**In Vitro Antioxidant activity Free Radical Scavenging Abilities of *Ocimum gratissimum* Leaf Extract**

**ABSTRACT**

In recent years, researchers have intensified the search for effective, non-toxic, natural edible plants with free radical scavenging activity whose effects are cushioned by antioxidant molecules. This study evaluated the free radical scavenging activities of the leaf extract of *Ocimum gratissimum*. *In vitro* antioxidant (Nitric oxide, Hydrogen Peroxide, Ferric Reducing property and DPPH) activities were determined using standard methods. Results showed that the leaf extract of *Ocimum gratissimum* considerably scavenges radicals of nitric oxide, hydrogen peroxide and DPPH in a concentration dependent manner. From the results of the study, it could be concluded that the leaf extract of *Ocimum gratissimum* could help to ameliorate oxidative stress and consequently improve health.

**Keywords:** Oxidative stress, Free radical, *Ocimum gratissimum,* Health.

**INTRODUCTION**

“Reactive oxygen species (ROS) are unstable molecules containing oxygen that play a crucial role in various physiological and pathological processes. In biological system, ROS are continuously produced in numerous processes such as mitochondrial respiration, metabolism of Xenobiotics by cytochromes P450, inflammation, and phagocytosis” [1]. “However, excessive production of ROS can lead to oxidative stress, causing damage to cellular components, including DNA, proteins, and lipids. This oxidative stress is implicated in the pathogenesis of various diseases, such as cancer, diabetes, neurodegenerative disorders, cardiovascular diseases and hemorrhoids”[1, 2]. “Antioxidants are molecules that neutralize or mop up ROS, thereby preventing oxidative damage. The human body has an endogenous antioxidant system, but dietary antioxidants such as polyphenols, flavonoids, and terpenoids from plants play a vital role in maintaining the balance between ROS production and antioxidant defenses” [3].

Most medicinal plants, including *Ocimum gratissimum* are rich in antioxidants and their usage and consumption can provide the body with sufficient antioxidant that can help the system to combat oxidation and consequently improve health [4]. The increased search for effective, local, natural and edible plants with free radical scavenging activity has been intensified in recent years. Hence, this study aims to evaluate the free radical scavenging activities of *Ocimum gratissimum* leaf extract.

*Ocimum gratissimum* (*O. gratissimum*) is an herbaceous plant that belongs to the Lamiaceae family and is sometimes referred to as smell leaf, African basil, camphor basil, basil leaf, or ram tulsi [4, 5]. This particular species of tropical plant is popularly known as "scent leaf," a term that Nigerians, particularly the easterners and westerners find endearing [2]. It is known as “*Nchanwu*” in Igbo, “*Daidoya*” in Hausa, and “*Efirin*” in Yoruba in native Nigeria [2, 6]. It is a small to average-sized plant with leaves that is comparable to cloveslike flavor and aroma, making it a significant herb in various cuisines [7]. In Nigeria, the plant is commonly grown for both culinary and medicinal reasons [4, 8], usually in gardens around community huts. *O. gratissimum* is used as a culinary ingredient in salads, soups, pastas, vinegars, and jellies in many parts of the world. Nutritionally, the plants have been documented to be rich in microelements sufficient for improved health and vitality [2]. It also contains several phytochemicals such as flavonoids and polyphenols [9, 10], responsible for its hypoglycemic [11], anti-inflammatory [12], anti-anaemic, hepatoprotective [13], anti-hypertensive [14], antibacterial [10], antifungal [15] as well as anti-hemorrhoidal activities [2, 8]. Although there have been several reports on its other pharmacological activities, there is need for research to focus on its free radical scavenging activities. Hence, this study evaluates the free radical scavenging activities of *Ocimum gratissimum*.

**MATERIALS AND METHODS**

**Plant Materials**

Fresh leaves of *O. gratissimum* were purchased from a daily market, Mgbakwu in Awka North Local Government Area of Anambra State, Nigeria and were authenticated by a Taxonomist from the Department of Botany, Nnamdi Azikiwe University, Awka. A voucher specimen was also deposited with Herbarium no NAUH 35B.The leaves were then detached from the stalk, rinsed with distilled water and were oven-dried at 40 0C and weighed (300 g). The weighed powdered sample (300 g) was then used for the extraction with a solvent combination of ethanol and water (7:3) (2500 ml) for 48 hr via maceration in an unheated medium. The mixture was decanted and filtered using sterile Whatman paper No. 1. The filtrate was there after evaporated to dryness with the aid of a rotary evaporator set at 50 0C to obtain crude ethanol extract (25.2 g) which was carefully preserved for further analysis.

