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Research Article

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

Akwetey WY[1]*, Adzitey F[2] and Teye GA[2]

[1]*Department of Animal Science, College of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

[2]*Department of Animal Science, Faculty of Agriculture, Food and Consumer Sciences, University for Development Studies, Nyankpala, University for Development Studies, Tamale, Ghana*

***Corresponding Author:** Akwetey WY, Department of Animal Science, College of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Received: February 18, 2021; **Published:** April 30, 2021

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 It is 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:

$$I\% = \{(Ablank - Asample)/(Ablank)\}\, x\ 100$$

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

Extract concentration providing 50% inhibition (IC_{50}) 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.

Type of phytochemical	Type of test	Inference
Saponins	Foam test	+++
Flavonoids	Ammonium and Aluminium Chloride	+++
Alkaloids	Mayer's /Wgner's	-
Terpenoids	Salkowski	++
Tannins	Ferric Chloride / Lead sub acetate	++
Phenols	Keller-Kiliani	++
Quinones	conc. Sulphuric acid test	-
Cardiac glycosides	Kedde test	-
Steroids	Salkowski/Liebermann-Burchurt test	++
Carbohydrate	Molish/Fehlings solution test	+++
Protein	Biuret solution test	+++

Table 1: *Phytochemicals in OG leaf extract.*

*"+++": Very Strongly Present, "++": Strongly Present, "+": Weakly Present, "-": Absent *: Not phytochemicals.*

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. pefringens*. The zone of inhibition observed at 50.0 mg/ml of *OG* against both *S. typhi* and *C. pefringens* was 13.50 mm Microorganisms against which the highest inhibitions were observed were *S. typhi* and *C. pefringens* 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].

Organism	Concentrations of *OG* (mg/ml) Mean Zone of inhibition (mm) (± SD)			P-Value
	200	100	50	
S. aureus	16.50 (0.70)a	14.50 (0.70)b	0.00 (0.00)c	<0.01
E. coli	19.50 (2.12)a	16.50 (0.700)b	0.00 (0.00)c	<0.01
S. typhi	19.00 (0.70)a	16.50 (0.70)b	13.50 (0.70)c	0.02
C. perfringens	17.50 (0.70)a	16.00 (1.41)b	13.50 (0.70)c	0.04
L. monocytogenes	18.00 (1.41)a	15.00 (1.41)b	0.00 (0.00)c	<0.01
C. botulinum	17.60 (1.71)a	16.00 (0.61)b	0.00 (0.00)c	<0.01
K. pneumonia	16.01 (1.80)a	14.13 (0.78)b	0.00 (0.00)c	0.02
P. aeruginosa	16.78 (0.47)a	14.67 (0.12)b	0.00 (0.00)c	<0.01

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.

Type of Organism	Concentration of OG (mg/ml)							
	100	50	25	12.5	6.25	3.125	1.5625	0.78125
S. aureus	-	-	-	-	-	-	+	+
E. coli	-	-	-	-	-	-	-	+
S. typhi	-	-	-	-	-	-	+	+
C. perfringens	-	-	-	-	-	-	-	+
L. monocytogenes	-	-	-	-	-	-	-	+
C. botulinum	-	-	-	-	-	-	+	+
K. pneumonia	-	-	-	-	-	-	+	+
P. aeruginosa	-	-	-	-	-	+	+	+

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. pefringens 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.

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

Table 4: *Percentage DPPH scavenging activity.*
Means in same row with different letters are significantly different ($p < 0.05$).

Conclusion and Recommendations

AAlkaloids, 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.

Bibliography

1. Farombi EO., *et al*. "Interference of common antimalarial drugs with some hepatic microsomal components and drug metabolism: potential implications for toxicity". *South African Journal of Science* 94 (1998): 303-304.

2. Akinmoladun AC., *et al*. "Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*". *Scientific Research and Essay* 2.5 (2007): 163-168.

3. Ijeh II., *et al*. "Antimicrobial effects of aqueous and ethanolic fractions of two spices, *Ocimum gratissimum* and *Xylopia aethiopica*". *African Journal of Biotechnology* 4.9 (2005): 953-956.

