

**Ocimum gratissimum** (OG) Leaf Extract to Offer Antimicrobial and Antioxidant Properties in Food

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**Abstract**

Aqueous leaf extract of *Ocimum gratissimum* (OG) was screened for phytochemical, antimicrobial and antioxidant properties. Saponins and flavonoids were "very strongly present"; Terpenoids, Tannins, Phenol, Steroids were "strongly present" while Alkaloids, Glycosides and Quinines were absent. Microbial activity of OG leaf extracts reduced with reducing concentrations from 200 mg/ml to 50 mg/ml for all organisms tested and significant antimicrobial activities were observed against all the organisms at 100 mg/ml. The organisms susceptible at 50 mg/ml extract were *S. typhi* and *C. pefringens* at a zone of 13.50 mm. Most of the organisms studied did not show any visible growth at 3.125 mg/ml OG, except *P. aeruginosa*. More so, *E. coli, C. pefringens and L. monocytogenes* had a common minimum inhibition concentration of 1.5625 mg/ml. OG extract exhibited significantly (p < 0.05) higher scavenging activity compared to standard ascorbic acid at 5.0 µg/ml DPPH. The results of this study suggest that aqueous OG extract has potential effects on the microorganisms studied. Aqueous OG extracts could therefore offer some benefits in microbial decontamination activities as well as preventing autoxidation reactions in foods. It is thus recommended to explore the potential use benefits of OG leaf extract in non-nitrite cured meat formulations.

**Keywords**: Ocimum gratissimum; Aqueous Leaf Extract; Phytochemical; Microbial Decontamination; Autoxidation

**Abbreviation**

OG: *Ocimum gratissimum*

**Introduction**

Plant portions have been suggested to have disease preventing abilities due to biologically active components with antioxidant and antimicrobial properties [1,2]. Some examples of such plants used against different ailments or diseases include *Cocos nucifera, Cola nitida, Mangifera indica, Moringa oleifera, Nicotiana tabacum, Parkia biglobosa, Tetrapleura tetraptera* and *Xylopia aethiopica*. *Ocimum gratissimum* is herbaceous and belongs to the family *Lamiaceae* and is indigenous to tropical areas especially India and West Africa, where it is commonly known differently in the different locations. It is believed to have originated from Central Africa and South East Asia. Reference

[3] reported that *Ocimum gratissimum* has active ingredients which provide several medicinal benefits and [4] suggested the use of *OG* in the treatments of high fever, influenza, rheumatism, gonorrhea while [5] reported antinociceptive properties of essential oils of *O. gratissimum*. Generally, the suitability of any, or a combination of plants in providing benefits to human health and or nutrition would be based on the presence of secondary metabolites or phytochemicals which function to deter predation; or presence of others components like pheromones used to attract insects for pollination. It is therefore very necessary to determine the phytochemical, antioxidant and or antimicrobial properties of such plants prior to being utilized for any benefit for mankind in any form. Reference [6] suggested potential utilization of the essential oil of *O. gratissimum* as an aid to the control of gastrointestinal helminths in small ruminants, and this was corroborated by [7], while [8] suggested its use in toothpastes and mouth washes due to germicidal properties.

**Objective of the Study**

The objectives of this study were to determine phytochemical, antimicrobial and antioxidant properties of *O. gratissimum* leaf extract in an attempt to evaluate its potential utilization in food formulations as a natural cure ingredient.

**Materials and Methods**

**Collection of plant material, air drying and aqueous extraction**

*OG (Ocimum gratissimum)* leaves were collected from the Forestry Research Institute of Ghana (FORIG) in Kumasi, carefully washed with tap water, rinsed with distilled water and air dried to 10% moisture. They were ground into powder and stored at room temperature. Aqueous extract of samples was prepared by soaking 100 gm of dried powder in 200 ml distilled water (DW) in 500 mL beaker overnight and filtered using Whatman No. 2 filter paper and concentrated under reduced pressure at 45°C to dryness using Buchi Rotavapor (R-200, Germany) to obtain crude *OG* residue which was stored in airtight bottles in a refrigerator at 4°C for further studies.

**Phytochemical screening of aqueous *OG* extract**

The *OG* residue obtained was screened for the presence of phytochemicals, namely Saponin, Flavonoid, Alkaloid, Terpenoids, Tannin, Phenol, Quinine, Glycoside, and Steroids according to the methods described by [9].

**Preparation of test organisms, antimicrobial testing and determination of minimum inhibition concentration (MIC)**

Isolates for antimicrobial susceptibility study were tested for vigor in buffered peptone water broth, sub-cultured on nutrient agar and incubated at 37°C for 24h. Prior to susceptibility testing, single isolates were sub-cultured in nutrient broth for 8h at 37°C at the Microbiology Laboratory of Department of Pharmaceutics, KNUST.

