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# Evaluation of Antibacterial Activity of *Ocimum gratissimum* and *Zingiber officianale* on Bacteria Isolated from the Environment

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author OOA designed the study, wrote the protocol and approved the final draft of the manuscript. Author OAOE performed the statistical analysis and prepared the manuscript. Authors TO and NDA performed all assays described in this study. All authors read and approved the final manuscript.*

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## ABSTRACT

**Background:** Antibiotic resistance continues to be an important public health issue and the environment constitutes a major source of antibiotics and antibiotic-resistant bacteria. This is because large amounts of antibiotics are deposited into the environment as a result of several human activities.

**Aim:** In this study, the antibacterial properties of *Ocimum gratissimum* and *Zingiber officianale* were evaluated against bacteria present in effluents from a University sewage treatment plant, health center and horticultural garden.

**Methods:** Enteric bacterial species and *Staphylococcus aureus* were isolated and assessed for resistance to different antibiotics. In addition, the ethanolic extracts of two plants *Ocimum gratissimum* and *Zingiber officianale* were tested for their antimicrobial activity against some selected antibiotic-resistant bacteria from the environment.

**Results:** A total of 215 organisms including 76 *Escherichia coli*, 56 *S. aureus*, 40 *Klebsiella spp*, 28

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*Salmonella spp* and 15 *Shigella spp*, were isolated with more than half possessing resistance to at least one of the antibiotics tested. 57% of *S. aureus* isolates were resistant to at least one of the antibiotics tested with the most resistance recorded against penicillin (82.22%) and Erythromycin (91.28%). 53.22% of all enterobacterial isolates possessed resistance to at least one antibiotic with Salmonella (91.28%) and *E. coli* (61.15%) as the most resistant. High levels of antibiotic resistance (65%) were found among isolates from the University horticultural garden. Phytochemical analysis showed the presence of tannins, saponins and cardiac glycosides in both plant fractions. Both plants showed antimicrobial activity against most of the isolates tested with *Ocimum gratissimum* showing better activity than *Zingiber officianale* at concentrations lower than 25 mg/ml.

**Conclusion:** This study adds credence to the local application of both plants as herbal remedies.

*Keywords: Antibiotic resistance; medicinal plants; Ocimum gratissimum; Zingiber officianale; phytochemicals.*

## 1. INTRODUCTION

Infectious diseases of bacterial origin have been controlled, primarily through commercially available antibiotics [1]. This heavy dependence on antibiotics has, in turn, led to the emergence of multi-drug resistant strains, prompting the development of novel compounds to add to the drying antibiotic pipeline. The problem, however, with drug discovery is the amount of time and resources needed to develop new compounds. Further complicating the issue is the presence of a crucial, but often ignored reservoir of antibiotic resistance genes; the environment. Large amounts of antibiotics are released from the gut of man and animals into the environment due to incomplete metabolism or disposal of unused antibiotics. As a result, both antibiotic resistant bacteria and genes are found in urban effluents [2-8]. It is important to study this effluent in a bid to assess the prevalence of antimicrobial resistance inherent in them.

Medicinal plants have been used for thousands of years in many settings. They provide a suitable palliative to the current problem of antibiotic resistance hence the need to study them, giving credence to their medicinal properties and ultimately promoting their proper use and widespread application.

*Ocimum gratissimum* is abundant in tropical Africa and India where it is used primarily as a food flavoring. It is also used in the treatment of high fever, cholera, and diarrhea [9,10]. *Zingiber officianale* is well distributed across many tropical regions including Nigeria, India, and Brazil. Besides being used for culinary purposes, it is used traditionally in the treatment of digestive problems and intestinal infections [11].

In this study, we investigated the resistance pattern of bacteria isolated from the environment and assessed the antimicrobial properties of two medicinal plants, *Ocimum gratissimum* and *Zingiber officianale* against some selected bacterial isolates.

## 2. MATERIALS AND METHODS

### 2.1 Study Area and Sample Collection

This study was conducted at Covenant University in Ado-Odo Local Government area of Ogun State, Nigeria. Three sampling stations in the University premises were chosen; University Health Centre, Sewage Treatment Plant, and University Horticultural Garden. Run-off waters samples were obtained from the three sampling sites.

### 2.2 Plant Materials

Fresh *O. gratissimum* leaves were obtained from the horticultural garden in the University while fresh rhizomes of *Z. officinale* were obtained from a market in Mushin, Lagos State. Both plants were identified in the Department of Biological Sciences, Covenant University. Upon collection, the plant's materials were air dried and then powdered in a blender.