4. Oliver B. "Medicinal Plants in Nigeria". *Nigerian College of Arts, Science and Technology* (1980): 90-94.

5. Rabelo M., *et al*. "Antinociceptive properties of the essential oil of *Ocimum gratissimum* L. (Labiatae) in mice". *Brazilian Journal of Medical Biology Resources* 36 (2003): 521-524.

6. Pessoa., *et al*. "Anthelmintic Activity of Essential Oil of Ocimum gratissimun Linn. and Eugenol against Haemonchus Contortus". *Veterinary Parasitology* 1.2 (2002): 59-63.

7. Chitwood DJ. "Phytochemical based strategies for nematode control". *Annual Review of Phytopathology* 40 (2003): 221-249.

8. Holets F., *et al*. "Effect of essential oil of *Ocimum gratissimum* on the trypanosomatid Herpetomonas samuelpessoai". *Acta Protozoologica* 42.4 (2003): 269-276.

9. Trease GE and Evans WC. "Phytochemicals". In: Pharmacognosy, Saunders Publishers, London (2002).

10. Adeniyi BA., *et al*. "Antimicrobial potentials of Diospyros Mespiliformis (Ebenace4e)". *African Journal of Medicinal and Medical Science* 25 (1996): 221-224.

11. Hatipoglu G., *et al*. "Automated and standard extraction of antioxidant phenolic compounds of Hyssopus officinalis L. ssp. Angustifolius". *Industrial Crops and Products* 43 (2013): 427-433.

12. SPSS Statistical Package for Social Sciences, version 15.0.1 for Windows. USA: Microsoft Inc (2006).

13. Szakiel A., *et al*. "Influence of environmental abiotic factors on the content of saponins in plants". *Phytochemical Review* 10 (2011): 471-491.

14. Mugford ST and Osburn A. "Isoprenoid Synthesis in Plants and Microorganisms". *Isoprenoid Biosynthesis in Prokaryotic Organisms: In: New Concepts and Experimental Approaches* (2013): 1-16.

15. Usman H., *et al*. "Phytochemical and antimicrobial screenings of the leaf extracts of Celtis integrifolia Lam". *Journal of Tropical Bioscience* 5 (2005): 72-76.

16. Palou L., *et al*. "Alternatives to conventional fungicides for the control of citrus postharvest green and blue molds". *International Journal of Stewart Postharvest Review* 4.2 (2008): 1-16.

17. Centeno S., *et al*. "Antifungal activity of extracts of Rosmarinus officinalis and Thymus vulgaris against Aspergillus flavus and A. ochraceus". *Pakistan Journal of Biological Sciences* 13.9 (2010): 452-455.

18. Prakash B., *et al*. "Plant essential oils as food preservatives to control molds, mycotoxin contamination and oxidative deterioration of agrifood commodities - Potentials and challenges". *Food Control* 47 (2015): 381-391.

19. Hendel N., *et al*. "Antioxidant activity of rosemary (Rosmarinus officinalis L.) and it's in vitro inhibitory effect on Penicillium digitatum". *International Food Research Journal* 23.4 (2016): 1725-1732.

20. Wiegand I., *et al*. "Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances". *Nature Protocols* 3 (2008): 163-175.

21. Tekwu EM., *et al*. "Antibacterial activity of selected Cameroonian dietary spices ethno-medically used against strains of Mycobacterium tuberculosis". *Journal of Ethnopharmacology* 142.2 (2012): 374-382.

22. Kuete V. "Potential of Cameroonian plants and derived products against microbial infections: a review". *Planta Medica* 76 (2010): 1-13.

23. Ogunniran KO. "Antibacterial effect of extracts of *Ocimum gratissimum* and Piper guineense on E. coli and S. aureus". *African Journal of Food Science* 3.3 (2009): 77-81.

24. Effraim KD., *et al*. "Histopathological studies on the toxicity of *Ocimum gratissimum* leave extract on some organs of rabbit". *African Journal of Biomedical Research* 6 (2000): 21-25.

Volume 16 Issue 5 May 2021