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Antibacterial Efficacy of Medicinal Plants on Multidrug Resistant Bacteria from Potable Water in Calabar, Cross River State

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Abstract

The presence of multidrug resistant (MDR) bacteria in water poses severe health challenges because of high risks of infection and limited treatment choices. This study was conducted to determine the antibacterial activity of *Aframomum melegueta* and *Ocimum gratissimum* on MDR bacteria from drinking water. Membrane filtration, Gram staining and biochemical test were used to analyse water samples and identify the Bacterial isolates. The susceptibility test and minimum inhibitory concentration carried out employed the agar well diffusion method and microbroth dilution technique respectively. The bacteria isolated include *Escherichia coli*, *Salmonella* sp. *Staphylococcus aureus*, *Klebsiella* sp. and *Proteus* sp. The physical and chemical evaluation of the water indicates that acidic pH numbers fall below WHO standard and turbidity levels was above the standard. Phytochemical parameters of both plants reveal the presence of saponins, tannins, flavonoids, glycosides, and alkaloids. The susceptibility test result shows that *Escherichia coli* had the highest susceptibility to the extracts of *O. gratissimum*, with zones of inhibition of 27.5 ± 3.5 mm and 23.5 ± 2.8 mm respectively at 100 mg/mL, while *Klebsiella* spp. showed the least susceptibility. Hot and cold extracts of *A. melegueta* demonstrated lower antimicrobial activity, with highest diameter zone of inhibition observed against *E. coli* (12.5 ± 0.7 mm at 100 mg/mL). The result from the present study revealed that hot and cold extracts of *O. gratissimum* exhibited better antimicrobial activity compared to *A. melegueta* against MDR bacteria isolated from drinking water sources. Routine surveillance of antimicrobial resistance in environmental water sources is highly recommended.

Keywords: *Ocimum gratissimum*, *Aframomum melegueta*, Susceptibility, Water, Quality

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Introduction

Alexander Fleming's discovery of antibiotics in the 1920s was one of the most significant medical breakthroughs that were utilized to save lives and improve the quality of human life [1]. The medical community felt that the finding would eventually lead to the annihilation or extermination of infectious pathogens [2]. In recent decades, however, the health advantages of these crucial treatments have been threatened by the development of antibiotic resistance in pathogenic microbes, which has rendered many routinely used antibiotics ineffective [3, 1]. And there is a growing global concern that present antibiotics will become less effective and more expensive against these deadly germs [4]. Consequently, it is of the utmost need to identify new antimicrobial agents, despite the fact that the global spread of multidrug-resistant isolates has grown. The widespread and rapid growth of resistance to newly manufactured antimicrobial medications in the past indicates that these new compounds may have a limited life expectancy or become less effective in no time [5]. Resistance strains continue to spread, posing a danger to the efficacy of treatments for diseases caused by microorganisms. Literature implies that the widespread emergence of antibiotic resistance in pathogenic microorganisms is due to improper or excessive medicine or antibiotic use [6, 7]. People in third world countries have easy access to antibiotics due to unregulated sales and distribution of medications without a valid prescription from a healthcare expert [2]. Literature indicates that antibiotic resistant genes that transfer resistance to many antibiotics are present in drinking water sources in both developing and developed nations [8]. The transferability of resistance genes from environmental bacteria to human infections poses a bigger threat to public health, as demonstrated by this study. In countries at various stages of economic development, it has been demonstrated that drinking water can transmit disease-causing microorganisms to bigger populations [9]. Furthermore, adequate and safe drinking water is the most effective means of preventing the spread of epidemic diseases and enhancing the quality of life. According to the World Health Organization, approximately 80 % of all

