ISSN No. 2456-4400
Int J Med Lab Res 2018, 3(3): 39-51

IJMLR International Journal of Medical Laboratory Research

ORIGINAL ARTICLE

ANTIBACTERIAL EFFICACY OF *OCIMUM GRATISSIMUM* ON MULTIDRUG RESISTANT *STAPHYLOCOCCUS AUREUS* AND *KLEBSIELLA PNEUMONIAE* ISOLATED FROM CLINICAL AND ENVIRONMENTAL SAMPLES IN ONDO STATE.

*OJOKOH, Anthony Okhonlaye[1], OJO, Martha Oluwatosin[2]

[1,2]*Department of Microbiology, Federal University of Technology, Akure, PMB 704, Nigeria*

Received: 1 December, 2018/ Accepted: 10 December, 2018

ABSTRACT: The rise in antibiotic resistance among pathogenic bacteria is a major public health concern. Hence, the need for natural safe and effective antimicrobial agents. This study therefore assayed the antibacterial efficacy of *Ocimum gratissimum*on multidrug resistance *Klebsiella pneumoniae* and *Staphylococcus aureus* isolated from clinical and environmental samples in Ondo State. Absolute ethanol, n-hexane and distilled water were used as extraction solvent, agar well diffusion method was used for the antibacterial efficacy of the extracts and phytochemical composition of the extracts was carried out using Analytical Methods Committee of Royal Society of Chemistry. The results showed that aqueous extract has the highest percentage yield and there was significant difference ($p \leq 0.05$) in quantitative phytochemical components of the extracts. N-hexane extract has the least phytochemical constituents. Among the tested *K .pneumoniae* and *S. aureus* isolated from clinical and environmental sources. *S. aureus* isolated from urine and *K. pneumoniae* isolated from wound samples were inhibited most by the extract. *K. pneumoniae* and *S. aureus* isolated from the air showed the least inhibition by the extract. Also, n-hexane extract showed the least inhibitory effect compared to the other extracts. However when extracts of the plant from different solvents were combined the inhibition surpassed that of single extract. Therefore it could be recommended that the extract of this plant be used for the treatment of infection caused by *K. pneumonia* and *S.aureus* since the extract has demonstrated anti-Staphylococcal and anti-Klebsiella activity irrespective of the sources *in-vitro.*

KEY WORDS: solvent, extract, multidrug resistant, phytochemical, inhibition

INTRODUCTION:

Klebsiella pneumoniae is a gram negative, bacilli, non- motile, encapsulated, lactose fermenting facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin and intestines and causative agent of many diseases, such as pneumonia, burns, urinary tract infection, wound infection and pyogenic liver abscesses.[1]*Klebsiella pneumoniae* have become important pathogens in nosocomial infections, It is clinically the most

Corresponding Author:
OJOKOH, Anthony Okhonlaye,
Department of Microbiology, Federal University of Technology, Akure, PMB 704, Nigeria

important member of klebsiella genus of *Enterobacteriaeceae*. *Enterobacteriaceae* are ubiquitous organisms found worldwide in soil, water, and vegetation and are part of the normal intestinal flora of most animals, including humans. These bacteria cause a variety of human diseases, including one quarter to one third of all bacteremia, more than 70% of urinary tract infections (UTIs), and many intestinal infections. It naturally occurs in the soil and about 30% of strains can fix nitrogen in anaerobic condition. *Klebsiella* infections tend to occur in people with weekened immune system. It is an opportunistic pathogen for patients with chronic pulmonary disease. Antibiotics treatment of *Klebsiella pneumonia* should be always guided by in vitro susceptibility test.[2] Multidrug resistant bacteria cause serious nosocomial and community acquired infections that are hard to eradicate by using available antibiotics. Moreover, extensive use of broad-spectrum antibiotics in hospitalized patients has led to increased prevalence of *Klebsiella pneumoniae*as well as development of multidrug resistant strains of *Klebsiella pneumoniae*. Pathogenicity of *Klebsiella pneumoniae* is due to the presence of many virulence genes which encode virulence factors that allow it to attack the immune system of mammalians and cause many kind of diseases. Some of these virulence factors are biofilm formation, hypermuco-viscosity, capsule synthesis, adhesions, iron uptake and lipopolysaccharides formation.[3] Many clinical features of *Klebsiella pneumoniae* infection are related with virulence gens according to number and mode of action of these genes.[4] Recently *Klebsiella pneumoniae*is found causing acute liver abscess as reported in many Asian countries like China, Kuwait and Iraq.[3,5]

Staphylococcus aureus is a commensal and major pathogen of human. The bacterium is important in human infections ranging from minor skin- infections to serious life threatening infections that may include endocarditis, deep seated abscesses, septicaemia, food borne illness, toxic shock syndrome and many other infections. Infection caused by multi resistant strains of multi resistant strains of *Staphylococcus aureus*are characteristically resistant to three or more classes of antimicrobial agents other than beta lactams. These strains have been recognized as the most common pathogen identified in wound infections.[6]The environment has been determined as a factor in transmission of resistant strains of *Klebsiella pneumoniae* and *Staphyloccous aureus* especially via air and air formites. The ability of *Klebsiella pneumoniae* and *Staphyloccous aureus* to survive in various environments for extended period of time without loss in viability or virulence enables it to spread to other sources and also community.[7]