### 2.3 Extraction of Plant Materials

Extraction for both plants was performed as described by Ayepola and Adeniyi [12]. 62.65 g of *O. gratissimum* and 110 g of *Z. officinale* were dissolved in 500 ml and 800 ml of absolute ethanol respectively. Using a Soxhlet extractor, extraction was carried out for 72 hours at room temperature. The resulting extract was then concentrated to dryness in a rotary evaporator and prepared to give the following concentrations; 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml. All extract preparations stored in stoppered bottles and refrigerated until needed.

### 2.4 Phytochemical Screening

Plant extracts were screened for the presence of different secondary metabolite classes including Alkaloids, Saponins, Tannins, Phlobotanins, Anthraquinones and Cardiac glycosides, using methods described by Trease and Evans [13].

### 2.5 Isolation and Identification of Bacterial Isolates

Serial dilutions of obtained water samples was performed. Dilutions $10^{-3}$ and $10^{-5}$ were plated, using the spread plate method, onto prepared plates of Mannitol salt agar (MSA), Eosin methylene blue agar (EMB), MacConkey agar (MCA) and Salmonella-Shigella agar (SSA). Plates were incubated at 37°C for 24 h. Following incubation, bacterial isolates were identified by morphological and biochemical methods according to standard microbiological procedures.

### 2.6 Antibiotic Susceptibility Testing

The antibiotic susceptibility profile of isolates was assayed using the disk diffusion method [14]. The antibiotics tested are Tetracycline, Gentamycin, Clindamycin, Penicillin, Trimethoprim/Sulphamethoxazole, Erythromycin, and Ciprofloxacin. An inoculum containing $10^6$ CFU of each isolate was prepared and standardized using the 0.5 McFarland's standard. The inoculum was then introduced onto Mueller Hinton agar plates with a sterile swab stick. Sterile discs containing test antibiotics were placed on the plates and incubated at 37°C for 18 h. Following incubation, the zones of inhibition were measured in millimeters using a ruler. Results were interpreted according to the Clinical and Laboratory Standard Institute (CLSI) guidelines [15].

### 2.7 Evaluation of Antimicrobial Activity of Plant Extracts

A total of 12 enteric bacterial isolates and 3 *S. aureus* isolates were randomly selected for the evaluation of antimicrobial activity of the plant extracts. This was carried out using the agar well diffusion method as previously described [16]. 0.1 ml of test isolates, cultured overnight, was seeded onto already prepared Mueller Hinton (MH) agar plates. Plates were rocked gently to ensure uniform distribution of inoculum and then allowed to set. A 7 mm sterile cork borer was used to make wells into which 0.1 ml of prepared extract concentrations were added. Plates were incubated at 37°C for 18-24 h and thereafter observed for zones of inhibition.

## 3. RESULTS

Phytochemical screening of both plants used in this study revealed the presence of Saponins, Tannins and Cardiac Glycosides (Table 1). *Zingiber officinale* showed the presence of Alkaloids in addition to the aforementioned phytochemicals.

**Table 1. Phytochemical screening of plant extracts**

| Phytochemical compound | *Ocimum gratissimum* | *Zingiber officinale* |
|---|---|---|
| Alkaloids | - | + |
| Saponin | + | + |
| Tannins | + | + |
| Phlorotannins | - | - |
| Anthraquinones | - | - |
| Cardiac glycosides | + | + |

*Key: Present (+), Absent (-)*

**Table 2. Frequency of isolation**

| Organism | STP | UHC | UHG | Total |
|---|---|---|---|---|
| *E. coli* | 48 | 13 | 15 | 76 |
| *S. aureus* | 23 | 15 | 18 | 56 |
| *Klebsiella spp* | 29 | 8 | 3 | 40 |
| *Salmonella spp* | 15 | 12 | 1 | 28 |
| *Shigella spp* | 5 | 6 | 4 | 15 |
| Total | 120 | 54 | 41 | 215 |

*STP - Sewage Treatment Plant; UHC - University Health Centre; UHG - University Horticultural Garden*

A total of 215 bacterial strains were isolated and identified to the genus level. They include 159 enterobacterial isolates (*E.coli*-76, *Klebsiella spp* -40, *Salmonella spp* -28, *Shigella spp*- 15) and 56 *Staphylococcus aureus* isolates (Table 2).