diseases are caused by drinking contaminated water [10]. Over 10 % of the disease burden in developing nations is attributable to water-related illnesses, such as diarrhoea [11].
The varied groups of organisms on Earth are bacteria, which occupy nearly all available habitats and, as a result, exhibit a remarkable range of metabolic processes. Recently, the discovery of multidrug-resistant bacteria has become a dilemma for researchers tasked with preventing bacterial infections and epidemics.
Natural remedies derived from plants have been utilized in traditional medicine worldwide for many decades, long before the discovery of antibiotics and other modern pharmaceuticals. The effectiveness of plants used to treat infectious disorders cannot be overstated. Approximately 10 % of local groups on Earth have employed flowering plants to heal various diseases, but only 1 % has been acknowledged by modern scientists [2]. Due to their widespread usage as potential treatments for all types of infectious disorders, the search for plants containing antimicrobial compounds has been initiated [13]. Secondary metabolites like alkaloids, tannins, and flavonoids, phenols, terpenoidswhich have demonstrated antibacterial activity in vitro, are expected to be abundant in medicinal plants [14]. Several medicinal plants currently used to treat infectious diseases have been reported and are believed to have fewer adverse effects and lower toxicity [15]. This has increased the use of medicinal plants and phytochemicals in the development of antimicrobial medications. The antibacterial effects of plants are attributable to the existence of secondary metabolites, according to scientific research. According to the World Health Organization [29], more than 80 percent of the global population relies on plant-based traditional medicine [16]. The WHO indicates that medicinal plants are the finest source for the development of new antibiotic types [17]. This information has prompted scientists to prioritize the screening of more plant-derived natural compounds for the development of new medicinal medicines. *Aframomum melegueta* (Alligator pepper) and *Ocimum gratissimum* (Scent leaves), both members of the Zingiberaceae family, are highly regarded for their antioxidant and antibacterial properties. The majority of antibiotics available on the market today are derived from plant extracts and herbs. Therefore, it is vital to investigate their utility to humanity.
The study was conducted to examine the antibacterial activity of hot and cold-water extracts of *Aframomum melegueta* (Alligator pepper) and *Ocimum gratissimum* (Scent leaves) against multidrug-resistant bacteria isolated from drinking water sources in Calabar, Cross River State, Nigeria.

Materials and Methods

Study area

The study was conducted at the Cross River State Water Board Limited and the Department of Microbiology Laboratory, Federal University of Lafia, to study the antibacterial activity of *O. gratissimum* (scent leaves) and seed of *A. melegueta* (alligator pepper) against multidrug resistant bacteria isolated from domestic-use (potable water) sources from public water supply in Calabar.

Materials used

The materials used were autoclave, safety control universal hot plate, 99-microwell plates, petri dishes, Whatmann no 1 filter paper, plants, Sterilin^{TM} disposable spreaders, Eppendorf, micropipette, glass, separation funnel and loops.

Plants

Fresh plants part of *O. gratissimum* (scent leaves) and seed of *A. melegueta* (alligator pepper) were both procured at Lafia modern market, Nasarawa State, Nigeria during the rainy season. The plants materials were transported to the Federal University of Lafia. They were identified and classified at Botany Department.

Sample collection

Approximately 10L of water from point of use were collected from domestic-use (potable water) sources from private water supply in Calabar following standard procedures for examining wastewater and water as outlined by WHO [21, 24].

Physico-chemical analysis

The Physico-chemical properties of the water was determined and performed by the method outlined by Peter *et al*. [19]. The physicochemical test carried out consists of the temperature, pH, turbidity, conductivity level, total dissolve, and chlorine contents (free and total).

Bacteria isolation and identification

The membrane filtration method was employed using membrane filter paper of 0.45 µm pore size and thickness of 150 µm. After filtration, a sterile forceps was used to pick the filter paper and place onto appropriate agar and the plates were incubated for 18-24 h at 37 ^{o}C. Presumptive isolates from the various agar plates were picked and streaked on nutrient agar and then incubated for 18-24 h at 37 ^{o}C. Pure colonies from the nutrient agar plates were inoculated into Luria broth (5 mL) and incubate for 18-24 h at 37 ^{o}C. The colonies were identified using Gram stain and biochemical methods.

