COMPARATIVE ANTIOXIDANT ACTIVITY, TOTAL PHENOL AND TOTAL FLAVONOID CONTENTS OF TWO NIGERIAN OCIMUM SPECIES

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Abstract

Antioxidants are compounds which act as a major defense against oxidative stress caused by free radicals. In this study, a comparative evaluation of the antioxidant properties, phenolic and flavonoid contents of the methanolic extracts of *Ocimum gratissimum* Linn and *Ocimum canum* Sims was carried out.

Crude extracts of both plants were obtained by maceration of powdered plant materials in methanol (80%) for 24hrs. The phenolic and flavonoid contents were determined using standard methods while the antioxidant capacities were evaluated using six different *in vitro* radical scavenging assays: total antioxidant potential, reductive potential, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging, lipid peroxidation inhibition, hydroxyl radical and nitric oxide scavenging activity.

The total phenolic content in *O. gratissimum* and *O. canum* were found to be 32.66 ± 6.21 and 17.19 ± 2.54 mg GAE/g dw while total flavonoid content gave 1.94 ± 0.23 and 0.67 ± 0.01 mg QUE/g dw, respectively. Both extracts had effective reductive potential as well as exhibited strong total antioxidant capacity with increasing concentration of extract. Comparatively, *O. gratissimum* exhibited a significantly ($p < 0.05$) higher capacity to quench the DPPH free radical with IC_{50} value of 26.01 ± 3.2 µg/ml than *O. canum*, which has an IC_{50} value of 60.45 ± 5.22 µg/m. *O. gratissimum* also significantly inhibited membrane lipid peroxidation and hydroxyl radical formation with IC_{50} of 99.37 ± 8.56 µg/ml and 465.33 ± 21.62 µg/ml, respectively while *O. canum* correspondingly gave IC_{50} values of 447.5 ± 35.61 µg/ml and 868.16 ± 43.05 µg/ml. In the nitric oxide scavenging activity, however, *O. canum* showed a stronger inhibitory effect than *O. gratissimum* as indicated by their IC_{50} values of 277.22 ± 15.09 µg/ml and 731 ± 56.99 µg/ml, respectively. These activities are however several folds lower than those of butylated hydroxyl toluene (BHT), ascorbic acid and quercetin used as antioxidant standards.

The results demonstrate that *O. gratissimum* has greater antioxidant capacity than *O. canum* because of its relatively higher radical scavenging activity in all antiradical tests carried out except the nitric acid scavenging test and higher contents of flavonoid and phenolic compounds. *O. gratissimum* is therefore more beneficial therapeutically than *O. canum* since it provides better defense against free radical induced oxidative stress, and this attribute probably explains its relative preference in ethnomedicine for both culinary and medicinal purposes amongst the *Ocimum* species widely cultivated in South Western Nigeria.

Key words: Antioxidant activity, phenolic content, DPPH, *Ocimum gratissimum, Ocimum canum.*

1. Introduction

It has long been recognized that oxidative stress elicited by oxygen-derived free radicals in biological systems plays a major role in the pathogenesis of several chronic disorders in humans, including coronary heart disease, type II diabetes, arthritis, ischemia-reperfusion injury, cancer, neurodegenerative diseases and even in aging process (Kumpulainen and Salonen, 1999; Buluca *et al.*, 2000; Arouma, 2003). Free radicals due to environmental pollutants, radiation, chemicals, toxins, deep-fried and spicy foods as well as physical stress cause depletion of immune system antioxidants, change in gene expression and induce the synthesis of abnormal proteins (Gulcin, *et al.*, 2007). There is increasing evidence that normal oxidative metabolism in living system and inflammatory processes remain one of the major routes for producing reactive oxygen free radicals, which may act as toxins, mediators and modulators of inflammatory gene activation (Gulcin *et al.*, 2004). The ineffective scavenging of such reactive oxygen species (ROS) has been reported to play an adverse role in determining the extent of tissue

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dysfunction (Halliwell, 1994; Arouma, 1998). The levels of intermediate reduction products of oxygen metabolism such as superoxide anion radical, hydroxyl radical and hydrogen peroxide are modulated by various cellular radical-scavenging systems consisting of enzymatic superoxide dismutase (SOD), catalyse (CAT), glutathione reductase (GHS-Rd) and glutathione peroxidase (GSH-Px) and non-enzymatic (glutathione, vitamins C and E, flavonoids, ubiquinol-10 and albumin) scavenger components (Halliwell and Gutterigde, 1989).