**Phytochemical Screening**

Following the method described by Thusa and Mulmi[16], phytochemical screening was carried out using a BUCK M910 Gas Chromatography fitted with a flame ionization detector. The amounts per gram of the various phytochemical concentrations are indicated in µg/g.

***IN VITRO* ANTIOXIDANT CAPACITY**

**Determination of Ferric Reducing Antioxidant Property**

The reducing property of the extract was determined as described by Pulido *et al.* [17]. The principle of the assay is the quantification of ferric degradation product, by its condensation with the extract.Mixture of 0.25ml of the extract with 0.25ml of 200mM Sodium phosphate buffer pH 6.6 and 0.25ml of 1% Potassium ferrocyanide was made. The mixture was incubated at 50oC for 20min, thereafter 0.25ml of 10% trichloroacetic acid was added and centrifuged at 2000rpm for 10 min, then 1ml of the supernatant was mixed with 1ml of distilled water and 0.2ml of ferric chloride and the absorbance was measured at 700nm.

**Determination of Superoxide Scavenging Activity**

The Superoxide scavenging ability of the sample was assessed by the method of Winterbourn *et al.* [18].This assay is based on the inhibition of the production of Nitroblue tetrazolium formation of the Superoxide ion by the sample and is measured spectrophotometrically at 560nm.Superoxide anions were generated in samples that was constituted to 3.0ml; 0.02ml of the leaf sample (20mg), 0.2ml of EDTA, 0.1ml of NBT, 0.05ml of riboflavin and 2.64ml of phosphate buffer. The control tubes were also set up where DMSO was added instead of the sample. All the tubes were vortexed and the initial optical density was measured at 560nm in a spectrophotometer (Genesys, 10-S, USA). The tubes were illuminated using a fluorescent lamp for 30 minutes. The absorbance was measured again at 560nm. The difference in absorbance before and after illumination was indicative of superoxide anion scavenging activity.

Superoxide scavenging activity = × 100

A0 – Absorbance before illumination

A1 - Absorbance after illumination

**Determination of Total Antioxidant Capacity (TAC)**

The Total Antioxidant Capacity (TAC) of extract in different extracting solvents (absolute ethanol, 70% and 50% ethanol) was determined by the phosphomolybdate method according to Jayaprakasha *et al*. [19]. An aliquot (30ml) of different concentrations (20, 40, 60, 80 and 100 mg/ml) of the test extract was mixed with 3ml of the reagent solution (0.6M sulphuric acid, 28mM sodium phosphate, 4mM ammonium molybdate) taken in test tubes. The tubes were covered with aluminum foil and heated to 95 degrees Celsius for 90minutes of incubation. When the reaction mixture reached room temperature, it was tested for absorbance at 695nm in comparison to a blank that contained 3ml of the reagent solution and the necessary amount of the dissolving solvents. The test sample and the blank were incubated together under the same circumstances to compare the effect of the extract, ascorbic acid was employed as a standard reference ingredient.

**Determination of Nitric Oxide Scavenging Activity**

According to the procedure described by Green *et al*. [20], the degree of suppression of Nitric oxide radical production in vitro was monitored. At physiological pH, Sodium nitroprusside in aqueous solution spontaneously produces nitric oxide, which reacts with oxygen to form nitrite ions, which are measured spectrophotometrically at 546nm. The reaction was initiated by adding 2.0ml of sodium nitroprusside, 0.5ml of PBS, 0.5ml of leaf sample (50mg) and incubated at 250C for 30minutes. Griess reagent (0.5ml) was added and incubated for another 30minutes. Control tubes were prepared without the sample. The absorbance was read at 546nm against the reagent blank, in a spectrophotometer (Genesys 10-S, USA).

**DPPH Spectrophotometric Assay**

The scavenging ability of the natural antioxidants of the leaves towards the stable free radical DPPH was measured by the method of Mensor *et al*. [21]. The leaf sample (20µl) was mixed with 0.48ml of methanol and 0.5ml of a 0.1mM methanolic solution of DPPH. The combination was given 30minutes to react at room temperature. Butylated hydroxytoluene (BHT) served as the reference, while DPPH in methanol, without the leaf sample, served as the positive control. The purple color's discoloration was detected at 518nm in a spectrophotometer after 30minutes of incubation (Genesys 10-S, USA). This is how the radical scavenging activity was computed;

