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ORIGINAL ARTICLE

Antibacterial Activity of *Ocimum gratissimum* (Scent Leaf) Extracts on Selected Bacteria Isolates

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ABSTRACT

Background: *Ocimum gratissimum* is a plant rich in bioactive compounds believed to possess antimicrobial, anti-inflammatory, analgesic, and antioxidant effects. This study was carried out to determine the antibacterial activity of *Ocimum gratissimum* extracts on selected bacteria isolates.

Methods: This was a prospective clinical and laboratory study in which bacteria swabs were collected from patients who presented with bacterial conjunctivitis at the Eye Clinic, Department of Optometry, Federal University of Technology, Owerri, Nigeria and transported to the Microbiology Laboratory at Federal University Teaching Hospital, Owerri for culturing and isolation. The zones of inhibition of ethanolic and aqueous extracts of *Ocimum gratissimum* on three bacteria isolates from the swab samples was tested for antibacterial activity.

Results: The mean zones of inhibition for 100 mg/ml ethanolic extract of *Ocimum gratissimum* was 18.96 ± 0.20 mm *on S. aureus*, 16.35 ± 0.28 mm on *E. coli*, and 17.80 ± 0.37 mm on *K. pneumonia*. For 100 mg/ml aqueous extract of *Ocimum gratissimum*, the mean zones of inhibition was 5.17 ± 0.17 *on S. aureus*, 3.82 ± 0.21 mm on *E. coli*, and 3.80 ± 0.20 mm on *K. pneumonia*. There was a significant antibacterial effect ($p < 0.001$) of both the ethanolic and aqueous extracts of *Ocimum gratissimum* on the selected bacteria isolates.

Conclusion: Both ethanolic and aqueous extracts of *Ocimum gratissimum* showed antibacterial activity on the selected bacteria isolates. These findings support the potential of *Ocimum gratissimum* as alternative therapeutic agent against drug-resistant bacteria.

Keywords: Bacteria, Zone of Inhibition, *Ocimum gratissimum*, Ethanolic extract, Aqueous extract, Ciprofloxacin

INTRODUCTION

The field of antibacterial research is of utmost importance in the fight against infectious diseases. With the rise of antibiotic resistance, there is a growing need to explore alternative sources of antibacterial agents. One such source is plant extracts, which have been used in traditional medicine for centuries.[1] Plants have been found to be a major source of medicine and humans have relied on them over the years. In present days, most people in developing countries continue to rely on plants as a primary form of medicine. This is due to antimicrobial resistance of microorganisms which has become a major global problem and a threat to the successful treatment of infectious diseases.[2] Plants have proven to be a potential solution to this problem. They are fortified with defense mechanisms which they use to fight against pathogenic and harmful microbes and prevent them from causing serious damage.[2] A few antimicrobial drugs have been obtained from numerous plant species so far, and some of these include Quinine (antimalarial) from Cinchona ledgerian, the antimalarial compound Artemisin, derived from Artemisia annua, ginseng which has been observed to have antibacterial activity against *Escherichae coli* and *Staphylococcus*.[3] Despite these big discoveries and developments, resistance to antimicrobials by microorganisms, remain on the increase. This can be attributed to the unnecessary prescription of these drugs incessantly, inappropriate use and inadequate dosing, poor patient compliance as well as genetic plasticity of microorganisms.[4]

Scent leaf, scientifically known as *Ocimum gratissimum*, is a plant that belongs to the Lamiaceae family. It is commonly found in tropical regions of Africa and Asia, and it is known by various names such as clove basil,

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African basil, and effirin in Nigeria. Scent leaf has a distinctive aroma and is widely used as a culinary herb and for medicinal purposes.[5] In traditional medicine, scent leaf has been used for centuries due to its various medicinal properties. It is believed to possess antimicrobial, anti-inflammatory, analgesic, and antioxidant effects. The leaves of the plant are rich in bioactive compounds such as phenols, flavonoids, alkaloids, and essential oils, which contribute to its therapeutic potential.[6] The antimicrobial activity of scent leaf has been a subject of interest in scientific research. Several studies[1,7,8] have investigated its efficacy against various bacterial strains. For instance, research has shown that scent leaf extracts exhibit antibacterial activity against pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa*.[8] These findings suggest that scent leaf may have the potential to be used as a natural alternative to conventional antibiotics. Moreover, scent leaf extracts have been studied for their antifungal properties. They have demonstrated inhibitory effects against fungi like *Candida albicans*, which is responsible for causing oral thrush and other fungal infections.[9] This antifungal activity further highlights the therapeutic potential of scent leaf in combating microbial infections. Apart from its antimicrobial properties, scent leaf has also been investigated for its antioxidant and anti-inflammatory effects. The presence of bioactive compounds in the plant contributes to its antioxidant activity, which helps neutralize harmful free radicals in the body. Additionally, scent leaf extracts have exhibited anti-inflammatory activity in various experimental models[4,10], suggesting its potential use in managing inflammatory conditions.

