Int. J. Chem. Sci.: 6(4), 2008, 1742-1751

COMPARATIVE EVALUATION OF ANTIOXIDANT CAPACITY AND CYTOTOXICITY OF TWO NIGERIAN OCIMUM SPECIES

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ABSTRACT

This study focused on a comparative evaluation of the antioxidant capacities and cytotoxicity of two ocimum species (*O. gratissimum* and *O. basillicum*). The methanolic extracts of the two species were investigated for antioxidant properties using standard methods. *Ocimum gratissimum* possessed the highest antioxidant capacity and highest percentage of polyphenolic contents than *O. basillicum*. The antioxidant capacities of the two ocimum species are not comparable to the reference standards used. These two ocimum species possessed very low cytotoxicity to brine shrimps and are relatively safe for the purpose utilized.

Key words: Ocimum species, Antioxidant, Cytotoxicity, Polyphenols.

INTRODUCTION

Ocimum basilicum and *Ocimum gratissimum* belong to the family of plant known as Lamiaceae. They are erect herbs and have characteristic pleasant aroma due to their volatile oil[1,2]. The genus *ocimum* is represented by over 50 species of herbs and shrubs in Nigeria[3]. *Ocimum gratissimum* leaf or the whole herbs are popular treatments, for diarrhoea[4]. The plant is rich in voltile oils, which contain up to 75 percent of thymol, the antimicrobial activity of which is well known. Infact, the antimicrobial activity of the water-saturated oil had been shown to be proportional to the thymol content[5]. The two *Ocimu*m species have been well known in Nigerian folk medicine to manage different diseases. *Ocimum basilicum* due to its high fragrance is often used to flavour meats, sauce, and vinegar, serving as food additives. It has been suspected to be useful medicinally in the

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treatment of ulcer, heartburns, and cold[2, 6].

Ocimum gratissimum is particularly effective in the management of upper respiratory tract infection, diarrhoea, headache, skin disease, pneumonia, fever, and conjuctivities[7]. Since reactive oxygen species (ROS) have been implicated in some of the disorders associated with the traditional uses of *Ocimum* species, such as gastrointentional tract disorders, diabetes mellitus and inflammatory injury[8]. Since polyphenolic compounds are able to bolster biological resistance against ROS, the antioxidant capacities of these species and their cytotoxicity need to be investigated.

Therefore, in this present research, we compare the antioxidant capacities, polyphenolic contents as well as relative cytotoxicities of the methanolic extracts of two ocimum species flourishing in the North Central of Nigeria.

EXPERIMENTAL

Materials and methods

Plant materials

The leaves and stems of *Ocimum basilicum* and *Ocimum gratissimum* were obtained from Kogi State University Staff Quarters, Anyigba, Nigeria. The plant materials were washed with water to remove dirts and were air dried in the laboratory for two weeks. The dried plant materials were pulverized using pestle and mortar.

Preparation of crude methanolic extracts

Cold extraction method was employed. 20 g of the powdered samples were weighed into conical flasks. 150 mL of pure methanol was added and left for 72 hours. The mixtures were filtered and the filtrate were concentrated using rotary evaporator.

Chemicals

DPPH (2,2- diphenyl- 1-picrylhydrazyl) and quercetin were purchased from Sigma Chemical Company (Sigma, Germany). Vitamin C used was a product of Glaxo Smithkline, methanol, Folin-ciocalteu reagent, potassium dichromate, ferric chloride, and amyl alcohol were products of BDH. The tannic acid used was M & B product.

Cytotoxicity to brine- shrimps

Modified methods of Solis et al.[9] and Potduang et al.[10] were used to determine the

inhibitory activity on *Artemia salina*. 50 µL of different concentrations of crude methanolic extracts (1000, 500, 250 and 125 µg/mL) and control (methanol) was added into graduated vial bottles containing 10 newly hatched brine- shrimps in 5 mL of artificial sea water and then incubated at room temperature for 24 hours. All samples were repeated in two wells to make the overall tested organism of 20 for each. The living brine- shrimps were counted under a hand-magnifying lens. Same procedure was followed using potassium dichromate as the reference standard or positive control and data analysed based on U.S. E.P.A probit analysis programme version 1.5[11] to determine the LC_{50} at 95% confidence limit.

