 2.5 ml of distilled water and 0.5 ml of 0.1%  $FeCl_3$  were then added and the absorbance measured at 700 nm. Higher absorbance values indicated higher reducing power. Vitamin C served as a positive control.

#### **Estimation of Thiobarbituric Acid Reactive Substances (TBARS).**

TBARS was estimated according to the method of Ohkawa *et al* (1979). Egg yolk homogenate (0.5 ml of 10 % v/v) and 0.1 ml of extract were added to a test tube and made up to 1ml with distilled water. 0.05 ml of  $FeSO_4$  (0.07 M) was added to induce lipid peroxidation and incubated for 30 min. Then 1.5 ml of 20 % acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 ml of

0.8% (w/v) TBA in 1.1% sodium dodecyl sulphate and 0.05 ml 20% TCA were added and the resulting mixture was vortex and then heated at 95 °C for 60 min. The generated colour was measured at 532 nm.

Inhibition of lipid peroxidation (%) =  
 $(C-E)/C \times 100\%$ ;

where C is the absorbance value of the fully oxidized control and E is ( $Ab_{532} + TBA - Ab_{532-TBA}$ ).

**Statistical analysis.** All analyses were carried out in triplicate and results expressed as mean  $\pm$  SEM. The data were subjected to one-way analysis of variance (ANOVA), where applicable. Differences between means were determined by Duncan's multiple range tests using Graph Pad Prism statistical package version 6. P values of  $< 0.05$  were regarded as significant. The  $IC_{50}$  values were calculated using linear regression graphs.

## RESULTS

Results from the present study showed a significantly higher concentration of total phenolic and flavonoid in *O. gratissimum*

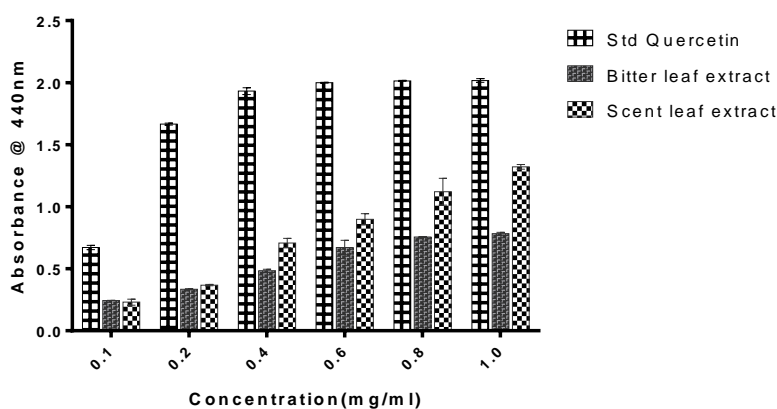
extract ( $168.5 \pm 5.50$  mg gallic acid equivalent /g of extract and  $126.7 \pm 1.00$  mg quercetin equivalent, respectively) when compared with *V. amygdalina* extract ( $97.0 \pm 0.01$  mg gallic acid equivalent /g of extract and  $65.4 \pm 4.42$  mg quercetin equivalent /g of extract, respectively) (Table 1). Similarly, the *O. gratissimum* extract showed a higher concentration - dependent increase in flavonol content than the *V. amygdalina* counterpart. However, the flavonol content of the extracts were lower than that of the standard antioxidant, quercetin (Figure 1).

The result of DPPH radical scavenging activity is shown in Figure 2. The DPPH scavenging ability was higher in the *O. gratissimum* extract than in the *V. amygdalina* extract with corresponding  $IC_{50}$  values of  $105.7 \mu\text{g/ml}$  and  $111.8 \mu\text{g/ml}$ , respectively (Table 2). The reference antioxidant compound, vitamin C showed the highest ability to scavenge DPPH radicals, at all concentrations, when compared with the two extracts ( $IC_{50}$  value of  $91.0 \mu\text{g/ml}$ ).

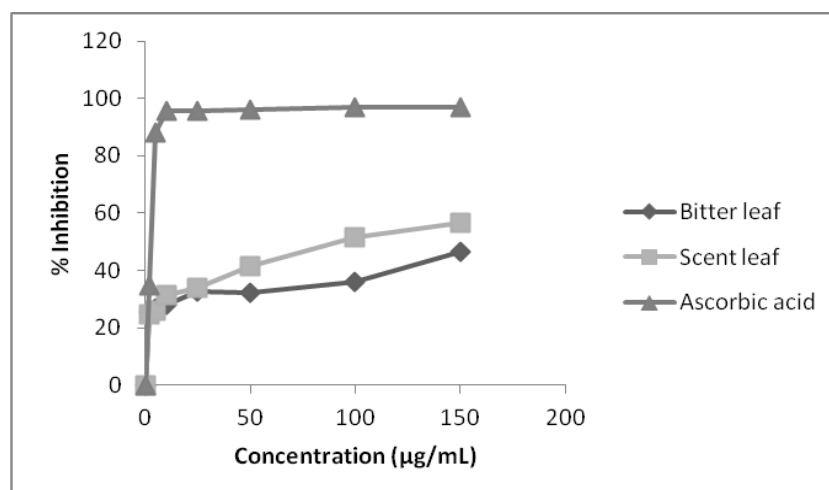
**Table 1:** Total phenolic and flavonoid content of ethanolic extracts of *V. amygdalina* and *O. gratissimum* leaves