Antibiotics are chemical substances obtained from various species of microorganisms that suppress the growth of other microorganisms and eventually may destroy them. The probable points of difference amongst the antibiotics may be physical, chemical, pharmacological properties, antibacterial spectra, and mechanism of action. They have made it possible to cure diseases caused by bacteria, such as pneumonia, tuberculosis, and meningitis, and they save the lives of millions of people around the world. Antibiotics are important to treat infections and have saved countless lives. However, anytime antibiotics are used, they can cause side effects and contribute to antibiotic resistance, one of the most urgent threats to public health. Antibiotics are used to treat or prevent some types of bacterial infection. They work by killing bacteria or preventing them from reproducing and spreading.[11] Ciprofloxacin is an antibiotic agent in the fluoroquinolone class used to treat bacterial infections. It exerts its effect by binding to and inhibiting bacterial DNA-gyrase. This enzyme produces supercoiling of cellular DNA which is needed for bacterial DNA synthesis.[12] It has an in vitro activity against many gram-negative and gram-positive organisms. Ciprofloxacin is indicated for treatment of several bacterial infections, including bacterial bronchitis, pneumonia, sinusitis, urinary tract infections, septicemia, joint and bone infections, soft tissue and skin infections, typhoid fever, anthrax, bacterial gastroenteritis, urethral and gynecological infections, bacterial conjunctivitis, pelvic inflammatory disease and several other infectious conditions.[13]

The emergence of resistant bacterial strains against the commonly prescribed antibiotics in hospitals is a worldwide problem. Most ocular bacterial infections are primarily treated with broad spectrum antibiotics. However, widespread and misuse of these antibiotics for bacterial and viral infections or prophylactics has resulted in emerging global increase of antibiotic resistance.[14] Antibiotic resistance for ocular bacteria has also been observed to be caused by factors such as empirical prescribing of antibiotics, short-term exposure to antibiotics, and repeated exposure to the same antibiotic identified as contributing to resistance of ocular pathogens, as well as leading to changes in resident ocular flora.[15] Most often, people stick to self-medication and empirical treatments with broad spectrum antibiotics and these contribute to antibiotic resistance. Diagnosis of most external ocular infections are given without proper laboratory culture and confirmation. In this case, these infections are treated empirically, with a failure to take into account the possibility of multidrug resistant bacteria, such as Methicillin resistant *Staphlyococcus aureus* being a problem.[16] New and emerging antibiotics are being developed to help with this antibiotic resistance, but are not very affordable for people in developing and under developed countries. To counteract the rise of antibiotic resistance, plants, as herbal medicines, could represent a potential solution. Herbal medicines, although more nutritionally safe, may vary in their efficacy, function and toxicology and safety. About 60% of the globe populations entirely rely on traditional medicinal plants and their extracts for different healthcare requirements.[17] Silva and Don[18] evaluated the antibacterial and antibiotic-enhancing effects of the essential oil obtained from *Ocimum gratissimum* and reported antibacterial activity against *E. coli* and *S. aureus*. Another study[19] also reported antibacterial activity of the essential oil extract of *Ocimum gratissimum* on selected bacteria. Hence, it is paramount to investigate this traditional belief that some plants and herbs treat various infections and therefore, may offer a plethora of interesting possibilities to combat drug resistance. This study aims to determine the antibacterial activity of *Ocimum gratissimum* extracts on selected bacteria isolates.

METHODOLOGY

This was a prospective clinical and laboratory study carried out at Federal University Teaching Hospital, Owerri, Imo State, Nigeria, from September 20, 2023, to March 26, 2024.

