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Antibacterial Activity of *Ocimum gratissimum* Leaf Extract Against Pathogenic Enterobacteriaceae

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Abstract

Medicinal plants have bioactive constituents that are used for treating and healing various human ailments. This study aimed to investigate the antibacterial potentials of Ocimum gratissimum leaves against some pathogenic members of Enterobacteriaceae. Fresh leaves of Ocimum gratissimum were collected from a farm in Unguwan Rimi, Kaduna State, Nigeria. The leaves were thoroughly washed, shade-dried at room temperature, and ground into a fine powder. **Ethanolic and methanolic extracts were obtained via cold maceration.** ***The phytochemical screening was carried out and the antibacterial activity was determined using agar well diffusion at different concentrations. The result of phytochemical analysis revealed the presence of phenol, tannins, saponins, cardiac glycosides and steroids for both ethanolic and methanolic extracts. The ethanolic extract of*** **Ocimum gratissimum** ***exhibited antibacterial activity against*** **Escherichia coli**, ***with a mean zone of inhibition ranging from 20.8 ± 4.71 mm to 13.8 ± 1.84 mm, while the methanolic extract showed a range of 21.6 ± 4.90 mm to 15.2 ± 1.97 mm. Against*** **Klebsiella spp.**, ***the ethanolic extract produced inhibition zones ranging from 25.5 ± 4.40 mm to 18.2 ± 1.50 mm, whereas the methanolic extract showed a more variable range from 20.5 ± 5.00 mm to 6.00 ± 0.00 mm. For*** **Pseudomonas aeruginosa**, ***the zone of inhibition for the ethanolic extract varied from 19.5 ± 2.76 mm to 14.2 ± 1.70 mm, while the methanolic extract ranged from 21.0 ± 7.00 mm to 14.5 ± 5.00 mm. The minimum inhibitory concentration (MIC) of the ethanolic extract against*** **E coli** ***was 250 mg/mL, while that of the methanolic extract was 125 mg/mL. The minimum bactericidal concentration (MBC) for both extracts was 250 mg/mL. For*** **Klebsiella spp.**, ***the MIC values were 500 mg/mL for the ethanolic extract and 250 mg/mL for the methanolic extract. Corresponding MBC values were 500 mg/mL (ethanolic) and 250 mg/mL (methanolic). In the case of*** **Pseudomonas aeruginosa**, ***the MICs were 500 mg/mL for the ethanolic extract and 250 mg/mL for the methanolic extract, and MBC followed the same pattern. The result suggests that O. gratissimum could be considered as a good antibacterial agent and can be used as an alternative cure to infections caused by these pathogens. Future studies should explore its therapeutic potential and safety profile.***

Keywords: Antibacterial activity, E coli, Klebsiellaspp, Ocimum gratissimum, P aeruginosa

INTRODUCTION

The use of herbs in traditional and complementary medicine for the treatment, management, or prevention of different types of diseases is as old as the origin of mankind (Yuan *et al.*, 2016; Ekweogu *et al.*, 2019). Globally, approximately 80% of the world's population depends mainly on medicinal plants for the treatment of various diseases (Magashi *et al.*, 2018). The increasing preference for the use of plant-based medicines over conventional medicines may be attributed to the efficacies of the bioactive compounds present in herbal medicine, which serve as healing agents and are readily available, accessible, affordable and their less or non-toxic effects (Ositadinma *et al.*, 2021).

Medicinal plants and their bioactive constituent have attracted the attention of many researchers because of their usefulness in the treatment and prevention of life-threatening and chronic diseases (WHO, 2019), such as cancer, diabetes, stroke, and arthritis (Bernell and Howard, 2016), as an alternative the treatment of mental disorders (Venuprasad *et al.*, 2014), and in meeting the health requirements of the elderly (WHO, 2019). These medicinal plants have not only been used in the treatment of many diseases, but also serve as a source of new drugs for use in traditional or orthodox medicine(Mbanaso *et al.*, 2020). Drugs such as quinine, digoxin, aspirin, and morphine were produced from medicinal plants such as *Cinchona officinalis*, *Digitalis purpurea*, *Saix alba*, and *Papaver somniferum*(Mbanaso *et al.*, 2020).