In the previous study, it was observed that *Staphylococcus aureus* and *Klebsiella pneumonia* counts in Ondo North (wound; $50.20\pm0.00 \times 10^4$cfu) and Ondo central (well water; $42.33 \pm 0.03\times10^4$cfu/ml) senatorial district respectively. *K. pneumonia* recovery rate are;23(23.71%) from market soil, 20(11.30%) from post-operative wound, 26(10.36%) from urine, 40(38.36%) from market well water and 13 (21.31%) hospital air while *S. aureus* was most prevalent in post-surgical wound 50 (28.25%). In Ondo north, *K. pneumonia* isolates were at least 68% resistant to septrin, chloramphenicol, amoxacillin, and sparfloxacin while in south they were 70% resistant to septrin, chloramphenicol and amoxicillin however, all *S. aureus* isolates were 100% resistant to amoxicillin and were at least resistant to five different antibiotics. *K. pneumonia* isolated from post-operative wound have resistance pattern of Septrin (71%), Chloramphenicol (13%), Amoxacillin (56%) and Sparfloxacin (56%) and in market soil the resistance pattern are; Augumentin (98%), Pefloxacin (99%), Septrin (98%), Chloramphenicol (97%),

Gentamycin (100%), Ofloxacin (98%), Amoxacillin (98%), ciprofloxacin (98%), Sparfloxacin (98%) and Streptomycin (100%).All *S. aureus* isolated from urine samples were 100% resistant to streptomycin, chloramphenicol, ceftriazone, erythromycin, cotrimoxazole and gentamycin.[8] As a result of this, there is quest to salvage the menace that may have emanated from this antibiotic resistance exhibited by these isolates.

The rise of antibiotic resistance has been linked with the widespread use of antibiotics in humans and now recognised as a global problem, this antibiotic resistance among pathogenic bacteria is a major public health concern.[9]Hence, there is need to look for safe and effective antimicrobial agent. It is therefore worthwhile to study the antibacterial activity of *Ocimum gratissimumon* multidrug resistance *Klebsiella pneumoniae* and *Staphylococcus aureus*isolated from clinical and environmental samples in Ondo State.

In the coastal area of Nigeria, the plant *Ocimum gratissimum*is used in the treatment of epilepsy, high fever and diarrhea. *Ocimum gratissimum* (Scent leaf) is a perennial plant which is widely distributed in the tropics of Africa and Asia. It belongs to the family Labiatae and it as the most abundant of the genus *Ocimum.* In the southern part of Nigeria, it is called "Efirinnla" by the Yoruba speaking tribe. "Nichonwu" in Igbo while in the northern part of Nigeria, it is called "Daidoga".[10]

MATERIALS AND METHODS:

Collection of sample, isolation and occurrence of Klebsiella pneumonia and Staphylococcus aureus

- ***Sample collection***

A total of 690 clinical and environmental samples were collected from three senatorial districts in Ondo State (Ondo north, south and central). Clinical samples (urine and post-surgical wound) and environmental samples (market soil, market well water and outdoor hospital air) were collected across Ondo state, Nigeria. Methods used in sample collection, isolation of *Klebsiella pneumonia* and *Staphylococcus aureus*, the occurrences of the isolates and antimicrobial susceptibility to commercially available antibiotics were reported in the previous study.[8]

- ***Standardization of inoculum (McFarland Turbidity standard)***

The McFarland 0.5 turbidity standard which was used to measure the density of bacterial cells.[11]In this method, fifty milliliter (50ml) of a 1.175% (wt/vol) dehydrates Barium chloride ($BaCl_2.2H_2O$) solution was added to 99.4ml of 1% (vol/vol) sulfuric acid. McFarland standard tube was then sealed with Paraffin to prevent evaporation and stored in the dark at room temperature. The accuracy of the density of a prepared McFarland standard was checked by using a spectrophotometer with a 1cm light path. The 0.5 McFarland standards were vigorously agitated before use.

- ***Antimicrobial susceptibility test***

Antibiotic susceptibility test of bacteria was determined by the single disc diffusion method with the use of Mueller-Hinton agar, according to the Bauer-Kirby method.[12] The suspension of the test organism in nutrient broth was matched with 0.5 McFarland turbidity standards to give concentration of 1.5×10^8 CFU/ml, 0.5ml of the suspension was transferred to prepared Mueller-Hinton agar and spread with a sterilized glass spreader, excess suspension was drained. The surface of the agar was allowed to dry and antibiotic disc was aseptically picked and gently placed on top of agar plate by sterile forceps. The inoculated plates were incubated at 37^{o}C for 18 hours, after incubation a clear zone of no growth in the immediate vicinity of an antibiotic disk was measured and recorded as zone of inhibition in millimeter (mm) and interpreted as resistant, susceptible or intermediate.[13]

- ***Quality control for Antimicrobial Susceptibility Test***

Typed culture (*Klebsiella pneumoniae* ATCC 33495 and *Staphylococcus aureus* ATCC 25923) was used as quality control for antimicrobial susceptibility testing as recommend by Clinical and Laboratory Standards Institute.[13]

