International Journal of Medicinal Plants and Natural Products (IJMPNP)
Volume 9, Issue 3, 2023, PP 1-10
ISSN 2454-7999(Online)
DOI: https://doi.org/10.20431/2454-7999.0903001
www.arcjournals.org

Chemical Composition and Antibacterial Potential of Essential Oil of *Cymbopogon Citratus* against Fluoroquinolone-Resistant *Staphylococcus Aureus* and Escherichia Coli Isolates from Humans in Enugu State, South-East Nigeria.

Adonu Cyril Chekwube[1*],OnwusobaRestus Chibuike[2]U, jam Treasure Nonye[1] Ali Ibeabuchi Jude[3] OkorieNdidiamaka Hannah[3]Omeh Romanus[4] and Esimone Charles[5]

[1]*Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria*

[2]*Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, Enugu State, Nigeria.*

[3]*Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria*

[4]*Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria*

[5]*Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, NnamdiAzikiwe University Awka, Anambra State, Nigeria.*

Corresponding Authors:** ***Adonu Cyril Chekwube, *Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu State, Nigeria*

Abstract: *Cymbopogon citratus (DC.) Stapf. (Poaceae) is an herb used in traditional medicine and its essential oil (EO) constituents could be a potential source of therapeutic agent with a broad-spectrum of activity against pathogenic microbes. The study was conducted to investigate the chemical component and antibacterial activity of essential oil of Cymbopogon citratus against fluoroquinolone-resistant Staphylococcus aureus and Escherichia coli isolates from humans in Enugu State, South-East Nigeria. Hydro-distillation method was used to extract the EO. Gas Chromatography-Mass Spectrometric (GC-MS) method was used to identify the chemical constituents. The agar diffusion cup-plate and broth dilution techniques were used for evaluation of antibacterial activity. Sixteen (16) constituents were identified in the essential oil of C. citratus based on retention time and peak area. Major constituents identified were citral, citral derivatives and fatty acids. The C. citratus EO exhibited significant antibacterial activity ($p < 0.05$) against test organisms. The MBC values ranged from 0.01 - 0.094 mg/ml and 0.11 – 0.160 mg/ml against FQRSA and FQREC isolates respectively. The C. citratus EO contains therapeutic compounds which can be used to treat infections caused by fluoroquinolone- resistant S. aureus and E. coli*

Keywords: *Essential oil, Cymbopogon citratus, Staphylococcus aureus, Escherichia coli, Fluoroquinolone*

1. INTRODUCTION

The emergence of resistance to fluoroquinolones in virtually all species of bacteria was recognized soon after the introduction of these compounds for clinical use (Dalhoff, 2008). Presently, there are several reports showing increase in resistance to fluoroquinolones among bacteria causing community-acquired infections, such as *E. coli* and *S. aureus.*(Adonu *et al.,* 2018). This increasing occurrence of drug resistant *E. coli* and *S. aureus* isolates of human and animal origin is a global public health problem. The increased prevalence of bacteria resistance to fluoroquinolone antibiotics has necessitated the search for new and alternative approaches.

Cymbopogon citratus (DC.) stapf, commonly known as lemongrass is a tall, coarse grass with a strong lemon taste. It is a genus of Asian, African, Australian, and tropical island plants in the grass family, designates two different species, East Indian *C. flexuosus* (DC.) stapf and West Indian, *C. citratus*

(DC.) stapf. (Naik*et al.*, 2010]. *C. citratus* (DC) stapf. is vastly farmed for its essential oils in various parts of the world (Majewska *et al*., 2019). The use of lemongrass was found in folk remedy for coughs, consumption, elephantiasis, malaria, ophthalmia, pneumonia and vascular disorders (Naik *et al.*, 2010). Researchers have found that lemongrass is cultivated in the subtropical and tropical regions of the world and widely used in the agriculture, cosmetics, flavor, food, pharmaceutical industries. (Wifek *et al.*, 2016) Furthermore, many workers had reported about the antibacterial activity of lemongrass oil against a diverse range of organisms comprising Gram positive and Gram negative organism, yeast and fungi (Shigeharu*et al.* 2001,Cimanga *et al*. 2002). Therefore, the present study was carried out to find out GC-MS profiling and antibacterial activity of *C. citratus* EO against fluoroquinolone-resistant *S. aureus* (FQRSA) *and* fluoroquinolone-resistant *E. coli* (FQREC).

2. MATERIALS AND METHODS

Plant material.

The fresh leaves of *C. citratus* were collected in the morning in December 2018 from Nsukka, Enugu State, Nigeria. The identity of the plant was authenticated by a botanist - Mr. A .O .Ozioko- of the Bioresource Development and Conservation programme (BDCP) Nsukka, Enugu State and voucher specimens was deposited at the Herbarium of the same institution.

