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# Determination of Phytochemical Compositions and Antioxidant properties of *Ocimum Basilicum* and *Ocimum Gratissimum* Plant Leaves

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## ABSTRACT

*O. basilicum* and *O. gratissimum* leaves are among the important nutritious and medicinal plants used in Nigeria and other parts of the world. The aim of this work was to determine the phytochemical compositions and antioxidant properties of *O. basilicum* and *O. gratissimum* leaves. n-hexane and methanol solvents were used to extract leaves of *O. basilicum* and *O. gratissimum* for phytochemical screening (for carbohydrate, cardiac glycoside, tannins, terpenoid and flavonoid) and antioxidant properties (for DPPH, FRAP, $H_2O_2$ scavenging, total phenol content and phosphomolybdic acid assay) at concentration of 50 µg/cm$^3$, 100 µg/cm$^3$, 150 µg/cm$^3$, 200 µg/cm$^3$ and 250 µg/cm$^3$. The n-hexane extracts of both leaves of *O. basilicum* and *O. gratissimum* showed presence of all the phytochemical compositions except tannins, while the methanol extracts of both leaves of *O. basilicum* and *O. gratissimum* showed presence of all the phytochemicals analyzed. The antioxidant assays showed that the n-hexane extracts of *O. gratissimum* leaves has maximum reducing effect of 88.97% in FRAP and 98.03% in Phosphomolybdic acid assay at 50 µg/cm$^3$ while *O. basilicum* leaves has maximum value of 99.19% in $H_2O_2$ scavenging and value of 102.00 mgGAC/g in total phenol content assay at 50 µg/cm$^3$. The methanol extract of *O. basilicum* leaves has maximum reducing effect of 98.56% in DPPH assay at 250 µg/cm$^3$. n-hexane extracts had high inhibition property in FRAP, $H_2O_2$ scavenging, and Phosphomolybdic acid assay and higher phenolic contents in both extracts of *Ocimum basilicum* than *Ocimum gratissimum* at 50 µg/cm$^3$ concentration while the methanol extract showed high inhibition in DPPH assay only at 250 µg/cm$^3$ concentration in both of the leaves. These results indicate the potential effectiveness of both leaves in health and medicinal applications. Statistically both leaves showed no differences in all the assays checked in respect to the antioxidant properties.

**Keywords:** Antioxidant properties, *O. basilicum*, *O. gratissimum* leaves and phytochemical screening

## INTRODUCTION

*Ocimum* plants (sent leaf) originated from the tropical regions of Asia, Africa, and Central and South America [1, 2]. Generally, these o*cimum* plants have square stems, fragrant leaves, and whorled flowers [2]. Herbs are generally used to add a distinctive aroma and flavour to food and for medicinal purposes [3]. Scent leaf is one of the important nutritious and medicinal plants mostly used in Nigeria. In the northern part of Nigeria the leaf is called "Daidoya" by the Hausas while in the southern part of Nigeria it is called "Nchanwu" by the Igbos [4]. *Ocimum basilicum* is found in North-Eastern Nigeria and is recognized by its strong scent and short leaves. It contains useful elements and is highly nutritious and is used as seasoning in cooking. It also consists of phytochemical contents and is highly medicinal [5]. It has been reported to be hepato-protective, immunomodulatory, anti-hyperglycermic, anti-toxic, anti- inflammatory, and anti-fungal [6]. *Ocimum gratissimum* is recognized by its broad leaves and has milder aroma than the *O. basilicum*. It is found in the South-Eastern part of Nigeria. It is used all through West Africa as anti-malarial and anti-convulsant and for the management of stomach pain, and catarrh. Oil from the leaves has been found to possess antiseptic, anti-bacterial and anti-fungal activities [7, 8]. This present research was focused on the type of scent leaves (*Ocimum* leaves) found in the North-Eastern and South-Eastern regions of Nigeria. The aim was to determine phytochemical composition and antioxidant properties of *Ocimum* leaves so as to assess their medicinal importance in order to know the different constituents which might affect human health.

## MATERIALS AND METHODS

### Collection, identification and preparation of plants samples

Fresh samples of *Ocimum basilicum* leaf was collected from Government Residential Area along Damboa road and 707 Housing Estate at Maiduguri town, Borno state in North-Eastern Nigeria and *Ocimum gratissimum* leaf at Nsukka and Awgu towns, Enugu State in South-Eastern part of Nigeria. Both leaves were examined and authenticated at the herbarium laboratory in the Department of Biological Sciences, Ahmadu Bello University, Zaria. The voucher numbers: 1285 for *Ocimum gratissimum,* and 044 for *Ocimum basilicum* were given to the plants. Fresh samples of *O. basilicum* and *O. gratissimum* leaves were separately washed with distilled water and dried at room temperature for 3 days and crushed in a mortar. The resulting powder of *O.*

*basilicum* and *O. gratissimum* leaves were sieved, weighed and stored in black polyethene leather for extraction.

**Sample Extraction**

The powdered samples of 250 g of each of *O. basilicum* and *O. gratissimum* leaves were successively extracted separately by using 1000 $cm^3$ of n-hexane and methanol as solvent in soxhlet apparatus for 48 hr. The resulting extracts were separately filtered through a Whatman filter paper No.11 and solvent were removed by using a rotator evaporator and were subjected to drying.

**Phytochemical Analysis**

Phytochemical screening of both *Ocimum gratissimum* and *Ocimum basilicum* leaves extracts of n-hexane and methanol were carried out to determine the presence of carbohydrate, cardiac glycoside, tannins, terpenoid, and flavonoid.