- ***Processing and Extraction of Ocimum-gratissimum Leaves***

The fresh leaves were washed with sterile distilled water and air dried on a mat until they turned brittle and fully crispy. The dry leaves were crushed manually using clean mortar and pestle, then pulverized into fine powder by a blending machine (Philips HR2001). They were separately kept in an airtight container to avoid the absorption of moisture. The powdered samples were soaked for 72 hours (3 days) in absolute ethanol, n-hexane and distilled water in the ratio 1:10 each (500 g of the powdered sample in 5000 ml of solvent) as solvents used for the extraction of the bioactive compounds from the plants, and this crude extraction was done at a room temperature. After 72 hours it was sieved using muslin cloth and then filtered using Millipore filter paper. The filtrates were vaporized to dryness using rotary evaporator (Union Laboratories England). The extracts were preserved in a sterile bottle at 4 °C ready for use. Each extract was tested for sterility by introducing 2ml of the supposed sterile extract into 10ml of sterile nutrient broth. Incubation was done at 37°C for 24 hours. A sterile extract was indicated by absence of turbidity or clearness of the broth after the incubation period.[14,15]

- ***Phytochemical Screening***

The phytochemical analysis was carried out according to the standard methods of analysis by Analytical Methods Committee of Royal Society of Chemistry.[16]

Antibacterial efficacy of Ocimum gratissimum extracts on multidrug resistant Staphylococcus aureus and Klebsiella pneumoniae

The antimicrobial activity of the ethanol, n-hexane and water extracts was determined by the agar well diffusion method.[17] Stock cultures were maintained at 4°Con nutrient agar slope. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Nutrient broth (NB) for bacteria and were incubated without agitation for 24hours at 37^0C. To 5ml of NB, 0.2 ml of culture was inoculated and incubated till it reached the turbidity equal to that of the standard 0.5 McFarland solution at 600nm which is equivalent to 10^6-10^8 CFU/ml.

Molten Muller Hinton agar (20ml) cooled to 45^0Cwas poured into sterilized petri dishes and left to solidify. An aliquot of culture (0.1ml) was evenly spread on the surface of the solidified Muller Hinton agar plates. Wells of 8mm were bored in the agar with sterile cork borers (7mm). The crude extracts (2grams) were dissolved in 20% dimethylsulfoxide (DMSO) of 200mg/ml and filtered through 0.22µm membrane filter and then introduced into each wells with the aid of a micropipette. Reference antibiotic Ciprofloxacin and DMSO (30%) was used as positive and negative control respectively. The plates were allowed to stand for one hour at room temperature (26 ± 2°C) to allow a proper diffusion of extracts. The plate were then incubated 37°C for 24hours. Inhibition zones were measured with a ruler in triplicates (three plates per indicator organism).

Minimum Inhibitory Concentration (MIC) of Ocimum gratissimum extracts on multidrug resistant Staphylococcus aureus and Klebsiella pneumoniae

The tube dilution method was used. The MIC was determined by serially diluting extracts. 5ml

of each of the dilution representing a known concentration of the extract was introduced into 5ml of sterile Nutrient broth in test tubes. The mixture was then inoculated with 0.1ml of test organism previously standardardized to 10^6 and then incubated at 37^0C for 24hours. The least concentration of the extract with no turbidity was taken as the minimum inhibitory concentration (MIC).[18]

Minimum Bactericidal Concentration (MBC) of Ocimum gratissimum extracts on multidrug resistant Staphylococcus aureus and Klebsiella pneumoniae

This is an off shoot of the previously determined MIC. The MBC of the *O. gratissimum*extracts was determined by subculturing from all tubes that showed no turbidity from MIC into a sterile nutrient plate, the least concentration in which no growth was observed and taken as the MBC. DMSO solution (30%) was used as a negative control. The tests were performed in triplicates.[18]

Screening for synergetic effect of Ocimum gratissimum extracts on multidrug resistant Klebsiella pneumoniae and Staphylococcus aureus

Different ratios of ethanol, n-hexane and aqueous extracts of *Ocimum gratissimum*was combined where ethanol and n-hexane extracts, ethanol and aqueous extracts, n-hexane and water extracts were combined respectively. The combination added to different wells was noted. Standard antimicrobial agent, ciprofloxacin (100mg/ml) and DMSO (20%) were used as positive and negative control for the isolated bacteria respectively. The plates were then incubated at 37^0Cfor 24hours. Antimicrobial activity was determined by measuring the diameter of the clear inhibition zone around each well by standard procedures.[13]After incubation, the zones of inhibition were measured. This was done in triplicate.

Ethical Approval

To carry out this study, authors were given ethical approval by the Ondo State Hospital Management board to carry out microbiological analysis on urine and wound swab collected from different hospitals in the state. Prior to the collection of the sample, the nature of the study was explained to the patients and they were assured that their identity will not be revealed, after which those that are not willing to participate were allowed to withdraw willingly and others that are willing to participate gave a verbal consent to participate in the study.