Chemicals and reagents

All chemicals used were of analytical reagent grade. All reagents and culture media were purchased from Sigma-Aldrich-Fluka (Saint-Quentin, France).

Ethical approval

Ethical approval was obtained from the Ethical committee of the State Ministry of Health and the clearance reference number is MH/MSD/EC/O2182.2

Extraction of EO of *C. citratus*

Freshly harvested leaves of the plant were hydro-distilled in a Clevenger apparatus for 3 h to get essential oil using standard method (Verma *et al*., 2011). A 1.0 kg of the plant material were chopped and loaded into a 4 liters round bottom flask containing 2500ml of water. The temperature of the condenser was kept low by connecting it to a water-circulators loaded with ice blocks. The condensate was collected in a burette and essential oil floated on the surface of the water in the burette. The essential oil was recovered and stored in the refrigerator.

Gas Chromatography/Mass Spectrometry Analysis

The essential oil of *C. citratus* was subjected to GC-MS analysis using QP2010 PLUS model (SHIMNADZU, JAPAN). The capillary column type was DB-IMS (30.0m (length) X 0.25mm (diameter) X 0.25µm (film thickness)). The carrier gas used was helium at constant flow rate of 0.99 L/min and average velocity of 36.2cm/s; the pressure was 56.2KPa. The initial column temperature was set at 6°C 0 for 1 min and increased by 3°C /min up to 180°C and to the final temperature of 280°C at the rate of 6°C /min; volume injected was 1.0 µl at 250 °C and split ratio was 41.6:1. The relative abundant of individual components of the total essential oil was expressed as percentages peak relative to total peak area. Qualitative identification of the different constituents was performed by comparison of the GC-MS data with published mass spectral database (NIST02.L) from literature.

Preparation of concentrations of EO of *C. citratus*.

Different concentrations of EO were prepared in sterile dimethyl sulphoxide (DMSO) ranging from 25 - 1.62 mg/ml and 1.6 – 0.013 mg/ml for preliminary sensitivity test and MIC/MBC determinations respectively.

Preparation and standardization of test isolates.

The test isolates were prepared and then standardized as previously described. (Iyevhobu, 2022; Murray, 2005). The tops of 5-10 similar appearing, well isolated colonies on an agar plate were touched with a sterilized straight wire and then inoculated in a nutrient broth medium. These broth bottles were incubated at 37 ^{0}C for 4 – 6 h to obtain the growth at logarithmic phase. The density of the organisms was adjusted to approximately 10^8 colony – forming units (CFU)/mL by comparing its turbidity with that of 0.5 McFarland opacity standards.

Antibiotic sensitivity test.

Antibiograms were prepared for the *S. aureus and E. coli* isolates against ciprofloxacin (5 µg), ofloxacin (5 µg), levofloxacin (5 µg), pefloxacin (5 µg) gentamicin (30 µg), ceftriaxone (30 µg),

amoxicillin (25 µg), erythromycin (15 µg) and doxycycline (30 µg) by disk diffusion method following the guidelines ofClinical and Laboratory Standards Institute (CLSI, 2013). Within 10 – 20 min after adjusting the turbidity of the inoculum suspension to that of standard, a sterile nontoxic cotton swab was dipped into the inoculum and rotated several times with firm pressure on the inside wall of the tube to remove excess fluid. The dried surface of Mueller-Hinton agar plate 100mm in diameter containing 20 ml Mueller-Hinton agar was inoculated by streaking the swab three times over the entire agar surface. The lid of the dish was then replaced and the dish was allowed to stand at room temp for 3 min to allow the surface of the agar to dry before the antibiotics discs were applied using sterile forceps. After placement, the disc on the surface of medium was pressed to provide uniform contact. The plates were allowed to stand at room temperature for 1 h to allow for diffusion of drug and then incubated aerobically at 37ºC for 24 h and the zones of inhibition developed were measured and recorded. The *S. aureus and E. coli* isolates were considered as sensitive or resistant to the test antibiotics based on the inhibition zone diameter (IZD) they produced following the guidelines of the CLSI (2013). *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used as the reference strain for the susceptibility studies.

Antibacterial evaluation of EO of *C. citratus*

The preliminary sensitivity test of EO of lemongrass was evaluated against fluoroquinolone-resistant *Escherichia coli* and *S. aureus* by the method of the agar well diffusion as previously described (Naik *et al*., 2010). Doubling dilutions of EO were prepared in DMSO ranging from 25 to 1.62 mg/ml. A 20 ml of molten Mueller-Hinton agar was seeded with 0.1 ml of standardized broth cultures of the test bacteria. A total of 6 wells, 8 mm in diameter were made in the agar using a sterile cork borer. A 0.05 ml each of the two-fold dilutions of the EO was added into each labeled hole using a sterile pipette. As a control, a 0.05 ml DMSO was put in the centre well. Similarly, doubling dilutions of 0.120 mg/ml of ciprofloxacin (PCCA^{Tm} Houston-USA) were added into respective agar-wells for comparison. The plates were left for 1 h at room temperature for diffusion after which they were incubated at 37ºC for 24 h. Diameters of the zones of inhibition (IZD) were measured at the end of the incubation period. The mean of triplicate determinations was recorded.

