Research Article

Antimicrobial and Antioxidant Potentials of Aqueous Extracts of *Ocimum gratissimum* Leaves

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Abstract

***Ocimum gratissimum*, commonly known as scent leaf, is a medicinal plant with a long history of use in traditional medicine due to its therapeutic properties. This study evaluates the antimicrobial and antioxidant potentials of aqueous extracts of *O. gratissimum* leaves. The antimicrobial activity was assessed using the agar well diffusion assay, and the antioxidant activity was determined using 2,2-Diphenyl-1-Picrylhydrazy (DPPH), nitric oxide scavenging assay (NOSA), ascorbic acid content, and total phenolic content assays. The results revealed that the *O. gratissimum* leaf extract exhibited significant antimicrobial activity against both gram-positive and gram-negative bacteria, with the highest inhibition observed against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In the antioxidant assays, the extract demonstrated moderate DPPH free radical scavenging activity, with a half-maximal inhibitory concentration (IC_{50}) of 40.21 ± 0.01 μg/mL, and high nitric oxide scavenging activity, with an IC_{50} of 47.62 ± 0.02 μg/mL. The extract also contained a notable amount of ascorbic acid (15.20 ± 2.20 mg/100 g) and phenolic compounds (0.80 ± 0.01 mg/100 g). These findings highlight the dual therapeutic potential of *O. gratissimum* in both animal and human health. The antimicrobial properties suggest its use as a natural alternative to synthetic antibiotics, especially in the management of bacterial infections in livestock. The antioxidant activity indicates the potential of the plant to mitigate oxidative stress, thereby improving overall animal health, productivity, and resistance to diseases. Moreover, the consumption of animal products derived from animals fed with antioxidant-rich plants like *O. gratissimum* could contribute to human health by reducing the risk of oxidative stress-related diseases.**

Keywords: Antimicrobial, Antioxidants, *Ocimum gratissimum*, Phytogenics

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Introduction

Ocimum gratissimum L., commonly known as scent leaf or African basil, is a widely distributed medicinal plant belonging to the Lamiaceae family. It has been extensively utilized in traditional medicine for the treatment of various ailments, including bacterial and fungal infections, respiratory diseases, and gastrointestinal disorders (Talabi and Makanjuola 2017). Scientific reports have shown that *O. gratissimum* has a wide range of bioactive compounds such as flavonoids and polyphenols (Venuprasad et al., 2014; Irondi et al., 2016) and essential oils with several beneficial effects (Benitez et al., 2009; Melo et al., 2019). Phytochemicals (oleanolic acid, caffeic acid, ellagic acid, epicatechin, sinapic acid, rosmarinic acid, chlorogenic acid, luteolin, apigenin, nepetoidin, xanthomicrol, nevadensin, salvigenin, gallic acid, catechin, quercetin, rutin, and kaempfero) and essential oils (camphene, β-caryophyllene, α- and β-pinene, α-humulene, sabinene, β-myrcene, limonene, 1,8-cineole, trans-β-ocimene, linalool, α- and δ-terpineol, eugenol, α-copaene, β-elemene, p-cymene, thymol, and carvacrol) are among the bioactive substances extracted from *O. gratissimum*. Numerous *in vitro* and *in vivo* investigations have demonstrated the pharmacological characteristics of *O. gratissimum* and its bioactive components, including antimicrobial, anti-inflammatory, anticancer, hepatoprotective, antidiabetic, antihypertensive, antidiarrheal, and antioxidant qualities (Ugbogu et al., 2021).

The rise in antibiotic resistance among pathogenic microorganisms has necessitated the search for alternative antimicrobial agents from natural sources (World Health Organization [WHO], 2020). Plant-derived antimicrobials are increasingly recognized for their potential to combat multidrug-resistant pathogens due to their diverse mechanisms of action (Angelini 2024). Similarly, oxidative stress has been implicated in the pathogenesis of several chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders. The antioxidant properties of plant extracts are attributed to their ability to scavenge free radicals and prevent oxidative damage (Maury et al., 2020). The antioxidant potentials of many plant extracts have been studied in poultry and livestock species. Akintunde et al. (2023) reported the antioxidant potentials of *Parquetina nigrescens* leaf extracts in broiler chicken production. The use of several plants in animal production have been reported and widely recommended because of their rich profiles of bioactive substances and the potential replacements of these phytogenics have been recommended (Akintunde et al., 2024; Adebisi et al., 2024). Considering the rich phytochemical composition of *O. gratissimum*, this study aims to evaluate the antimicrobial and antioxidant potentials of its aqueous leaf extracts. The findings from this research could provide insights into the therapeutic applications of the aqueous extracts of *O. gratissimum* leaves and contribute to the development of novel natural health products especially in animal production.