RESULTS :

Effects of extraction solvent on Percentage yield of Ocimum gratissimum extracts and phytochemical analysis.

Table 1 revealed the effect of extraction solvents on the percentage yielded of *O. gratissimum extracts* after extractions. Aqueous extract has the highest percentage yielded. **Table 2a and 2b** revealed the presence and absence of some phytochemical constituent in ethanol, n-hexane and aqueous extracts and the quantitative analysis of each extract. The result revealed that there was significant difference ($p \leq 0.05$) in quantitative analysis of the extracts. N-hexane extract has the least phytochemical constituents.

Table 1: Percentage yield of extracts

Solvents	Percentage yield (%)
Ethanol	2.33
n-Hexane	2.6
Water	3.65

Table 2a: Effect of Extraction Solvent on Qualitative Phytochemical Constituents of *Ocimum gratissimum* leaves

Phytochemicals	Ethanol extract	n-Hexane extract	Water extract
Tannins	+	-	+
Saponins	+	-	-
Flavonoids	+	-	+
Alkaloids	+	-	+
Steroids	+	++	+
Terpenoids	+	-	+
Phlobatannins	+	-	+
Glycoside	+	-	+
Cardiac compound	+	+	+

Key: - = absent, + = present

Table 2b: Effect of Extraction Solvent on Quantitative Phytochemical Constituents of *Ocimum gratissimum* leaves

Phytochemicals	Ethanol extract	n-Hexane extracts	Water extracts
Tannins	4.75±0.27^{c}	0.00±0.00^{a}	3.43±0.09^{b}
Saponins	3.03±0.24^{b}	0.00±0.00^{a}	0.00±0.00^{a}
Flavonoids	1.05±0.29^{b}	0.00±0.00^{a}	1.89±0.16^{c}
Alkaloids	3.89±0.82^{b}	0.00±0.00^{a}	4.56±0.49^{b}
Polyphenol	1.97±0.14^{b}	0.26±0.07^{a}	1.43±0.35^{b}

Values are means ± SE of samples. Values in the same row carrying the same superscript are not significantly different at ($p \leq 0.05$) using Duncan's New Multiple Range test

Multidrug resistant patterns of the bacterial isolates used for this study

The result showed in **Table 3** revealed that all the bacterial isolates used in this study were resistant to at least three different antibiotics except the isolate of *Klebsiella pneumoniae* isolated from urine sample.

Table 3: Antibiotic resistant patterns of *Staphylococcus aureus* and *Klebsiella pneumoniae* isolated from different sources in Ondo state, Nigeria

Isolates	AMX	OFL	STR	CHL	CEF	PEF	ERY	COT	CPX	GEN	AUG	SP
SS	R	R	S	S	S	S	S	R	S	R	ND	ND
SW	R	S	R	R	R	S	R	R	S	R	ND	ND
SU	R	S	R	R	R	R	R	R	S	R	ND	ND
SWW	R	S	R	S	S	S	S	R	S	R	ND	ND
SA	R	R	R	R	S	S	S	R	S	S	ND	ND
KS	R	R	R	R	ND	R	ND	R	R	R	R	R
KW	R	S	S	R	ND	S	ND	R	S	S	S	R
KU	S	S	S	S	ND	S	ND	S	S	S	S	S
KWW	R	S	S	R	ND	S	ND	R	S	R	R	R
KA	R	S	S	R	ND	S	ND	R	S	R	R	R

Keys: R= resistant, S= susceptible, ND = not determined, SS= *Staphylococcus aureus* isolated from market soil, SW= *Staphylococcus aureu s*isolated from post-operative wound, SU= *Staphylococcus aureus* isolated from urine, SWW= *Staphylococcus aureu s*isolated from well water, SA= *Staphylococcus aureus* isolated from hospital air, KS= *Klebsiella pneumonia e*isolated from market soil, KW= *Klebsiella pneumonia* isolated from post-operative wound, KU= *Klebsiella pneumoniae isolated* from urine, KWW=*Klebsiella pneumoniae isolated* from well water, KA= *Klebsiella pneumoniae isolated* from hospital air, AMX- Amoxycillin, OFL- Ofloxacin, STR- Streptomycin, CHL- Chloranphenicol CEF- Ceftriazone, PEF-Pefloxacin ERY- Erythromycin COT-Cotrimoxacin CPX- Ciprofloxaci, GEN- Gentamycin, AU- Augumentin, SP- Sparfloxacin

Antibacterial efficacy of Ocimum gratissimum extracts on antibiotic resistant Klebsiella pneumoniae and Staphylococcus aureus different sources in Ondo State