Minimum inhibitory concentration (MIC) determination

The MIC of the EO was determined using broth dilution method with little modification (). Eight (8) different dilutions (0.013 – 0.16 mg/ml) of the oil in DMSO were prepared by two-fold dilution. The fresh culture of the test isolate was prepared by inoculating the FQREC and FQRSA each in a sterilized test tube containing 5 ml nutrient broth. The inoculated tubes were incubated overnight at 37ºC. Then, test tubes containing 10 ml of sterilized tryptic soy broth (TSB) were prepared and inoculated with different concentration of lemongrass oil ranging from 0.013 – 1.6 mg/ml. TSB with DMSO less oil was used as positive control. A 25 µl each of the bacterial suspension was added to each tube of TSB-lemongrass oil and incubated at 37ºC for 24 h. At the end of the incubations, the tubes were observed for turbidity. The MIC was determined as the lowest concentration of oil to inhibit the growth of microorganism.(Naik *et al.*,2010).

Minimum bactericidal concentration (MBC) determination

After taking the MIC readings, the tubes with no visible growth (no increase in turbidity) were sub-cultured on freshly prepared Mueller–Hinton agar by streaking method. The culture media were incubated appropriately for 48 h and then observed for growth. After 24 h, the lowest concentration from which the microorganisms did not recover and grow when transferred to the fresh media was recorded as the minimum bactericidal concentration (MBC) (Cheesbrough 2002).

Data analysis

Data resulting from the study were analysed and evaluated on the basis of averages and percentage values. Tables were used for the presentation of results. Statistically, a descriptive analysis was performed using SPSS version 23.

3. RESULTS

Extraction yield.

The yield (%) of the essential oil was 1.10 % of the mass of the leaves.

Chemical composition of *C. citratus* EO

GC-MS analysis of the essential oil of the leaves of *C. citratus* showed sixteen identified constituents comprising hydrocarbons, terpenes and terpenes derivatives (Figure 1). The most abundant constituents of the essential oil were found to be citral (3,7-dimethyl-2,6-octadienol) and citral derivatives (2,6-dimethyl-3,7-octadie-2-ol, 2,7-dimethyl-2,7-octanediol, and 2,6-octadienoic acid) which occurred at moderate to higher level (5-30%). The remaining components mostly hydrocarbon occur at a very low level, < 4%, (Table 1).