Exactly 0.1 grams of each the extracts of n-hexane and methanol of *O. basilicum* and *O. gratissimum* leaves were prepared in 30 $cm^3$ of ethanol solvent for the following tests:

**Test for Carbohydrate**

**Molischs Test**

Few drops of molischs reagent was added to 2$cm^3$ of each samples extracts in test tubes, followed by the addition of concentrated sulphuric acid by the side of each test tube. The mixture was allowed to stand for 2 min and then diluted with 5$cm^3$ of distilled water. Formation of a red or dull violet colour at the interphase of two layers indicates the presence of carbohydrate [9].

**Barfoed's Test (test for monosaccharide)**

A solution of each sample extracts of 1$cm^3$ was mixed with Barfoed's reagent in the test tubes and then heated on a water bath for 2 min. Red precipitate of cuprous oxide indicates the presence of monosaccharide like fructose and glucose [9]

**Test for soluble starch**

An already prepared 2$cm^3$ solution of each sample extracts were boiled with 1 $cm^3$ of 5% potassium hydroxide (KOH), cooled and acidified with sulphuric acid ($H_2SO_4$) for each sample extracts. A yellow coloration indicates the presence of soluble starch [10].

**Test for Tannins**

The leaves sample extracts of 0.1g each were stirred with 10cm$^3$ of distilled water and filtered. The filtrate was used for following test;

**Ferric chloride test**

Exactly 2cm $^3$ of 1% ferric chloride solution was added to 2cm$^3$ of each filtrate of the sample extracts. A blue-black, green or blue-green precipitate shows the presence of tannins [9].

**Test for cardiac glycoside**

Exactly 0.1g of each sample extracts were dissolved in 3cm$^3$ of chloroform separately, and sulphuric acid was carefully added by the side of each test tube to form a lower layer. Appearance of a reddish-brown color or yellow at the interphase indicates the presence of steroid ring (i.e a glycone portion of cardiac glycoside) [11].

**Test for Terpenoid**

Exactly 2cm $^3$ of each sample extracts was prepared in 1cm$^3$ of acetic anhydride followed by the addition of conc. sulphuric acid. A colour change from pink to violet indicates the presence of terpenoids [11].

**Test for Flavonoids**

***Shinoda's Test***

Exactly 4 cm $^3$ of each sample extracts was warmed on water bath and filtered and three pieces of magnesium chips was added to the each filtrated of sample extracts. A few drops of conc. hydrochloric acid were added and a change in color from pink, orange to purple will indicate the presence of flavonoids [12].

**Ferric chloride Test**

Exactly 2 cm $^3$ of each sample extract were boiled with distilled water and filtered. Few drops of exactly 10% ferric chloride solution were added to 2cm$^3$ to each filtrated of sample extracts. A blue-green colouration will indicate the presence of a phenolic hydroxyl group [9].

**Antioxidant properties determination**

The determination of antioxidant properties for *Ocimum gratissimum* and *Ocimum basilicum* leaves extracts of n-hexane and methanol were determined for the following antioxidants: 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, ferric ion reducing power (FRAP) assay, hydrogen

peroxide scavenging assay, phosphomolybdic acid assay and total phenol content. Exactly 0.1g of each of the extracts were prepared in different fractions of 50, 100, 150, 200 and 250 µg/cm $^3$ using ethanol, 20%DMSO and 50% methanol for the following assays:

***2,2-diphenyl-1-picrylhydrazyl (DPPH) assay***

Exactly 1g of 2, 2-diphenyl-1-picrylhydrazyl was dissolved in 1000 cm$^3$ of 50% methanol and stored. Exactly 3 cm$^3$ of each aliquot of the solution of DPPH were mixed with 2 cm$^3$ of each different fraction of sample extracts. The reaction mixtures were shaken well and incubated at 40 $^o$C in dark for 15 min at room temperature. A blank solution was prepared also and the absorbance of both the blank and the fractions of 50, 100, 150, 200 and 250 µg/cm$^3$ solutions prepared were taken at 517 nm using a spectrophotometer [13]. The scavenging effect was calculated based on the percentage of DPPH radical scavenged using the following equation:

$$\text{Scavenging effect (\%)} = \frac{blankabsorbance - sampleabsorbance}{blankabsorbance} x\ 100$$

***Ferric ion reducing Antioxidant power (FRAP Assay).***

Exactly 2 cm $^3$ of prepared fractions of sample solution were mixed with 2 cm$^3$ of 0.2 M phosphate buffer (pH 6.6) and 2 cm$^3$ of potassium ferric cyanide (10mg/ml) separately. The mixed fractions were incubated at 50$^o$C for 20 min and cooled. Exactly 2cm$^3$ of trichloroacetic acid (100 mg/cm$^3$) were added to each fraction and centrifuged at 3000 rpm for 10 min to collect the separated liquid solution from the precipitate of the mixture. A volume of exactly 2cm $^3$ from each of the decant fractions solution were mixed with 2cm $^3$ of distilled water and 0.4 cm $^3$ of 0.1% fresh ferric chloride each. Also a blank solution was prepared. After 10 min of reaction, the absorbance was measured at 700nm [13]. The scavenging effect was calculated base on the percentage of FRAP radical scavenged using the following equations below.

$$\text{Scavenging effect (\%)} = \frac{blankabsorbance - sampleabsorbance}{blankabsorbance} x\ 100$$

