Antioxidant Properties of *Ocimum gratissimum* (Scent Leaf)

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ABSTRACT: *Ocimum gratissimum* (Scent leaf) is plant species mainly found in the tropics and widely used as spices. A study was carried out with the leaves of the plant to determine its antioxidant activities and phytochemicals present as well as toxicity levels. The acute toxicity of the plant extract on mice was found to be 2450mg/kg body weight. Phytochemical analysis on both the fresh and dried leaves of the plant revealed the presence of terpenes, flavonoids, tannins, alkaloids, steroids, proteins, carbohydrate, fats and oils with the dried samples having higher concentrations. The antioxidant properties of ethanol extract of the leaves was determined *in vitro* using rat liver homogenate. A significant inhibition at different concentration was observed with nitric oxides, ferrous sulphate and carbon tetrachloride induced lipid peroxidation activities when compared with ascorbic acid. This study revealed that *Ocimum gratissimum* may serve as a good scavenger of free radicals and thus reduces the effects of oxidative stress in the body.

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INTRODUCTION

Current studies on the formation of reactive oxygen species (ROS) has shown that normal body metabolism cannot function well without these species (Nelson and Cox, 2005). They are useful to the body when moderately produced or strictly regulated (Manoj *et al*., 2009). The highly reactive free radicals continuously produced within healthy human cells include hydroxyl radicals, superoxide radicals as well as excited or single state oxygen radicals.

To sustain life, there are some enzymes in the human system like catalase, superoxide dismutase (SOD), glutathione systems that help to regulate and control the escalation of these radicals in the body, a situation known as oxidative stress. Complementary to these enzymes are some plant species that have been found useful in controlling or regulating the over-production of these free radicals in the body (Manoj *et al*., 2009).

Ocimum gratissimum is popularly known as scent leaf. It is a full developed flowering plant with root, stem and leaves systems (Iwu, 1993). The plant is naturally used in the treatment of different diseases like diarrhea, headache, fever, ophthalmic, skin disease and pneumonia (Onajobi, 1986; Ilori *et al*.,1996).

All plant and animal cells contain antioxidants that prevent damage due to the action of reactive oxygen species (ROS). These highly reactive oxygen species when left uncontrolled are capable of causing cell death or may form DNA adducts that could cause cancer-promoting mutations (Eze, 1991; Robin, 2002).

To prevent uncontrolled propagation of these free radicals, cells normally contain a dozen or more antioxidant control systems that regulate the many necessary and desirable free radicals presents (Linus, 1991). Those mechanisms include endogenous enzymes like catalase, superoxide dismutase, thioredocxin and glutathione peroxidase (Aloh and Ozougwu, 2010). When functioning properly, antioxidant system suppresses and control excessive free radicals production, allowing control oxidative energy metabolism to proceed normally without cellular or molecular damage.

Oxidative stress occurs where there is an imbalance between the production of reactive oxygen and the biological system's ability to readily detoxify the reactive intermediate or easily repair the resulting damage (Halliwell, 1994). All forms of life maintain a reducing environment within their cells by enzymes through a constant input of metabolic energy. Disturbances in this normal redox state could lead to the production of peroxides and free radicals that damage all compounds of the cell, including proteins, Lipids and DNA (Rimbach *et al*., 1999).

Many phytochemicals have been identified as components of food and more are still being discovered (Ajali, 2004). Some of the phytochemicals of greater importance are plant steroids, flavonoids, tannins, glucosides, saponins and alkaloids.

This study is to investigate the possible secondary plant metabolites present in the ethanol leave extract of *Ocimum gratissimum* with a view of

evaluating the acute toxicity of the leave extract and its antioxidant status *in vitro.*

MATERIALS AND METHODS

Plant Materials

The leaves of *Ocimum gratissimum* were used for this study. The leaves were bought from the Nsukka market and were identified by Mr. Njokuocha of the Department of Botany, University of Nigeria, Nsukka where a voucher has been deposited.

Animals

Six (6) Adult albino rats were used for the lipid peroxidation scavenging tests. Twenty one (21) adult albino mice were used for the acute toxicity study. All the animals used were bought from the Animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka. The rats were fed (p.o.) with pellets and water.

Preparation of Plant Material

The freshly collected leaves and stem of *Ocimum gratissimum* were chopped and dried milled to coarse powder using the hammer mill.

Extraction of Plant Material

A weighed quantity, 50g of the powdered plant was extracted with 250ml of absolute ethanol to obtain the extract. The extract was used for the lipid peroxidation scavenging tests.