It is probable therefore that the alleviation of oxidative stress could result in decreasing the incidence or progression of oxidation-associated diseases. Consequently, several approaches have been carried out to decrease oxidative stress in order to manage oxidation-associated disorders. Several studies have shown that the therapeutic effects of some medicinal plants, fruits and even vegetables commonly used in ethnomedicine against many diseases could be attributed to the antioxidant properties of their phytochemicals (Pietta, 1998; Pourmorad *et al.*, 2006). Consequently, the search for natural antioxidants has been intensified because of their therapeutic potential for maintaining human health. Numerous medicinal plants and their formulations have been used for treating several oxidative stress related diseases in ethnomedical practice in Nigeria (Gbile and Adesina, 1987; Sofowora, 1996).

The genus Ocimum, Lamiaceae, collectively called basil, has long been acclaimed for its diversity. Ocimum is represented by over 30 species of herbs and shrubs from the tropical and subtropical regions of Asia, Africa and Central and South America (Martins *et al.*, 1999; Simon, 1999), with Africa being the main centre of diversity (Paton, 1992). However, only about three of these species are prevalent in Nigeria; *O. gratissimum*, *O. basilicum* and *O. canum* (Famurewa, 2003). Ocimum species have long been known to contain phytochemicals with significant preservative properties and health benefits (Oke and Hamburger, 2002; Exarchou *et al*, 2002). Furthermore, a number of phenolic compounds with strong antioxidant activity have been identified in some members of this genus (Nakamura *et al.*, 1997; Javanmardi *et al.*, 2003; Gulcin *et al.*, 2007). *O. gratissimum*, in particular, has been used in Nigeria ethnomedicine as a remedy for treating different human diseases. These include upper respiratory tract infection, diarrhea, headache, skin diseases, pneumonia, fever, conjunctivitis and diabetes (Onajobi, 1986, Ilori *et al.*, 1996, Aguiyi *et al.*, 2000; Orafidiya *et al.*, 2004). *O. canum* on the other hand is used for managing diabetes mellitus and for the treatment of conjunctivitis in Ghana ethnomedicine (Nyarko *et al.*, 2002; Ngassoum *et al.*, 2004). Studies on the essential oils from both plants have shown that they possess potent antimicrobial (Janssen *et al.*, 1989; Dubey *et al.*, 2000; Bassole *et al.*, 2005), insecticidal and cytotoxic properties (Dubey *et al.*, 1997).

Given the implication of reactive oxygen species (ROS) in many diseased conditions such as diabetes mellitus and cardiovascular diseases (Halliwell, 1991) and the prospect of polyphenolic compounds providing biological resistance against the deleterious effects of reactive oxygen species, it was therefore worthwhile to evaluate the possible antioxidant properties of the Ocimum. Thus, in continuation of our research on this genus (Onajobi, 1986; Osoniyi and Onajobi, 1998; Famurewa, 2003), the present study was carried out to evaluate the antioxidant capacities and determine the phenolic and flavonoid content of the methanolic extracts of *O. gratissimum and O. canum,* the two prevalent Ocimum species cultivated in the South Western Nigeria.

2. Materials and Methods

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), gallic acid, ferrous sulphate heptahydrate ($FeSO_4.7H_2O$), ethylene diaminetetraacetate (EDTA), potassium ferricyanide, ascorbic acid, riboflavin, nitro blue tetrazolium (NBT), thiobarbituric acid (TBA) were purchased from Sigma Chemical Co (St., Louis, USA).Tert-butyl-4-hydroxy toluene (BHT), Folin Ciocalteu reagent, methanol were purchased from Merck Co. (Germany). Quercetin was a donation from Dr. M. Aderogba, Department of Chemistry, O.A.U., Ile-Ife. All other reagents used were of Analytical grade and products of the British Drug Houses (BDH), Ltd., U.K.

Plant materials and extraction procedures

Leaves of *O. gratissimum* were collected from the Town planning way, Ilishan-Remo, Ogun State while that of *O. canum* were collected from Obafemi Awolowo University, Ile-Ife and were authenticated by Mr Oladele of the Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University. Voucher specimens were deposited at the Herbarium of the Faculty of Pharmacy.