The result of the antimicrobial susceptibility testing showed that 53.22% of the enterobacterial isolates possessed resistance to at least one of the antibiotics tested. About 91.28% of the enterobacterial isolates were resistant to Trimethoprim/Sulphamethoxazole. Gentamycin and Ciprofloxacin were the most effective having inhibited the growth of 72.9% and 66.67% enterobacterial strains respectively (Fig. 5). The highest levels of resistance were obtained from strains obtained from the University Horticultural Garden (65%) followed by University Health Centre (54.39%) and the Sewage Treatment Plant (50.11%) (Figs. 2-4).

Among the *S. aureus* strains isolated, 57.07% possessed resistance to at least one of the antibiotics. High levels of Resistance were observed to Penicillin (82.22%) and Erythromycin (86.30%). Gentamycin and Ciprofloxacin inhibited the growth of 88.65% and 67.50% of test isolates respectively (Fig. 1).

The results of the evaluation of antimicrobial activity of the plant extracts revealed that all isolates except one were susceptible to either plant extract at the highest concentration of 50 mg/ml. The ability of the extract, however, decreased with decreasing concentration. No activity was observed against most isolates at 12.5 mg/ml and 6.25 mg/ml concentration of both extracts. *O. gratissimum* performed better against selected isolates than *Z. officinale* (Table 4).