GC-MS analysis of *C. citratus* EO

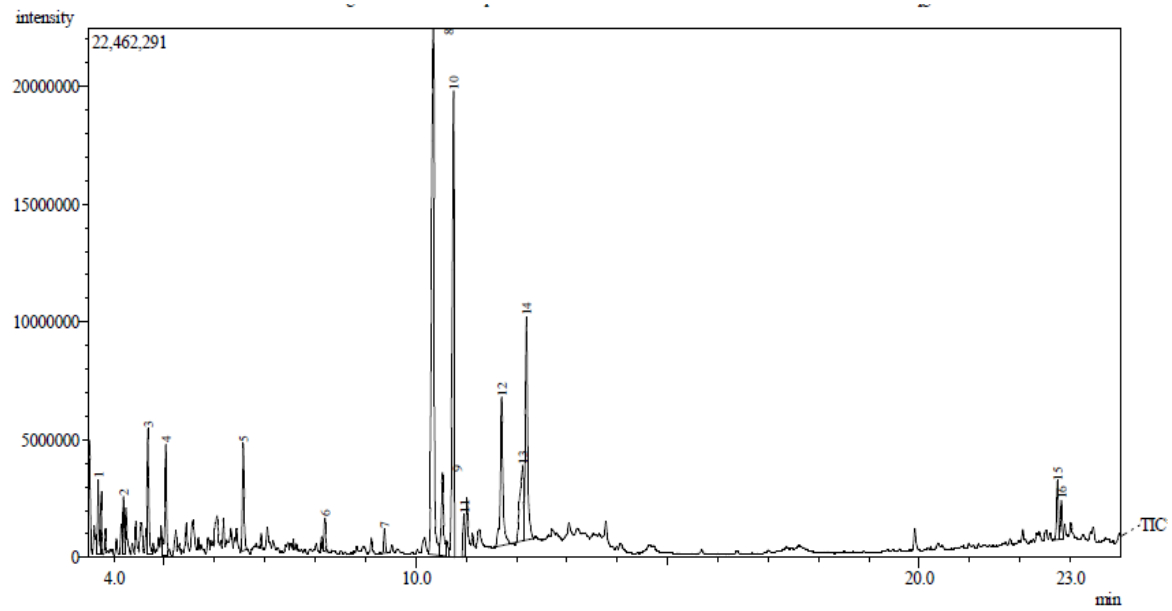

Fig1. *Gas chromatographic chart of the C. citratus **EO***

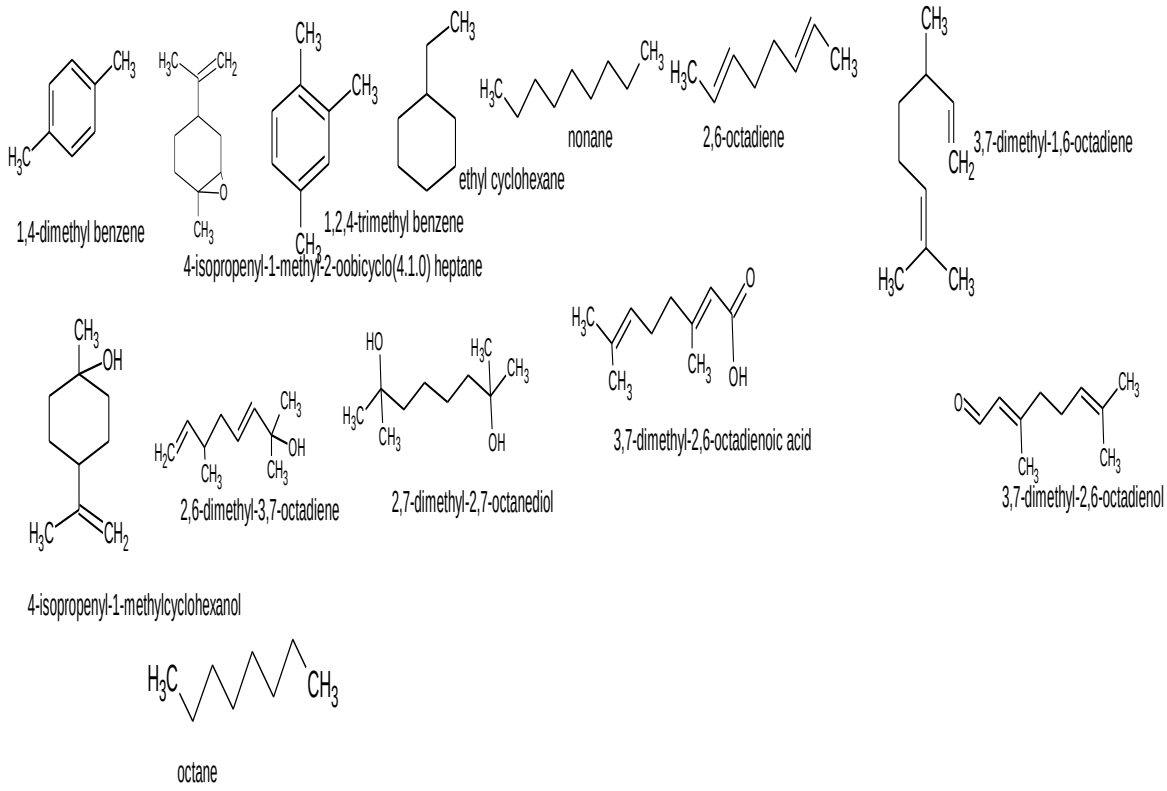

Figure2. *Compounds identified in the EO of C. citratus*

Table 1: Composition of *C. citratus* EO

S/N	Compounds Name	Percentage peak area	Molecular formular	Molecular weight	RT (min
1	Octane	1.91	C_8H_{18}	114	3.693
2	Ethylcyclohexane	1.58	C_8H_{16}	112	4.193
3	1,4-di methyl benzene	4.19	C_8H_{10}	106	4.677
4	Nonane	3.83	C_9H_{18}	128	5.034
5	1,2,4-tri-methyl benzene	3.87	C_9H_{12}	120	6.573
6	4-isopropenyl-1-methylcyclohexanol	1.06	$C_{10}H_{18}O$	154	8.193
7	3,7-dimethyl-1,6-octadiene	0.80	$C_{10}H_{18}$	138	9.379
8	Neral	30.20	$C_{10}H_{16}$	152	10.350
9	Nerol	4.25	$C_{10}H_{17}O$	154	10.527
10	Geranial	18.45	$C_{10}H_{16}O$	152	10.748
11	Prenderol	1.65	$C_{10}H_{18}O_3$	186	10.995
12	Nerolic acid	8.25	$C_{10}H_{22}O_2$	174	11.05
13	Geranic acid	5.59	$C_{10}H_{16}O_2$	168	12.121
14	2,6-dimethyl-3,7-octadien-2-ol	10.76	$C_{10}H_{17}O$	154	12.198
15	3,7- Dimethyl 2, 6- octadienoic acid	2.29	$C_{10}H_{16}O_2$	168	22.746
16	3,7-dimethyl-2,6-octadiene	1.3	$C_{10}H_{18}$	138	22.825

Antibiotic resistant profile of FQREC and FQRSA.