The leaves of both plants were dried in an oven at 45 °C for 72 hrs and then ground to a fine powder using a Kitchen mill. Twenty gram (20 g) portions of the powdered materials were macerated in 80% methanol (1:10, w/v) with magnetic stirring for 24 hrs and then filtered. The marc was re-extracted twice under same conditions and the combined extract obtained was then filtered over Whatman No 1 paper and concentrated under vacuum in a rotavapor (Buchi, Flawil Switzerland) at 45 °C to obtain the crude extracts.

Total phenolic content

The total phenolic content of crude extracts was determined by using the Folin-ciocalteu assay method

of Singleton and Rossi (1965) as described by Pourmorad *et al*, (2006). A 1 ml aliquot of each extract was added into a 25 ml volumetric flask, containing 9 ml of distilled water. Folin-ciocalteu's phenol reagent (1 ml) was then added to the mixture and vortexed. After 5 min, 10 ml of 7% sodium carbonate solution was added to the mixture and then incubated for 90mins at room temperature. The absorbance was then read at 765nm against a reagent blank. The standard curve was prepared using (50, 100, 150, 200, and 250 mg/L) solution of gallic acid in methanol: water (1:1, v/v). The total phenol content of the extracts were expressed as milligrams of gallic acid equivalents (GAE) per gram dry weight of plant material.

Total flavonoid content

The determination of the total flavonoid content of the plant extracts was based on the aluminium chloride colorimetric assay method described by Neergheen *et al*. (2006). An aliquot (1 ml) of each extract was added to 4 ml of distilled water in a 10 ml volumetric flask. This was followed by the addition of 0.3 ml 5% sodium nitrite. After 5 min, 0.3 ml of 10% aluminium chloride was added and vortexed. This was immediately followed by the addition of 2 ml of 1 M sodium hydroxide solution and the volume then made up to 10 ml with distilled water. The resulting assay mixture was vortexed and the absorbance was then measured at 510 nm against a reagent blank. A calibration curve was prepared by using quercetin solutions at concentrations 12.5 to 100 µg/ml in methanol.

Total antioxidant capacity

This method is based on the reduction of molybdenum (VI) to molybdenum (V) by the extract and the subsequent formation of a green phosphate/ molydenum (V) complex at acid pH (Prieto *et al*., 1999). To 0.3 ml of the extract or standard solutions of ascorbic acid and gallic acid (100, 200, 300, 400 500 and 600 µg/ml) in a test tube was added, 3 ml of the reagent solution which consisted of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The tubes containing the reacting mixture were incubated in a water bath at 95 °C for 90 min. The mixture was then allowed to stand and cool to room temperature and the absorbance measured at 695 nm against a blank, which consisted of the reacting mixture and distilled water in place of the extract. The antioxidant activity was expressed as the number of equivalent ascorbic acid and gallic acid.

Determination of DPPH radical scavenging activity

The hydrogen donating or radical scavenging properties of the extracts was determined using the stable radical DPPH· (2,2-diphenyl-2-picrlhydrazyl hydrate) as described by Brand-Williams *et al*. (1995). When DPPH reacts with an antioxidant compound which can donate hydrogen, it is reduced (Blois, 1958). The change in color from deep violet to light yellow was measured spectrophotometrically at 517 nm. To 1ml of different concentrations (125, 98.75, 62.5, 46.8, 31.25 µg/ml) of the extract or standard (vitamin C) in a test tube was added 1ml of 0.3mM DPPH in methanol. The mixture was vortexed and then incubated in a dark chamber for 30mins after which the absorbance was measured at 517 nm against a DPPH control containing only 1ml of methanol in place of the extract. The percent inhibition of DPPH scavenging activity was calculated using the equation:

$$\text{DPPH (\%) inhibition} = [(A_o - A_i)/ A_o] \times 100)$$

Where A_o is the absorbance of control with methanol and A_i is the absorbance with extract samples. A plot of the percentage DPPH radical scavenging versus log concentration of each extract was prepared and the concentration at 50% radical inhibition (IC_{50}) was determined from the linear regression equation. Regression equations had correlation coefficients ≥ 0.91.