Antibacterial susceptibility testing

All standardized isolates were subjected to different classes of antibiotic using the Kirby–Bauer method and were assess by the NCCL standards. The grown isolates were standardized to a turbidity equivalent to 0.5 Mcfarland standards (5 x 10^5 CFU/mL). The standardize isolates were spread evenly on the surface of Mueller–Hinton-agar plates.The isolates were tested on ten antibiotics disc which includes ampicillin (10 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), erythromycin (15 μg), penicillin (10 IU), cefuroxime (30 μg), cefotaxime (30 μg), nalidixic acid (30 μg), tetracycline (30 μg), and gentamicin (10 μg). Antibiotic disks to determine MDR isolates were placed using a sterile forceps, and the plates were labelled and incubated at 37 °C for 18-24 h [23]. And interpretation of results was done using NCCLS [22].

Preparation of plant extracts

Hot extract: The fresh leaves of *O. gratissimum* (scent leaves) and seed of *A. melegueta* (alligator pepper), were shade dry at room temperature for seven (7) days and were separately crushed into fine powder using a grinder. Twenty gram (20 g) was weighed and dissolved in a 500 mL beaker that contains 200 mL of distilled water. The extract was boiled for approximately 60 minutes using a universal hot plate. After filtration, the extracts were oven dried at 50-60 ^{o}C to obtain a semi solid mass and the yield of the extract was calculated based on the weight of the dried plants. Each of the extract was re-suspended in distilled water to the desired concentrations and stored in the fridge at 4 ^{o}C prior to analysis.

Cold extract: Twenty (20 g) was weighed and dissolved in a 500 mL beaker that contain 200 mL of distilled water and allow to stand for 3 days. After filtration, the extracts were oven dried at 50-60 ^{o}C to obtain a semi solid mass and the yield of the extract was calculated based on the weight of the dried plants. Each of the extract was re-suspended in distilled water to the desired concentrations and stored in the fridge at 4 ^{o}C prior to analysis.

Antibacterial susceptibility assay

Both hot and cold extracts of *O. gratissimum* and *A. melegueta* were tested for antibacterial activity against the multidrug resistant isolates using agar well diffusion technique as described by Peter *et al*. [19]. A sterile cork borer was used to make a 4-6 mm wells from Mueller Hinton agar plates. From the different concentrations of the extract, 100 µL was added to the wells. All plates were labelled accordingly and incubated for 18-24 h at 37 ^{o}C

Determination of minimum inhibitory concentration (MIC)

The Micro-broth dilution method was used as describe by Pratiwi and Elin [18] to determine the minimum inhibitory concentration using a 96 well microtitre plates.

Determination of minimum bactericidal concentration (MBCs)

MCB was determined to check the concentration that just killed the bacteria. This was done by transferring a drop of the preparation from MIC result onto a freshly prepared Mueller hinton agar and then incubated at 37 ^{o}C for 18-24 h.

Phytochemical screening

The plants from the study were screened for the presence of various classes of secondary metabolites including tannins, alkaloids, flavonoids, saponins, steroids, glycosides, phlobatannins, reducing sugars, anthraquinones, and amides as described by Wadood *et al*. [20]

Statistical analysis

The test of significance was carried out on data obtained from the diameter zones of inhibition of plant extracts on all the test organisms using appropriate statistical package SPSS (IBM version 25). Results obtained were expressed as Mean ± SD and level of significant set at $P<0.05$.

Results and Discussion

Accessibility to potable water in Nigeria is a major concern, were many residents struggle to access safe and clean water. According to the Nigerian Standard for Drinking Water Quality, the country's drinking water standards are aligned with the World Health Organization (WHO) guidelines. Despite these standards, many water sources in Nigeria are contaminated, and the enforcement of water quality regulations is often weak.

The physicochemical assessment of the drinking water samples as presented in Table 1 provides an important background for understanding the isolation of multidrug-resistant (MDR) bacteria from the water samples. Although temperature values fall within ambient range, some sampling pointssuch as flash mixer, settled water, filtered water, ground level reservoir, and elevated water tank showed acidic pH values below the Nigerian Industrial Standard for Drinking Water Quality, alongside turbidity levels exceeding recommended limits in raw water and flash mixer with turbidity values of 9.5 and 14.3 respectively. Condition like this could lead to microbial persistence which in turns makes the disinfection processes ineffective. This is usually common where residual chlorine is very low or very minute to be detected. The present study suggests that the water sources examined may present a public health risk and could contribute to the survival and dissemination of resistant bacteria in the community.

