Antibacterial Activity of *Ocimum gratissimum* Against Drug Resistant Bacteria Isolated from Drinking Water Quality in Calabar, Cross River State, Nigeria

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Abstract: This study was conducted to investigate the potential of *Ocimum gratissimum* against drug resistant bacteria isolated from drinking water quality in Calabar, Cross River State, Nigeria. Water samples were collected from 20 locations following standard procedure. Membrane filtration technique was adopted for isolation of the bacteria. The physicochemical analysis was all within World Health Organisation (WHO) standard and Nigeria Industrial Standard for Drinking Water Quality (NISDWQ). The phytochemical analysis carried out revealed the presence of amides, tannins, saponins, phlobatanins, steroids, reducing sugars, glycosides, flavonoids, anthraquinones, and alkaloids. Out of 66 bacteria isolated, 6 species where identified of which *Staphylococcus aureus* was the dominant organisms with percentage frequency of 19 (28.79%), *Escherichia coli* 14 (21.21%), *Salmonella* spp 13 (19.70%), *Pseudomonas aeruginosa* 9 (13.63%), *Proteus* spp 8 (12.12%), and the least *Klebsiella* spp 3 (4.55%). The ethanol, methanol and water extract of *Ocimum gratissimum* show activity against the test organisms. *Staphylococcus aureus* was more susceptible to the extract with mean zone of inhibition of (24.5±0.7mm) and (22.5±2.1 mm) in 100mg/mL respectively. *Escherichia coli* (17.5±0.7mm), and *Klebsiella* spp (15±0.0 mm), no activity was recorded in water extract of *Ocimum gratissimum* against *Pseudomonas aeruginosa* in 25mg/mL. There was a significant difference in the various concentrations of the extract at $P<0.05$ against the test organisms. The minimum inhibitory concentration of *Ocimum gratissimum* against the test organisms in ethanol, methanol and water extract ranges between 0.39-50mg/mL, 0.78-50mg/mL, and 12.5-50mg/mL respectively. The study revealed that all extraction solvents show activity against the test organisms but ethanol had better activity. Hence, the plant can be an alternative source of drug for the treatment of water related diseases.

Keywords: *Ocimum gratissimum*, Physicochemical, Antibacterial, Steroids.

INTRODUCTION

There is no gainsaying that quality and availability of water is of utmost importance to man's continuous existence and human body physiology. Its significance to public health cannot be overemphasised [1-3]. Available, accessible, adequate and safe supply of water – vital for life sustenance – must be made available to all [4]. To prevent health hazards in urban and rural settlements, the provision of "potable" water is necessary [5,6]. To be regarded as potable, water has to meet compliance to specific standards – physical, chemical and microbiological – as regulated and recommended by WHO and other regional and international standard organisations; designed to make water ingestible and free from harmful substances [7].

Water can be obtained from different sources (rain, streams, lakes, ponds, etc.). However, only a few sources of water are pure and safe in nature and these are frequently polluted by environmental factors as well as anthropological activities. Therefore, water is mostly unfit for immediate and direct consumption without undergoing purification processes [8,9]. Reservoirs of water are rich in water-surviving microorganisms which are dangerous to human health. An assessment of microbial qualities of water supplies in Nigeria showed an unacceptable level of microbial contaminants, far exceeding W.H.O. recommended levels [8, 10-12].

Numerous diseases – termed waterborne diseases – have been attributed to the consumption of untreated water and constitute a significant burden to public and human health. These diseases have been reported to be responsible for the death of 5 million children annually and morbidity of one-sixth of the global population [4,13,14]. Some of the pathogens that have been

reported to be isolated from water include *E. coli, V. cholera, Pseudomonas, Proteus, Shigella, Hepatitis A and E viruses, and Toxoplasma gandii* which have been clinically proven to cause a range of diseases including cholera and typhoid [12,15,16].