Ocimum gratissimum is an herbaceous shrub found in tropical countries, including Nigeria, where it is commonly called clove basil, sweet basil, teabush, scent leaf or fever plant.In Nigeria, *it* is popularly known by different local names(Mann, 2012). Nupe called it Tanmotsungi-wawagi, Ebira called it Ireru, in Hausa it is known as Dai doya ta gida, the Yorubas called it Efinrinajase, while the Ibos called it Nchanwu (Mann, 2012).It is a widespreadviable perennial plant with a very strong aromatic smell belonging to the family *Lamiaceae* found in different parts of the world (Ositadinma *et al.*, 2021). The leaf of *Ocimum gratissimum* is widely used as a food seasoning because of its aromatic appeal (Abubakar *et al.*, 2023). In West Africa, the leaves are also used as anti-cancer, anti-diabetic, mosquito repellent and for the treatment of malaria, convulsion, catarrh, gastrointestinal disorders, stomach pains, cold, dysentery, high fever and diarrhea (Mann, 2012; Adebolu and Oladimeji, 2020; Abubakar *et al.*, 2023). In the Southern part of Nigeria, the plant is used in the treatment of seizures, fever, and diarrhoea, while in the Northern part, decoction of the leaves is used to treat mental diseases. In North-Central Nigeria, the Ebira people of Kogi State use the leaves of *Ocimum gratissimum* to locally prepare a dish that is given to a woman after childbirth (Daniel and Nuhu, 2016). Scientific reports have shown that *O. gratissimum* contains a wide range of bioactive compounds, including tannins, saponins, alkaloids, glycosides, phenols, and flavonoids, which have therapeutic and preservative effects (Edem *et al.*, 2023).This plant possesses various pharmacological properties, which include: anti-hyperglycaemic, hypoglycaemic, anti-inflammatory, anti-diarrhoeal, anti-anaemic, hepatoprotective, anti-hypertensive, antibacterial, antifungal, and anti-oxidative effect, as well as many other pharmacological activities (Ositadinma *et al.*, 2021).

Antimicrobial resistance (AMR) is the capacity of a microbe to survive and grow when exposed to dosages of antibiotics that were once thought to be effective against it (Regassa *et al.*, 2025). On a worldwide scale, this is a growing issue. Antimicrobial resistance is currently considered a serious developing danger to global public health and food security since it has reached alarming levels in the majority of the world, both in human and in veterinary medicine (Regassa *et al.*, 2025). Multiple drug resistance has developed due to the misuse of commercial antimicrobial drugs, usually used in the treatment of infectious diseases. Antibiotics are sometimes associated with adverse side effects on the host, such as hypersensitivity, immune suppression, and allergic reactions (Tijjani *et al.*, 2018). This situation has thus engaged researchers in the search for novel antimicrobial substances (Muhammad *et al.*, 2021).

Enterobacteriaceae is a genus of gram-negative bacteria that are inhabitants of the normal gut flora. The most common pathogens are *Escherichia coli*, *Klebsiella species*, and *Enterobacter species*. Conventional antibiotics are not effective against most *Enterobacteriaceae* (Venkataraman *et al.*, 2023). There is a rapid increase in bacterial resistance to antibiotics; other options need to be explored, such as natural products that may inhibit bacterial pathogens (Xie *et al.*, 2023). The need arises to continually explore medicinal plants for possible exploitation by scientifically validating the folkloric claims.

The aim of this study was to determine the antibacterial activity of the leaves of *Ocimum gratissimum*(Scent leaf) against some *Enterobacteriaceae*.

MATERIALS AND METHODS

Sample Collection, Preparation, and Extraction

Fresh specimens of *Ocimum gratissimum*were collected from a garden in Unguwan Rimi, Kaduna State, Nigeria (latitude 10° 31' 44" N longitude 7° 27' 40"E), and subsequently identified and authenticated at the Herbarium Unit, Department of Biology, Faculty of Life Sciences, Kaduna State University, with voucher number KASU/BSH/398. The leaves were plucked, rinsed with tap water, and shade-dried at room temperature for nine days. The dried leaves were then ground into a fine powder using an electric blender, yielding 400 g of plant material as measured on a weighing balance. For extraction, 100 g of the powdered material was subjected to cold maceration by soaking in 500 mL each of ethanol and methanol for 48 hours at room temperature with intermittent shaking. The mixtures were filtered using Whatman No. 1 filter paper, and the filtrates were concentrated using a water bath at 60 °C. The concentrated extracts were stored at 4 °C until further use (Amolegbe *et al.*, 2021).