***Hydrogen Peroxide scavenging assay***

Exactly 2 mmol/dm$^3$ hydrogen peroxide was mixed with exactly 50 mmol/dm$^3$ of phosphate buffer (pH7.4). Exactly 0.1cm$^3$ aliquots of the different fractions of sample extracts were transferred into different test tubes and their volume was made up to 0.4cm$^3$ with 50 mmol/dm$^3$phosphate buffer (pH 7.4). Addition of 0.6 cm$^3$ hydrogen peroxide solutions were put

into the fraction in the tubes and vortexed, for ten minutes. Blank solution was prepared An absorbance of the hydrogen peroxide at 230 nm of the fractions was determined after 10 min against blank using spectrophotometer and was analyzed in duplicate [13]. The scavenging effect was calculated using the following equation:

$H_2O_2$ scavenging effect %= $\frac{blankofabsorbance - Absorbanceofsample}{blankofAborbance} x\ 100$

***Phosphomolybdic acid assay***

An aliquot solution of exactly 2 $cm^3$ of each fractions of sample extracts were mixed with 2 $cm^3$ of reagent solution (0.6 mM sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in different test tubes. The test tubes were each capped with aluminum foil and incubated in a water bath at 95ºC for 90 min. Blank solution was prepared. After the sample had been cooled to a room temperature the absorbance of the mixtures were measured at 695 nm against blank using spectrophotometer [13]. The scavenging effect was calculated based on the percentage of phosphomolybdic radical scavenged using the following equation:

$$\text{Scavenging effect (\%)} = \frac{(blankabsorbance - sampleabsorbance}{blankabsorbance} x\ 100$$

***Total phenol content estimation assay***

Exactly 1 $cm^3$ of each prepared sample of 50% methanol only was mixed with 1 $cm^3$ of folin-ciocalteu's phenol reagent. After 5 min, exactly 10 $cm^3$ of 7% $Na_2CO_3$ solution was added to the mixture followed by the addition of exactly 13 $cm^3$ of deionized distilled water and mixed thoroughly. The mixture was kept for 90 min at 23ºC, after which the absorbance was read at 750 nm using spectrophotometer. The phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of phenolic compound was carried out in duplicate and the total phenolic content was expressed as milligrams of Gallic acid equivalent per g of dried sample [13].

**Statistical Treatment of Data**

The data collected were expressed as mean and standard deviation which were subjected to statistical analysis using Microsoft spreadsheet and statistical package for social science (SPSS) software version 20 for the analysis of variance (ANOVA).

## RESULTS AND DISCUSSION

### Phytochemical composition of crude extracts of n-hexane and methanol extracts of *Ocimum basilicum* and *Ocimum gratissimum* leaves

Phytochemical composition of extracts of n-hexane and methanol of *O. basilicum* and *O. gratissimum* leaves were obtained through screening the extracts of both leaves in which carbohydrate test, tannins test, cardiac glycoside test, terpenoid test, and flavonoid test. The results showed the presence of carbohydrate, cardiac glycoside, terpenoid and flavonoid content. Absence of tannins and monosaccharide in carbohydrate were recorded in n-hexane extracts of both *O. basilicum* and *O. gratissimum* leaves. The methanol extracts of *O. basilicum* and *O. gratissimum* leaves showed the presence of carbohydrate, tannins, cardiac glycoside, terpenoid and flavonoid, but monosaccharide (Barfoeds test for carbohydrate) in both the extracts of the leaves were absent as illustrated in Table 1.

### Antioxidant properties of extracts of n-hexane and methanol of *Ocimum basilicum* and *Ocimum gratissimum* of plant leaves.

Antioxidant properties was determined using 2,2-diphenyl-1-picrylhyrazyl assay, hydrogen peroxide scavenging assay, ferric ion reducing power assay, phosphomolybdic acid assay and phenolic content assay of both extracts of n-hexane and methanol of *O. basilicum* and *O. gratissimum* plant leaves were determined as follows;

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The antioxidant properties of *O. basilicum* and *O. gratissimum* leaves extracts was examined using 2,2-diphenyl-1-picrylhyrazyl assay, based on the ability of extracts to reduce the free radical in DPPH solution using ascorbic acid as a reference standard. The ability of n-hexane extracts of *O. basilicum*, *O. gratissimum* and ascorbic acid at various concentrations (50, 100, 150, 200 and 250 μg/cm$^3$) were determined and the values are shown in Figure 1. The maximum reducing property of both the n-hexane extract of *O. basilicum*, *O. gratissimum*, and ascorbic acid in DPPH solution was obtained in 250 μg/cm$^3$ concentration at 94.38 ± 0.53, 95.63 ± 0.18 and 99.56 ± 0.09 %, respectively. The maximum reducing property of methanol extracts of *O. basilicum*, *O. gratissimum* and ascorbic acid in DPPH solution was obtained in 250 μg/cm$^3$ concentrations at 98.59 ± 0.09, 98.13 ± 0.18 and 99.56 ± 0.09% respectively shown in Figure 2.

Hydrogen peroxide scavenging assay

The reducing property of *O. basilicum, O. gratissimum* and ascorbic acid as an antioxidant ingredient in reducing hydrogen peroxide generated free radicals in solution was examined. The reducing ability of n-hexane extracts of *O. basilicum, O. gratissimum* and also ascorbic acid as a reference standard at various concentrations (50, 100, 150, 200 and 250 μg/cm$^3$) are presented in Figure 3. The maximum mean percentage inhibitions were obtained in 50 μg/cm$^3$ concentration of *O. basilicum, O. gratissimum* and ascorbic acid in $H_2O_2$ solution at 99.19 ± 0.60%, 97.58 ± 0.60% and 88.91 ± 0.86% respectively. The mean percentage reducing ability of methanol extracts of *O. basilicum, O. gratissimum* and ascorbic acid at different concentrations (50, 100, 150, 200 and 250 μg/cm$^3$) were determined and shown in Figure 4. The maximum percentage reducing ability of *O. basilicum, O. gratissimum* and ascorbic acid were in 50μg/cm$^3$ concentration of *O. basilicum, O. gratissimum* leave extracts and ascorbic acid in $H_2O_2$ solution at 94.15 ± 3.70%, 95.97 ± 0.60% and 88.91 ± 0.86% respectively.