The result (Figure 1) revealed the antibacterial activity of *Ocimum gratissimum* extracts of three solvents (ethanol, n-hexane and water extract) against *K. pneumoniae isolated* from clinical and

environmental sources. The ethanol extract showed greatest antibacterial activity at different concentration while n-hexane showed the least antibacterial activity on *K. pneumoniae*. *K. pneumoniae* isolated from urine samples, ethanol extract showed zones of inhibition that ranges from (8mm-18mm), n-hexane extracts ranges from (0.1mm-6mm), and water extract ranges from (4mm-13mm). *K. pneumoniae* isolated from post-operative wound, ethanol extract showed zones of inhibition ranges from (6mm-23mm), n-hexane ranges from(0.1mm-7mm) and no zone of inhibition was found at concentration of 25mg/ml, and water extract ranges from (5mm-16mm). *K. pneumoniae* isolated from market soil, ethanol extract showed zone of inhibition ranges from (6mm-16mm), n-hexane ranges from (0.1mm-7mm) and no zone of inhibition was observed at concentration of 25mg/ml, and water extracts ranges from (4mm-14mm). *K. pneumoniae* isolated from well water, ethanol extract zones of inhibition ranges from (5mm-16mm), n-hexane extract ranges from (0.2mm-7mm) and no zone of inhibition was observed at concentration of 25mg/ml, and water extract ranges from (8mm-23mm), n-hexane ranges from (4mm-7mm), no zone of inhibition was found at concentration of 25mg/ml and 50mg/ml. *K. pneumoniae* isolated from hospital air sample, ethanol extract ranges from(6mm-16mm), n-hexane extract (4mm-9mm) and water extract (4mm-14mm). The result revealed that the effect of the extract on *K. pneumoniae* is concentration dependent. *K. pneumoniae* ATCC 33495 was used as quality control for antibacterial testing. The n-hexane extract showed the least inhibitory effect compared to the other extracts. The inhibition produced by ciprofloxacin (0.05%) was significantly higher ($p \leq 0.05$) than that produced by each of the extracts.

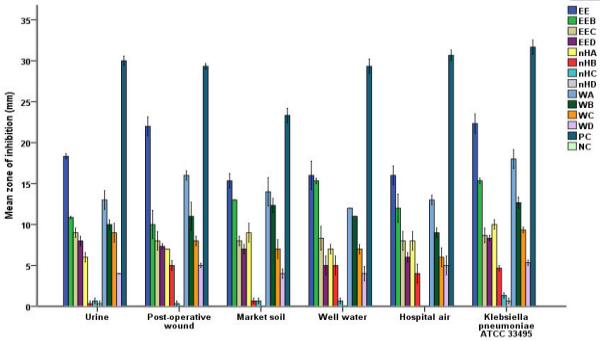
The result (Figure 2) revealed the antibacterial activity of *O. gratissimum* extracts against *S. aureus* isolated from clinical and environmental sources. The ethanol extract showed the greatest antibacterial activity of *O. gratissimum* extracts against *S. aureus* at different concentrations, followed by water extract, n-hexane showed the least antibacterial activity. *S.aureus* isolated from the urine samples, ethanol extract revealed zones of inhibition that ranges from (14mm-28mm), n-hexane revealed zone of inhibition only at concentration of 200mg/ml (10mm), and water extract ranges from (6mm-17mm). *S.aureus* isolated from post-operative wound, ethanol extract showed zones of inhibition that ranges from (14mm-24mm), zone of inhibition for n-hexane extract was observed only at concentration of 200mg/ml (12mm), and water extract ranges from (6mm-19mm). *S. aureus* isolated from market soil, ethanol extract showed zones of inhibition that ranges from (7mm-19mm), n-hexane extract only showed zone of inhibition at concentration of 200mg/ml (13mm), and water extract ranges from (6mm-17mm). *S. aureus* isolated from well water, ethanol extract revealed zones of inhibition ranges from (6mm-14mm), n-hexane revealed zone of inhibition at concentration of 200mg/ml (9mm), and water extract ranges from (5mm-13mm). *S. aureus* isolated from hospital air, ethanol extract ranges from (5mm-16mm), n-hexane extract (5mm-7mm) and water extract (4mm-14mm). Among the different solvent used, ethanol extract showed highest degree of inhibition followed by water extract. The n-hexane extract showed the least inhibitory effect compared to the other extracts. The inhibition produced by ciprofloxacin (0.05%) was significantly higher ($p \leq 0.05$) than that produced by each of the extracts.

Among the tested *K .pneumoniae* and *S. aureus* isolated from clinical and environmental sources. *S. aureus* isolated from urine and *K. pneumoniae* isolated from wound samples were inhibited most by the extract. *K. pneumoniae* and *S. aureus* isolated from the air showed the least inhibition by the extract.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Ocimum gratissimum extracts.

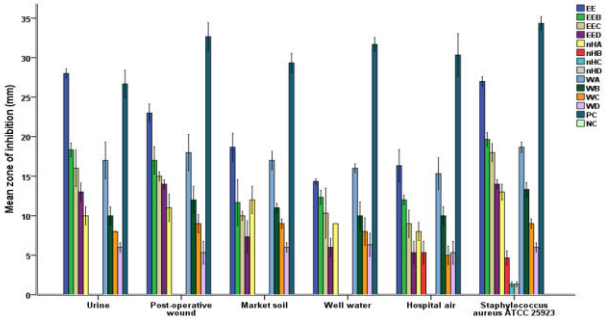
Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Ocimum gratissimum* extracts on multiple antibiotic resistant *K. pneumoniae* isolated from clinical and environmental sources are presented in **Table 4.** The range of MIC and MBC observed for *K. pneumoniae* in ethanol extract (18.75mg/ml) and (18.75mg/ml to 75.00mg/ml), n-hexane extract (18.75mg/ml to 75.00mg/ml) and (37.50mg/ml to 150mg/ml), and water extract (18.75mg/ml to 37.50mg/ml) and (37.50mg/ml) respectively while the range of MIC and MBC observed for *S. aureus* in ethanol extract (18.75mg/ml to 37.50mg/ml) and (18.75mg/ml to 75.00mg/ml), n-hexane extract (37.50mg/ml to 75.00mg/ml) and (75.00mg/ml to 150mg/ml), and water extract (18.75mg/ml to 37.50mg/ml) and (37.50mg/ml) respectively.