Materials and Methods

Plant collection and preparation

Fresh leaves of *O. gratissimum* were collected from Babcock University Campus, Ilishan-Remo, Ogun State, Nigeria. The plants were authenticated by a botanist from the Department of Basic Sciences, Babcock University, Ilishan-Remo, Ogun State, Nigeria. The plant was deposited at the herbarium unit of the Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, Kwara State with the voucher number UILH/001/1356/2021. Collected leaves were washed thoroughly under running tap water.

Extraction of plant material

The fresh leaves of *O. gratissimum* were harvested around 6:00 hrs and 6:30 hrs; thereafter, they were washed. 50 g of the fresh leaves harvested were blended with 100 mL of distilled water using a blender. The blending was done for about 3 minutes, after which the blended samples were filtered using filter papers (Whatman paper No.1). The filtrate was then analyzed for chemical compositions. This research was conducted at the Animal Science Laboratory, Babcock University, Ilishan-Remo, Ogun State, Nigeria. Ilishan-Remo is located in Nigeria's rainforest zone, with an annual rainfall of about 1,500 mm and a mean temperature of 27 °C.

Antimicrobial assay for *O. gratissimum* leaf extract

The antimicrobial activity was assessed using the agar well diffusion assay. The antimicrobial activity of the extracts was assessed against selected Gram-negative (*Escherichia coli* MTCC 585, *Klebsiella pneumoniae* MTCC 3040, *Pseudomonas aeruginosa* MTCC 424) and gram-positive (*Bacillus subtilis* MTCC 441, *Staphylococcus aureus* MTCC 3160) using the agar well diffusion method (Prescott et al., 1999; Shahidi, 2004). All bacterial cultures were plated out on Nutrient agar plates and were incubated for 24 h at 37 ± 0.5 °C and colonies from this fresh culture were used for making suspension. 10 ml of sterile nutrient broth were aseptically inoculated with test cultures and incubated at 37 ± 0.5 °C for 18 hours. Fresh inoculum of approximately 10^6 CFU / mlMcFarland Turbidity Range of tested drug resistant microorganisms was used for the study. 100 µl of the bacterial suspension was uniformly spread on sterile Muller Hinton Agar plates. After solidification of the agar, wells were made with a 6 mm sterile cork borer. The extracts were made with 99% (v/v) DMSO (Dimethyl sulfoxide) and 100 µl of the extracts were poured into separate wells. The plates were incubated for 24 hours at 37 ± 0.5 °C and antibacterial activity was observed by measuring the zone of inhibition as diameter in millimeters. Negative controls were made by DMSO alone and positive controls were made by the antibiotic streptomycin (25 µg /disc).

Antioxidant assay

DPPH (2,2-Diphenyl-1-Picrylhydrazy) radical scavenging activity: The procedure outlined by Pejin *et al.* (2013) was utilized to ascertain the impact of the crude ethanolic extract on free radicals. As 4 g of DPPH, were dissolved in 100 mL of methanol. The extract was combined with 2 mL of DPPH solution. Using a Spectrumlab 752S UV Vis Spectrophotometer at 517 nm, the decrease in the DPPH free radical was assessed after the 30 min incubation period. The half-maximal inhibitory concentration (IC_{50}) value was used to calculate the scavenging capacity of the aqueous extract. It is described as a sample concentration that causes a 50% decrease in oxidative radicals. The strength of the antioxidant scavenging activity increases with decreasing IC_{50} values. The extract's scavenging activity was evaluated using the percentage of decolorization. The sample's scavenging activity was calculated using the sample's percentage of decolorization.