***Ferric ion reducing antioxidant power (FRAP) assay.***

The percentage inhibition of *O. basilicum, O. gratissimum* and ascorbic acid as an antioxidant against ferric ion reducing solution was examined based on the property to reduce ferric ion generating free radical in its solution. The mean percentage reducing ability of n-hexane extracts of *O. basilicum, O. gratissimum* and also ascorbic acid as a reference standard at various concentrations (50, 100, 150, 200 and 250 μg/cm$^3$) were determined and results given in Figure 5. Maximum mean percentage inhibition was obtained in 50 μg/cm$^3$ concentration of *O. basilicum, O. gratissimum* and ascorbic acid in FRAP solution at 88.53±0.21, 88.97±0.12 and 83.39±0.36% respectively. The mean percentage inhibition of methanol extracts of *O. basilicum, O. gratissimum* and also ascorbic acid as a reference standard at different concentrations (50, 100, 150, 200 and 250 μg/cm$^3$) were determined and shown in Figure 6. The maximum mean percentage inhibition of *O. basilicum, O. gratissimum* and ascorbic acid were at 50μg/cm$^3$ of *O. basilicum, O. gratissimum* leave extracts and ascorbic acid at 87.84 ± 0.19, 85.63 ± 0.09 and 83.39 ± 0.36% respectively.

***Phosphomolybdic acid assay***

The free radical scavenging properties of *O. basilicum, O. gratissimum* leaves extracts as an antioxidant ingredient in reducing Phosphomolybdic solution (Total Acid Concentration (TAC)) was examined. The reducing ability of n-hexane extracts of *O. basilicum, O. gratissimum* and

ascorbic acid as a reference standard at various concentrations (50, 100, 150, 200 and 250 μg/cm$^3$) are illustrated in Figure 7. The maximum mean percentage reducing ability was obtained in 50μg/cm$^3$ concentration of *O. basilicum, O. gratissimum* and ascorbic in phosphomolybdic acid solution at 96.03 ± 0.21, 98.03 ± 0.21 and 91.84 ± 0.86% respectively. The reducing ability of methanol extracts of *O. basilicum, O. gratissimum* and ascorbic acid at different concentrations (50, 100, 150, 200 and 250 μg/cm$^3$) were also determined as shown in Figure 8. The maximum mean percentage inhibition of *O. basilicum, O. gratissimum* and ascorbic acid were in 50μg/cm$^3$ concentration of *O. basilicum, O. gratissimum* leave extracts and ascorbic acid and at 96.67 ± 0.00, 97.67 ± 0.21 and 91.84 ± 0.86% respectively.

***Total phenol content Assay***

The Mean amount of total phenolic content for the extracts of n-hexane and methanol of both *Ocimum basilicum* and *Ocimum gratissimum* plant leaves at 50μg/cm$^3$ were determined shown in Figure 9. The result shows that n-hexane and methanol extract of *O. basilicum* has a concentration of phenol of 102.0 ± 0.02 and 99.0 ± 0.02 mgGAC/g respectively. Also *O. gratissimum* has concentration of phenol content of 11.75 ± 0.00 and 10.25 ± 0.00 mgGAC/g in n-hexane and methanol extract respectively.

**Statistical Analysis of antioxidant properties of *Ocimum basilicum* and *Ocimum gratissimum* plant leaves.**

Analysis of variance (ANOVA) was explore and impact of n-hexane and methanol extracts of *Ocimum basilicum* and *Ocimum gratissimum* leaves samples against the mean percentage inhibition for Antioxidant assays of DPPH, ferric ion reducing assay, $H_2O_2$, Phosphomolybdic acid and total phenolic content. The following significant of antioxidant assays were obtained at $p \leq 0.05$ shown in Table 2.

Table 1: Phytochemical screening of n-hexane and methanol extracts of *Ocimum basilicum* and *Ocimum gratissimum* leaves extracts

| Phytochemical screening | | | *Ocimum basilicum* | | *Ocimum gratissimum* | |
|---|---|---|---|---|---|---|
| | | | n-hexane Extract | Methanol Extract | n-hexane Extract | Methanol Extract |
| Carbohydrate test | I. | Molishs test | + | + | + | + |
| | II. | Barfoeds test | _ | _ | _ | _ |
| | III. | Starch test | + | + | + | + |
| Test for tannins | | | _ | + | _ | + |
| Test for Cardiac glycoside | | | + | + | + | + |
| Test for Terpenoid | | | + | + | + | + |
| Test for Flavonoid | I. | Shinoda's test | + | + | + | + |
| | II. | Ferric Chloride test | + | + | + | + |