Figure 1: Antibacterial activity of *Ocimum gratissimum* extracts on antibiotic resistant *Klebsiella pneumonia* isolated from clinical and environmental sources.



Key: EE= ethanol extract 200mg/ml, EEB= ethanol extract 100mg/ml, EEC= ethanol extract 50mg/ml, EED= ethanol extract 25mg/ml, nHA= n-Hexane extract 200mg/ml, nHB= n-Hexane extract 100mg/ml, nHC= n-Hexane extract 50mg/ml, nHD= n-Hexane extract 25mg/ml, WA= water extract 200mg/ml, WB= water extract 100mg/ml, WC= water extract 50mg/ml, WD= water extract 25mg/ml, PC= positive control (ciprofloxacin 5mg/ml), NC= negative control (DMSO).

Figure 2: Antibacterial activity of *Ocimum gratissimum* extracts on antibiotic resistant *Staphylococcus aureus* isolated from clinical and environmental sources.



Key: EE= ethanol extract 200mg/ml, EEB= ethanol extract 100mg/ml, EEC= ethanol extract 50mg/ml, EED= ethanol extract 25mg/ml, nHA= n-Hexane extract 200mg/ml, nHB= n-Hexane extract 100mg/ml, nHC= n-Hexane extract 50mg/ml, nHD= n-Hexane extract 25mg/ml, WA= water extract 200mg/ml, WB= water extract 100mg/ml, WC= water extract 50mg/ml, WD= water extract 25mg/ml, PC= positive control (ciprofloxacin 5mg/ml), NC= negative control (DMSO)

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of extracts on multiple antibiotic resistant *Klebsiella pneumonia* and *Staphylococcus aureus* isolated from different sources in Ondo State

Isolates	MIC/ MBC (mg/ml)		
	Ethanol extract	n-Hexane extract	Water extract
SS	37.50/75.00	37.50/75.00	37.50/37.50
SW	18.75/37.50	75.00/150.00	37.50/37.50
SU	37.50/75.00	37.50/75.00	18.75/37.50
SWW	18.75/37.50	75.00/150.00	37.50/37.50
SA	18.75/37.50	75.00/150.00	37.50/37.50
KS	18.75/75.00	37.50/75.00	37.50/37.50
KW	18.75/37.50	75.00/75.00	37.50/37.50
KU	18.75/37.50	75.00/150.00	37.50/37.50
KWW	18.75/18.75	37.50/75.00	37.50/37.50
KA	18.75/37.50	37.50/75.00	37.50/37.50
*Staphylococcus aureus*ATCC 25923	18.75/18.75	18.75/37.50	18.75/37.50
*Klebsiella pneumoniae*ATCC 33495	18.75/37.50	37.75/75.00	37.50/37.50

Keys: SS= *Staphylococcus aureus* isolated from market soil, SW= *Staphylococcus aureus* isolated from post-operative wound, SU= *Staphylococcus aureus* isolated from urine, SWA= *Staphylococcus aureus* isolated from well water, SA= *Staphylococcus aureus*isolated from hospital air, KS= *Klebsiella pneumoniae* isolated from market soil, KW= *Klebsiella pneumoniae* isolated from post-operative wound, KU= *Klebsiella pneumoniae* isolated from urine, KWA= *Klebsiella pneumoniae* isolated from well water, KA= *Klebsiella pneumoniae* isolated from hospital air

Synergistic interaction of combined Ocimum gratissimum extracts against multiple antibiotic resistant Klebsiella pneumoniae and Staphylococcus aureus isolated from different sources in Ondo State

The result (**Figure 3a**) revealed antibacterial activity of combined *Ocimum gratissi-mum*extracts (Ethanol and n-hexane extract, ethanol and water extract, n-hexane and water extract) against multi drug resistance *Klebsiella pneumoniae* isolated from clinical and environmental sources. Combination of ethanolic and water extract revealed the best antibacterial activity on multi drug resistance *Klebsiella pneumonia* and highest zone of inhibition was observed at 200mg/ml. Antibacterial activity of combined *Ocimum gratissimum* extracts against multi drug resistance *S. aureus* isolates from clinical and environmental sources, combination of ethanol and water extract also revealed the best antibacterial activity on multidrug resistance *S. aureus* and highest zone of inhibition was observed at 200mg/ml. The detail is shown in **Figure 3b**.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Synergistic interaction of combined Ocimum gratissimum extracts from different sources

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Ocimum gratissimum* extracts on multiple antibiotic resistant *Klebsiella pneumonia* and *Staphylococcus aureus* isolated from clinical and environmental sources are presented in **Table 5.** The range of MIC and MBC observed in ethanol extract and n-hexane extract, ethanol and water extract, n-hexane and water ranges from (12.5mg/ml to 25mg/ml and 25mg/ml to 50mg/ml), (12.5mg/ml and 25mg/ml) and (25mg/ml to 100mg/ml and 50mg/ml to 100mg/ml) respectively.