Nitric oxide scavenging assay (NOSA): 0.5 mL of the extract was combined with 2 mL of sodium nitroprusside in 0.5 ml of phosphate buffer saline, and the mixture was incubated for 150 minutes at 25 °C. A volume of 0.5 mL was extracted from the incubated mixture and added to 1.0 mL of sulfanilic acid reagent. The absorbance at 540 nm was then measured after 1.0 mL of naphthylethylenediamine dihydrochloride had been combined and allowed to sit at room temperature for 10 minutes. Using the Badami et al. (2003) method, NOSA was measured in triplicate and expressed as IC_{50} (µg/ml).

Determination of ascorbic acid

A 2, 6- dichlorophenol titrimetric method was used to determine the ascorbic acid content of the sample according to the methods labeled by AOAC (2010) and Okwu (2004). Ascorbic acid standard solution was prepared by dissolving 50 mg standard ascorbic acid tablet in water. Two grams (2 g) of the resulting sample is then extracted by mixing properly in acetic acid solution and diluting to 100 ml to ensure a homogenous mix. The solution obtained was then filtered. Thereafter, 10 ml of the clear filtrate was pipetted into a 2.6 ml solution of acetone contained in a conical flask. The resulting solution was then titrated with indophenoldye solution (2, 6-dichlorophenol indophenols) until a faint pink colour appeared. The procedure was also repeated for the standard. All the analyses were done in triplicates.

Determination of phenolic content

Standard spectrophotometric methods as described by Obadoni and Ochuko (2001) were used in the determination of total phenolic content of the aqueous extracts of *O. gratissimum* leaf. The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for fifteen minutes. 5 ml of the extract was pipetted into a 50 ml flask and then 10 ml of distilled water was added. Two 2 millilitres of ammonium hydroxide solution and 5 ml of amyl alcohol were added to the sample and made up to the mark. It was left to react for 30 minutes for colour development; the absorbance was measured at 550 nm.

Statistical analysis

Data were conducted in triplicate, and results were expressed as mean ± standard deviation. Data obtained from the study were subjected to statistical analysis using appropriate Statistical Package for Social Sciences (SPSS Version 20).

Results

The antimicrobial assay results of *O. gratissimum* leaf extract against selected bacterial strains are presented in Table 1. The extract exhibited varying degrees of inhibition against both gram-positive and gram-negative bacteria, demonstrating its broad-spectrum antimicrobial potential. The highest zone of inhibition was observed against *S. aureus* (16.31 ± 0.54 mm) and *P. aeruginosa* (16.29 ± 0.06 mm), while the lowest inhibition was recorded for *E. coli* (11.03 ± 0.02 mm). Comparatively, the positive control (streptomycin) exhibited significantly larger inhibition zones against all test organisms, confirming its superior antimicrobial efficacy. The negative control (DMSO) exhibited no inhibition, indicating that the observed antimicrobial activity was solely due to the bioactive components in the plant extract.

Table 1. Antimicrobial assay for *Ocimum gratissimum* leaf extract.

Microorganisms	Zone of inhibition (Diameter in mm)		
	Sample	Streptomycin	DMSO
		Positive control	Negative control
Escherichia coli MTCC 585	11.03 ± 0.02	22.60 ± 0.01	-
Klebsiella pneumoniae MTCC 3040	14.75 ± 0.61	19.27 ± 0.13	-
Pseudomonas aeruginosa MTCC 424	16.29 ± 0.06	20.21 ± 0.03	-
Bacillus subtilis MTCC 441	12.34 ± 0.04	18.14 ± 0.02	-
Staphylococcus aureus MTCC 3160	16.31 ± 0.54	20.43 ± 0.01	-

The antioxidant activity of *O. gratissimum* (scent leaf) leaf extract was measured using various assays, and the results are presented in Table 2. These assays include DPPH activity and NOSA. The leaf extract of *O. gratissimum* contained ascorbic acid and phenolic compounds. The ascorbic acid content was found to be 15.20 ± 2.2 mg per 100 g of the sample extract, while the total phenolic content was 0.80 ± 0.01 mg per 100 g of the sample extract.

Table 2. Antioxidant assay for *Ocimum gratissimum* leaf extract.

Antioxidant assay	IC_{50} (μg/mL)
DPPH	40.21 ± 0.01
NOSA	47.62 ± 0.02

The half-maximal inhibitory concentration, IC_{50}.