Phytochemical Screening

The methanolic and ethanolic extracts of *Ocimum gratissimum* (scent leaf) were subjected to phytochemical screening using standard procedures as described by Edeoga *et al.* (2005). For the test of tannins, 2 mL of 5% ferric chloride was added to 1 mL of the extract; the formation of a dark blue or greenish-black color indicated the presence of tannins. To detect phenols, 1 mL of extract was mixed with 2 mL of distilled water and a few drops of 10% ferric chloride; a blue or green coloration confirmed the presence of phenols. Saponins were identified by mixing 2 mL of the extract with 2 mL of distilled water and shaking vigorously in a graduated cylinder for 15 minutes; the formation of a 1 cm layer of foam indicated a positive result. The presence of steroids was confirmed by mixing 1 mL of extract with an equal volume of chloroform and adding a few drops of concentrated sulphuric acid; a brown ring at the interface signified a positive result. For cardiac glycosides, 0.5 mL of extract was treated with 2 mL of glacial acetic acid containing a few drops of ferric chloride and under-layered with 1 mL of concentrated sulphuric acid; the appearance of a brown ring at the interface indicated their presence. Alkaloids were tested by adding an equal volume of concentrated hydrochloric acid to 2 mL of the extract, followed by a few drops of Mayer's reagent; a green coloration indicated a positive result. Glycosides were detected by adding 3 mL of chloroform and 10% ammonia solution to 2 mL of the extract; the formation of a pink color confirmed their presence. For terpenoids, 0.5 mL of the extract was treated with 2 mL of chloroform and concentrated sulphuric acid; a red-brown coloration at the interface indicated a positive result. Finally, the presence of quinones was confirmed by adding 1 mL of concentrated sulphuric acid to 1 mL of the extract, which resulted in the formation of a red color.

Collection and Preparation of *Enterobacteriaceae* Isolates

Clinical isolates of *Escherichia coli*, *Klebsiella*, and *Pseudomonas aeruginosa* were collected from the Medical Laboratory of the Nigerian Army Reference Hospital, Kaduna (NARHK). The isolates were then sub-cultured on their respective selective media.*E. coli* were cultured on Eosine Methylene blue agar for 24 hours. Colonies with a green metallic sheen were observed, indicating a positive result for *E. coli*. *Klebsiella spp.* were cultured on MaConkey Agar, which ferment lactose and appear pink.*P.aeruginosa* were identified by culturing on CLED Agar for 24hrs, bluish, lactose non-fermenting colonies were observed. Discrete colonies of fresh cultures of the bacterial isolates were picked and suspended in 2 mL of nutrient broth in labeled, sterile test tubes covered with foil paper, and incubated for 24 hours at 37°C prior to antibacterial susceptibility testing (Amengialue *et al.*, 2013).

Standardization of the inoculum

A standard wire loop was drop to pick a loopful of 24-hour-old culture and suspended into a test tube containing normal saline. This tube containing the suspension of the test organism was compared against another tube containing 0.5 McFarland solution. Normal saline was added until the turbidity of the suspension matches that of McFarland as described by Ado *et al.*(2018).

Bioassay Procedure

The working samples were of four values, 500 mg/ml, 250 mg/ml, 125mg/ml and 62.5mg/ml, which were achieved by weighing 1g of the extract and dissolving in 1ml of Dimethyl Sulphoxide (DMSO) to yield 1000mg/ml stock solution and then double diluting to have other working values (Amolegbe *et al.*, 2021). The agar well diffusion method was employed for the bioassay of the different extracts (ethanolic and methanolic). A loopful of the bacterial suspension was swabbed on the surface of solidified Mueller-Hinton agar plates in duplicate. Wells were made by using a sterilized cork borer (6mm) and various concentrations of the extracts were introduced into the wells, one well containing ciprofloxacin (25mg/ml) as a positive control. The plates were incubated at 37 °C for 24 hours (Ahmed *et al.*, 2015). Activity of the plant extracts was determined by measuring the diameter of the zone of inhibition around each well in millimeters.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) of the extracts was determined using the broth dilution method, as described by Owuama (2017). Serial dilutions of the extracts were prepared at concentrations of 500 mg/mL, 250 mg/mL, 125 mg/mL, and 62.5 mg/mL in labeled test tubes, each inoculated with the test microorganisms. The tubes were incubated at 37 °C for 24 hours. The MIC was recorded as the lowest concentration of the extract that visibly inhibited microbial growth. The minimum bactericidal concentration (MBC) was subsequently determined following the MIC assay. A loopful of the content from test tubes showing no visible growth was sub-cultured onto Mueller-Hinton agar plates and incubated at 37 °C for 24 hours. The MBC was defined as the lowest concentration of the extract that prevented any visible bacterial growth on the agar surface, indicating bactericidal activity (Owuama, 2017).