Note: + means present and – means absent

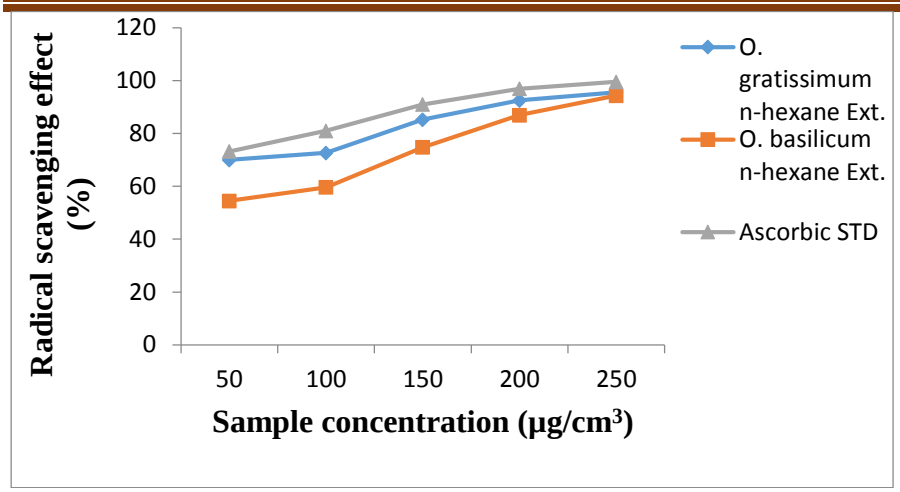


Figure 1: Mean percentage of radical scavenging effect of n-hexane extracts of *Ocimum basilicum*, *Ocimum gratissimum* leaves and Ascorbic acid as a reference standard in DPPH solution at various concentrations of extract.

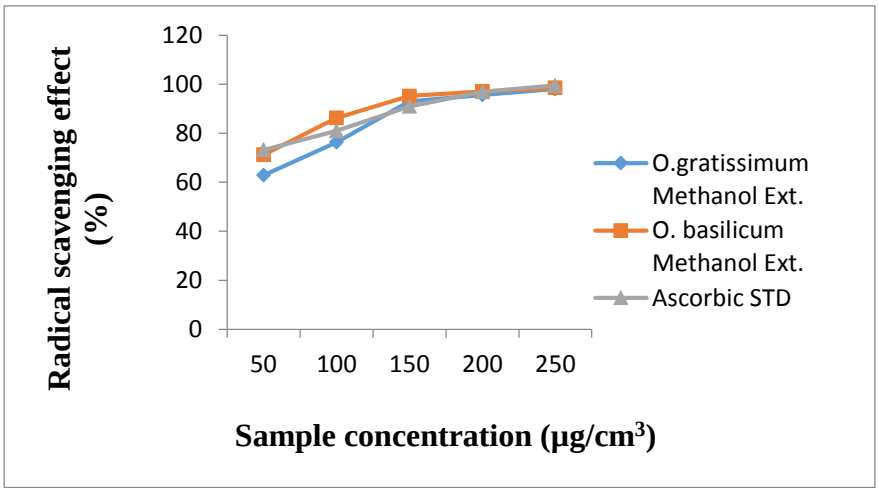


Figure 2: Mean percentage of radical scavenging effect of Methanol extracts of *Ocimum basilicum*, *Ocimum gratissimum* leaves and Ascorbic acid a reference standard in DPPH solution at various concentrations of extract.

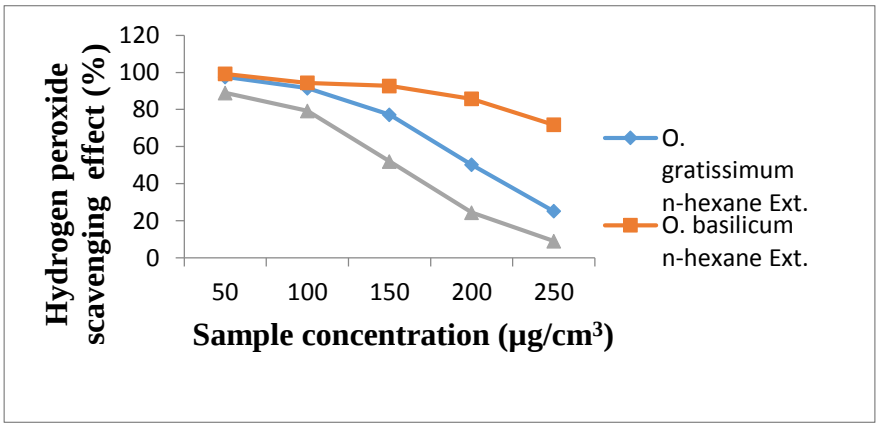

Figure 3: Mean percentage of hydrogen peroxide scavenging effect of n-hexane extracts of *Ocimum basilicum*, *Ocimum gratissimum* leaves and Ascorbic acid as a reference standard in $H_2O_2$ solution at various concentrations of extract.

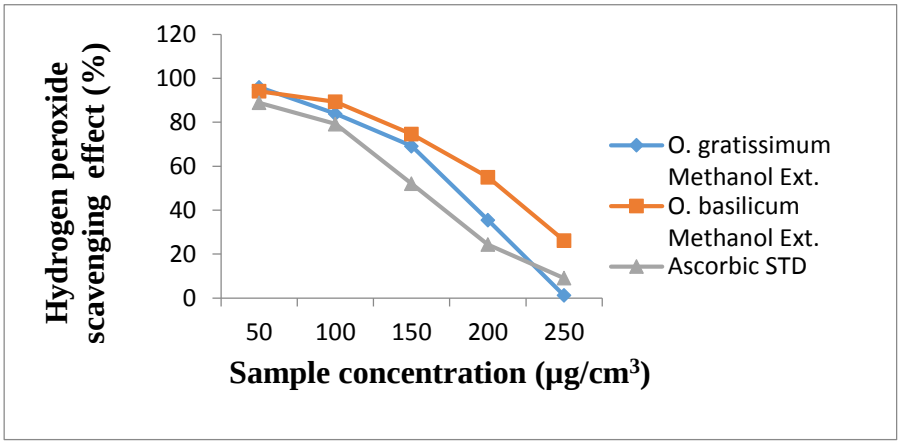


Figure 4: Mean percentage of hydrogen peroxide scavenging effect of Methanol extracts of *Ocimum basilicum*, *Ocimum gratissimum* leaves and ascorbic acid as a reference standard in $H_2O_2$ solution at various concentrations of extract.