The percentage of the test isolates resistant to common antibiotics in the study area is shown in Table 2. All FQREC isolates resisted erythromycin. About 92 – 100% of FQREC isolates were resistant to amoxicillin and doxycycline. Furthermore, large proportions (35.6 – 82.4 %) of the test isolates were also resistant to ceftriaxone and gentamicin. All FQREC isolates from stool of healthy volunteers and wound swab of patients suffering from chronic wound infections were resistant to doxycyclines. Other FQREC were resistant in varying degrees to the rest of the test antibiotics. All FQRSA isolates were resistant in varying degrees to the rest of the test antibiotics. Hundred percent of the FQRSA isolated from urine samples collected from both symptomatic and asymptomatic healthy carriers exhibited resistance to amoxicillin and doxycyclines. Similarly, 100 % of the FQRSA isolated from wound swab and patient nasal swab showed resistance to amoxicillin.

Statistically, there was no statistical significant difference in the antibiotic resistance of the FQREC isolates from both the healthy carriers and the patients. The isolates recovered from the healthy volunteer and patient samples showed significant difference in the resistance pattern of the FQRSA against the test antibiotics. Significantly, all the FQRSA were resistant to amoxicillin and a large proportion was resistant to each of the remaining test antibiotics.

Table 2: The proportion (%) of the test isolates that are resistant to other antibiotics in use in the study area

Bacteria	Specimen source	Antibiotics					
		FQ	GN	Cef	Am	Ery	Doxy
FQREC	HVUS	100	66.7	44.4	100	100.0	92.3
	PSU	100	61.0	54.2	96.6	100	93.0
	HVSS	100	82.4	47.1	94.1	100	100
	PS	100	79.5	56.4	92.3	100	97.4
	W/S	100	35.7	64.3	92.9	100	100
FQRSA	HVUS	100	64	64	100	64	100
	PSU	100	83	61	100	70	100
	HVnS	100	47.1	38.2	97.1	38.2	91.2
	PnS	100	68.8	75	100	93.8	100
	W/S	100	70	80	100	67.6	85

HVUS = healthy volunteer urine specimen, PSU = patient specimen of urine, HVSS= healthy volunteer stool specimen. HVnS= healthy volunteer nasal specimen. PS = Patient stool specimen,W/S=wound swab Fq = Fluoroquinolones,Gn= Gentamicin, Cef = Ceftriaxone, Amx =Amoxicillin, Ery= Erythromycin, Doxy = Doxycycline.

Antibiotic susceptibility test against the *C. citratus* EO.

The results of the preliminary susceptibility tests carried out are shown in Tables 3 and 4. The respective inhibition zone diameters (IZD) of the EO against the *S. aureus* and *E. coli* isolates ranged from 10.33-38.99 mm and 9.11-34.22 mm depending on the concentration of the EO used. The antibacterial activity of the oil was found to be progressively increasing with the increase in EO concentration. The maximum effect was noted at 50 mg/ml concentration and minimum effect was found at 6.25 mg/ml. Fluoroquinolone-resistant *S. aureus* isolates were found to be more susceptible than FQREC to lemongrass EO. The control antibiotic, ciprofloxacin (5µg) was found to be less active than each of the concentration of EO against FQRSA. However, it was found to produce greater IZD than lemongrass oil at concentration ≤ 6.25 mg/ml against FQREC. Statistically, there was a significant difference ($P< 0.05$), between the FQRSA isolates from the nasal swabs of the patient and that of the corresponding healthy carrier. In broth dilution experiment, the test isolates were found to be inhibited by lemongrass oil at very low concentration. The minimum inhibitory concentration (MIC) values of the EO against FQRSA and FQREC ranged from 0.010 – 0.048 mg/ml and 0.069 – 0.140 mg/ml respectively. The minimum bactericidal concentration (MBC) values against FQRSA and FQREC ranged from 0.01 - 0.094 mg/ml and 0.11 – 0.160 mg/ml respectively (Tables 5 and 6). The MIC and MBC of the oil against FQRSA isolates from the healthy volunteer's nasal swab were 0.044 $\pm$ 0.02 mg/ml and 0 .094 $\pm$ 0.03 mg/ml respectively. The MIC and MBC results showed that the activity of the oil was greater among the isolates from the patients than the isolates from the healthy carriers (Tables 5 and 6).