Collection of Plant Material

The fresh scent leaves were obtained from the local market at Owerri and were identified and authenticated by a botanist at the Department of Crop Science and Technology, Federal University of Technology, Owerri. The leaves were then taken to the Microbiology Laboratory, Federal University Teaching Hospital, Owerri for processing. The leaves were washed thoroughly with distilled water and dried. The leaves were then ground into fine powder under laboratory conditions using a sterile electric blender, weighed and stored in an air tight container and was then stored in dry plastic container until time of extraction.[20]

Ethanolic Extraction of *Ocimum gratissimum*

The ethanolic extraction of *Ocimum gratissimum* leaf was done using the soxhlet extraction process as described by Fairbrother[21]. Three hundred milliliters (300 ml) of ethanol was poured into a Soxhlet flask, and 100 grams of the scent leaf was placed into the extractor, and the reflux arm plugged with cotton wool to avoid flow of particles. The Soxhlet apparatus was mounted on a heating mantle set at the boiling point of the solvent (78°C). When the solvent was boiling, the vapor evaporated through the extractor arm into the extraction chamber, while the condenser at the top condensed the vapor. The liquid condensate dripped into the center which contained the *Ocimum gratissimum* leaf sample to be extracted. The extract seeped through the reflux arm and filled the siphon tube, where it flowed back down into the soxhlet flask. This was allowed to continue to circulate until the extraction was completed. It was then removed from the tube, and then the ethanol was evaporated to remain only the *Ocimum gratissimum* extract in the soxhlet flask.

Aqueous Extraction of *Ocimum gratissimum*

The aqueous extraction of *Ocimum gratissimum* was done using the cold maceration process as described by Hidayat and Wulandari.[22] Thirty grams (30 grams) of *Ocimum gratissimum* sample was weighed into a sterile 250 ml conical flask. One hundred milliliters (100 ml) of distilled water was poured into the scent leaf, corked, and allowed to soak for 24 hours. The mixture was filtered after 24 hours using sterile cheese filter cloth and the *Ocimum gratissimum* extract was then evaporated using hot air oven or rotary vacuum evaporator at 60°C to remain only the extract.

Collection and Transportation of Bacteria Swabs

Bacteria swabs were collected from patients who presented with signs and symptoms of bacterial conjunctivitis at the Eye Clinic, Department of Optometry, Federal University of Technology, Owerri, Nigeria. The patients signed an Informed Consent Form to participate in the study. Ethical approval for the study was obtained from the Ethics Committee of the School of Health Technology, Federal University of Technology, Owerri, Nigeria, with reference number FUT/SOHT//REC/vol 3 dated September 8, 2023. The Amies transport medium was used to transport swab samples to the Microbiology Laboratory at Federal University Teaching Hospital, Owerri for culturing and identification of microorganisms. [23] The systematic sampling technique was used to select the patients for collection of bacteria swabs. In this technique, starting with the first case with signs and symptoms of bacterial conjunctivitis at the start of the study, every fourth case was chosen, skipping three cases within the study group. This process continued for 6 months, which was the duration of the study, and provided a sample size of 23 subjects and 23 bacterial swabs.

Identification of Bacteria Isolates

The media preparation was done according to the manufacturer's specification[21] prior to collection of swabs. The swab samples underwent inoculation on Blood agar, Mac-Conkey agar, and Chocolate agar plates to isolate and grow individual bacterial colonies from the mixed microbial population. The bottom of the agar plate was labelled with relevant information, including sample identification number, specimen source, and date. Starting at one edge of the agar plate, the swab was streaked over the surface of the agar in a zigzag motion while rotating the plate slightly. After completing the first streak, without touching the initial streak, a second quadrant of the plate was streaked by spreading the bacteria from the first streak into the second quadrant. The streaking process was repeated for the third and fourth quadrants. The agar plate lid was closed immediately after streaking to prevent contamination. The inoculated agar plate was then placed in an incubator for 24 hours. Bacterial isolates were identified based on standard biochemical characteristics, employing both microscopic and macroscopic analy-ses.[24] Gram staining and motility tests were conducted. For gram-negative identification, biochemical tests such as indole, citrate, oxidase, catalase, H_2S production, lysine decarboxylase, lactose fermentation, urea hydrolysis, and gas production were employed. Gram-positive bacteria were identified using catalase, coagulase tests, and observing haemolysis patterns on blood agar. The sterility of culture media was verified by incubating 3–

5% of the batch at 37°C overnight and observing for bacterial growth. The media that produced growth were examined for color, shape, elevation and pattern of growth.