Lipid peroxidation assay

The lipid peroxidation inhibition potential of the extracts was determined using a modified thiobarbituric acid reacitive species (TBARS) assay of Ohkowa *et al* (1979). The end product of lipid peroxidation, using egg yolk homogenate as lipid-rich media (Ruberto *et al*., 2000), was quantified by determining the formed malondialdehyde (MDA) which react with the thiobarbituric acid (TBA) under acidic condition to form an MDA-TBA adduct. To 0.5 ml of a 10% (v/v) egg homogenate was added 0.1ml of varying concentrations of the extract (1000, 500, 250, 125, 62.5, 31.25 µg/ml) in a test tube followed by the addition of 1ml distilled water. Then 50 µl of FeS04 (0.07 M) was added to the reaction mixture followed by 50 µl of ascorbate (1 mM) to induce lipid peroxidation. The mixture was vortexed and allowed to stand for 30min at room temperature after which 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% (w/v) thiobarbituric acid in 1.1% sodium dodecyl sulphate were added. The resulting mixture was then heated in a water bath at 95°C for 60 min. After cooling, 4.0 ml of butan-1-ol were added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm. Percent inhibition of lipid peroxidation was calculated as expressed above with DPPH radical scavenging.

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging potential of the extracts was determined using the deoxyribose assay of Halliwell *et al*. (1987) as described by Goncalves *et al*. (2005). This method is based on the competition between deoxyribose and the test compounds for

hydroxyl radicals generated from the Fe^{3+}/ascorbate/ EDTA/H_2O_2 system. The reacting mixture contained, in a final volume of 1 ml, the following reagents: 200µL KH_2PO_4-KOH (100 mM) pH 7.4, 200µL deoxyribose (15 mM), 200 µL $FeCl_3$ (500 µM), 100 µL EDTA (1 mM), 100µL sample, vehicle (blank) or D-manitol (reference compound), 100 µL H_2O_2 (10 mM) and 100 µL ascorbic acid (1 mM). The reaction mixture was vortexed and then incubated in a water bath at 37 °C for 1 h. At the end of the incubation period, 1 ml of 1% (w/v) thiobarbituric acid (TBA) in 1.1% sodium dodecyl sulphate (SDS) was added to each mixture followed by the addition of 1ml of 20% (w/v) acetic acid. The solutions were heated in a water bath at 95 °C for 60 min to develop the pink color. The absorbance was measured at 532 nm. Percent inhibition of hydroxyl radicals was calculated as expressed above with DPPH radical scavenging.

Nitric oxide radical inhibition activity

The nitric oxide radical inhibiting activity of the extracts was carried out according to the method of Green *et al.* (1982) as described by Marcocci *et al.* (1994). Nitric oxide, generated from sodium nitroprusside in aqueous solution at physiological pH, interacts with oxygen to produce nitrite ions which was measured by Griess reaction. The reaction mixture (3ml) containing sodium nitroprusside (10mM) in phosphate buffered saline (PBS) and the varying concentrations (10000, 5000, 2500, 625 and 312.5µg/ml) of the extract were incubated in a water bath at 25°C for 150min. After incubation, 1.5ml of the reaction mixture was removed and 1.5ml of Griess reagent was then added. The absorbance of the chromophore formed was evaluated at 546nm (Marcocci *et al.*, 1994). The concentration of nitrite formed was derived from a regression analysis using serial dilutions of sodium nitrite as a standard. Percentage inhibition of nitric oxide radical formation was calculated as expressed above with DPPH radical scavenging.

Reductive potential

The reductive potential of the extracts was determined according to the method described by Oyiazu *et al.* (1986). To 1 ml of different concentrations (500, 250,125, 62.5, 31.25 and 15.625 µg/ml) of the extracts or standard vitamin C (10 µg/ml) and BHT 10 µg/ml) was added 1ml of phosphate buffer (0.2 M, pH 6.6) and vortexed after which 2.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$] was added. The reacting mixture was then incubated in a water bath at 50 °C for 20 min. 2.5 ml of 10% trichloroacetic acid was then added to the mixture and centrifuged for 10 min. To 2.5 ml of the supernatant was added 2.5 ml of distilled water and then 0.5 ml of 1% $FeCl_3$ after which the mixture was vortexed. The absorbance was then measured at 700 nm. The increase in absorbance of the reaction mixture indicated higher reductive potential and was expressed as reductive potential index (REI) relative to the reductive power of 10 µg/ml vitamin C standard.