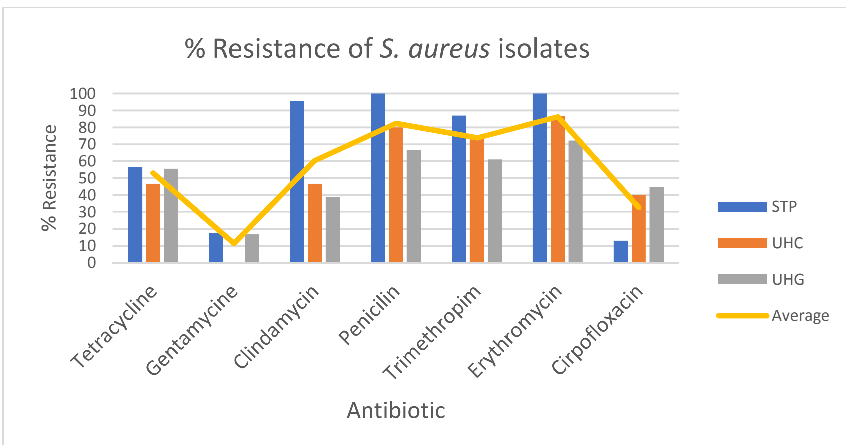


**Fig. 1. Percentage resistance of *S.aureus* isolates**

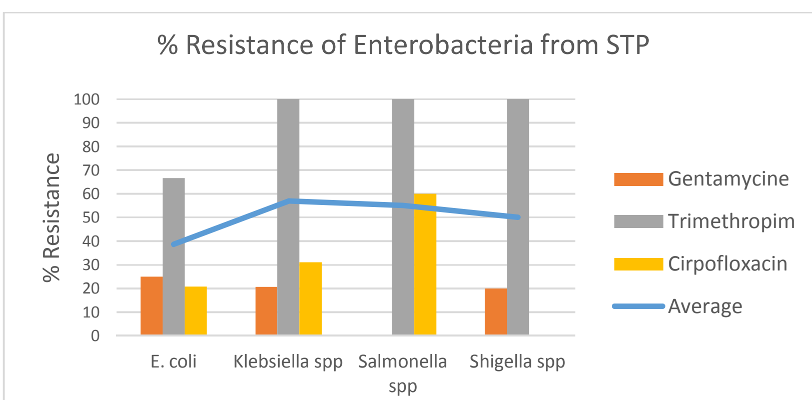


**Fig. 2. Percentage resistance of enterobacteria from STP**

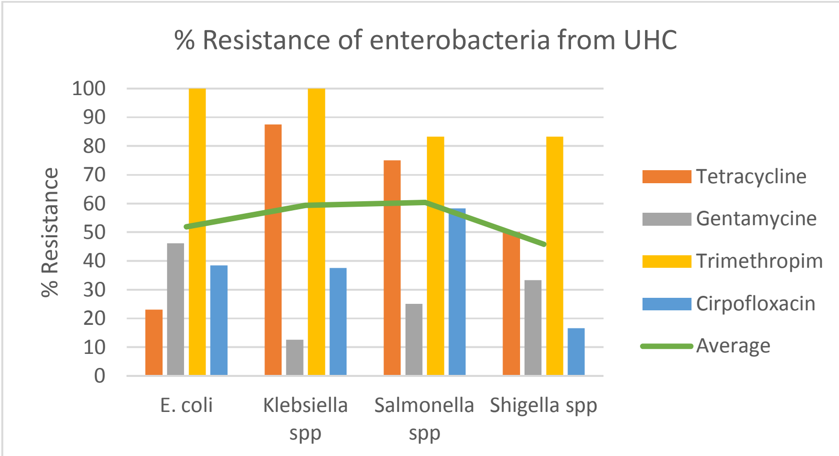


**Fig. 3. Percentage resistance of enterobacteria from UHC**

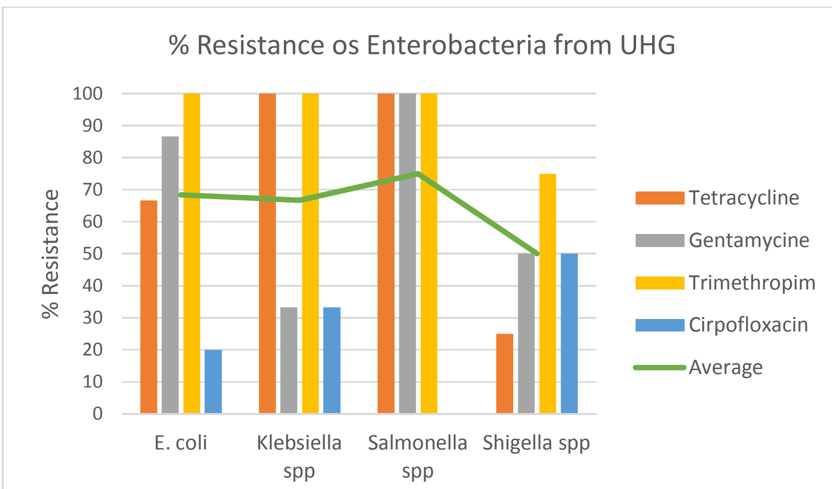


**Fig. 4. Percentage resistance of enterobacteria from UHG**

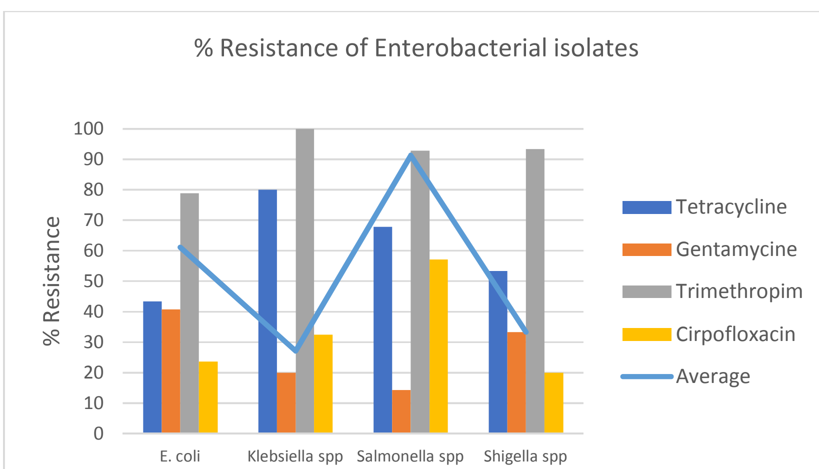


**Fig. 5. Percentage resistance of enterobacterial isolates**

**Table 3. Resistance phenotype of selected isolates**

| Code | Organism | Resistance Profile |
|---|---|---|
| SA01 | *S. aureus* | TE, DA, P, E |
| SA02 | | Nil |
| SA03 | | TE, DA, P, SXT, E, CIP |
| EC01 | *E. coli* | TE, SXT |
| EC02 | | TE, SXT, CIP |
| EC03 | | TE, SXT |
| EC04 | | Nil |
| EC05 | | Nil |
| EC06 | | TE, SXT |
| KS01 | *Klebsiella spp* | TE, CN, SXT, CIP |
| KS02 | | TE, SXT |
| KS03 | | TE, SXT |
| KS04 | | TE, SXT |
| KS05 | | SXT |
| KS06 | | TE, SXT |

*KEY: TE -Tetracycline; CN- Gentamycin; DA- Clindamycin; P- Penicillin SXT- Trimethoprim-Sulphamethoxazole; E- Erythromycin; C- Ciprofloxacin*