Test for Antibacterial Activity of Extracts

The antibacterial efficacy of the extracts was done using the disc diffusion method.[25] The bacteria isolates were streaked on Mueller Hinton agar. A stock solution of extract was prepared by dissolving 10 grams of the extract in 100 ml of their respective solvents (distilled water and ethanol) to produce a concentration of 100 mg/ml. The stock solution was then prepared at concentrations of 50 mg/ml, 25 mg/ml and 12.5 mg/ml by dissolving 10 grams of the extract in 200 ml, 400 ml, and 800 ml of the solvent respectively. Sterile paper disc were impregnated with *Ocimum gratissimum* extracts (ethanol and aqueous) at varying concentrations and placed onto the agar plates inoculated with bacterial cultures using sterile forceps. The set up was incubated aerobically at 37°C for 24 hours. The zone of inhibition diameters was measured using meter rule after 24 hours incubation and the results recorded in millimeters (mm).

Antibiotic Sensitivity Testing

Antibiotic sensitivity testing was carried out with Ciprofloxacin which served as control in this study. Ciprofloxacin 500 mg tablets were bought from a reputable pharmacy in Owerri. It was diluted in distilled water to prepare different concentrations. The 500 mg was dissolved in 5 ml distilled water to produce 100 mg/ml. To produce other concentrations of 50 mg/ml, 25 mg/ml and 12.5 mg/ml, the 500 mg was dissolved in 10 ml, 20 ml and 40 ml of distilled water respectively. Antibiotic sensitivity testing was done using the disc diffusion method.[25] By plating out, the test organism was seeded on Mueller Hinton agar. Sterile paper discs were impregnated with the different concentrations of ciprofloxacin. The antibiotic sensitivity disc was then placed onto agar plates inoculated with bacterial cultures with sterile forceps. The set-up was incubated aerobically at 37°C for 24 hours. The zone of inhibition diameters (the clear region around an anti-microbial agent on the agar surface) were measured using meter rule after 24 hours incubation and recorded in millimeters (mm).

Statistical Analysis

Data collected from this study were uploaded onto the Statistical Package for Social Sciences (SPSS) version 23 software for analysis. The antibacterial activity of *Ocimum gratissimum* extracts was tested using the one sample T test at 0.05 level of significance. The Analysis of Variance (ANOVA) was used to compare the antibacterial activities of *Ocimum gratissimum* extracts and Ciprofloxacin at 0.05 level of significance.

RESULTS

The distribution of the mean zones of inhibition of *Ocimum gratissimum* on *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* is shown in table 1. Ciprofloxacin served as a control and the mean zones of inhibition on the bacteria isolates is also shown in the table. Out of 23 swab samples taken to the laboratory for microbial analysis, *S. aureus* was isolated in all 23 samples, *E. coli* was isolated in 17 samples, and *K. pneumoniae* was isolated in 5 samples. The ethanolic extract of *Ocimum gratissimum* produced zones of inhibition with all the bacteria isolated at 100 mg/ml, 50 mg/ml and 25 mg/ml concentrations. For *S. aureus*, there was a mean (± standard error mean) zone of inhibition was 18.96± 0.20 mm with 100 mg/ml concentration, 11.52 ± 0.31 mm with 50 mg/ml, and 3.39 ± 0.27 mm with 25 mg/ml. There was no zone of inhibition with 12.5 mg/ml. For *E. coli*, the mean zone of inhibition was 16.35 ± 0.28 mm with 100 mg/ml concentration, 8.12 ± 0.19 mm with 50 mg/ml, and 0.76 ± 0.25 mm with 25 mg/ml. There were no zones of inhibition with 12.5 mg/ml. For *K. pneumoniae*, the mean zone of inhibition was 17.80 ± 0.37 mm with 100 mg/ml concentration, 8.40 ± 0.40 mm with 50 mg/ml, and 0.60 ± 0.24 mm with 25 mg/ml. There were no zones of inhibition with 12.5 mg/ml. Data analysis using the one sample T-test showed a significant antibacterial activity [p(0.00)<0.05] of the ethanolic extract of *Ocimum gratissimum* on the selected bacteria isolates. The aqueous extract of *Ocimum gratissimum* also produced zones of inhibition especially at a higher concentration of 100 mg/ml with all the bacteria isolated. They were however lower in diameter when compared to the ethanolic extract of *Ocimum gratissimum*. For *S. aureus*, there was a mean zone of inhibition of 5.17 ± 0.17 mm with 100 mg/ml concentration and 0.78 ± 0.17 mm with 50 mg/ml. There was no zone of inhibition with 25mg/ml and 12.5 mg/ml. For *E. coli*, the mean zone of inhibition was 3.82 ± 0.21 mm for 100 mg/ml. There were no zones of inhibition with 50 mg/ml, 25 mg/ml and 12.5 mg/ml concentrations. For *K. pneumoniae*, the mean zone of inhibition was 3.80 ± 0.20 mm with 100 mg/ml. There were no zones of inhibition with 50 mg/ml, 25 mg/ml and 12.5 mg/ml. Data analysis also showed antibacterial activity [p(0.00)<0.05] of the aqueous extract of *Ocimum gratissimum* on the selected bacteria isolates. With Ciprofloxacin which served as a control, the mean zone of inhibition for *S. aureus* was 24.09 ± 0.29 mm with 100 mg/ml concentration, 17.17 ± 0.20 mm with 50 mg/ml, 9.17 ± 0.20 mm with 25 mg/ml and 1.43 ± 0.20 mm with 12.5 mg/ml. For *E. coli*, the mean zone of inhibition was 24.12 ± 0.21 mm with 100