Discussion

The evaluation of antimicrobial activity using plant extracts is typically performed through the agar well diffusion or disc diffusion method, where the zone of inhibition (ZOI) around the sample application area on the agar plate reflects the extent of microbial growth suppression. In this study, the antimicrobial activity of *O. gratissimum* leaf extract was assessed against five bacterial strains, and results were compared to both a positive control (streptomycin) and a negative control (DMSO). Breakpoints provide reference points for determining the efficacy of antimicrobial agents. Although standard clinical breakpoints do not exist for plant extracts, a ZOI ≥ 15 mm is typically considered indicative of strong antimicrobial activity (Oluduro 2012).

The antimicrobial efficacy of *O. gratissimum* suggests its potential as a natural alternative to conventional antibiotics in livestock production. The growing concern over antibiotic resistance in animal husbandry necessitates the exploration of phytogenic alternatives (Wang et al.,

2024). The inhibition of *E. coli* and *K. pneumoniae*, which are commonly associated with gastrointestinal infections in livestock, indicates that *O. gratissimum* could serve as a natural feed additive or therapeutic agent in managing bacterial enteritis in poultry and ruminants (Wang et al., 2024). This is in line with the observations of Koche et al. (2012) who reported the antibacterial activity of chloroform solvent of the root extract of *O. gratissimum* to be high in *E. coli*. The results were however in contrast with the conclusion of Oladele and Ologundudu (2022) that bacterial isolates Klebsiella oxytoca, *K. pneumoniae*, *E. coli*, *Proteus mirabilis*, and *Salmonella pullorum* were all resistant to the plant extract. The results from the present study further confirms the observation of Anugom and Ofongo (2019) that aqueous *O. gratissimum* leaf extract improved growth performance and reduced gut pH and *E. coli* counts.

Additionally, the significant inhibition of *P. aeruginosa* and *S. aureus-both* of which are known to cause mastitis and respiratory infections in cattle and small ruminants-implies that *O. gratissimum* could be integrated into herbal veterinary medicine to manage bacterial infections in dairy and meat-producing animals (Wilson et al., 1997; Bergonier et al., 2014; Chen et al., 2018).

The antibacterial potential of *O. gratissimum* also has important implications for human health, particularly in addressing antibiotic-resistant bacterial infections. The presence of bioactive compounds such as eugenol, thymol, and flavonoids in *Ocimum* species has been widely reported to contribute to their antimicrobial properties (Dharsono et al., 2022). The inhibition of S. aureus, a major cause of skin infections, food poisoning, and pneumonia in humans, suggests that the plant extract could be explored for the development of herbal antiseptics and functional foods to boost immune defense (Wertheim et al., 2005; Hanselman et al., 2009).

Moreover, with the rise of antimicrobial resistance (AMR) due to excessive antibiotic use in both humans and livestock, the use of *O. gratissimum* as a natural antimicrobial agent could play a role in reducing dependency on synthetic antibiotics, thereby mitigating the emergence of drug-resistant strains (Rahman et al., 2022). The results demonstrate the antimicrobial efficacy of *O. gratissimum* leaf extracts against common pathogenic bacteria, highlighting its potential applications in animal production, veterinary medicine, and human health.

The DPPH assay is widely used to assess the free radical scavenging ability of antioxidants, indicating their potential to mitigate oxidative stress. The result shows a moderate scavenging activity of *O. gratissimum* leaf extract in contrast to *Moringa oleifera* leaf extracts have shown a DPPH inhibition at 100 μg/ml, with an IC value of 53.95 μg/ml (Aziz et al., 2021). This suggests that the extract contains compounds capable of neutralizing free radicals. Antioxidants such as these can play a significant role in animal health by preventing oxidative damage to cells, tissues, and organs. Oxidative stress is known to contribute to various diseases in animals, including those affecting growth, reproduction, and immune function (Ponnampalam et al., 2022). Therefore, the incorporation of antioxidant-rich plants like *O. gratissimum* in animal diets could promote better health, productivity, and resistance to diseases. The NOSA indicates the ability of the plant extract to neutralize nitric oxide radicals, which are involved in inflammatory processes and various pathophysiological conditions in animals. Excessive nitric oxide production is associated with chronic diseases, including cardiovascular diseases and immune dysfunctions (Roy et al., 2023). The high NOSA value in *O. gratissimum* suggests that its inclusion in animal feed could help reduce inflammation and oxidative stress, thereby improving animal health and