Table 1: Physicochemical properties of drinking water

Parameters	NISDWQ	RW	FM	SW	FW	GLR	EWT
Temperature (^{0}C)	Ambient	25.3	24.3	23.3	25.2	25.1	24.2
pH	6.5-8.5	6.16	4.8	4.8	4.6	4.7	5.5
Conductivity (µs/cm)	500	22.7	42.5	49.3	57.7	59.4	53.6
Turbidity (NTU)	5	9.5	14.3	2.6	1.3	1.2	1.4
Total Dissolved Solid (mg/L)	500	13.2	26.1	30.2	31.4	32.0	33.3
Free Chlorine (mg/L)	0.2					0.1	0.03
Total chlorine (mg/L)	0.5					0.08	0.09

NISDWQ = Nigerian Industrial Standard for Drinking Water Quality, RW = raw water, FM= flash mixer, SW = settled water, FW = filtered water, GLR = ground level reservoir, EWT = elevated water tank

Table 2: Phytochemical component of water extract of *A. melegueta* (alligator pepper) and *O. gratissimum* (scent leaves)

Parameters	*O. gratissimu* (water extract)	*A. melegueta* (water extract)
Amides	+	+
Tannins	++	+
Saponins	++	+
Steroids	+	-
Glycosides	+	+
Flavonoids	++	+
Alkaloids	+	+

+ = present, – = absent

Phytochemical screening shows that multiple bioactive constituents in both *O. gratissimum* and *A. melegueta* extracts as presented in Table 2. Compounds that were detected include tannins, saponins, flavonoids, alkaloids, steroids, and glycosides; however their distribution varied with plant species. The richer phytochemical profile observed in *O. gratissimum*, particularly the consistent presence of flavonoids, tannins, and saponins across extracts, may partly explain its comparatively stronger antibacterial activity. These classes of compounds have been widely associated with antimicrobial effects through mechanisms such as membrane disruption, enzyme inhibition, and interference with microbial metabolism [25].

The antibacterial activity showed a clear-difference in activity involving the two plants and the methods of extraction. The hot water extraction of *O. gratissimum* showed better inhibitory-activity to all the organisms, with larger diameter zones of inhibition across concentrations as shown in Table 3 compared with *A. melegueta* shown in Table 4. However, *A. melegueta* indicates reduced activity, specifically against *Salmonella* spp. as well as *Staphylococcus aureus*, mostly at reduced concentrations. This present study indicates that *O. gratissimum* displays an improved and stronger-antibacterial-properties against MDR bacteria.

Table 3: Diameter zone of inhibition of bacterial isolates on hot extract of *O. gratissimum*

Isolate	mg/mL (100)	mg/mL (75)	mg/mL (50)	mg/mL (25)	mg/mL (PC water)	F-value	P-value
Salmonella sp	17.5±2.1	14.5±2.1	12.5±0.7	7±1.4	00±0.0	13.609	<0.014
Escherichia coli	27.5±3.5	25±1.4	20.5±0.7	18.5±2.1	00±0.0	6.932	<0.046
S. aureus	17.5±0.7	13.5±0.7	13±2.8	8.5±3.5	00±0.0	5.047	0.076
Proteus sp	13.5±2.1	12±1.4	10±1.4	6.5±0.7	00±0.0	8.148	<0.035
Klebsiella sp	25±2.8	20.5±0.7	19.5±7.7	10±1.4	00±0.0	4.479	0.091

P<0.05, PC= Positive control

Table 4: Diameter zone of inhibition of bacterial isolates on hot extract of *A. melegueta*