Although these waterborne diseases are not lethal, there have been reports of drug resistance of microbes isolated from water. Lack of compliance to drug prescription, unauthorised and continuous and improper use of antibiotics have been reported to be responsible for the emergence of drug resistance. With about 17 million deaths globally, drug resistance has been regarded as the greatest threat to world health [17-20]. The use of medicinal plants for the treatment of diseases is quite common in Africa and Asia. *Ocimum gratissimum* commonly known as Scent leaf is a plant that is common in Nigeria and is believed to contain important volatile oil and chemical compounds which has been reported to be active against pathogenic microorganisms. *O. gratissimum* has also been reported to be a barrier against some species of fungi and bacteria [21]. It is therefore essential to regularly screen water for microbial contaminants and the antibiotic spectrum of waterborne pathogens. It is in this regards that this study aims to investigate the potential of *Ocimum gratissimum* against drug resistant bacteria isolated from drinking water quality in Calabar, Cross River State, Nigeria.

MATERIALS AND METHODS

Study Area, Collection of Plants and Water Samples

The study was conducted in Calabar, Cross River State and Lafia, Nasarawa State. Both towns are located in Nigeria. Fresh leaves of *Ocimum gratissimum* (Scent leaf) was purchased in Lafia Modern Market of Nasarawa State, Nigeria and then transported to the Department of Plant Science and Biotechnology for authentication. Water samples were collected from 20 different locations in Calabar following standard procedure by WHO [22]. Samples were collected using a sterile 500mL bottle separately at 20 sites throughout the sampling period.

PHYSICOCHEMICAL ANALYSIS

The Physical and chemical parameters of the water samples were performed as described by Hill *et al*., [23] and this includes temperature, pH, conductivity, turbidity, total dissolved solid, total and free chlorine.

DETERMINATION OF PHYTOCHEMICALS

Qualitative phytochemical analysis was done with the use of standard phytochemical methods as described by Harborne [24].

PREPARATION OF PLANT MATERIALS

The leaves of *Ocimum gratissimum* were washed using tap water from the laboratory and air dried at room temperature for 10 days and then ground into fine powder using a blender. The ground sample was extracted using ethanol, methanol and water as solvents. 40g of the powdered sample was dissolved in 400mL each of the extraction solvents for 72hrs. The dissolved sample was filtered using Whatman grade 1 filter paper (5 times each to get rid of any debris) and was concentrated using a rotary evaporator and then oven dried at 60%. It was then resuspended by dissolving them in ethanol, methanol, and water to the concentration of 100mg/mL, 50mg/mL, and 25mg/mL, and stored in a refrigerator prior to analysis.

MEDIA PREPARATION.

All media were prepared based on manufacturer's instruction and autoclaved at 121^0C for 15mins.

BACTERIAL ISOLATION

Membrane filtration technique was adopted using membrane filters (0.45µm pore size). The filter papers were picked using sterile forceps and placed onto appropriate agar plates. The plates were incubated for 24hrs at 37^0C. Presumptive colonies of *Staphylococcus aureus, Escherichia coli, Proteus* spp*, Klebsiella* spp*, Pseudomonas aeruginosa and Salmonella* spp were picked and streaked onto nutrient agar plates and then incubated for 24hrs at 37^0C. Pure colonies from NA plates were inoculated into 5mL of peptone broth, and incubated for 24hrs at 37^0C. Susceptibility test by Kirby-Bauer was performed using appropriate antibiotics to determined drug resistant bacteria.

ANTIMICROBIAL ACTIVITY

The three extracts were tested for antimicrobial activity against the test organisms using the agar well diffusion technique as described by Ubafie and Ejale, [25]. A 6mm Wells were made on mueller Hinton agar plates using a sterile cork borer. 40µL each of 100mg/mL, 50mg/mL, and 25mg/mL of the extract was added to the wells. The plates were properly labelled and incubated for 24hrs at 37^0C.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATIONS (MIC)

Broth dilution technique was adopted making use of 96-well microtitre plates. The minimum inhibitory concentration was the least concentration of the extract that inhibited the bacteria growth.

RESULTS

Table 1: *Mean Physicochemical Quality of drinking water samples*

S/N	PARAMETERS	WHO/NISDWQ	RW	FM	SW	FW	GLR	EWT
1	Temperature (^{0}C)	Ambient	25.19	26.3	23.7	24.9	25.1	24.3
2	pH	6.5-8.5	7.2	6.4	5.93	5.77	5.67	5.7
3	Conductivity (µs/cm)	500	22.7	53.5	56.3	57.1	54.3	51.6
4	Turbidity (NTU)	5	11.8	14.7	1.2	1.1	0.6	1.7
5	Total Dissolved Solid (mg/l)	500	51.6	41.7	36.3	32.5	30.8	30.6
7	Free Chlorine (mg/l)	0.2					0.3	0.07
8	Total chlorine (mg/l)	0.5					0.4	0.05