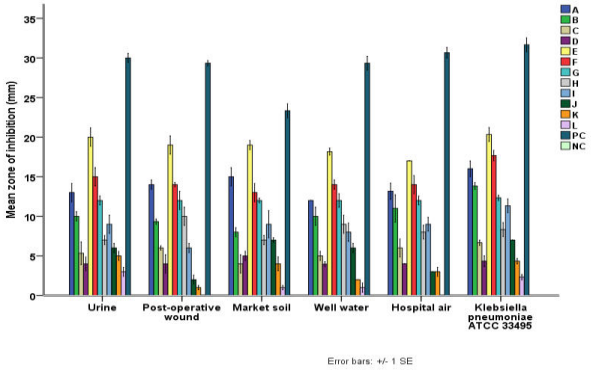


Figure 3a: Synergistic interaction of combined *Ocimum gratissimum* extracts against multiple antibiotic resistant *Klebsiella pneumoniae isolated* from different sources in Ondo State

Key: A= ethanol+n-Hexane extract 200mg/ml, B= ethanol+n-Hexane extract 100mg/ml, C= ethanol+n-Hexane extract 50mg/ml, D= ethanol+n-Hexane extract 25mg/ml, E= ethanol+water extract 200mg/ml, F= ethanol+water 100mg/ml, G= ethanol+water extract 50mg/ml, H= ethanol+water extract 25mg/ml, I= n-Hexane+water extract 200mg/ml, J= n-Hexane+water extract 100mg/ml, K= n-Hexane+water extract 50mg/ml, L= n-Hexane+water extract 25mg/ml, PC= positive control (ciprofloxacin 5mg/ml), NC= negative control (DMSO)

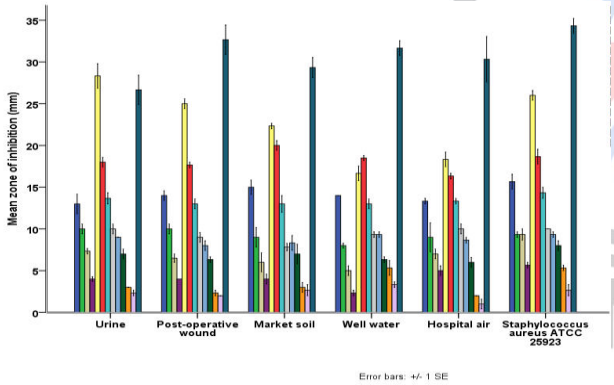


Figure 3b: Synergistic interaction of combined *Ocimum gratissimum* extracts against multiple antibiotic resistant *Staphylococcus aureus* isolated from different sources in Ondo State

Key: A= ethanol+n-Hexane extract 200mg/ml, B= ethanol+n-Hexane extract 100mg/ml, C= ethanol+n-Hexane extract 50mg/ml, D= ethanol+n-Hexane extract 25mg/ml, E= ethanol+water extract 200mg/ml, F= ethanol+water 100mg/ml, G= ethanol+water extract 50mg/ml, H= ethanol+water extract 25mg/ml, I= n-Hexane+water extract 200mg/ml, J= n-Hexane+water extract 100mg/ml, K= n-Hexane+water extract 50mg/ml, L= n-Hexane+water extract 25mg/ml, PC= positive control (ciprofloxacin 5mg/ml), NC= negative control (DMSO)

Table 5: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Synergistic interaction of combined *Ocimum gratissimum* extracts from different sources

	MIC/ MBC (mg/ml)		
Isolates	**EE +n-H**	**EE+W**	**n-H+W**
SU	12.5/25	12.5/25	50/100
SW	12.5/25	12.5/25	50/100
SS	12.5/25	12.5/25	50/100
SWW	25/50	12.5/25	25/50
SA	12.5/25	12.5/25	50/100
KU	12.5/25	12.5/25	25/50
KW	12.5/25	12.5/25	100/100
KS	12.5/25	12.5/25	25/50
KWW	12.5/25	12.5/25	50/100
KA	12.5/25	12.5/25	100/100
*Staphylococcus aureus*ATCC 25923	12.5/25	12.5/25	100/100
*Klebsiella pneumoniae*ATCC 33495	12.5/25	12.5/25	50/100

Key: SU= *Staphylococcus aureus* isolated from urine, SW= *Staphylococcus aureus* isolated from post-operative wound, SS= *Staphylococcus aureus* isolated from market soil, SWA= *Staphylococcus aureus* isolated from well water, SA= *Staphylococcus aureus* isolated from hospital air, KU= *Klebsiella pneumoniae isolated* from urine, KW= *Klebsiella pneumoniae* isolated from post-operative wound, KS= *Klebsiella pneumoniae* isolated from market soil, KWA= *Klebsiella pneumoniae isolated* from well water, KA= *Klebsiella pneumoniae* isolated from hospital air.