Statistical analysis

Results are expressed as mean value ± SEM. Simple regression analysis was performed to calculate the dose-response relationship of standard solutions and the test samples. The statistical significance between antioxidant activities was evaluated using a Mann-Whitney U test. P values less than 0.05 were considered to be statistically significant.

3. Results

The percentage yield of the methanol (80%v/v) extract from the dried plant material is shown in Table 1. *O. gratissimum* contained more methanol-soluble compounds than *O. canum*. Furthermore, the Folin-Ciocalteu assay showed that the concentration of phenolic compounds in *O. gratissimum* is twice that of *O. canum*. Similarly, the concentration of total flavonoids in *O. gratissimum* was 1.94 mg QUE/g dry wt plant material, which is thrice that observed in *O. canum* (0.67 ± 0.11 mg QUE/g dry wt plant material) as shown in Table 1.

The total antioxidant capacity of the plant extracts is shown in Table 2. At a concentration of 250 µg/ml, the total antioxidant capacity for *O. canum* was 679.29 ± 21.63, (vitamin C equivalent ug/g dw) and 270.91 ± 9.21 (GAE ug/g dw) while *O. gratissimum* gave relatively higher values of 1157.31 ± 30.52 (vitamin C equivalent µg/g dw) and 474.31 ± 12.98 (GAE µg/g dw), respectively.

Figure 1.illustrates the reductive potential of the plant extracts. At the lowest concentration of 31.25 µg/ml, the two plant extracts apparently show the same reductive potential; but at higher concentrations, *O. gratissimum* was observed to have much higher reductive potential than *O. canum*.

The scavenging potential of the plant extracts was assayed by the DPPH radical scavenging, lipid peroxidation inhibition, hydroxyl radical and nitric oxide scavenging assays as shown in Table 3.

In the DPPH radical scavenging assay, extracts of both plants were observed to exhibit a concentration dependent decrease in the concentration of DPPH radical owing to their scavenging properties. *O. gratissimum* showed a stronger DPPH scavenging activity with an IC_{50} of 26.01 ± 3.31 µg/ml than *O. canum*, which gave an IC_{50} of 60.4 ± 5.67µg/ml. However, DPPH scavenging activity in both plant samples was less than that of the reference samples, L-ascorbic acid, BHT and quercetin.

Similarly, both extracts inhibited lipid peroxidation induced by Fe^{2+} ascorbate system in a concentration dependent manner with *O.gratissimum* exhibiting a potency of approximately 5 times that of *O. canum*.

Table 1: Percentage yield of total phenolic and flavonoid content of the methanolic extract of *O. canum* and *O. gratissimum*

Tested material	% Yield	Total phenolic content (mg GAE /g of dry wt)	Total flavonoid content (mg QUE /g of dry wt)
O. canum	4.84 ± 0.79	17.19 ± 2.54	0.67 ± 0.11
O. gratissimum	7.18 ± 1.12	32.66 ± 6.21	1.94 ± 0.23

Values are expressed as mean ± SD of at least three experiments

Table 2: Total antioxidant capacity of methanolic extract *O. canum* and *O. gratissimum*

Concentration (µg/ml)	Vitamin C equivalent (µg/g)		Gallic acid equivalent dry wt (µg/g)	
	O. gratissimum	*O. canum*	*O. gratissimum*	*O. canum*
500	2227.38 ± 13.65*	1421.47±39.44	929.84 ± 56.32	586.19 ± 17.55
250	1157.32 ± 31.07*	679.29 ± 21.26	474.31 ±13.14	270.91 ± 9.23
125	704.07 ± 11.24*	333.57 ± 33.81	281.45 ± 15.03	123.75 ±14.11

Values are expressed as mean ± SD of at least three experiments

Table 3: *In vitro* antioxidant activities of the methanolic extracts of *O. canum* and *O. gratissimum* in different antiradical test systems

Tested material	DPPH radical scavenging	Lipid peroxidation inhibition	Hydroxyl radical inhibition	Nitric oxide inhibition
	IC_{50} µg/ml			
O. .canum	60.43 ±4.71*	447.55 ± 32.47*	868.16±74.17*	277.22 ± 14.18
O. gratissimum	26.01 ± 2.33	99.37 ± 7.97	465.33± 55.96	731.10 ± 21.67*
(L-ascorbic acid)	10.56 ± 3.52	ND	ND	32.48 ± 6.22
(BHT)	14.16 ± 2.78	28.34 ± 7.88	ND	26.42 ± 10.54
(Quercetin)	18.06 ± 3.19	45.78 ± 8.38	ND	17.45 ±6.41
D-mannitol	ND	ND	74.31 ±8.11	

Values are expressed as mean ± SD of triplicate experiments.
IC_{50} values were calculated from regression equations of extract concentrations against % inhibition of free radical formation /prevention of lipid peroxidation.