Total phenol and polyphenol determination

The total phenol composition was determined using the Folin-Ciocalteu reagent as described by McDonald et al.[12] The method of Harbone[13] was followed in the determination of the total flavonoid content. The colorimetric method of Van-Burden and Robinson[14] was employed in the determination of tannin composition.

Antioxidant assay

Scavenging of DPPH radicals

The free radical scavenging activity of the plants extracts were determined using the modified method of Blois[15]. 1 mL of different concentration (500, 250, 62.5, 31.25 µg/mL of extracts or standard (Vitamin C and quercetin) in a test tube was added to 1 mL of 0.3 mM DPPH in methanol. The mixture was vortexed and then incubated in a dark chamber for 30 minutes after which the absorbance was measured at 517 nm against a DPPH control containing only 1 mL of methanol in place of the extract. Percentage scavenging activity was calculated using the expression.

$$\% \text{ Scavenging activity} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absrobance of control}} \text{ x } 10$$

IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radical and this was computed using Jandel Scientific sigma plot for windows version 1.2

Rapid radical scavenging screening

The methods of Mensor et al.[16], Burits and Bucar[17] and Adebayo et al.[18] were followed in screening the antioxidant property of the extracts. With the aid of a capillary

tube, stock solutions (1 mg/mL) of extracts were spotted on silica gel thin layer chromatographic (TLC) plate and developed with a solvent system of ethanol : methanol (90 : 10). After development, the chromatograms were dried and sprayed with a 0.3 mM solution of the stable radical DPPH. Yellow spot formed against purple background were taken as positive results. The duration for the development of yellow colour indicated whether the antioxidant activity was strong or not.

RESULTS AND DISCUSSION

Table 1 shows the phenol and polyphenol contents of the two *ocimu*m species studied. *Ocimum gratissimum* leaf contains the highest percentage crude yield for flavonoid (6.267 $\pm$ 0.235%) and tannins ((0.765 $\pm$ 0.127) compared to *Ocimum basillicum* leaf extract (2.700 $\pm$ 0.637%) and (0.659 $\pm$ 0.042%) respectively.

Table 1: Phenol and polyphenol contents of two *Ocimum* species studied

Plant Species	Phenol (%)	Tannins (%)	Flavonoids (%)
O. *gratissimum* stem	0.641 ± 0.018	0.304 ± 0.000	2.700 ± 0.173
O. *gratissimum* leaf	0.669 ± 0.013	0.765 ± 0.127	6.267 ± 0.235
O. *basilicum* leaf	1.971 ± 0.465	0.659 ± 0.042	2.700 ± 0.637
O. b*asillicum* stem	1.406 ± 0.331	0.421 ± 0.017	0.900 ± 0.268

Each value in the table was obtained by calculating the average of three experiments ± standard error of mean (SEM).

The phenol content is higher in both; stem and leaf of *Ocimum basillicum* than *O. gratissimum.* IC_{50} of 206.27 and 233.79 (µg/mL) on DPPH radical scavenging was observed for the leaf and stem of O. *gratissimu*m, respectively, but *O. basillicum* showed very weak activity. Percentage radical scavenging activity was concentration dependent (Table 2).

Table 2. DPPH radical scavenging activities of the two *Ocimum* species compared to reference standards

Sample	Concentration (µg/mL)	% Scavenging activity	IC_{50} µg/mL
*O. gratissimu*m leaf	500	86.2	206.27
	250	68.5	
	125	53.7	
	62.5	38.3	
	31.25	20.6	
O. gr*atissimum* Stem	500	80.1	233.79
	250	59.3	
	125	46.5	
	62.5	29.7	
	31.25	19.2	
O. *basillicum* leaf	500	27.2	-
	250	22.9	
	125	21.6	
	62.5	21.3	
	31.25	21	
O. basillicum stem	500	35.8	-
	250	34.5	
	125	31.9	
	62.5	26.7	
	31.25	26.4	
Quercetin	500	93.8	166.506
	250	75.9	

Cont…

Sample	Concentration (µg/mL)	% Scavenging activity	IC_{50} µg/mL
Vitamin C	125	65.8	178.24
	62.5	51.9	
	31.25	44.7	
	500	68.6	
	250	62.8	
	125	59.5	
	62.5	54.3	
	31.25	48.4	

The intensity of spots and reaction time of DPPH radical scavenging abilities of the two-*ocimum* species extracts using the TLC method showed that *O. gratissimum* leaf and stem extracts gave high antioxidant activities (Table 3), while *Ocimum basillicum* showed weak antioxidant capacity.