Antimicrobial sensitivity testing was performed using the agar well diffusion method as described by [10] with slight modifications. Two (2) mL of 8h broth cultured isolates of test organisms were seeded in each of 18 mL Mueller Hinton agar plates (MHA Difaco, France) in sterile Petri dish. Isolates were uniformly distributed by slow rotation of the Petri dishes and allowed to set. Eight (8) mm sterile cork borer was used to make uniform wells on the agar and filled with 2 mL respectively of the *OG* extract using sterile Pasteur pipette and allowed to stand at room temperature for 45 minutes for sufficient diffusion to take place.

Control experiments were also setup with 2 mL distilled deionized water in separate wells.

All plates were incubated for 24h at 37°C and the assay was conducted at regular intervals while clearance round each well was measured in millimeter (mm), to represent the zones of inhibition. Large clearance zones imply high susceptibility while small zones indicated
low susceptibility of the organism to the tested OG extract.

Minimum inhibition concentration (MIC) is the lowest concentration of an antimicrobial agent which prevents visible growth of a given microbial strain. Serial dilutions of OG extract (100 - 0.7512 mg/mL in 2:1 progressive dilutions) were prepared and tested against the 8 microbes investigated earlier for antimicrobial activity using broth dilution method. Each setup was observed for microbial growth, and the least concentration of OG that showed no growth was selected as the MIC for the specific microbe.

**Antioxidant testing by DPPH scavenging activity**

Free radical scavenging potential of OG extract was determined using 2,2-diphenyl-1-picrylhydrazy (DPPH) as described by [11]. The mixture of DPPH (0.1 mM) in methanol was prepared and 4 mL of this solution was added to 1 mL of sample solutions of OG extract (50.0 mg/ml; 100.0 mg/ml; 200.0 mg/ml) and stored for thirty (30) min after which absorbance was measured at 517 nm at room temperature using a spectrophotometer (Shimadzu UV-1800, Japan). Lower absorbance of the reaction mixture indicates a higher free radical scavenging activity. Ascorbic acid was used as a standard.

The percent inhibition of DPPH (I%) was calculated as follows:

\[
I\% = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100
\]

Where Ablank is the absorbance of control and Asample is the absorbance of OG sample.

Extract concentration providing 50% inhibition (IC50) was calculated from the graph of inhibition percentage plotted against extract OG concentration. Tests were carried out in triplicates.

**Statistical analysis**

Data were analysed statistically by [12] using a completely randomized design and significant differences between means were determined at 5% using Duncan’s test.

**Results and Discussion**

**Phytochemical components determined in OG leaf extract**

Table 1 shows types of phytochemicals inferred from aqueous extracts of OG leaf. It was observed that Alkaloids and Quinones were absent while Glycosides were “weakly present”. Terpenoids, Tannins and Steroids were “strongly present” while Saponins, Flavonoids, Phenols, Carbohydrates and proteins were “very strongly present”. Similar observations have been reported by [2], in both methanolic and aqueous extracts of OG, but their work recorded no Saponins probably due to soil differences between the two geographical regions of these studies. According to reference [13] such environmental variations substantially tend to impact on the quality and properties of wild and cultivated plants exploited for pharmaceutical, nutritional and industrial applications. The “very strong” presence of Saponins and Flavonoids in particular, observed in this study is not surprising because they are generally considered as some of the most abundant and diverse groups of plant natural compounds performing various ecological functions including defence against fungi and bacterial attack or disease, and against herbivores. They are also thought to be responsible for providing superior competitive edge during plant interactions, strong flavour and possibly colour [14]. The “very strong presence” of carbohydrates and proteins make OG a possible candidate for further research as livestock feed additives.
Antimicrobial activity

Results obtained for the potential antimicrobial activity of OG leaf extracts against 8 microbes are shown in table 2. It was observed that extract activity reduced with reducing concentration from 200.0 mg/ml to 50.0 mg/ml for all the organisms studied and significant (p < 0.05) activity was shown against all of the organisms at 100.0 mg/ml, but the 50.0 mg/ml extract showed activity against only two organisms, namely S. typhi and C. perfringens. The zone of inhibition observed at 50.0 mg/ml of OG against both S. typhi and C. perfringens was 13.50 mm Microorganisms against which the highest inhibitions were observed were S. typhi and C. perfringens at 200.0 mg/ml of OG with 19.50 mm and 19.00 mm respectively. In general, antimicrobial activity of plant extracts results from their inherent ability to cause structural and functional damages to microorganisms through disruption of cell membrane permeability and osmotic balance [15]. According to reference [15] inhibition zones ≥ 10 mm can be considered active. The antibacterial activity of OG extract is possibly due to the "strong presence" of Saponins, and Flavonoids in addition Tannins, Terpenoids and Steroids as shown in table 1. Several studies have shown plant extract activity against some microorganisms, and it was suggested that such activity were due to the phytochemicals observed during phytochemical screening [16-19].