Phytochemical Analysis of *Ocimum gratissimum*

The phytochemical analysis of the plant was carried out on both fresh and dried samples according to the method of Harborne, (1973) to identify its active constituents of *Ocimum gratissimum.*

Acute Toxicity Study (LD_{50}) of the Extract

The acute toxicity study (LD_{50}) of the extract was determined using the method of Lorke (1983).

Preparation of Extract of Celery for Nitric Oxide/Lipid Peroxidation-Induced Scavenging Test

A quantity, 1g of the ethanol extract of *Ocimum gratissimum* was suspended in 100ml of 20% tween 80 in standard phosphate buffer (0.025M, pH 7.4) to give the stock solution containing 10,000µg/ml of the extract. The stock solution was then used for the entire nitric oxide/lipid peroxidation-induced scavenging tests.

Nitric Oxide Scavenging Activity

Nitric oxide scavenging assay was performed as described by Sreejayan (1997)

Carbon Tetrachloride-Induced Lipid Peroxidation Scavenging Test

Carbon tetrachloride induced lipid peroxidation scavenging test was performed using the method of Comporti (1989).

Ferrous Sulphate-Induced Lipid Peroxidation Scavenging Test

The method of Okhawa *et al.* (1979) was used with minor modification by Tripathi and Sharma (1998).

Statistical Analysis

The results were analyzed with one way ANOVA expressed as Mean ± SEM. The Fischer LSD post hoe test was used to test the difference between mean of treated and control groups were regarded significant at $P<0.05$.

RESULTS

Percentage Yield of Extract

TABLE 1: Percentage yield of the ethanol extract of the leaves stems of *Ocimum gratissimum*

EXTRACT (g)	Percentage (%)
50.0	15.50

The result of the percentage yield from the table 1 is found to be 15.50%

Phytochemical Analysis

Table 2: Phytochemical analysis of both the fresh and dried leaves of *Ocimum gratissimum.*

PHYTOCHEMICAL TEST	RESULTS	
	Fresh Sample	**Dried Sample**
1. Alkaloid	+	++
2. Flavonoids	+	+
3. Glycosides	ND	ND
4. Reducing Sugars	ND	ND
5. Saponin	+	+
6. Protein	+	+
7. Carbohydrates	+	++
8. Fats and Oils	+	ND
9. Tannins	+	++
10. Steroids	+	+
11. Terpenoids	ND	ND
12. Test for Resins	+	+

KEY:

ND	Not Detected
+	Present in little amount
++	Moderately present
+++	present in large amount

From the result of the phytochemical studies as shown in Table 2 above, alkaloids, flavonoids, resins, saponins, proteins, carbohydrate, fat and oils, tannins and steroids were present in the leaves and stems of both fresh and dried leaves of *Ocimum gratissimum.*

ACUTE TOXICITY STUDIES OF *OCIMUM GRATISSIMUM*

TABLE 3: Acute toxicity studies (LD_{50}) of the ethanol extract of *Ocimum gratissimum.*

Dose (mg/kg)	No. of Animals before administration	No. of deaths after weight body administration
250	3	–
500	3	–
750	3	–
1000	3	–
2000	3	1
3000	3	2
Control	3	–

LIPID PEROXIDATION-INDUCED SCAVENGING TESTS

Effects of Nitric oxide Scavenging Activity of the Extract of *Ocimum gratissimum*

The extract of *Ocimum gratissimum* showed significant free radical scavenging activity on nitric oxide (NO)-induced release of free radicals. Different concentrations (100µg/ml, 200µg/ml, 400µg/ml and 800µg/ml) exhibited different percentage of inhibition. Ascorbic acid was used as standard antioxidant drug.

From Table 3, the concentration of the extract increased, as the absorbance decreased and there was a corresponding increase in the inhibition of the nitric oxide produced.

TABLE 3: Percentage inhibitions of different concentrations of the extract of *Ocimum gratissimum* in the nitric oxide scavenging activity test.

	Concentration (µg/ml)	Absorbance 546nm (mean ± S.E)	Inhibition (%)
Ethanol extract of *Ocimum gratissimum*	100	0.365 ± 0.003	29.94
	200	0.343 ± 0.004	34.17
	400	0.309 ± 0.020	40.69
	800	0.253 ± 0.005	51.44
Ascorbic acid	100	0.426 ± 0.003	18.23
	200	0.421 ± 0.001	19.19
	400	0.403 ± 0.006	22.65
	800	0.333 ± 0.005	36.66
Control		0.521 ± 0.035	

Effect of the Extract on Carbon tetrachloride-induced Lipid Peroxidation in Rat Liver Homogenate

Lipid peroxidation induced by carbon tetrachloride was inhibited by the extract of *Ocimum gratissimum* at all tested doses (100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml). The percentage inhibition of peroxide formation was increased in a dose-dependent manner. Also, the standard, vitamin C (ascorbic acid), showed significant reduction in lipid peroxidation formation.