Table 3: Result of rapid radical scavenging screening of two *Ocimum* species extracts

Extracts	Reaction speed	Intensity of spots
O. gratissimum stem	Fast	+++
O. gratissimum Leaf	Fast	+++
O. basillicum Stem	Slow	+
O. basillicum Leaf	Slow	+

+++ = Strong intensity (immediate reaction)

++ = Intermediate intensity (1-15 minutes before colour develops)

+ = Weak intensity of yellow colouration (15-30 minutes before colour develops).

The cytotoxicity to brine shrimps showed that the LC_{50} (lethal concentration) of the plant extracts were higher than that of the reference standard (Potassium dichromate) indicating low toxicity (Table 4*)*. *Ocimum basillicum* appears to be more toxic than

O. gratissimum when both are compared to the reference standard.

Table 4: Inhibitory effects on brine- shrimps of two *ocimum* species crude methanolic extracts compared with reference standard.

Compound	Concentration (µg/mL)	% Lethality	LC50 (µg/mL)
Crude methanol stem extract of *O. gratissimum*	1000	55	5176.835
	500	35	
	250	25	
	125	10	
Crude methanol leaf extract of *O. gratissimum*	1000	60	1917.355
	500	50	
	250	30	
	125	10	
Crude methanol leaf extract of *O. basillicum*	1000	65	2693.355
	500	50	
	250	25	
	125	20	
Crude methanol stem extract of O. bassillicum	1000	70	1230.244
	500	60	
	250	45	
	125	30	
Potassium dichromate	1000	100	180.142
	500	80	
	250	70	
	125	70	

The two *Ocimum* species studied showed mild inhibition to *Artemia salina* when compared with the reference standard. However, *Ocimum basillicum* appears to be more

toxic to the cells than *O. gratissimum*.

On the general note, it can be inferred from this work that the two ocimum species are relatively non-toxic and are safe for the purposes utilized.

Ocimum basillicum had a higher content of phenolic compounds than *O. gratissicum* with the concentration in *O. basillicum* being almost twice higher (Table 1). The polyphenolic contents are higher in *O. gratisimum* than *O. basillicum*. The content of total flavonoid was observed to be more than two times higher in *O. gratissimum,* when compared to *O. basillicum*. These polyphenols have been known to show medicinal activities[19]. This probably could contribute to wider utilization of *O. gratissimum* in Nigerian folk medicine than *O. basillicum.* Free radical scavenging of phenolic and poly phenolic compounds is an important property underlying their various biological and pharmacological properties. It has been recognized that flavonoids show antioxidant activity and the effects on human nutrition and health are considerable.

The mechanism of action of flavonoids are through scavenging or chelating process[20, 21]. The present study shows that the presence of flavonoids in the leaf of O. *gratissimum* is in higher proportion than *O. basillicum,* which explains the higher antioxidant ability of the plant (Table 2 and 3).

Polyphenols possesses reductive potential. It is plausible that the antioxidant properties of *O. gratissimum* extracts may be attributed to the redox properties, which allow it to act as strong reducing agent, hydrogen donating ability as well as good scavengers of hydroxyl and single oxygen quenchers. The result of the rapid scavenging screening of the plant species confirm their antioxidant ability (Table 3). *O. basillicum* showed very weak antioxidant capacity. The antioxidant capacity of the two *Ocimum* species are not comparable with the reference standards. Free radicals are involved in many disorders like neurodegenerative diseases, arthritis, cancer, etc. Antioxidant through their scavenging power is useful for the management of these diseases. The radical scavenging activity in the plant parts decreased in the following order: *O. gratissimum* leaf > *O. gratissimum* stem > *O. basillicum* Stem > *O. basillicum* leaf. This probably correlates with the flavonoid contents.

CONCLUSION

The result of this investigation showed *that O. gratissimum* contains the highest amount of flavonoids and exhibited the greatest antioxidant capacity than *O. basillicum*. It

is apparent from this work that *O. basillicu*m is a very weak antioxidant source and both *Ocimum* speices are relatively non-toxic to cell.

ACKNOWLEDGEMENT

The technical assistance from Paul Ekeyi and Friday Emmanuel of Biochemistry Department Laboratory, Kogi State University, Anyigba is gratefully acknowledged.

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Accepted : 09.07.2008