KEY=**NISDWQ**-Nigerian Industrial Standard for Drinking Water Quality, **EWT**-Elevated Water Tank, **GLR**-Ground Level Reservoir, **SW**- Settled Water, **FW**-Filtered Water, **RW**- Raw Water, **FM**- Flash Mixer

Table 2: Phytochemical component *Ocimum gratissimum* (Scent leaves)

Parameters	*Ocimum. gratissimu* (water)	*Ocimum gratissimum* (ethanol)	*Ocimum gratissimum* (methanol)
Amides	+	+	+
Tanins	+	+	+
Saponins	+	+	+
Phlobatanins	-	+	-
Steroids	+	+	+
Reducing sugars	+	+	-
Glycosides	+	+	+
Flavonoids	+	+	+
Anthraquinones	-	-	-
Alkaloids	+	-	-

Key; + = present, -- =Absent

Table 3: Percentage occurrence of bacterial isolates from water sample

Bacterial isolates	Frequency (N)	Percentage occurrence (%)
Escherichia coli	14	21.21
Salmonella spp	13	19.70
Staphylococcus aureus	19	28.79
Proteus spp	8	12.12
Pseudomonas aeruginosa	9	13.63
Klebsiella spp	3	4.55
Total	**66**	**100**

Table 4: *Mean zone of inhibition of bacterial isolates in (mm) on ethanol extract of Ocimum gratissimum (Scent leaves)*

Conc.	*E. coli*	*Salmonella*	*S. aureus*	*Klebsiella*	*Proteus*	*P. aeruginosa*	*F-val*	*P-value*
100mg/mL	17.5±0.7	13±0.0	24.5±0.7	15±0.0	13±0.0	10.5±0.7	196.33	<0.000
50mg/mL	12.5±0.7	10.5±0.7	18.5±0.7	12±1.4	9.5±0.7	8±1.4	26.73	<0.000
25mg/mL	7.5±2.1	8±1.4	15.5±0.7	9±0.0	6±1.4	4.5±0.7	18.36	<0.001

P<0.05

Reference control = Streptomycin (32.5), Ciprofloxacin (36)

Table 5: *Mean zone of inhibition of bacterial isolates in (mm) on methanol extract of Ocimum gratissimum (Scent leaves)*

Conc.	*E. coli*	*Salmonella*	*S. aureus*	*Klebsiella*	*Proteus*	*P. aeruginosa*	*F-val*	*P-value*
100mg/mL	14.5±0.7	12±1.4	22.5±2.1	12±0.7	10±1.4	8±1.4	26.58	<0.001
50mg/mL	11.5±2.1	10±1,4	17±1.4	10.5±0.7	8±1.4	5.5±2.1	11.56	<0.005
25mg/mL	6±1.4	6.5±0.7	13.5±0.7	7±1.4	5±1.4	2.5±0.7	21.56	<0.001

P<0.05

Reference control = Streptomycin (32.5), Ciprofloxacin (36)

Table 6: *Mean zone of inhibition of bacterial isolates in (mm) on water extract of Ocimum gratissimum (Scent leaves)*

Conc.	*E. coli*	*Salmonella*	*S. aureus*	*Klebsiella*	*Proteus*	*P. aeruginosa*	*F-val*	*P-value*
100mg/mL	8.5±2.1	7±1.4	13.5±2.1	7.5±2.1	6.5±2.1	2.5±0.7	7.40	<0.01
50mg/mL	5.5±0.7	5±1.4	11.5±0.7	4.5±2.1	4.5±0.7	0.5±0.7	17.75	<0.002
25mg/mL	2.5±0.7	3.5±0.7	9.5±0.7	3±1.4	1.5±0.7	00±0.0	32.00	<0.000

P<0.05

Reference control = Streptomycin (32.5), Ciprofloxacin (36)

Table 7: Minimum inhibitory concentration of bacterial isolates in (mg/mL) on ethanol, methanol, and water extract of *Ocimum gratissimum*

Organisms	Ethanol extract	Methanol extract	Water extract
Escherichia coli	0.78	1.56	25
Salmonella spp	1.56	6.25	50
Staphylococcus aureus	0.39	0.78	12.5
Klebsiella spp	6.25	12.5	50
P. aeruginosa	50	-	-