Table 3: Sensitivity of EO of *C. citratus* and ciprofloxacin against FQRSA

Specimen source	IZD of different Concentration of Essential oil used (mg/ml)				Ciprofloxacin
	50	25	12.5	6.25	5µg
HVns	36.33±2.10	27.56 ± 2.31	19.33 ± 1.72	10.56 ± 1.88	6.11 ± 1.64
PNS	28.9 ± 2.78	15.82 ± 2.08	9.64 ± 1,22	4.64 ± 1.13	6.75 ± 1.46
HVUS	31.6 ± 2.29	16.40 ±1.17	9.00 ± 0.45	4.00 ± 1.67	6.20 ±2.65
PSU	27.78 ±2.05	18.43 ± 1.74	10.07 ± 1.16	3.79 ± 0.94	6.14 ± 1.36
PWS	33.17 ± 2.23	19.61 ± 1.59	10.11± 0.83	4.33 ± 0.76	5.56 ±1.16

HVns = Healthy Volunteer nasal Specimen, PNS = Patient Nasal Swab. HVUS = Healthy Volunteer urine Specimen .PSU = Patient Specimen of Urine. PWS = Patient wound swab.

Table 4: Sensitivity of EO of *C. citratus* and ciprofloxacin against FQRSA

Specimen source	IZD of different Concentration of Essential oil used (mg/ml)				Ciprofloxacin
	50	25	12.5	6.25	5µg
HVUS	25.37 ±1.79	16.0 ± 1.30	9.10 ± 1.09	4.57 ±0.97	4.74 ± 1.21
PSU	25.36 ± 1.20	14.68 ± 0.93	6.88 ±0.85	2.84 ± 0.72	5.7 ± 0.95
HVSS	22.36 ± 1.64	11.00 ± 1.14	6.29 ± 0.98	2.886 ± 0.93	8.57 ±0.63
PSS	25.53 ± 1.78	14.93± 1.12	9.8 ± 0.80	4.67 ± 1.0	8.2± 1.0
PWS	23.80 ±1.77	14.20 ± 1.74	9.6 ± 0.85	4.0 ± 1.37	5.1 ± 1.6

HVUS =Healthy Volunteer urine Specimen, PSU =Patient Specimen of Urine, HVSS = Healthy Volunteer Stool Specimen, PSS= Patient specimen of stool. PWS =Patient wound swab.

Table 5: MIC and MBC (MIC ± SEM) mg/ml of EO of *C. citratus* against FQRSA

Source	Initial MIC	Final MIC	MBC
HVNS	0.044 ± 0.02	0 .094 ± 0.03	0 .094 ± 0.03
PNS	0.054 ± 0.01	0.064± 0.02	0.064± 0.02
HVUS	0.035± 0.01	0.048 ± 0.01	0.048 ± 0.01
PSU	0.040 ± 0.01	0.090 ± 0.02	0.09 ± 0.02
PWS	0.047 ±0.01	0.08 ± 0.02	0.08 ± 0.02

HVns =Healthy Volunteer nasal specimen, PNS= Patient Nasal Swab, HVUS= Healthy Volunteer Urine Specimen, PSU= Patient Specimen of Urine, PWS= Patient wound swab.

Table 6. MIC and MBC (MIC ± SEM) mg/ml of EO of *C. citratus* against FQREC

Source	Initial MIC	Final MIC	MBC
HVUS	0.069 ± 0.01	0.133± 0.015	0.133± 0.015
PSU	0.140 ± 0.05	0.143 ± 0.01	0.143 ± 0.01
HVSS	0.097 ±0.016	0.160 ±0.08	0.160 ±0.08
PSS	0.097 ± 0.016	0.160 ±0.08	0.160 ±0.08
PWS	0.144 ± 0.031	0.24 ±0.027	0.24 ±0.027

HVUS= Healthy Volunteer urine Specimen, PSU= Patient Specimen of Urine, HVSS= Healthy Volunteer Stool Specimen, PSS= Patient specimen of stool, PWS= Patient wound swab.

4. DISCUSSION

The antibiotic susceptibility tests conducted on the test FQRSA and FQREC against the commonly used antibiotics showed that these isolates are multi-drug resistant. Therefore, treatment of infections caused by these isolates is a difficult task as the clinicians will be left with very few or no alternative drugs. In the present study, sixteen compounds were identified by GC-MS analysis of the essential oil of *C. citratus* as seen in the chromatographic chart in figure 1. The GC-MS analysis identified constituents comprising hydrocarbons, terpenes and terpenes derivatives as structurally represented in figure 2. It is important to note that other researchers had recorded more than 16 constituents, some of which were different from the components identified in this study. (Amit and Anushere 2010, Saleem *et al.*, 2003). The reason for this disparity may be as results of geographical, seasonal and edaphic variations. Similar to our findings, several reports confirmed citral and citral derivatives as the major components of the oil (Amit and Anushere 2010, Saleem*et al.*, 2003).