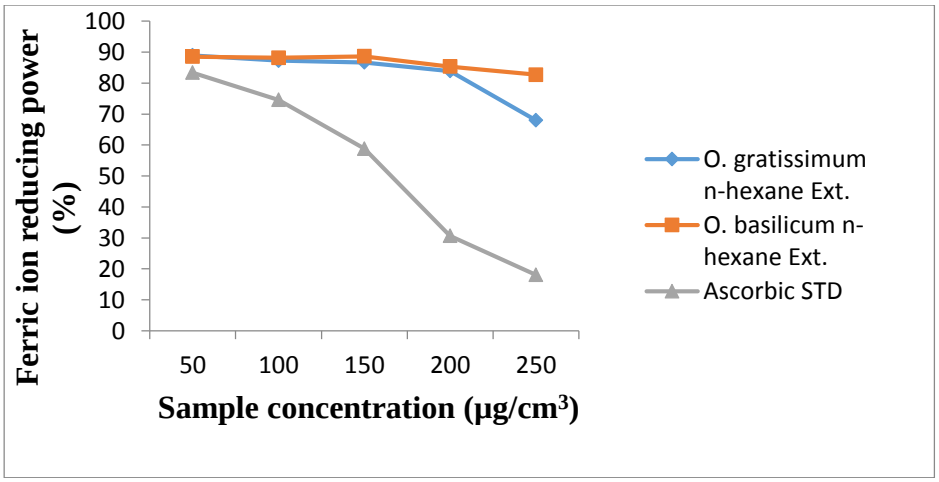


Figure 5: Mean percentage of ferric ion reducing power of n-hexane extracts of *Ocimum basilicum*, *Ocimum gratissimum* leaves and Ascorbic acid as a reference standard in ferric ion solution at various concentrations of extracts.

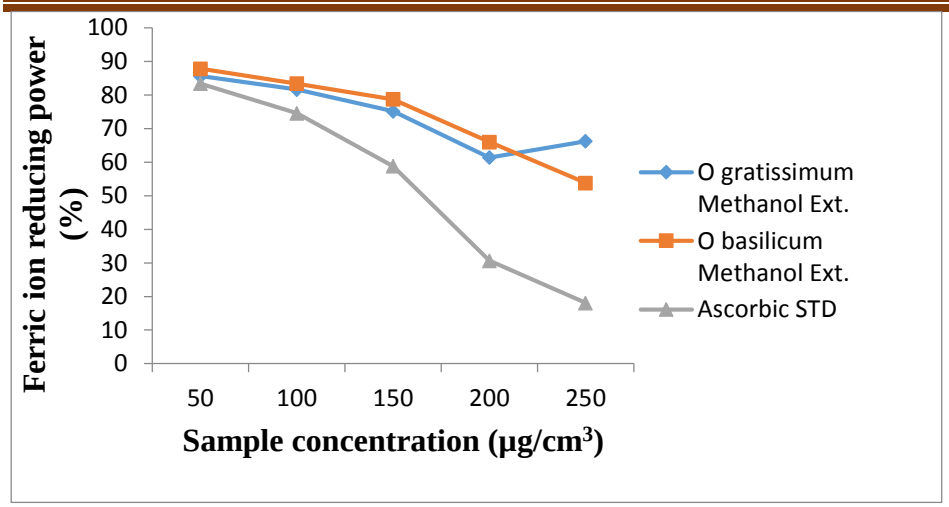


Figure 6: Mean percentage of ferric ion reducing power of Methanol extracts of *Ocimum basilicum*, *Ocimum gratissimum* leaves and Ascorbic acid as reference standard in ferric ion solution at various concentrations of extract.

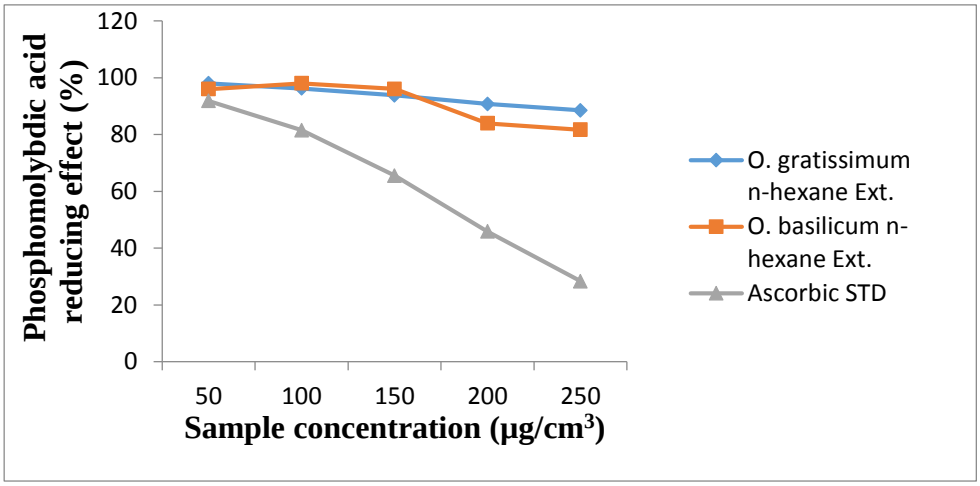


Figure 7: Mean percentage of Phosphomolybdic acid reducing effect of n-hexane extracts of *Ocimum basilicum*, *Ocimum gratissimum* leaves and Ascorbic acid as a reference standard in Phosphomolybdic solution at various concentrations of extract.