productivity, particularly in livestock with inflammation-based conditions such as mastitis and arthritis. Vitamin C (ascorbic acid) is a potent antioxidant that is critical for immune function, collagen synthesis, and the maintenance of healthy tissues. The concentration of ascorbic acid in *O. gratissimum* leaves can contribute to enhancing the immune system of animals, particularly in poultry and livestock (Hieu et al., 2022). A diet rich in ascorbic acid has been shown to improve the resistance of animals to infectious diseases. Ascorbic acid has been found to improve poultry performance, especially in heat stress conditions. The immune system was also improved with the addition of ascorbic acid to the diet. In particular, ascorbic acid improves the responses to infection and inflammation (Hieu et al., 2022). Furthermore, animals under stress, such as those in intensive farming systems, benefit from increased vitamin C intake to support optimal growth and health. Phenolic compounds are another class of bioactive molecules with strong antioxidant properties. They are known to play a role in protecting cells from oxidative damage and reducing the risk of chronic diseases in animals. The phenolic content of *O. gratissimum* is relatively modest, but it still contributes to the overall antioxidant capacity of the leaf extract. Phenolic compounds have been reported to improve digestive health, enhance immune response, and reduce the incidence of diseases like gastrointestinal disorders in livestock (Mahfuz et al., 2021; Formato et al., 2022). Their inclusion in animal nutrition could therefore have broader implications for animal welfare and productivity.

The results of the antioxidant assays demonstrate that *O. gratissimum* leaf extract contains significant antioxidant properties, which could offer valuable health benefits for animals. Incorporating antioxidant-rich plants into animal diets can help in the management of oxidative stress, a major contributor to poor health outcomes such as reduced growth rates, immune suppression, and increased susceptibility to diseases (Ponnampalam et al., 2022; Akintunde et al., 2023, 2024a). This, in turn, could enhance productivity in animal farming by reducing mortality rates and improving feed conversion efficiency (Akintunde et al., 2024b). In a study on growth performance and carcass characteristics of broiler chickens administered oral aqueous extracts of *O. gratissimum* as replacement of synthetic antibiotics by Olumide et al. (2022), it was observed that the administration of *O. gratissimum* leaf extracts up to 0.6 ml improved growth performance of broiler chicks and they however concluded that oral administration of *O. gratissimum* leaf extracts in the production of broilers had no detrimental effect on the performance of broilers. In another study by Olumide and Akintola (2020), it was reported that the inclusion of *O. gratissimum* in the diets of broiler chicken has no detrimental effect on performance and carcass characteristics but better acceptability of the meat and improved livability as birds fed with diet containing 300 g/ 100 kg *O.gratissimum* leaf meal had the best livability of 100%.

In terms of human health, the consumption of animal products from animals fed with antioxidant-rich plants like *O. gratissimum* may provide health benefits to humans as well. Animal-derived products such as milk, meat, and eggs contain bioactive compounds that can influence human health outcomes (Kussmann et al., 2023). Antioxidant-rich products may offer protective effects against oxidative stress-related animal diseases, including cardiovascular diseases, digestive disorder and neurodegenerative disorders. The results indicate that *O. gratissimum* leaf extract has substantial antioxidant potential, with significant implications for animal production and health. By incorporating this plant into animal diets, it is possible

to improve overall health, productivity, and resistance to diseases. These benefits also extend to human health, particularly through the consumption of animal products enriched with antioxidants.

Conclusion

The antimicrobial and antioxidant activities of *O. gratissimum* leaf extracts demonstrate its promising role as a natural agent in both animal and human health. The plant's significant antibacterial properties indicate its potential as an alternative to synthetic antibiotics, which is particularly important in the context of rising antibiotic resistance in both animals and humans. The moderate antioxidant activity, coupled with high levels of ascorbic acid and phenolic compounds, suggests that *O. gratissimum* could be utilized in animal nutrition to reduce oxidative stress, promote better health, and enhance productivity in livestock. Furthermore, the consumption of animal-derived products enriched with antioxidants from plants like *O. gratissimum* could provide valuable health benefits to humans, offering protective effects against various oxidative stress-related diseases. This study underscores the importance of exploring plant-based alternatives in managing health challenges in both animals and humans and highlights the potential of *O. gratissimum* as a functional ingredient in natural health products.

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