Extract	Total phenol (mg GAE/g of extract)	Total flavonoid (mg QE/g of extract)
<i>V. amygdalina</i>	$97.0 \pm 0.01^a$	$65.4 \pm 4.42^a$
<i>O. gratissimum</i>	$168.5 \pm 5.50^b$	$126.7 \pm 1.00^b$

Values are expressed as mean  $\pm$ SEM ( $n = 3/\text{group}$ ). Values in a column with the same superscript letters are not significantly different ( $p < 0.05$ ). GAE = Gallic acid equivalent, QE = Quercetin equivalent.



**Figure 1:** Total flavonol content of ethanolic extracts of *V. amygdalina* and *O. gratissimum* leaves  
 Values are expressed as mean  $\pm$  SEM ( $n = 3/\text{group}$ )



**Figure 2:** DPPH radical scavenging activities of ethanolic extracts of *V. amygdalina* and *O. gratissimum* leaves. Values are expressed as mean  $\pm$  SEM ( $n = 3/\text{group}$ ).

**Table 2:**  $IC_{50}$  values of ethanolic leaf extracts of *V. amygdalina* and *O. gratissimum*

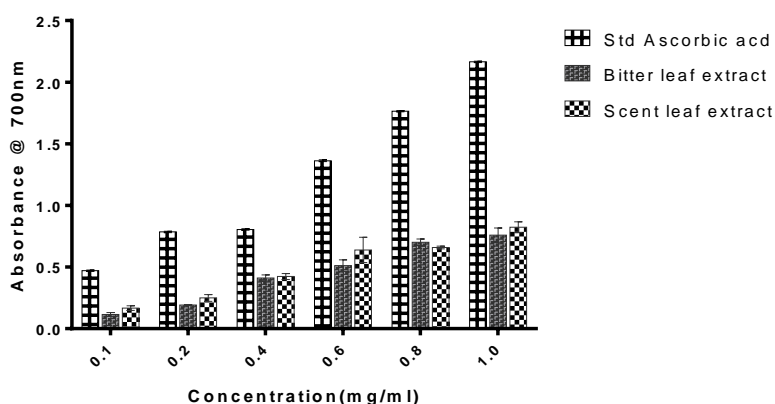
Plant extract	$IC_{50}(\mu\text{g/mL})$
Ascorbic acid	91.0
Bitter leaf	111.8*
Scent leaf	105.7*

Values in a column with asterisk (\*) are significantly different from the standard ( $p < 0.05$ )

**Table 3:** Ferric Reducing Antioxidant Potential (FRAP) of ethanolic leaf extracts of *V. amygdalina* and *O. gratissimum*

Plant extract	FRAP value(mg Fe(II)/g of the extract)
Ascorbic acid	140.5 $\pm$ 5.61
Bitter leaf	85.0 $\pm$ 0.01*
Scent leaf	110.0 $\pm$ 0.02*

Values are expressed as mean  $\pm$  SEM ( $n = 3/\text{group}$ ). <sup>a</sup> $P \leq 0.05$  compared to ascorbic acid. Values in a column with asterisk (\*) are significantly different from the standard ( $p < 0.05$ )



**Figure 3:** Reducing power of ethanolic extracts of *V. amygdalina* and *O. gratissimum* leaves. Values are expressed as mean  $\pm$  SEM ( $n = 3/\text{group}$ ).

**Table 4:** Thiobarbituric Acid Reactive Substances (TBARS) of Ethanolic Extracts of *V. amygdalina* and *O. gratissimum* Leaves

Plant extract	Lipid peroxidation inhibition (%)
Bitter leaf	31.4 ± 0.15 <sup>a</sup>
Scent leaf	42.8 ± 1.00 <sup>b</sup>

Values are expressed as mean ± SEM,  $n = 3$ /group. Different uppercase letters represent significant difference between means at  $P \leq 0.05$ .

FRAP value (expressed as mg Fe (II) / g of extract) was significantly lower ( $p < 0.05$ ) in the *V. amygdalina* extract ( $85.0 \pm 0.01$ ) when compared with that of *O. gratissimum* extract ( $110.0 \pm 0.02$ ) (Table 3). However, the reference antioxidant (ascorbic acid) had a significantly higher FRAP value than both extracts ( $140.5 \pm 5.61$ ). The ferric reducing power of both extracts was not significantly different ( $p > 0.05$ ) and increased with their concentrations (Figure 3). The ferric reducing power of vitamin C was however higher than that of the extracts. The ability of the extracts to inhibit lipid peroxidation induced by ferrous sulphate in egg-yolk homogenates are shown in table 4. The percentage lipid peroxidation inhibition was significantly higher ( $p < 0.05$ ) in *O. gratissimum* leaf extract ( $42.8 \pm 1.00\%$ ) in contrast to the *V. amygdalina* extract ( $31.7 \pm 0.15$ ).