From Table 4, the concentration of the extract increase as the absorbance decreased and there was a corresponding increase in the inhibition to the carbon tetrachloride lipid peroxidation.

TABLE 4: Inhibition of the extract on carbon tetrachloride induced lipid peroxidation test.

	Concentration (µg/ml)	Absorbance 543nm (mean ± S.E)	Inhibition (%)
Ethanol extract of *Ocimum gratissimum*	100	0.869 ± 0.026	48.76
	200	0.745 ± 0.012	56.07
	400	0.565 ± 0.054	66.69
	800	0.267 ± 0.034	84.26
Ascorbic acid	100	0.402 ± 0.0055	76.30
	200	0.348 ± 0.0033	79.48
	400	0.267 ± 0.0069	84.26
	800	0.187 ± 0.042	88.95
Control		1.696 ± 0.013	

Effect of the Extract on Ferrous sulphate-Induced Lipid Peroxidation in Rat Liver Homogenate

The extract showed significant reduction in the lipid peroxidation formation against ferrous sulphate-induced lipid peroxidation in a dose-dependent manner. Ascorbic acid, the standard antioxidant used also showed significant reduction in the lipid peroxidation.

From Table 5, as the concentration of the extract increased, the absorbance decreased and there was a corresponding increase in the inhibition to the ferrous sulphate lipid peroxidation.

TABLE 5: Inhibition of the extract on ferrous sulphate induced lipid peroxidation test.

	Concentration (µg/ml)	Absorbance 543nm (mean ± S.E)	Inhibition (%)
Ethanol extract of *Ocimum gratissimum*	100	0.178 ± 0.030	62.92
	200	0.168 ± 0.006	65.00
	400	0.141 ± 0.027	70.63
	800	0.120 ± 0.015	75.00
Ascorbic acid	100	0.324 ± 0.027	32.50
	200	0.236 ± 0.021	50.83
	400	0.200 ± 0.041	58.33
	800	0.147 ± 0.005	69.38
Control		0.480 ± 0.069	

DISCUSSION

In many parts of the world, especially Africa and Asia, plant parts are used for the treatment of various ailments such as inflammation, fever, gout (Krawinkel). The leaves of *Ocimum gratissimum* is used for prevention and treatment of gout, catarrh, fever and malaria which has been found to be associated with free radical generation (Pamplona-Roger, 2004).

The result of the acute toxicity study of the extract shows that *Ocimum gratissimum* is safe for human consumption (Table 1). Investigation on the phytochemical constituents of the plant extract reveals that the plant contains alkaloids, tannins, terpenes, flavonoids, steroids, proteins, carbohydrates, fats and oils. The result also revealed higher quantities of the phytochemical in the dried leaves than in the wet leaves which have higher percentage of moisture present in the plant.

These phytochemicals have a lot of properties and are widely used in the manufacturing of many pharmaceutical and nutritional supplements and food. Reactive oxygen species generated endogenously as well as exogenously during normal human metabolism leads to various diseases like diabetes, cancer, ageing process, artheriosclerosis (Guyon *et al.*, 1987). The consumption of fruits and vegetable containing flavonoids, terpenes, carotenoid s and other natural antioxidants have consistently been shown to reduce many forms of cancer including scavengers and inhibiting lipid peroxidation (Iwu *et al*., 2003).

The antioxidant activities of *Ocimum gratissimum* as reactions shown by the various thiobarbituric acids (TBA) assays methods were found to act as scavengers of nitric oxide. The result also showed that the plant has antioxidant properties and as the antioxidant activity was dose-dependent.

The plant extract also exhibitede protection against lipid peroxidation induced by carbon tetrachloride and ferrous sulphate and this activity was also found to be dose- dependent compared with the standard used. It is evident that the *Ocimum gratissimum* has antioxidant property and this compliments our earlier studies on the antioxidant properties of this plant (Okonkwo and Njoku, 2011).

CONCLUSION

From the studies, *Ocimum gratissimum* is a good antioxidant and also has some medicinal and nutritive values. Further studies could be carried out with the plant aimed at isolating the plant active compound that is responsible for the antioxidant activity, as numerous studies have pointed out the effect *in vitro.*

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