Key: -- = no activity

RESULTS AND DISCUSSIONS

The physicochemical quality of drinking water samples from Calabar, Cross River State revealed that the pH values were in accordance with World Health Organisation Standard (WHO) and Nigeria Industrial standard for Drinking Water Quality (NISDWQ). Although slightly acidic. The present study is consistent with the findings by Udom *et al.,* [26] who recorded similar pH. Temperature values ranged between 23.7-25.19^{0}C which is in line with the recommended value of 25±2^{0}C. Braide *et al.,* [27] also recorded similar temperature values. The conductivity level values ranged between 22.7-57.1µs/cm. The turbidity and total dissolve solid all fall within the standard by WHO and NISDWQ.

The microbial analysis indicates high microbial contamination of the water distribution channels in Calabar. Out of 66 bacteria isolated, 6 species were identified of which *S. aureus* was the dominant organisms with percentage frequency of (28.79%), *E. coli* (21.21%), *Salmonella* spp (19.70%), *Pseudomonas aeruginosa* (13.79%), *Proteus* spp (12.12%), and the least was *Klebsiella* spp (4.55%). This present study shows the presence of coliform and faecal coliform in the drinking water quality which revealed the quality of the water samples at different locations in Calabar. The phytochemical parameters revealed the presence of amides, phlobatanins, reducing sugars, anthraquinones, alkaloids, tannins, saponin, steroids, flavonoids and glycosides. This agrees with the findings of Agholor *et al.,* [28].

The ethanol, methanol and water extract of *Ocimum gratissimum* show activity against the test organisms. *Staphylococcus aureus* was more susceptible to the extract with mean zone of inhibition of 24.5±0.7mm and 22.5±2.1mm in 100mg/mL respectively. *Escherichia coli* 17.5±0.7mm, and *Klebsiella* spp 15±0.0mm, and no activity was recorded in water extract of *Ocimum gratissimum* against *Pseudomonas aeruginosa* in 25mg/mL. The result of antibacterial activity of ethanol, methanolic and water extract of *Ocimum gratissimum* on the test organisms revealed decreased in mean zone of inhibition with decreased concentrations of the extract. As the concentrations decreases, the antibacterial activity decreases. This conforms with the work of Agholor *et al*., [28], who observed a decrease in antibacterial activity with decrease in concentration of the extract as the concentration decreases from 0.20-0.025mg/mL. The present study shows that ethanol extract of *Ocimum gratissimum* was a better extraction solvent than water and methanol. This also corroborate with the study conducted by Amjad, [29] who recorded a high mean zone of inhibition using ethanol extract of *Ocimum gratissimum*. A similar result was obtained by Adebolu and Oladineji [30] against *Staphylococcus aureus* and *Escherichia coli*.

The minimum inhibitory concentration of *Ocimum gratissimum* against the test organisms in ethanol, methanol and water extract ranges between 0.39-50mg/mL, 0.78-50mg/mL, and 12.5-50mg/mL respectively. The low minimum inhibitory concentration values in the present study suggest the antibacterial efficacy of the plant. *Staphylococcus aureus* had the least MIC value of 0.39mg/mL followed by *Escherichia coli* and *Salmonella* spp with MIC values of 1.56mg/mL respectively. While *Klebsiella* spp, *Proteus* and *Pseudomonas aeruginosa* had the highest MIC values of 50mg/mL respectively. This work supports the traditional use of the leaves of *Ocimum gratissimum* (Scent leaves) for the treatment of water related diseases.

The present study shows the effectiveness of the leaves extract of *Ocimum gratissimum* against the test organisms, suggesting that the plant can be used as an agent against bacterial infections. The antibacterial

effect of *Ocimum gratissimum* is due to the phytochemical present in it.

CONCLUSION

The study revealed that *Staphylococcus aureus* was more susceptible to the extracts of *Ocimum gratissimum* and the ethanolic extract was a better extraction solvent. The plant contains important parameters that possess antibacterial property which could be used as an alternative therapy for the management of ailment from water related diseases. Faulty equipment in water treatment plant should always be replaced immediately especially leakages in other to ensure seamless production of quality water in conformity with NISDWQ and WHO standards.

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