The preliminary susceptibility study carried out on essential oil of *Cymbopogon citratus* against fluoroquinolone-resistant *S. aureus and E. coli* isolates showed that the oil has a promising antibacterial activity against the test bacteria. The antibacterial activity of the oil was found to be progressively increasing with increase in EO concentration. The maximum effect was noted at 50 mg/ml concentration and minimum effect was detected at 6.25 mg/ml. Fluoroquinolone-resistant *S. aureus* isolates were found to be more susceptible than FQREC to lemongrass EO. The control antibiotic, ciprofloxacin (5µg) was found to be less active than each of the concentrations of EO against FQRSA. However, the control drug was found to produce more antibacterial effect than lemongrass oil when the concentration of the oil was low. Statistically, there was a significant difference ($P < 0.05$), between the FQRSA isolates from the nasal swabs of the patient and that of the corresponding healthy carrier. The present study showed that the sensitivity of these FQRSA isolates was greater among isolates from the healthy volunteers than the patients isolates The reason for this is unclear, but, may be that the isolates from the patients might have acquired drug resistance as a result of antibiotic selective pressure or inappropriate use of antibiotics. This is in agreement with the results of the works done by other researchers (Shigeharu *et al*. (2001) and Cimanga *et al*. (2002)). Similarly, the MIC and MBC results of this work showed that the essential oil has a good antibacterial activity against both FQREC and FQRSA. Sensitivity of the *S. aureus* to EO of lemongrass was higher than that of the *E. coli* isolates to the same agent. Similar observations have been made by other workers (Aber *et al*, 2021; Torris *et al*.,2002; Naik *et a*l.,2010). There were no significant differences in the antibacterial activities of the oil among the FQRSA and FQREC isolates from different specimen sources (urine, nasal swab and wound swabs). The result of MIC values of the EO against the test isolates showed that the activity of the oil was more appreciated when the sensitivity

test was done using broth dilution techniques, probably, due to the problem of solubility of this EO. The MBC values of the essential oil on FQREC and FQRSA were significantly higher than that of the MIC values on the same isolates. This shows that the EO is both bacteriostatic and bacteriocidal in nature, but, the bacteriocidal effect is detected at higher concentration. This can be confirmed by the MBC/MIC ratio of less than four as could be deduced from Tables 5 and 6 (Benjamin *et al*., 2012). The high antibacterial activity of this EO could be correlated to the presence of high level of citral as its major components. Citral is a mixture of two isomeric acyclic monoterpene aldehydes, which includes, geranial (citral A or trans citral) and neral (citral B or ciscitral) (Hasim *et al*., 2015) These two constituents have same molecular formula ($C_{10}H_{16}O$) but different structures (Hartatie *et al*., 2014, Manvitha *et al.*, 2018). The mechanisms of action of citral component of essential oil of *C. citratus* have been investigated to include reduction of intracellular ATP, hyperpolarization of plasma membrane and intracellular P^H lowering (Shi *et al*., 2016). These actions distort cellular functions that are necessary for growth, replication and survival of bacteria. Therefore, the observed antibacterial activity of the EO of *C. citratus* against both FQRSA and FQREC demonstrated its therapeutic potential on multidrug-resistant bacteria.

5. CONCLUSION

The EO of *C. citratus* exhibited a promising antibacterial activity against both FQRSA and FQREC isolates. The significant effect produced by the EO was due to the components with proven antibacterial property. Citral and citral derivatives being the most abundant compounds in the essential oil might be responsible for the observed antibacterial activity. The results of the present work have corroborated the potential of EO of *C. citratus* in the treatment of infections caused by fluoroquinolone-resistant *S. aureus and E. coli*

REFERENCES

[1] Abers M, Schroeder S, Goelz L Sulser A, St.Rose T, Puchalski K, Langland J (2021) Antimicrobial activity of the volatile substances from essential oils. *BMC Complement Med Ther* **21**, 124

[2] Adonu CC, Ejikeugwu C, Iroha I, Aguiyi J, Esimone CO. (2018). Prevalence and plasmid profile of fluoroquinolone-resistant *Staph. aureus* isolates from domestic animals in Enugu State, Nigeria.*Middle-east journal of scientific research,* 26(6):585- 591.