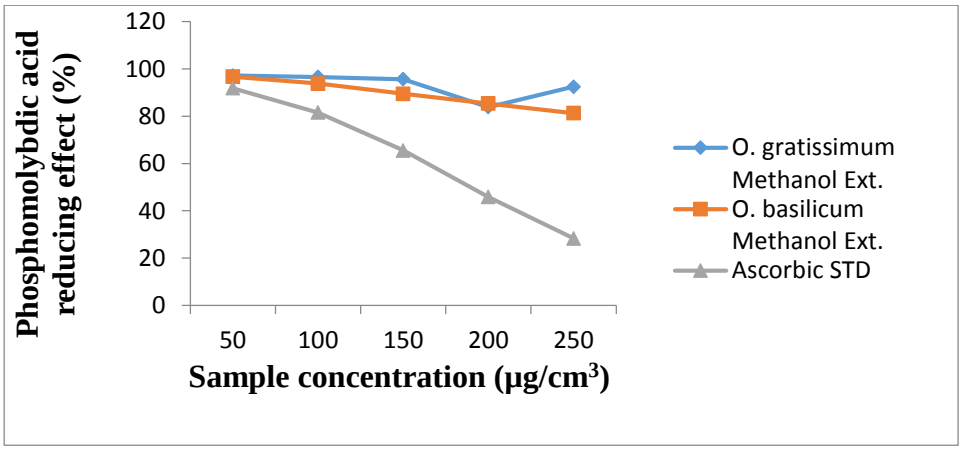

Figure 8: Mean percentage Phosphomolybdic acid reducing effect of Methanol extracts for *Ocimum basilicum*, *Ocimum gratissimum* leaves and Ascorbic acid as a reference standard in Phosphomolybdic solution at various concentrations of extract.

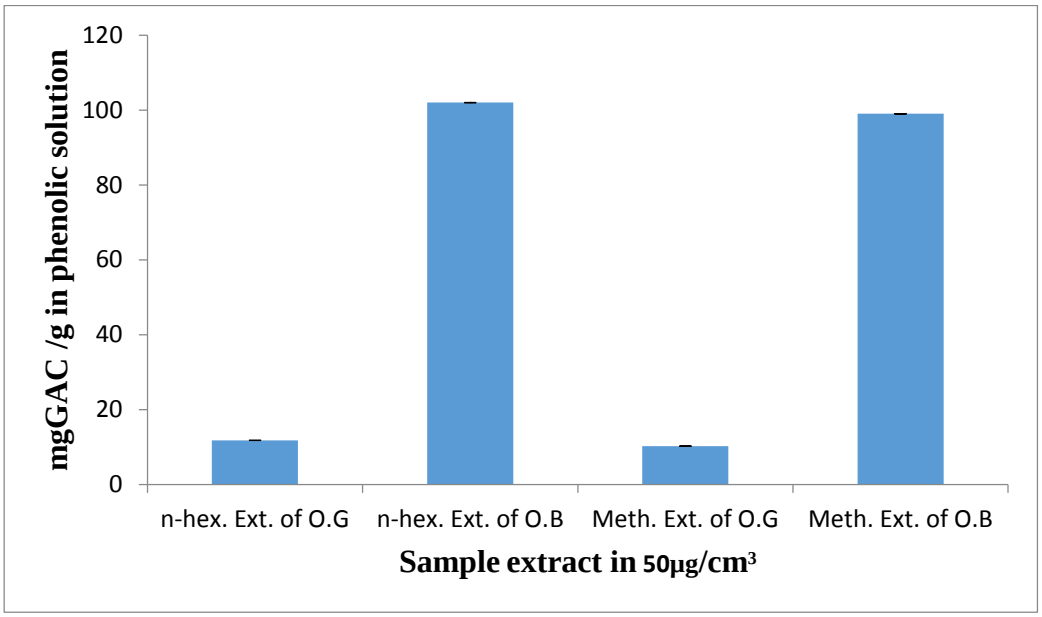


Figure 9: Mean concentration of phenolic content of n-hexane and methanol extracts in *O. basilicum* and *O. gratissimum* plant leaves.

Table 2: Significant difference of Antioxidant assay against n-hexane and methanol extracts of *Ocimum basilicum* and *Ocimum gratissimum* plant leaves.

| Antioxidant assay | p value |
|---|---|
| DPPH | 0.409 |
| $H_2O_2$ | 0.394 |
| FRAP | 0.129 |
| Phosphomolybdic acid | 0.676 |

Note: p value $\leq$ 0.05 significant

Phytochemicals in plants linked to information on the health benefits of foods [14]. From the result obtained, *O. basilicum* and *O. gratissimum* of methanol extracts showed the presence of the following phytochemical parameters such as carbohydrate, tannin's, cardiac glycoside terpenoid and flavonoid; while n-hexane extracts of both *O. basilicum* and *O. gratissmum* leaves had presence of carbohydrate, cardiac glycoside, terpenoid and flavonoid but tannins was absent as shown in Table 1. The Phytochemicals present in both extracts of *O. basilicum* and *O. gratissimum* leaves contain bioactive food composition and also will serve as antioxidant within the human body.

Antioxidant substance scavenges against free radicals within the body and also protects the body from different diseases which also act either by inhibiting the reactive oxygen species or stimulating the antioxidant defense mechanisms [15]. Antioxidants are also involved in autoimmune disorder such as rheumatoid arthritis, etc [16].