mg/ml, 17.53 ± 0.23 mm with 50 mg/ml, 9.24 ± 0.20 mm with 25 mg/ml, and 1.24 ± 0.24 mm with 12.5 mg/ml. For *K. pneumoniae*, the mean zone of inhibition was 22.60 ± 0.40 mm with 100 mg/ml, 16.00 ± 0.32 mm with 50 mg/ml, 8.40 ± 0.24 mm with 25 mg/ml, and 3.80 ± 0.37 mm with 12.5 mg/ml. Table 2 showed results of statistical comparison of the zones of inhibition of *Ocimum gratissimum* ethanolic and aqueous extracts, and Ciprofloxacin on the selected bacterial isolates using the Analysis of Variance (ANOVA). At 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml concentrations, there were significant differences [p(0.00)<0.05] in the zones of inhibition between Ciprofloxacin and both *Ocimum gratissimum* ethanolic and aqueous extracts on most of the bacterial isolates. With 12.5 mg/ml concentration, *Ocimum gratissimum* ethanolic and aqueous extracts showed no difference [p(1.00)>0.05] in the zones of inhibition across all bacterial isolates. For the bacterium *K. pneumoniae*, the ethanolic and aqueous extracts of *Ocimum gratissimum* ethanolic and aqueous extracts also showed no difference [p(1.00)>0.05] in the zones of inhibition with 25 mg/ml concentration.

DISCUSSION

Although well known for its nutritive value, *Ocimum gratissimum* (scent leaf) has been found to have the potential to exhibit microbial growth.[16] This study observed that the ethanolic and aqueous extracts of *Ocimum gratissimum* have some antibacterial activity, though not as much as ciprofloxacin, the conventional drug for treating bacterial infections.[2] Ciprofloxacin showed significant inhibition of bacterial growth, with higher concentrations exhibiting greater effectiveness. In contrast, the ethanol extract of *Ocimum gratissimum* demonstrated varying degrees of inhibition, with higher concentrations showing more pronounced effects. However, the aqueous extract of *Ocimum gratissimum* inhibited bacterial growth mostly with 100 mg/ml concentrations. There were no antibacterial effect with the lower concentrations of 25 mg/ml and 12.5 mg/ml. Overall, Ciprofloxacin displayed stronger anti-bacterial activity against *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae*, when compared to both the ethanol and aqueous extracts of *Ocimum gratissimum*. Ciprofloxacin kills the bacteria by disrupting their ability to create and repair their DNA.[11] On the other hand, *Ocimum gratissimum* contain various phytochemicals such as flavonoids, terpenoids, and phenolics, which may exert their antibacterial effects through different mechanisms, including disruption of bacterial cell membranes or inhibition of vital enzymes.[26] The antibacterial activity of *Ocimum gratissimum* is also believed to be due to the presence of eugenol, a phytoconstituent of *Ocimum gratissimum*.[8] In another study[1], the antibacterial activity of *Ocimum gratissimum* was attributed to one or more of its phytoconstituents which included alkaloid, anthraquinone, flavonoid, glycoside, phenol, saponin, steroid and tannins. The observed variations in the antibacterial activity of *Ocimum gratissimum* extract between the ethanol and aqueous extracts highlight the importance of extraction methods in determining the bioactivity of plant-derived compounds. In this study, the ethanolic extract presented more antibacterial activity than the aqueous extract. This increased antibacterial activity in the ethanol extract can be attributed to the bactericidal property of ethanol, highlighting the importance of the solvent choice in the extraction process. Other studies[10,27,28] also reported greater antibacterial effect of *Ocimum gratissimum* with the ethanol extract. The minimum inhibitory concentration of the ethanolic extract of *Ocimum gratissimum* in this study was 25 mg/ml in all the bacteria isolated. For the aqueous extract, the minimum inhibitory concentration was 50 mg/ml for *S. aureus*, and 100 mg/ml for *K. pneumoniae* and *E. coli*. Ciprofloxacin however inhibited bacterial growth at all the concentrations in this study. In comparison to another study[29], the minimum inhibitory concentration of the ethanolic extract of *Ocimum gratissimum* ranged from 100 mg/ml in *S. aureus* to 25 mg/ml in *Bacillus cereus*. For the aqueous extract, it ranged from 200 mg/ml in *S. aureus* to 100 mg/ml for *P. aeruginosa*.