- P< 0.05, *O. gratissimum* vs *O. canum*
- ND, not determined

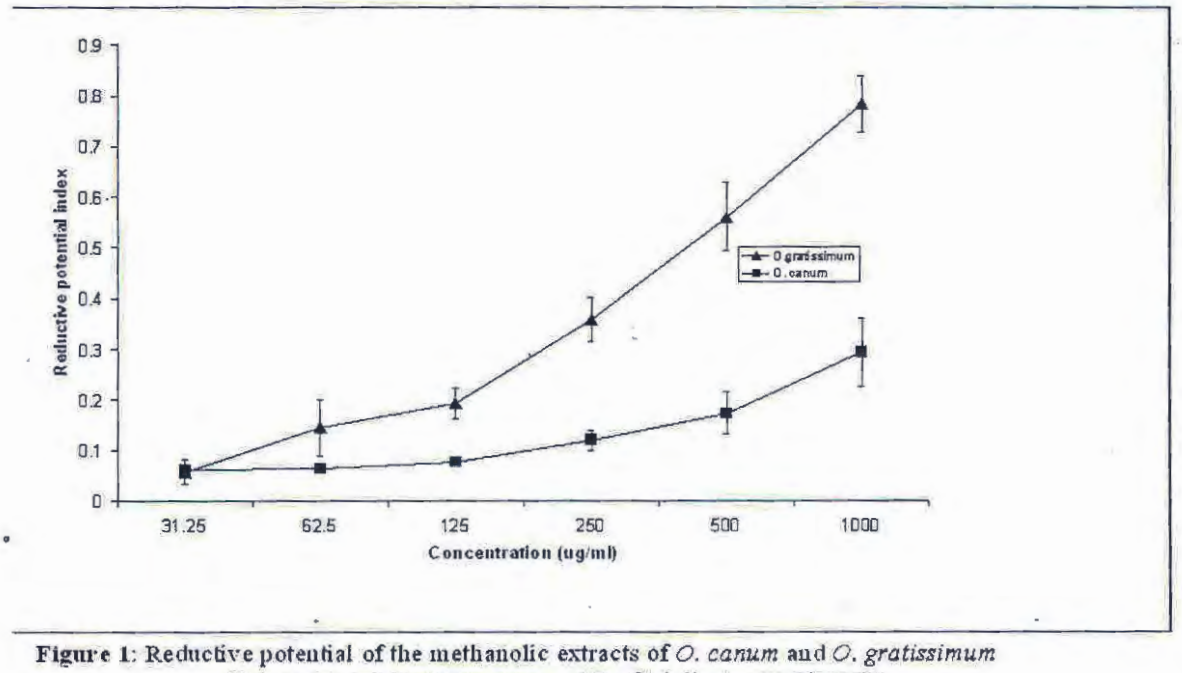

Figure 1: Reductive potential of the methanolic extracts of *O. canum* and *O. gratissimum*
Values are expressed as mean ± SD of triplicate experiments.
* P< 0.05, *O. gratissimum* vs *O. canum*

The IC_{50} values for the inhibition of non-enzymatic lipid peroxidation of egg yolk lipids were 99.37 ± 7.97 µg/ml and 447.55 ± 32.47 µg/ml for *O. gratissimum* and *O. canum*, respectively. Under the same experimental conditions, the IC_{50} values of BHT and quercetin were 28.34 and 45.78 µg/ml respectively. A study of the inhibitory action of the plant extracts on hydroxyl radicals dependent deoxyribose degradation showed that both extracts were generally weak inhibitors of hydroxyl radicals when compared to the standard antioxidant agent, D-mannitol (IC_{50} value of 74.31 ± 8.11). Comparatively, the IC_{50} values *O. gratissimum* and *O. canum* were 465.33 ± 55.96 µg/ml and 868.16 ± 74.17 µg/ml, respectively.