RESULTS

Phytochemical Constituents of *Ocimum gratissimum* Leaf Extract

The phytochemical screening of both ethanolic and methanolic leaf extract of *Ocimum gratissimum* reveals the presence of cardiac glycosides, saponins, tannins, phenols and steroids, while alkaloids, quinones and terpenoids were not detected as shown in Table 1.

Table 1: Phytochemical Constituents of *Ocimum gratissimum* Leaf Extract

Phytochemical Test	Ethanolic Extract	Methanolic Extract
Alkaloids	-	-
Cardiac glycosides	+	+
Saponins	+	+
Quinones	-	-
Tannins	+	+
Terpenoids	-	-
Phenols	+	+
Steroids	+	+

Key: + = Detected, - = Not detected

The ethanolic extract of *Ocimum gratissimum* exhibited antibacterial activity against *E. coli*, with a mean zone of inhibition ranging from 20.8 ± 4.71 mm to 13.8 ± 1.84 mm, while the methanolic extract showed a range of 21.6 ± 4.90 mm to 15.2 ± 1.97 mm. Against *Klebsiella* spp., the ethanolic extract produced inhibition zones ranging from 25.5 ± 4.40 mm to 18.2 ± 1.50 mm, whereas the methanolic extract showed a more variable range from 20.5 ± 5.00 mm to 6.00 ± 0.00 mm. For *P. aeruginosa*, the zone of inhibition for the ethanolic extract varied from 19.5 ± 2.76 mm to 14.2 ± 1.70 mm, while the methanolic extract ranged from 21.0 ± 7.00 mm to 14.5 ± 5.00 mm as shown in Table 2.

In Table 3, the MIC and MBC values are displayed for the ethanolic and methanolic leaf extracts, indicating their respective antibacterial potencies. For the ethanolic extract, *Escherichia coli* exhibited the lowest MIC and MBC values (250 mg/mL), whereas both *Klebsiella* spp. and *Pseudomonas aeruginosa* showed the highest MIC and MBC values (500 mg/mL). In the case of the methanolic extract, *E. coli* recorded the lowest MIC (125 mg/mL), while *Klebsiella* spp. and *P. aeruginosa* had higher MICs (250 mg/mL). The MBC values for all three bacterial isolates using the methanolic extract were consistent at 250 mg/mL.

DISCUSSION

The methanolic and ethanolic extract of *Ocimum gratissimum* leaves reveals the presence of steroids, tannins, saponins, phenols, and cardiac glycosides in both extracts. This is in line with the findings of Ladipo *et al*. (2010), which show that *O. gratissimum* contains similar phytochemicals, while Ajose and Okozi (2017) reported the presence of tannins and saponins only. The absence of alkaloids, quinones, and terpenoids in the study of Ajose and Okozi (2017). The difference in phytochemical constituents of the leaf extract of *O. gratissimum* could be due to the extraction method used to obtain the extract, the planting location, seasonal and environmental variations. According to Ajose and Okozi (2017), the presence of these bioactive compounds in the leaf extract accounts for its use as a medicinal plant. *Ocimum gratissimum* is recognized for its diverse applications in traditional medicine. The plant is known to be used in the treatment of gastrointestinal diseases (diarrhoea, dysentery), skin infections (dermatitis, eczema, scabies), upper respiratory tract infections associated with cough, asthma, and bronchitis, wounds and sores, insect bites, nosebleeds, stroke, and anaemia (Mann, 2012). Bioactive compounds give plants their color, flavor, and smell, and serve as the natural defense system of the plant, protecting it against insects, vertebrates, fungi, pathogens, and parasites (Magashi *et* al. 2018). Phenols found in the plant extract serve as antioxidants, which are molecules that can delay or prevent free radical-catalyzed oxidative reactions. Tannins are known to have the ability to inhibit microbial growth by precipitating and inactivating microbial adhesion enzymes and cell envelope proteins (Mann, 2012).

Table 2:Antibacterial Activity of Ethanolic and Methanolic Extract of *Ocimum gratissimum* Leaves against *E.coli, Klebsiella and P.aeroginosa*.