Staphylococcus aureus, Escherichia coli, and *Klebsiella pneumonia* represent clinically significant pathogens with varying degrees of resistance to antibiotics. The ability of Ciprofloxacin to exhibit potent activity against these bacterial strains suggests its continued relevance in clinical practice, especially in the management of infections caused by multidrug-resistant pathogens. However, the antibacterial activity of *Ocimum gratissimum* against clinically relevant bacterial isolates provides valuable insights into the potential therapeutic efficacy of natural plant extracts as alternative treatments for antibiotic-resistant infections. This study contributes to the growing body of evidence supporting the therapeutic potential of *Ocimum gratissimum* in the management of bacterial infections. By leveraging the bioactive properties of natural plant extracts, such as *Ocimum gratissimum*, novel antimicrobial agents can be developed to address the global challenge of antibiotic resistance. A study by Ugueri *et al.*[7] offers valuable insights into the antibacterial properties of *Ocimum gratissimum* and *Vernonia Amygdalina* extracts, providing further support for the potential of these natural remedies as alternative therapeutic agents against drug-resistant bacteria. Further research is warranted to elucidate the specific bioactive constituents responsible for the antibacterial

Table 1: Distribution of Mean Zones of Inhibition (mm) of *Ocimum gratissimum* and Ciprofloxacin on identified bacteria isolates

Bacteria	***Ocimum gratissimum* Ethanolic extract**							
	100 mg/ml		**50 mg/ml**		**25 mg/ml**		**12.5 mg/ml**	
	N	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**
Staphylococcus aureus	23	18.96 ± 0.20	23	11.52 ± 0.31	23	3.39 ± 0.27	23	0.00±0.00
Escherichia coli	17	16.35 ± 0.28	17	8.12 ± 0.19	17	0.76 ± 0.25	17	0.00±0.00
Klebsiella pneumonia	5	17.80 ± 0.37	5	8.40 ± 0.40	5	0.60 ± 0.24	5	0.00±0.00
	***Ocimum gratissimum* Aqueous extract**							
	100 mg/ml		**50 mg/ml**		**25 mg/ml**		**12.5 mg/ml**	
	N	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**
Staphylococcus aureus	23	5.17 ± 0.17	23	0.78 ± 0.17	23	0.00±0.00	23	0.00±0.00
Escherichia coli	17	3.82 ± 0.21	17	0.00±0.00	17	0.00±0.00	17	0.00±0.00
Klebsiella pneumoniae	5	3.80 ± 0.20	5	0.00±0.00	5	0.00±0.00	5	0.00±0.00
	Ciprofloxacin (Control)							
	100 mg/ml		**50 mg/ml**		**25 mg/ml**		**12.5 mg/ml**	
	N	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**
Staphylococcus aureus	23	24.09 ± 0.29	23	17.17 ± 0.20	23	9.17 ± 0.20	23	1.43 ± 0.20
Escherichia coli	17	24.12 ± 0.21	17	17.53 ± 0.23	17	9.24 ± 0.20	17	1.24 ± 0.24
Klebsiella pneumonia	5	22.60 ± 0.40	5	16.00 ± 0.32	5	8.40 ± 0.24	5	3.80 ± 0.37

SE = Standard Error of Mean

Table 2: Statistical comparison of mean zones of inhibition of Ciprofloxacin, and *Ocimum gratissimum* Ethanol and Aqueous extracts on identified bacteria isolates