Scavenging activity % = 100 - A518 (sample) - A518 (blank) × 100

A518

**Determination of Hydroxyl Radical Scavenging Activity**

According to Elizabeth and Rao [22] the amount of hydroxyl radical scavenging from the Fenton reaction was measured using 2'-deoxyribose oxidative degradation. The assay's basic premise is the measurement of Malondialdehyde, a 2'-deoxyribose breakdown product, by condensation with Thiobarbituric acid. The reaction mixture which had 20µl of sample in a final volume of 1.0ml, contains 0.1ml of deoxyribose, 0.1ml of FeCl3, 0.1ml of EDTA, 0.1ml of H2O2, 0.1ml of ascorbate and 0.1ml of KH2PO4-KOH buffer. For one hour, the mixture was incubated at 370C. After the incubation time, 1.0ml of TBA was added, and the colour was developed by heating the mixture for 20minutes at 950C. After cooling, the TBARS production was assessed using a suitable blank and a spectrophotometric instrument (Genesys 10-S, USA). By contrasting the absorbance of the control with the samples, the hydroxyl radical scavenging activity was identified. The relative percent TBARS was computed for the sample-treated groups after the percent TBARS generation for the positive control (H2O2) was set at 100%.

% hydroxyl radical = (A0 – A1) × 100

A0

A0 - Absorbance of control

A1 - Absorbance in the presence of sample

**Data Analysis**

The mean values obtained and the significance between the treated and control group was analyzed by one-way ANOVA using the SPSS version 17 and P< 0.05 was considered to be statistically significant.

**RESULTS AND DISCUSSION**

**Table 1.0: Phytochemical composition of the leaf extract of *Ocimum gratissimum***

|  |  |
| --- | --- |
| Parameters | Quantity |
| **Proanthocyanin (ug/g)** | 0.42±0.03 |
| **Rutin (ug/g)** | 6.02±1.56 |
| **Ribalidine (ug/g)** | 4.90±0.00 |
| **Quinine (ug/g)** | 19.95±5.11 |
| **Flavol-3-ol (ug/g)** | 81.88±9.14 |
| **Anthocyanin (ug/g)** | 67.13±6.63 |
| **Lunamarin (ug/g)** | 14.64±0.15 |
| **Sapogenin (ug/g)** | 26.36±5.68 |
| **Epihedrine (ug/g)** | 1.55±0.51 |
| **Phenol (ug/ml)** | 50.72±6.07 |
| **Flavonones (ug/g)** | 6.04±1.14 |
| **Steroids (ug/g)** | 81.91±14.16 |
| **Epicatechin (ug/g)** | 21.02±3.12 |
| **Kaepferol (ug/g)** | 2.97±0.07 |
| **Phytate (ug/g)** | 1.15±0.17 |
| **Oxalate (ug/g)** | 5.61±0.62 |
| **Resveratol (ug/g)** | 5.53±0.54 |
| **Flavones (ug/g)** | 3.32±0.22 |
| **Naringenin (ug/g)** | 0.70±0.03 |
| **Tannin (ug/g)** | 12.39±2.85 |

**Figure 1.0: Effect of *Ocimum gratissimum* extract on Nitric Oxide Assay**

**Figure 2.0: Effect of *Ocimum gratissimum* extract on Hydroxyl radical scavenging activity**

**Figure 3.0: Effect of *Ocimum gratissimum* extract on DPPH scavenging activity**

**Figure 4.0: Effect of *Ocimum gratissimum* extract on Ferric reducing antioxidant power (FRAP)**

**Figure 5.0: Effect of *Ocimum gratissimum* extract on superoxide scavenging activity**

**Figure 6.0: Effect of *Ocimum gratissimum* extract on Total Antioxidant Capacity**

“The use of medicinal plant for therapeutic purposes is gaining wide acceptance, and this is largely due to its many phytochemical constituents” [2]. These phytochemical constituents often referred to as bioactive compounds have been known to play a crucial role in the prevention and treatment of several ailments. In this study, about twenty (20) bioactive compounds were discovered from the leaf extract of *O. gratissimum* (table 1.0) including Steroids (81.91 ug/g), Flavol-3-ol (81.88 ug/g), Anthocyanin (67 ug/g), Phenol (50.72 ug/ml), Sapogenin (26.36 ug/g), Epicatechin (21.02 ug/g), Quinine (19.95 ug/g).