<table>
<thead>
<tr>
<th>Organism</th>
<th>Concentrations of OG (mg/ml)</th>
<th>Mean Zone of inhibition (mm) (± SD)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>S. aureus</td>
<td>16.50 (0.70)a</td>
<td>14.50 (0.70)b</td>
<td>0.00 (0.00)c</td>
</tr>
<tr>
<td>E. coli</td>
<td>19.50 (2.12)a</td>
<td>16.50 (0.70)b</td>
<td>0.00 (0.00)c</td>
</tr>
<tr>
<td>S. typhi</td>
<td>19.00 (0.70)a</td>
<td>16.50 (0.70)b</td>
<td>13.50 (0.70)c</td>
</tr>
<tr>
<td>C. perfringens</td>
<td>17.50 (0.70)a</td>
<td>16.00 (1.41)b</td>
<td>13.50 (0.70)c</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>18.00 (1.41)a</td>
<td>15.00 (1.41)b</td>
<td>0.00 (0.00)c</td>
</tr>
<tr>
<td>C. botulinum</td>
<td>17.60 (1.71)a</td>
<td>16.00 (0.61)b</td>
<td>0.00 (0.00)c</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>16.01 (1.80)a</td>
<td>14.13 (0.78)b</td>
<td>0.00 (0.00)c</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>16.78 (0.47)a</td>
<td>14.67 (0.12)b</td>
<td>0.00 (0.00)c</td>
</tr>
</tbody>
</table>

Table 2: Antimicrobial activity of OG extract against some bacteria.

ab: Means in the same row with same letters are not significantly different (p > 0.05).
Minimum inhibition concentration (MIC) of aqueous OG extract

Based on results reported in table 2 the MICs of OG extracts were tested at the 2:1 dilution concentrations for the 8 microorganisms in this study, and the result obtained are shown in table 3. MIC for the majority of the organisms ranged from 1.5625 - 100 mg/mL but *P. aeruginosa* seemed to be the only organism with MIC range of 3.125 mg/ml to 100 mg/ml. In general, antimicrobial activity of plant extracts results from their inherent ability to cause structural and functional damages to microorganisms through disruption of cell membrane permeability and osmotic balance. According to reference [15] inhibition zones ≥ 10 mm can be considered active. The antibacterial activity of OG extract is possibly due to the "strong presence" of Saponins, and Flavonoids in addition Tannins, Terpenoids and Steroids as shown in table 1.

<table>
<thead>
<tr>
<th>Type of Organism</th>
<th>Concentration of OG (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>-</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>-</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>-</td>
</tr>
<tr>
<td><em>C. perfringens</em></td>
<td>-</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>-</td>
</tr>
<tr>
<td><em>C. botulinum</em></td>
<td>-</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>-</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>-</td>
</tr>
</tbody>
</table>

*Table 3: Minimum inhibition concentration of OG against some bacteria species.*

"-": No Visible Growth; "+": Visible Growth.

The MIC of any substance with potential antimicrobial activity has been defined as the least concentration at which no visible growth could be achieved under a defined set of conditions for specific microorganisms of importance [20]. Thus, MIC is very important for establishing microbial resistance or otherwise against known antimicrobials in pharmacological studies as well as establishing potential inhibition of microbial growth in the presence of newly manufactured antimicrobials. From table 3 it was observed that most of the organisms did not show any visible growth at 3.125 mg/mL of OG, except *P. aeruginosa*; whereas *E. coli, C. perfringens* and *L. monocytogenes* had a common MIC of 1.5625 mg/mL. These findings suggest that OG could possibly have aggravated effects in microbiological decontamination compared to most plant spices studied by [21]. In their study "Antibacterial activity of selected Cameroonian dietary spices ethnomedically used against strains of *Mycobacterium tuberculosis*" very high MICs ranging from 32 mg/ml to > 1024 mg/ml were reported for roots of *Echinops giganteus A. Rich* and *Xylopia parviflora*. Also the range of MICs obtained in this study fall perfectly lower than ranges of plant extracts that have been considered as "significantly active" by [22]. OG in this study showed MICs against most of the organisms in the range of 3.125mg/mL to 100mg/mL while 1.5625 mg/mL was inhibitory to the growth of *E. coli, C. perfringens* and *L. monocytogenes*. The result of this study compare favourably with those reported by [23] with MICs of 2.50 mg/ml to 10.00 mg/ml as determined by agar diffusion method for OG and *Piper guineense* on *E. coli* and *S. aureus*.