Bacteria	Variable	Variable	Mean Difference	SE	P value
S. aureus	Ciprofloxacin (100 mg/ml)	OC Ethanol Extract (100 mg/ml)	5.13	0.32	0.00
		OC Aqueous Extract (100 mg/ml)	18.91	0.32	0.00
	OC Ethanol Extract (100 mg/ml)	Ciprofloxacin (100 mg/ml)	-5.13	0.32	0.00
		OC Aqueous Extract (100 mg/ml)	13.78	0.32	0.00
	OC Aqueous Extract (100 mg/ml)	Ciprofloxacin (100 mg/ml)	-18.91	0.32	0.00
		OC Ethanol Extract (100 mg/ml)	-13.78	0.32	0.00
	Ciprofloxacin (50 mg/ml)	OC Ethanol Extract (50 mg/ml)	5.65	0.33	0.00
		OC Aqueous Extract (50 mg/ml)	16.39	0.33	0.00
	OC Ethanol Extract (50 mg/ml)	Ciprofloxacin (50 mg/ml)	-5.65	0.33	0.00
		OC Aqueous Extract (50 mg/ml)	10.74	0.33	0.00
	OC Aqueous Extract (50 mg/ml)	Ciprofloxacin (50 mg/ml)	-16.39	0.33	0.00
		OC Ethanol Extract (50 mg/ml)	-10.74	0.33	0.00
	Ciprofloxacin (25 mg/ml)	OC Ethanol Extract (25 mg/ml)	5.79	0.27	0.00
		OC Aqueous Extract (25 mg/ml)	9.17	0.27	0.00
	OC Ethanol Extract (25 mg/ml)	Ciprofloxacin (25 mg/ml)	-5.78	0.27	0.00
		OC Aqueous Extract (25 mg/ml)	3.39	0.27	0.00
	OC Aqueous Extract (25 mg/ml)	Ciprofloxacin (25 mg/ml)	-9.17	0.27	0.00
		OC Ethanol Extract (25 mg/ml)	-3.39	0.27	0.00
	Ciprofloxacin (12.5 mg/ml)	OC Ethanol Extract (12.5 mg/ml)	1.43	0.16	0.00
		OC Aqueous Extract (12.5 mg/ml)	1.43	0.16	0.00
	OC Ethanol Extract (12.5 mg/ml)	Ciprofloxacin (12.5 mg/ml)	-1.43	0.16	0.00
		OC Aqueous Extract (12.5 mg/ml)	0.00	0.16	1.00
	OC Aqueous Extract (12.5 mg/ml)	Ciprofloxacin (12.5 mg/ml)	-1.43	0.16	0.00
		OC Ethanol Extract (12.5 mg/ml)	0.00	0.16	1.00

OC = *Ocimum Gratissimum*, SE = Standard Error of Mean

properties of *Ocimum gratissimum* and optimize extraction protocols to enhance its efficacy.

Conclusion: The higher concentrations of ethanolic and aqueous extracts of *Ocimum gratissimum* showed antibacterial activities against the selected bacteria isolates in this study. This highlights the therapeutic potential of *Ocimum gratissimum* as a natural alternative for the treatment of bacterial infections.

Disclosure of conflict of interest: None

Authors' contributions: AYC and UKN conceptualized and designed the study. AYC performed data analysis. EEC worked on the preparation of *Ocimum gratissimum* extracts. JCF cultured the bacteria and determined the zones of inhibition. AYC and EEC participated in the fieldwork. AYC, EEC and JCF interpreted the data. All authors contributed to the development of the final manuscript and approved its submission.

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Table 2 contd. Statistical comparison of mean zones of inhibition of Ciprofloxacin, and *Ocimum gratissimum* Ethanol and Aqueous extracts on identified bacteria isolates