“Steroids belong to a class of natural and synthetic organic compounds characterized by a rigid framework of 17 carbon atoms formed from four fused rings with varying levels of functionalization. They are widely distributed in nature, having diversity in the structures and possess a broad biological profile including anti-tumor, immunosuppressive, hepatoprotective, antibacterial, antihelminthic, cytotoxic and cardiotonic activitydue to their ability to penetrate cell membranes and bind to nuclear and membrane receptors” [23].

“Another bioactive compound of significant importance in the plant extract is flavol-3-ol. Flavan-3-ols are a class of flavonoids found in many fruits, vegetables, and beverages. They are a major source of polyphenols in the human diet.Increasing consumption of dietary flavan-3-ols has been shown to help in improving blood pressure, cholesterol concentrations, and blood sugar” [24].

“Similarly, Anthocyanins, a class of water-soluble flavonoids was also found in appreciable quantity (table 1.0). Like other bioactive compounds, anthocyanins have been shown to have a significant antidiabetic, anticancer, anti-inflammatory, antimicrobial, and anti-obesity effects, as well as prevention of cardiovascular diseases (CVDs)”[25].

“Free radicals are often generated in biological systems and they can cause extensive damage to tissues and biomolecules if not properly regulated, and this could lead to various disease conditions, especially hemorrhoids and other degenerative diseases. Although many synthetic drugs show protective effect against oxidative damage, their adverse side effects however place a limit to their global usage” [26]. “An alternative solution to the existing problem is to consume natural antioxidants from food supplements and traditional medicines. Researchers have shown that plant phenolics are highly effective free radical scavengers and antioxidants due to their hydrogen donating ability” [27], and consequently improve health. The scavenging ability of *O. gratissimum* leaf extract on nitric oxide, hydrogen peroxide and DPPH radicals were shown in Figures 1 to 3 respectively. The results showed a dose-dependent scavenging power for nitric oxide and Hydroxyl radical (fig 1 and 2), where activity increased as the concentration increased.

“Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and is involved in the regulation of various physiological processes” [28]. Excess concentration of NO is associated with several diseases [1, 29]. “Since oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals, the extract, in this study could have plausibly competes with oxygen to react with nitric oxide and thus inhibits generation of the anions.

Hydroxyl radicals are the major active species causing lipid oxidation and enormous biological damage” [30]. As depicted in this study, the plant extract was able to scavenge the hydroxyl radical to about 65% at 100 mg/ml. This value is consistent with the reports of Ahmad *et al*., [31] and Halliwell *et al*., [32].

“DPPH scavenging activity has been used by various researchers as a quick and reliable parameter to assess the in vitro antioxidant activity of crude plant extracts” [3]. In DPPH test, the ability of a compound to act as donor for hydrogen atoms or electrons was measured spectrophotometrically. The scavenging activities of DPPH exerted by the extract as well as ascorbic acid were summarized in figure 3. There was no significant difference in the DPPH scavenging activities of the extract in all the chosen concentrations (5mg/ml to 100 mg/ml). This is however at variance with the reports of Chaudhary *et al*., [30] and Ahmad *et al*., [31].

“As depicted in figure 4.0, the ferric reducing antioxidant power of the plant extract increased with increasing concentration. The ferric reducing antioxidant power (FRAP) assay is a colorimetric method that measures the ability of antioxidants to reduce ferric (Fe3+) ions to ferrous (Fe2+) ions. It is a quick and sensitive way to measure antioxidant capacity of a sample. Since the antioxidant activity of a sample has been reported to be concomitant with the development of reducing power” [3], the reducing power of the extract might be due to its hydrogen donating ability, as described by Oladejo and Osukoya, [27]. This further corroborate with the assertion of Chaudhary *et al*, [30].

The principle of superoxide free radical scavenging activity is the conversion of highly water soluble NBT salt into NBT diformazan dye upon reduction with superoxide anion. As evident in this study, the plant extract was able to scavenge superoxide radicals to about 70% at a concentration of 80mg/ml. Similar assertion was documented by Lalhminghlui *et al*., [33].

Similarly, the total antioxidant capacity of the plant extract was observed to increase as the concentration increases. Since total antioxidant capacity *(*TAC*)* is used to measure the scavenging of free radicals by a test solution or suspension, the increasing ability of the plant extract to scavenge this free radical implies that the plant could mitigate oxidative stress-related diseases and consequently improve general wellbeing.

**CONCLUSION**

Medicinal herbs are known to contain a variety of antioxidants; most notably associated with its bioactive compounds which could help to prevent the onset of a number of diseases. From the results of this study, *O. gratissimum* leaf extract is shown to contain considerable antioxidant properties and continuous use of this plant could be a therapeutic approach against diseases.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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