Organism	500	250	125	62.5	Control (25 mg/ml)	P-value
	Ethanolic Extract					
E. coli	20.8 ± 4.71	18.2 ± 2.24	17.6 ± 2.80	13.8 ± 1.84	36.0	0.078
Klebsiella spp.	25.5 ± 4.40	21.2 ± 3.87	19.3 ± 3.06	18.2 ± 1.50	51.0	0.146
P. aeruginosa	19.5 ± 2.76	17.4 ± 1.30	16.5 ± 1.62	14.2 ± 1.70	41.0	0.063
	Methanolic Extract					
E. coli	21.6 ± 4.90	19.9 ± 3.41	18.5 ± 2.46	15.2 ± 1.97	36.0	0.172
Klebsiella spp.	20.5 ± 5.00	18.2 ± 1.50	16.2 ± 1.68	6.0 ± 0.00	51.0	0.028
P. aeruginosa	21.0 ± 7.00	18.5 ± 2.45	16.0 ± 2.00	14.5 ± 5.00	41.0	0.107

Values with superscript are statistically significance at P-value (≤0.05).

Table3:Minimum Inhibitory Concentration (mg/ml) and Minimum Bactericidal Concentration (mg/ml)

Organism	Ethanolic Extract		Methanolic Extract	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
E. coli	250	250	125	250
Klebsiella spp.	500	500	250	250
P. aeruginosa	500	500	250	250

The inhibitory effects of spices are attributed to the volatile oils they contain. Antibacterial properties of the leaves of *Ocimum gratissimum* were studied. The data obtained from this study reveal that the ethanolic and methanolic extracts of the leaf of *Ocimum gratissimum* possess antibacterial activity against the three Enterobacteriaceae, *E. coli, P. Aeruginosa, and Klebsiella spp.* These established a solid foundation for the use of this plant in ethnomedicine and a basis for the development of novel drugs (Ajose and Okozi 2017). This is in line with the report by Muhammad et al. (2021), which has shown that Ocimum gratissimum extract exhibits antibacterial activity against E. coli, Klebsiella, and Pseudomonas aer*uginosa*. Muhammad *et al.* (2021) reported that the extract of *O. gratissimum* has antibacterial activity against *P. Aeruginosa*. Ladipo *et al.* (2010) reported that the methanolic extract of *Ocimum gratissimum* leaves has antibacterial activity against *Klebsiella*. Work of Amolegbe *et al.* (2021) revealed that both methanolic and ethanolic extracts of *O. gratissimum* has antibacterial activity against *E.coli*, which agreed with the findings of Ishiwu *et al. (*2014). It is also similar to the findings of Regassa *et al.*(2025) who stated that both *E. coli* and *P. aeruginosa* were sensitive the both the ethanolic and methanolic extract of the plant. The result of this finding contrasts with the finding of Oladele and Ologundudu (2022), who reported that *E. coli*, *Klebsiella,* and *Pseudomonas* were resistant to all the plant extracts (methanolic, ethanolic, and aqueous). It was observed that the antibacterial activity decreases with a decrease in the concentration of the extracts. The zone of inhibition decreases with a decrease in the concentration of the extracts. This is similar to the findings of Ishiwu *et al.* (2014), Hamma *et al.* (2020), and Lawrence and Olusola-Makinde (2023). According to Nsor *et al.* (2012), the antibacterial activity of the plant extracts is due to the presence of one bioactive constituent or the interaction of two or more of the bioactive constituents against the bacterial isolates. This also justifies the basis for the traditional use of the plant in the treatment of different bacterial infections, such as diarrhoea and dysentery.

The MBC result from the extract was shown to be higher than the MIC value. That is, the extracts inhibit (bacteriostatic) the test isolates at lower concentrations and kill (bactericidal) at higher concentrations. This can be due to the fact that the extracts are in their crude form and may contain a small amount of bioactive compounds. This agrees with Aliyu *et al*. (2019), who stated that MBC values are higher than MIC values for crude extracts against pathogens.

CONCLUSIONS

The study demonstrated that the ethanolic and methanolic extracts of *Ocimum gratissimum* leaves are rich in tannins, saponins, steroids, phenols, and cardiac glycosides, but do not contain alkaloids, quinones, or terpenoids. All three bacterial isolates tested responded to both types of extracts, and their antibacterial effects were found to increase with concentration, as reflected in the MIC and MBC results.

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