Isolate	mg/mL (100)	mg/mL (75)	mg/mL (50)	mg/mL (25)	mg/mL (PC water)	F-value	P-value
Salmonella sp	2.5±0.7	00±0.0	00±0.0	00±0.0	00±0.0	25.000	<0.005
Escherichia coli	12.5±0.7	4.5±2.1	2.5±0.7	00±0.0	00±0.0	42.515	<0.002
S. aureus	4.5±0.7	1.5±0.7	00±0.0	00±0.0	00±0.0	36.000	<0.002
Proteus sp	23.5±2.8	18.5±0.7	12.5±4.9	8.5±3.5	00±0.0	7.220	<0.043
Klebsiella sp	10.5±2.1	6.5±3.5	4±2.8	2.5±0.7	00±0.0	3.837	0.113

P<0.05, PC= Positive control

Table 5: Diameter zone of inhibition of bacterial isolates on cold extract of *O. gratissimum*

Isolate	mg/mL (100)	mg/mL (75)	mg/mL (50)	mg/mL (25)	mg/mL (PC water)	F-value	P-value
Salmonella sp	4.5±2.1	00±0.0	00±0.0	00±0.0	00±0.0	9.000	<0.030
Escherichia coli	23.5±2.8	11.5±3.5	2.5±0.7	00±0.0	00±0.0	41.302	<0.002
S. aureus	9.5±2.1	00±0.0	00±0.0	00±0.0	00±0.0	40.111	<0.002
Proteus sp	22.5±2.1	11.5±0.7	10±1.4	5.5±0.7	00±0.0	55.533	<0.001
Klebsiella sp	12.5±2.1	10.5±0.7	6.5±0.7	2.0±0.7	00±0.0	26.222	<0.004

P<0.05, PC= Positive control

Table 6: Diameter zone of inhibition of bacterial isolates on cold extract of *A. melegueta*

Isolate	mg/mL (100)	mg/mL (75)	mg/mL (50)	mg/mL (25)	mg/mL (PC water)	F-value	P-value
Salmonella sp	11±7.0	5.5±2.1	00±0.0	00±0.0	00±0.0	4.070	0.104
Escherichia coli	13.5±2.1	9.5±0.7	6±1.4	00±0.0	00±0.0	37.429	<0.002
S. aureus	1.5±0.7	00±0.0	00±0.0	00±0.0	00±0.0	9.000	<0.030
Proteus sp	19.5±0.7	14.5±0.7	12.5±0.7	8±2.5	00±0.0	19.140	<0.008
Klebsiella sp	7.5±0.7	3.5±0.7	1.5±0.7	00±0.0	00±0.0	56.333	<0.001

P<0.05, PC=Positive control

Different conditions including temperature contributed to the antibacterial efficacy. Both plants showed activity, however, hot water-extract showed a larger zones of inhibition as compared to the cold water extracts as shown in Tables 3–6, this shows that heat can contribute to the extraction of important chemicals in plants. This evidence was seen *O. gratissimum*, where the hot extracts continuously fed better compared to the cold extracts against organism tested. Study has shown that that heat assisted extraction can accelerate the solubility as well as availability of bioactive compounds that are polar in nature. Similar observations have been reported in earlier studies evaluating aqueous plant extracts, where extraction conditions greatly affected antimicrobial outcomes [26]. Differences in susceptibility between the MDR bacterial isolates were-an evident. The present study indicates that *E. coli* and *Proteus* spp. were more susceptible to both plant extracts, at higher concentrations of 100 mg/mL, while *Klebsiella* spp. and *S. aureus* showed reduced susceptibility. These differences may be as a result of differences in the structure of the cell wall, the permeability, and also the rate of resistant mechanisms that exist among the organisms. The reduced susceptibility of *Klebsiella* spp, an organism that is known for its hefty rate of resistance determinants, conform with existing reports on plant extract activity against Gram negative bacteria [27].

The present study indicates a concentration-dependent pattern on the extracts. This means that the higher the concentrations, the higher the diameter zone of inhibition and the lower the concentration, the lower the diameter zone of inhibition. The dose response association shows the existence of active antibacterial constituents whose effects decreases at a reduced-concentration. The statistical analysis indicates that some of the differences were significant, especially for *O. gratissimum* extracts, supports the credibility of the noticed trends.