The inhibition of nitric oxide generated from sodium nitroprusside at physiological pH was found to be concentration dependent by both extracts. However, *O. canum* with an IC_{50} of 277.22 ± 14.18 µg/ml showed a higher inhibitory effect than *O. gratissimum* with an IC_{50} of 731.10 ± 21..67 (Table 3). BHT and quercetin gave IC_{50} values of 26.42 and 17.45 µg/ml, respectively.

Discussion

Numerous studies have shown that a great number of aromatic, spicy and medicinal plants contain natural antioxidants which are responsible for their physiological activities such as the reduction of chronic degenerative diseases associated with oxidative stress (Wickens, 2001). Thus, research is continuing at various laboratories worldwide with the objective of screening bioactive plants for new sources of natural antioxidants. Several species and varieties of plants of the genus Ocimum have been reported to contain phytochemicals with a range of biological activities (Holetz *et al.*, 2003; Gulcin *et al.*, 2007). Given that the mechanisms of action of naturally occurring antioxidants can be diverse *in vivo*, a comprehensive determination of antioxidant efficacy *in vitro* will require different methods of evaluation in order to identify plants of therapeutic value for managing oxidative stress related pathologies (Arouma, 2003). In the present study, we have carried out a comparative evaluation of the total flavonoid and phenolic content of and the antioxidant activities of *O. gratissimum and O. canum*, using six different in vitro models.

Our results demonstrate that the methanolic extract of *O. gratissimum* exhibited greater antiradical activity than *O. canum*. This is evident in the relatively higher radical scavenging values obtained from all antiradical tests (except nitric acid scavenging test) for *O. gratissimum* when compared with *O. canum*. In addition, the concentrations of flavonoid and phenolic compounds in *O.* gratiisimum are comparatively higher than those of *O. canum*. The combination of better antiradical property and the occurrence of relatively higher concentrations of flavonoid and phenolic compounds in *O. gratissimum* make it more valuable than *O. canum* for the therapeutic management of oxidative stress related pathologies. These results compare favourably with previous reports which showed that *O. gratissimum* contained the greatest amount of phenolic compounds (13.81 mg tannic acid equivalent/g dry wt) amongst eight other Nigerian spices studied (Odukoya *et al.*, 2001). Other studies carried out on the polyphenolic composition of *O. gratissimum* (Grayer *et al.*, 1999) identified xanthomicrol, cirsimaritin, rutin, kaempferol 3-*O*-rutinoside and vicenin-2 as the major flavonoids while luteolin 5-*O*-glucoside, luteolin 7-*O*-glucoside, apigenin 7-*O*-glucoside, vitexin, isovitexin, quercetin 3-*O*-glucoside and isothymusin were detected as minor constituents. Similar studies on other related Ocimum species have indicated the occurrence of polyphenolic compounds. For example, Javanmardi *et al.* (2002) measured the relative antioxidant capacity of *O. basilicum* using the TEAC assay and observed varying levels of antioxidant activities that correlated positively with total phenolic contents amongst the tested accessions of *O. basilicum*. Javanmardi *et al.* (2002) also observed rosmarinic acid to be the predominant phenolic acid.

Phenolic acids such as caffeic, ferulic acid and vanillic, and flavonoids have repeatedly been implicated as natural antioxidants in fruits, vegetables and other plants (Rice–Evans *et al.*, 1995). The scavenging property of these compounds has been ascribed to the hydroxyl moiety in the chemical structures of the phenolic compounds (Gulcin *et al.*, 2002). The relatively stronger inhibitory action of *O. canum* against nitric oxide production *in vitro* at physiological pH could also be related to its polyphenolic constituents, which may significantly influence its anti-inflammatory properties. It is well known that nitric oxide plays an important role in the induction of immune responses and inflammatory reactions that cause cell damage. Studies in animal models have suggested a role for NO in the pathogenesis of inflammation and pain and NOS inhibitors have been shown to have beneficial effects on some aspects of the inflammation and tissue changes seen in models of inflammatory bowel disease and in diabetes mellitus (Aydin *et al.*, 2001).

In conclusion, the results from the different in vitro free radical scavenging tests and phytochemical analysis show that the methanolic extracts of two local species of Ocimum have varying degrees of antioxidant activity, which is traceable to the occurrence of endogenous antioxidant phytochemicals. However, *O. gratissimum* exhibited greater antioxidant properties than *O. canum*.

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