**Table 4. Antimicrobial activity of plant extracts against test organisms**

| Organism | *Ocimum gratissimum* | | | | *Zingiber officinale* | | | |
|---|---|---|---|---|---|---|---|---|
| | 50 mg/ml | 25 mg/ml | 12.5 mg/ml | 6.25 mg/ml | 50 mg/ml | 25 mg/ml | 12.5 mg/ml | 6.25 mg/ml |
| SA 01 | 18 | 0 | 0 | 0 | 20 | 0 | 0 | 0 |
| SA 02 | 15 | 14 | 14 | 12 | 10 | 0 | 0 | 0 |
| SA 03 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| EC 01 | 30 | 23 | 18 | 0 | 11 | 0 | 0 | 0 |
| EC 02 | 14 | 0 | 0 | 0 | 15 | 10 | 0 | 0 |
| EC 03 | 15 | 0 | 0 | 0 | 22 | 20 | 0 | 0 |
| EC 04 | 12 | 12 | 10 | 0 | 0 | 0 | 0 | 0 |
| EC 05 | 19 | 17 | 0 | 0 | 12 | 0 | 0 | 0 |
| EC 06 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| KS 01 | 30 | 17 | 0 | 0 | 25 | 10 | 0 | 0 |
| KS 02 | 17 | 14 | 12 | 0 | 15 | 15 | 0 | 0 |
| KS 03 | 20 | 0 | 0 | 0 | 20 | 18 | 0 | 0 |
| KS 04 | 10 | 0 | 0 | 0 | 22 | 20 | 0 | 0 |
| KS 05 | 22 | 18 | 12 | 0 | 11 | 0 | 0 | 0 |
| KS 06 | 17 | 17 | 0 | 0 | 0 | 0 | 0 | 0 |

## 4. DISCUSSION

Antibiotic-resistant bacteria remain a major global health challenge that could potentially roll back important advances in modern medicine. Effluents from hospitals, gardens, and wastewater treatment plants, accumulate both chemical and biological waste and these could constitute the interface where resistance genes are acquired by bacteria.

In this study, more than half of all organisms isolated from the environment were resistant to at least one of the antibiotics assayed. The highest levels of resistance (50-70%) were found among the isolates from the horticultural garden. This could have resulted from the manure used as fertilizer in the garden [17]. Studies have shown the presence of antibiotics and drug-resistant bacteria in animal feces and soil [18–23]. The lower levels of resistance found among the enterobacterial isolates from the treatment plant effluent (40-57%) could be as a result of the treatment of wastes. Some studies have also shown that selection of antibiotic resistance genes occurs downstream from the treatment plant [24,25]. This selection downstream is thought to be as a result of the release of bacterial DNA following lysis from effluent treatment and subsequent uptake by competent cells downstream [26,27]. More than half of all isolates from the health center effluent were resistant to one of the antibiotics tested. Furthermore, an average of 80% of the *S. aureus* isolates from the University Health center effluent possessed resistance to either Penicillin, Erythromycin or Trimethoprim-Sulphamethoxazole. This is indicative of the heavy usage of antibiotics in clinical settings.

Phytochemicals have been shown to be potent inhibitors of microbial growth, Tannins, for example, disrupt microbial growth by inhibiting extracellular microbial enzymes hence baring access to substrates [28]. Phytochemical analysis from this study showed that both plants are rich in phytochemicals. Secondary metabolites found in both plants were similar to those reported in other studies [11,29,30].

Both extracts showed activity against *S. aureus, E. coli,* and *Klebsiella spp.,* this is consistent with previously reported studies [31,32]. The ethanol extract of *Ocimum gratissimum* performed better than the ethanol extract of *Zingiber officinale,* inhibiting the growth of 14 of the 15 (93.33%) test isolates. *Z. officinale, on the other hand,* showed activity against 11 of the 15 (73.33%) isolates tested. *O. gratissimum* was also able to inhibit the growth of a multi-drug resistant isolate of *S. aureus* (SA 03). Findings from this study do not explain the difference in efficacy. However, it may be indicative of a higher phytochemical content in *Ocimum gratissimum.* Compared to the antibiotics, both extracts performed well against the test isolates showing activity against multi-drug resistant isolates at higher concentrations.

## 5. CONCLUSION

Results from this study have shown the presence of multi-drug resistant bacterial strains in the environment and have also shown the efficacy of *Ocimum gratissimum* and *Zinigiber officinale* against bacterial species thereby substantiating their application as medicinal plants. Further studies should seek to elucidate the origins of resistance of isolated bacteria.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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