DISCUSSION:

Drug resistant microbes of all kinds can move among people and animals, from one country to another without notice. In developing countries

where resistance is a prime issue, data are least available.[19,20] In this study, several clinical and environmental samples of urine, post-operative wound, market soil, well water and hospital air were examined for the presence of multidrug resistant *K. pneumonia* and *S. aureus* and antibacterial effects of *Ocimum gratissimum* leaf extracts using different extraction solvents.

High frequency of resistance of *K. pneumonia* and *S. aureus* to many antibiotics especially β-lactam and β-lactam inhibitor antibiotics may suggest that the bacterial isolates were Extended-spectrum β-lactamases (ESBLs) producer because ESBL-producing bacteria often exhibit multidrug resistance.[21] Also, the high level of bacterial resistance to fluoroquinolones may be influenced by the use of enrofloxacin which is the most used drug in Akure poultries. High resistance to fluoroquinilones drug may result to over use of the drug in treating infection, since the drug is highly toxic at high dose hence bacteria resistance may expose man to dose toxicity.
In this study, the phytochemicals present in the extracts of *Ocimum gratissimum* were alkaloid, saponin, tannin, flavonoid, phenolics and glycosides. These phytochemicals present in the extracts may be responsible for the antibacterial activities of the leave extracts as described by many researchers.[22,23,24] The phytochemicals in medicinal plants have been reported to be the active principles responsible for the pharmacological potentials of medicinal plants.[25]The presence of these chemicals in the leaves of *O. gratissimum* justifies the local use of this plant for the treatment of various ailments. The leaves are rich in flavonoids, saponins and tannins, with considerable amount of phenolics and alkaloids. Flavonoids are polyphenolic compounds that are biologically active against liver toxins, microorganisms, inflammation, tumor and free radicals.[26] They have also been reported to inhibit the growth of cataracts in diabetic patients.[27] The present of flavonoids in the leave might be responsible for the use of the plant by traditional healers to treat diabetes. Saponins are essential elements in ensuring hormonal balance and synthesis of sex hormones.[28]Thesaponin content of *O. gratissimum* extract (ethanolic extract) is considerably high. This substantiates its use as a local condiment for the nursing mothers in some tribes in Nigeria. Tannins are bitter polyphenolic compounds that hasten the healing of wounds. They also possess anti-diuretic and anti-diarrhea properties.[26]The amount of tannins in the ethanol and water extract of *O. gratissimum* leave (4.75 ± 0.27 and 3.43 ± 0.09) might be responsible for its use by the local herbalists to treat gastrointestinal disorders. Phenolic compounds are potent antioxidants and free radical scavengers with inhibitory activities against some pathogenic microorganisms.[29]

This present investigation revealed the antibacterial activities of ethanol, n-hexane and water extracts of *O. gratissimum* against *K. pneumoniae* and *S. aureus* isolated from clinical and environmental sources. The results clearly revealed the pronounced activity of ethanol and water extracts against tested bacteria. The activity of ethanol extracts was higher than that of the n-hexane and water extracts. The n-hexane extract showed the least inhibitory effect. The antibacterial activities of combined solvent extracts showed higher inhibitory effects (synergism) than when used singly.

However when extracts from different solvents were combined the inhibition surpassed that of single extract. The synergistic effect may be due to formation of certain complexes which become more effective in the inhibition of microoraganisms. Similar conclusions were drawn in other study, synergistic effect of *O. gratissimum* and *Solenostemon monostachyus* against *S. aureus, E.*

coli and *P. aeruginosa.*[30] Generally, it was observed that the extract was more effective against *S. aureus* than *K. pneumoniae*, this may be due to the differences in the cell wall components of the two bacteria, because cell wall enhance the permeability of the extract into the cell thereby causing cell death. Also, the difference in the susceptibility of *S. aureus* than *K. pneumoniae* from different sources to the extract might be because the bacteria isolated from different sources are of different strain and the environment in which they were isolated may have modified there susceptibility to the extract.

CONCLUSION :

This work has demonstrated the antibacterial effect of *Ocimum gratissimumon* multidrug resistance *K. pneumonia* and *S.aureus* isolated from environment and clinical samples. Therefore it could be recommended that the extract of this plant be used for the treatment of infection caused by *K. pneumonia* and *S.aureus* since the extract has demonstrated anti-Staphylococcal and anti-Klebsiella activity irrespective of the sources *in-vitro*.However, toxicological and *in vivo* assay may be incorporated to ascertain the safety of the extract when used.

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CONFLICT OF INTEREST: Authors declared no conflict of interest
SOURCE OF FINANCIAL SUPPORT: Nil

Cite of article: Anthony Okhonlaye O, Martha Oluwatosin O; Antibacterial efficacy of *ocimum gratissimum* on multidrug resistant *staphylococcus aureus* and *klebsiella pneumoniae* isolated from clinical and environmental samples in ondo state.Int. J. Med. Lab. Res. 2018. 3(3): 39-51