Antioxidant potential of OG extract by DPPH scavenging activity

In order to test the potential antioxidant activity of aqueous OG leaf extract, it was compared at similar concentrations with standard ascorbic acid (AA) to determine their respective abilities to bleach DPPH, the result of which are shown in table 4. The concentrations of DPPH used ranged from 0 µg/ml to 5.0 µg/ml. Significant differences (p < 0.05) were observed in scavenging activities between OG and standard ascorbic acid. The scavenging activity of standard ascorbic acid was significantly (p < 0.05) higher than OG extract up to 0.625

µg/ml, but at 1.25 µg/ml DPPH, both standard ascorbic acid and OG extract exhibited similar scavenging potentials. However, OG extract exhibited significantly higher (p > 0.05) scavenging activity at concentrations of 2.50 µg/ml to 5.0 µg/ml DPPH. The reductive potentials of both OG extract and standard ascorbic acid (AA) were concentration-dependent, and the potential of AA was clearly higher than that of OG at lower concentrations of DPPH except the 1.25 µg/ml. Reference [2] reported similar DPPH scavenging activity for methanolic extracts of OG. Results from this investigation also confirm that OG is rich in phytochemicals as observed in table 1. References [8] and [24] had previously reported important specific compounds in extracts from OG. Thus, this study corroborates the suggestion that extracts from leaves of OG possess good antioxidant potentials apparently due to their phytochemical constituents; especially the very strong presence of saponins and flavonoids (Table 1) which are responsible for scavenging and fighting off free radicals that cause oxidative stress.

<table>
<thead>
<tr>
<th>DPPH Conc. (µg/ml)</th>
<th>Standard Ascorbic Acid</th>
<th>OG Extract</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0.078125</td>
<td>67.91a</td>
<td>57.28b</td>
<td>0.023</td>
</tr>
<tr>
<td>0.15625</td>
<td>72.39a</td>
<td>67.31b</td>
<td>0.043</td>
</tr>
<tr>
<td>0.3125</td>
<td>89.50a</td>
<td>64.31b</td>
<td>0.031</td>
</tr>
<tr>
<td>0.625</td>
<td>78.44a</td>
<td>65.63b</td>
<td>0.041</td>
</tr>
<tr>
<td>1.25</td>
<td>67.36</td>
<td>67.63</td>
<td>0.104</td>
</tr>
<tr>
<td>2.5</td>
<td>75.88b</td>
<td>85.86a</td>
<td>0.030</td>
</tr>
<tr>
<td>5.0</td>
<td>87.02b</td>
<td>98.47a</td>
<td>0.041</td>
</tr>
<tr>
<td>*50% DPPH scavenging effect</td>
<td>0.06b</td>
<td>0.07a</td>
<td>0.031</td>
</tr>
</tbody>
</table>

**Table 4:** Percentage DPPH scavenging activity.

Means in same row with different letters are significantly different (p < 0.05).

Conclusion and Recommendations

A Alkaloids, Quinones and cardiac glycosides were absent in aqueous OG leaf extracts but terpenoids, tannins, phenol and steroids were strongly observed, while Saponins and Flavonoids were very strongly present.

Microbial activity of OG leaf extracts reduced with reducing concentration from 200 mg/ml to 50 mg/ml for all the organisms studied. But significant activities were observed against all the organisms at 100 mg/ml, while 50 mg/ml extract showed activity against only two microorganisms, namely *S. typhi* and *C. pefringens*. The zone of inhibition observed at 50 mg/ml OG against both *S. typhi* and *C. pefringens* was 13.50 mm and microorganisms against which the highest inhibitions were recorded were *S. typhi* and *C. pefringens* at 200 mg/ml with 19.50 mm and 19.00 mm zones of inhibition respectively. Most of the organisms studied did not show any visible growth at 3.125 mg/ml of OG, except *P. aeruginosa*; whereas *E. coli*, *C. pefringens* and *L. monocytogenes* respectively had a common minimum inhibition concentration of 1.5625 mg/ml, thus suggesting that OG extract could possibly have aggravated effects in microbiological decontamination activity. Also, OG extracts exhibited higher scavenging activity compared to standard ascorbic acid at 5.0 µg/ml DPPH. These findings suggest recommendations to explore the potential of OG leaf extract as antimicrobial and antioxidants in non-nitrite cured meat formulations as well as other foods.

Conflict of Interest

The authors have no conflicts of interest to be declared.
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**Bibliography**


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