The result of the minimum inhibitory and minimum bactericidal concentration results shown in Tables 7 and 8, offers an extra proof into the nature of the antibacterial effects. The MIC results were seen to be lower for hot extracts than cold extracts; this shows the influence of the method of extraction. The MBC results indicate no activity for all isolates and was recorded as zero, this suggest that the extract is bacteriostatic in nature instead of bactericidal effects on the various concentrations. The present study is significant, because bacteriostatic agents can still be important in other to control the growth of bacteria, in particular the MDR organisms, but this may need a combined strategies as well as exposing the organism for a long time for a complete eradication.

Table 7: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of hot and cold extracts of *O. gratissimum* against MDR bacterial from drinking water

Isolate	MIC (mg/mL) Hot *O. gratissimum*	MIC (mg/mL) Cold *O. gratissimum*	MBC (mg/mL) Hot *O. gratissimum*	MBC (mg/mL) Cold *O. gratissimum*
Salmonella sp	25	50	00	00
Escherichia coli	12.5	25	50	00
S. aureus	25	50	00	00
Proteus sp	25	50	00	00
Klebsiella sp	25	50	00	00

MDR: Multidrug Resistant

Table 8: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of hot and cold extracts of *A. melegueta* against MDR bacterial from drinking water

Isolate	MIC (mg/mL) Hot*A. melegueta*	MIC (mg/mL) Cold *A. melegueta*	MBC (mg/mL) Hot*A. melegueta*	MBC (mg/mL) Cold*A. melegueta*
Salmonella sp	25	50	00	00
Escherichia coli	25	25	00	00
S. aureus	25	50	00	00
Proteus sp	25	50	00	00
Klebsiella sp	25	50	00	00

MDR: Multidrug Resistant

The finding from this study conforms to previous reports that explain that the stronger antibacterial activity of *O. gratissimum* is relative to *A. melegueta*. Existing studies have reported the broad-spectrum antibacterial efficacy of *O. gratissimum* extracts against enteric as well as waterborne pathogens, is as a result of its rich bioactive components [28, 27]. The reduced activity observed in *A. melegueta* does not conform with previous reports that describe the activity of *A. melegueta* to be moderate to strong when organic solvents are used, this indicate that the choice of solvent can play an important role in extracting the bioactive compounds [26]. These differences explain the significant of the method of extraction in studying antimicrobial activity of plants against microorganisms.

The results from the present study indicates that use of hot water to extract *O. gratissimum*, revealed better activity compared to cold water against the organisms that were tested. The present study also supports the use of local medicinal plants in managing waterborne pathogens.

Conclusion

The findings from the present study indicate that some of the drinking water sources in Calabar possess physicochemical features that can accommodate microbial safety. This proof is seen by the suboptimal pH, turbidity, as well as low residual chlorine levels, also, with the isolation of multidrug-resistant bacteria. Aqueous extracts of *Ocimum gratissimum* and *Aframomum melegueta* showed antibacterial activity on the isolates, whereby, *O. gratissimum* showed strong inhibitory effects, especially when hot water was used to extract the plant. The present study also showed that hot water extraction was more efficient compared to cold-water extraction, this indicates the impact of the method of extraction on antibacterial performances. The findings from the MIC and MBC results showed that the extracts acted as bacteriostatic agent. In total, the existence of MDR bacteria in drinking water sources indicates an important public health concern. The inhibitory activity observed in the locally available medicinal plants indicates the possibility of using them as supportive value to addressing microbial contamination, without replacing the need for effective water treatment as well as surveillance.

Recommendations

1. There should be a routine monitoring of water Treatment Company to ascertain the level of compliance by the authority, the Water quality association of Nigeria.
2. There should be a proper and improved method of extraction as well as multiple concentrations in impending studies on *A. melegueta* and *O. gratissimum.*
3. There should toxicity level check and safety evaluations measures of the medicinal plants for practical applications.
4. There should be a proper implementation and routine surveillance of antimicrobial resistance in the environment and water sources.

Conflicts of interest: No conflicts of interest

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