[3] Amit KT, Anushere M. (2010). Liquid and vapour-phase antifungal activities of selected essential oils against *Candida albicans*: microscopic oberservations and chemical characterization of *Cymbopogon citratus BMC complementary and Alternative medicine;* 10:65-68

[4] Benjamin TT, Adebare JA, Remi Ramota R, Rachael K.(2012) Efficiency of some disinfectants on bacterial wound pathogens. Life Sci J. ;9:2012.

[5] Cheesbrough M. (2002). District Laboratory Practice in Tropical Countries Part 2 Cambridge University Press, Dock House, cape town 8001, S/ Africa. 157- 159

[6] Cimanga K, Tona L, Apers S, BruyneTde, Hermans N, Totte J .(2002). Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J Ethanopharmacol; 79 (2): 213-20.

[7] CLSI-Clinical and Laboratory Standards Institute (2013). Performance standards for antimicrobial susceptibility testing approved standard M100-S23.Clinical and Laboratory Standards Institute, Wayne, PA.

[8] Dalhoff, A .(2008).“Discovery and development of anti-infectives at bayer: a personal view. Part III: fluoroquinolones,” SIM News, 58 (3). 92–105.

[9] Hartatie ES, Prihartini I, Widodo W, Wahyudi A.(2018) Bioactive compounds of lemongrass (Cymbopogon citratus) essential oil from different parts of the plant and distillation methods as natural antioxidant in broiler meat. IOP Conference Series: *Materials Science and Engineering.* 2018;532:1-6

[10] Hasim SF, Ayunda RD, Faridah DN.(2015) Potential of lemongrass leaves extract (Cymbopogon citratus) as prevention for oil oxidation. *Journal of Chemical and Pharmaceutical Research.*;7(10):55-60

[11] Iyevhobu KO, Edo EO, Airefetalor A, Jabbo AA, Malgwi JM.(2022) In Vitro Antibacterial Activity of Garcinia Kola Aqueous Stem Extract on Bacterial Isolated from Patient Visiting a Tertiary Health Facility in South-South Nigeria. *Am J Biomed Sci & Res* 17(1).

[12] Majewska E, Kozowska M, Gruczyska-Skowska E, Kowalska D, Tarnowska K. (2019) Lemongrass (Cymbopogon citratus) essential oil: Extraction, composition, bioactivity and uses for food preservation: A review. *Poland Journal of Food Nutrition and Science.*69(4):327-341

[13] Manvitha K, Bidya B. (2014) Review on pharmacological activity of Cymbopogon citratus. *International Journal of Herbal Medicine*.1(6):5-7

[14] Murray PR., Rosenthal KS and Pfaller MAC. (2005). Medical Microbiology. Elsevier Mosby Publishers, Philadephia.

[15] Naik M, Fomda B, Jaykumar E, Bhat JA. (2010). Antibacterial activity of lemongrass (*Cymbopogoncitratus*) oil against some selected pathogenic bacteria *Asian Pacific Journal of Tropical Medicine*, 535-538.

[16] Saleem M, Afza N, Anwar MA, Hai SM, Ali MS (2003) Comparative study of essential oils of *Cymbopogoncitratus* and some members of the Genus Citrus. Nat Prod Res, 17:369–73.

[17] Shigeharu I, Toshio T, Hideyo Y (2001) Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. *J AntimicrobChemother;* 47: 565-73.

[18] Shi C., Song K., Zhang X., Sun Y., Sui Y., Chen Y., Jia Z., Sun H., Sun Z., Xia X(2016) Antimicrobial Activity and Possible Mechanism of Action of Citral against CronobacterSakazakii. *PLoS ONE*. 11.

[19] Torres RC, Otengco DC, Balgos NS, Villanuya MA, Lanto EA, Cruz MS (2002). Antibacterial essential oil from some Philippine plants. Laguna. *The Philippine society of Microbiol*Inc; 219-220.

[20] Verma SR, Verma KR, Padalia RC, chauhan A, Singh A, Singh HP (2011). Chemical diversity in the Essential oil of indian Valerian, *Chemistry and Biodiversity*, 8 (10):1921-1929.

[21] Wifek M, Saeed A, Rehman R, Nisar S. Lemongrass (2016). A review on its botany, properties, applications, and active components. *International Journal of Chemical and Biochemical Sciences.* 9:79-84

Citation: *Adonu Cyril Chekwube, et.al., (2023). "Chemical Composition and Antibacterial Potential of Essential Oil of Cymbopogon Citratus against Fluoroquinolone-Resistant Staphylococcus Aureus and Escherichia Coli Isolates from Humans in Enugu State, South-East Nigeria.". International Journal of Medicinal Plants and Natural Products (IJMPNP), 9(3), pp.1-10. https://doi.org/ 10.20431 /2454-7999.0903001*