## DISCUSSION

Fruits and vegetables are considered as important components of any diet. Fruits and vegetables are known to contain vitamins and minerals necessary for maintenance of good health. The good health benefits of vegetables are also ascribed to their inherent phytochemical compounds which help in counteracting the deleterious effects of free radicals and highly reactive species *in vivo* (Ola et al., 2009).

Nigeria is blessed with a rich array of green leafy vegetables that serve as vital ingredients in soups and medicine. *Vernonia amygdalina* ('bitter leaf') is one of the most popular vegetables used in cooking in Southern Nigeria while the popularity of *Ocimum gratissimum* ('scenting leaf') as a spice is due to the distinct, pleasant aroma it

releases when added to soups. These vegetables, though popular as cooking ingredients, are also known to possess several medicinal values (Iweala and Obidoa, 2010; Farombi and Owoeye, 2011).

The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytochemicals (Padmanabhan and Jangle, 2012). Total phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators. Flavonoids are the most widespread group of natural compounds and probably the most important natural phenolics as a result of their health-promoting benefits (Gil et al., 2002; Padmanabhan and Jangle, 2012). Results from this study showed the presence of phenols in both plants extracts with *O. gratissimum* containing higher amounts of total phenolics, flavonoids and flavonols compared to the *V. amygdalina* leaf extract. The antioxidant potential of phenols is believed to be conferred on them by their hydroxyl group (-OH), which is bonded directly to an aromatic hydrocarbon (phenyl) ring. This makes them donate electrons easily to electron-seeking free radicals, thus down-regulating their menace in living cells (Uyoh et al., 2013). The significantly higher amount of phenols in the *O. gratissimum* as compared to *V. amygdalina* is not surprising as freshly plucked *O. gratissimum* leaves give off a highly aromatic scent.

DPPH free radical scavenging ability is one of the most popular methods utilized in screening for anti-oxidative activity. The DPPH radical is employed as a substrate to evaluate antioxidant activity. Reduction in DPPH radical is measured by decrease in absorbance induced by antioxidants present in

the sample. An important variable estimated from DPPH free radical scavenging test is termed IC<sub>50</sub>. IC<sub>50</sub> expresses the amount of antioxidant required to decrease the DPPH radical concentration by 50% (Chanda *et al.*, 2011). The lower IC<sub>50</sub> value recorded in this study for *O. gratissimum* extract suggests that it may be a more potent antioxidant when compared to *V. amygdalina*.

The reducing capacity of an extract may be an important indicator of its potential antioxidative activity (Chanda *et al.*, 2011). In this study, we assessed the reducing capacity of our extracts by utilizing two (2) assay methods; ferric reducing antioxidant potential (FRAP) and reducing power. The principle of FRAP method is based on the reduction of a ferric-2,4,6-tri(2-pyridyl)-*s*-triazine [Fe (III)-TPTZ] complex to its ferrous 2,4,6-tri (2-pyridyl) -*s*-triazine [Fe(II)-TPTZ] complex coloured form in the presence of antioxidants. This complex has an intense blue colour that can be monitored at 593nm (Yim *et al.*, 2010). On the other hand, the reducing power of an antioxidant measures its ability to donate electrons. Both assays showed higher reducing capacity for the *O. gratissimum* extract compared to the *V. amygdalina* extract. These data in addition to the DPPH test suggests that *O. gratissimum* leaf extract may be a more potent antioxidant than the *V. amygdalina* leaf extract.

Lipid peroxidation has been implicated in the pathology of many diseases. Previous reports have demonstrated a significant reduction in lipid peroxidation and superoxide dismutase in rats fed diets supplemented with ground leaves of *O. gratissimum* (Iweala and Obidoa, 2010). These findings are not surprising as we have demonstrated in this study significant inhibition of lipid peroxidation by *O. gratissimum* leaf extract. Nwanjo (2005) observed reduction in malondialdehyde levels in diabetic rats fed *V. amygdalina* leaves. This also supports our findings of inhibition of

lipid peroxidation by *V. amygdalina* leaf extract. Inhibition of lipid peroxidation by *V. amygdalina* extract may be due to the flavonoids present in the leaves as flavonoids have been demonstrated to possess antioxidant activities (Igile *et al.*, 1994).

Gil *et al* (2002) asserted that fruits showing higher antioxidant capacity contained higher amounts of phenolics. Our results follow this trend as *O. gratissimum* extract revealed higher amount of all groups of phenolics estimated (including total phenol, flavonoids and flavonols) as well as a higher anti-oxidative capacity (in terms of the DPPH free radical scavenging capacity, FRAP and reducing power) when compared with the extract of *V. amygdalina*. These data do not in any way prejudice against the consumption of *V. amygdalina*. We however use the generated data to draw attention to the benefits accruable from consumption of these vegetables and recommend their sustained inclusion in human diet.

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