Antioxidant property in plants use as food and medicinal value can be determined using DPPH solution which decolorizes the DPPH solution (by electron donation) to releasing the free radical in the solution 13]. The less the absorbance of the reacting mixture indicates a high inhibition ability of free radical by the plant under test 17]. The results obtained showed that both n-hexane and methanol extracts of *O. basilicum, O. gratissimum* and ascorbic acid had maximum percentage inhibition at 250µg/cm$^3$ concentration. The n-hexane extracts of *O. gratissimum* leaves had maximum inhibition at 95.63 % than *O. basilicum* leaves at 94.38% as shown in Figure 1. The methanol extract of *O. basilicum* leaves showed maximum inhibition at 98.56% than *O. gratissimum* at 98.13% as shown in Figure 2. The ascorbic acid which was used as reference standard showed the maximum inhibition at 99.56%. Both leaves extracts showed good scavenging activity.

Hydrogen peroxide naturally occurs in small concentration in air, water, human body, plant microorganisms and food. It is toxic to cell membrane and damage DNA within the body of living Organisms [18, 19]. The results obtained showed that n-hexane extract of *O. basilicum* had maximum inhibition in 50 µg/cm$^3$ concentration at 99.19% than *O. gratissimum* at 97.58% as shown in Figure 3. The methanol extracts of showed that *O. gratissimum* had maximum inhibition in 50 µg/cm$^3$ concentration at 95.60% than *O. basilicum* at 94.15% as shown in Figure 4. The ascorbic acid used as reference standard showed minimal inhibition at 88.91%. The n-hexane extracts showed better inhibition than the methanol extract. This might be due to the high concentration of tannins within the methanol extract

In the reducing power assay the antioxidant reduced the ferric cyanide complex to ferrous form (Fe $^{3+}$ to Fe $^{2+}$) changing the colour of the test solution from yellow to green (breaking the free radical chain by donating a hydrogen atom) [13]. The result obtained showed that n-hexane extracts of *O. gratissimum* extracts had maximum inhibition in 50 µg/cm$^3$ concentration at 88.97% than *O. basilicum* at 88.53% as shown in Figure 5. The methanol extracts of *O. basilicum* had the maximum inhibition in 50 µg/cm$^3$ concentration at 87.84% than *O. gratissimum* at 85.63% as shown in Figure 6. The ascorbic acid used as reference standard had the low inhibition at 83.39%. The n-hexane extracts showed better inhibition than the methanol extract. This might be due to the high concentration of tannins within the methanol extract.

In the assay of phosphomolybdic solution the antioxidant agent reduced Mo(VI) to Mo(V) which form a colour of green from phosphomolybdenum (V) complex [15]. The n-hexane extract of *O.*

*gratissimum* had maximum inhibition in 50 μg/cm$^3$ concentration at 98.03% than *O. basilicum* at 96.82% as shown in Figure 7. The methanol extracts of *O. gratissimum* had maximum inhibition in 50 μg/cm$^3$ concentration at 97.13% than *O. basilicum* at 96.67% as shown in Figure 8. The ascorbic acid used as reference standard had minimal inhibition at 91.84%. The n-hexane extracts showed better inhibition than the methanol extract. This might be due to the high concentration of tannins within the methanol extract.

The presence of flavonoid and related polyphenol constituents in medicinal plant reduced the activities of phosphomolybdate [13, 20, 21]. Phenolic content are secondary metabolite in plant which possesses the ability to inhibit free radical. It is also used in therapeutic for anti-carcinogenic, anti-mutagenic and antioxidant activities [22]. The ability of phenol to inhibit is due to the presence of hydroxyl group and also the presence of flavonoid contents in plant [15]. n-hexane and methanol extracts of *O. basilicum* leaves had high concentration of phenolic content of 102 and 99 mgGAE/g respectively while n-hexane and methanol extracts of *O. gratissimum* leaves had low concentration of phenolic content of 11.75 and 10.25 mgGAE/g respectively in 50 μg/cm$^3$ concentration of the prepared samples leaves shown in Figure 9. The inhibition obtained within the antioxidant assays was not only due to the phenolic contents alone but there are some constituents within the leaves that also take part in the scavenging of these free radicals. The presence of tannins in the methanol extracts of both *O. gratissimum* and *O. basilicum* leaves also contributes the less inhibition properties because of the content of tannin which supports oxidation.

Statistical analysis of all antioxidant assays against the sample extracts of *O. basilicum* and *O. gratissimum* leaves shows no significant difference between the assays and the extracts of both leaves as illustrated in Table 2.

## CONCLUSION

The phytochemical compositions showed the presence of carbohydrate, tannin's, cardiac glycoside terpenoid and flavonoid in methanol extract of both leaves while in n-hexane extract only tannins and monosaccharide's in carbohydrate was absent but has good secondary metabolic compositions. n-hexane had high inhibition in FRAP, $H_2O_2$ and phosphomolybdic acid assay in *O. basilicum* leaves, high inhibition of both extracts in DPPH assay in *O. gratissimum* leaves and high phenolic contents in both extracts of *O. basilicum* leaves. Both leaves extracts

showed good potential to medicinal applications in the antioxidant assay. Statistically both leaves showed no differences in all the extracts check for antioxidant properties.

**Recommendation**

*O. basilicum* and *O. gratissimum* have good medicinal values with respect to their inhibition properties. Therefore the consumption of these leaves daily in meal will help in protecting the body system against oxidative stress which can lead to different kind of diseases.

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## Conflict of interest

There was no conflict of interest exist among the authors regarding the publication of this article.

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