Bacteria	Variable	Variable	Mean Difference	SE	P value
E. coli	Ciprofloxacin (100 mg/ml)	OC Ethanol Extract (100 mg/ml)	7.76	0.34	0.00
		OC Aqueous Extract (100 mg/ml)	20.29	0.34	0.00
	OC Ethanol Extract (100 mg/ml)	Ciprofloxacin (100 mg/ml)	-7.76	0.34	0.00
		OC Aqueous Extract (100 mg/ml)	12.53	0.34	0.00
	OC Aqueous Extract (100 mg/ml)	Ciprofloxacin (100 mg/ml)	-20.29	0.34	0.00
		OC Ethanol Extract (100 mg/ml)	-12.53	0.34	0.00
	Ciprofloxacin (50 mg/ml)	OC Ethanol Extract (50 mg/ml)	9.41	0.24	0.00
		OC Aqueous Extract (50 mg/ml)	17.53	0.24	0.00
	OC Ethanol Extract (50 mg/ml)	Ciprofloxacin (50 mg/ml)	-9.41	0.24	0.00
		OC Aqueous Extract (50 mg/ml)	8.12	0.24	0.00
	OC Aqueous Extract (50 mg/ml)	Ciprofloxacin (50 mg/ml)	-17.53	0.24	0.00
		OC Ethanol Extract (50 mg/ml)	-8.12	0.24	0.00
	Ciprofloxacin (25 mg/ml)	OC Ethanol Extract (25 mg/ml)	8.47	0.26	0.00
		OC Aqueous Extract (25 mg/ml)	9.24	0.26	0.00
	OC Ethanol Extract (25 mg/ml)	Ciprofloxacin (25 mg/ml)	-8.47	0.26	0.00
		OC Aqueous Extract (25 mg/ml)	0.76	0.26	0.01
	OC Aqueous Extract (25 mg/ml)	Ciprofloxacin (25 mg/ml)	-9.23	0.26	0.00
		OC Ethanol Extract (25 mg/ml)	-0.76	0.26	0.01
	Ciprofloxacin (12.5 mg/ml)	OC Ethanol Extract (12.5 mg/ml)	1.24	0.19	0.00
		OC Aqueous Extract (12.5 mg/ml)	1.24	0.19	0.00
	OC Ethanol Extract (12.5 mg/ml)	Ciprofloxacin (12.5 mg/ml)	-1.24	0.19	0.00
		OC Aqueous Extract (12.5 mg/ml)	0.00	0.19	1.00
	OC Aqueous Extract (12.5 mg/ml)	Ciprofloxacin (12.5 mg/ml)	-1.24	0.19	0.00
		OC Ethanol Extract (12.5 mg/ml)	0.00	0.19	1.00

OC = *Ocimum Gratissimum*, SE = Standard Error of Mean

Table 2 contd. Statistical comparison of mean zones of inhibition of Ciprofloxacin, and *Ocimum gratissimum* Ethanol and Aqueous extracts on identified bacteria isolates

Bacteria	Variable	Variable	Mean Difference	SE	P value
K. pneumonia	Ciprofloxacin (100 mg/ml)	OC Ethanol Extract (100 mg/ml)	4.80	0.48	0.00
		OC Aqueous Extract (100 mg/ml)	18.80	0.48	0.00
	OC Ethanol Extract (100 mg/ml)	Ciprofloxacin (100 mg/ml)	-4.80	0.48	0.00
		OC Aqueous Extract (100 mg/ml)	14.00	0.48	0.00
	OC Aqueous Extract (100 mg/ml)	Ciprofloxacin (100 mg/ml)	-18.80	0.48	0.00
		OC Ethanol Extract (100 mg/ml)	-14.00	0.48	0.00
	Ciprofloxacin (50 mg/ml)	OC Ethanol Extract (50 mg/ml)	7.60	0.42	0.00
		OC Aqueous Extract (50 mg/ml)	16.00	0.42	0.00
	OC Ethanol Extract (50 mg/ml)	Ciprofloxacin (50 mg/ml)	-7.60	0.42	0.00
		OC Aqueous Extract (50 mg/ml)	8.40	0.42	0.00
	OC Aqueous Extract (50 mg/ml)	Ciprofloxacin (50 mg/ml)	-16.00	0.42	0.00
		OC Ethanol Extract (50 mg/ml)	-8.40	0.42	0.00
	Ciprofloxacin (25 mg/ml)	OC Ethanol Extract (25 mg/ml)	7.80	0.28	0.00
		OC Aqueous Extract (25 mg/ml)	8.40	0.28	0.00
	OC Ethanol Extract (25 mg/ml)	Ciprofloxacin (25 mg/ml)	-7.80	0.28	0.00
		OC Aqueous Extract (25 mg/ml)	0.60	0.28	0.06
	OC Aqueous Extract (25 mg/ml)	Ciprofloxacin (25 mg/ml)	-8.40	0.28	0.00
		OC Ethanol Extract (25 mg/ml)	-0.60	0.28	0.06
	Ciprofloxacin (12.5 mg/ml)	OC Ethanol Extract (12.5 mg/ml)	3.80	0.31	0.00
		OC Aqueous Extract (12.5 mg/ml)	3.80	0.31	0.00
	OC Ethanol Extract (12.5 mg/ml)	Ciprofloxacin (12.5 mg/ml)	-3.80	0.31	0.00
		OC Aqueous Extract (12.5 mg/ml)	0.00	0.31	1.00
	OC Aqueous Extract (12.5 mg/ml)	Ciprofloxacin (12.5 mg/ml)	-3.80	0.31	0.00
		OC Ethanol Extract (12.5 mg/ml)	0.00	0.31	1.00

OC = *Ocimum Gratissimum*, SE = Standard Error of Mean

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