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Antimicrobial activities of volatile oils of *Ocimum gratissimum*, *Eucalyptus citriodora* and *Cymbopogon citratus* against organisms isolated from Nigerian currency notes

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Abstract
Naira notes are means of exchange for goods and services that may act as fomites in the transmission of pathogens. This study determined the identities of microorganisms isolated from notes and their susceptibilities to antibiotics and volatile oils of *Ocimum gratissimum*, *Cymbopogon citratus* and *Eucalyptus citriodora*. Fifteen notes (₦50-₦500) obtained from two Ilorin markets were soaked in sterile water and loopful of each sample was inoculated onto appropriate agar plates. Isolates were identified using standard methods, confirmed with Identification System and Mycological Atlas. Disc and agar diffusion methods were employed for susceptibility tests and positive controls were Ciprofloxacin and fluconazole. *S. aureus* (42%) was the most prevalent bacteria, *Serratia odorifera* (11.1%) including *Aspergillus niger* (1) while some had multiple bacteria. The isolates exhibited 100% resistance to amoxicillin/clavulanate (100%), ceftazidime (57%), sulphamethoxazole trimethoprim (43%) and 100% sensitivity to gentamicin. All *S. aureus* isolates were methicillin resistant – MRSA and vancomycin susceptible while *A. niger* exhibited 100% sensitivity to fluconazole. The volatile oil of *C. citratus* exhibited highest growth inhibitory effects with MBC and MIC of 3.13-0.39 and 1.56-0.39 mg/mL respectively; the oil of *E. citriodora* exhibited the least activity. This oil can be used as antiseptics against Naira fomites.

Keywords: Naira notes; Microbial contamination; Antimicrobial susceptibility; Volatile oils

INTRODUCTION

Medicinal plants are used for the prevention, treatment and management of various infections [1] and their use is gaining more recognition due to the emergence and spread of antimicrobial resistant isolates within the environment [2, 3]. Different plant parts namely; fruits, leaves, stems, bark, roots and flowers [4] as well as by-products such as gums, resins, waxes and volatile essential oils [5] have been reported to have antimicrobial characteristics. Volatile oils have been used by many cultures for various purposes such as personal beauty care, aromatherapy, perfume

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production, food preservation, antioxidant, insecticidal, antiviral and antibacterial [6].

All over the world, currencies serve as the major medium of exchange of value for goods and services. The circulatory mobility of currencies within different groups of people of varying occupations might act as indirect environmental vehicles for the transmission of potential pathogenic microorganisms [7,8]. Although several works have reported the isolation of various microorganisms from different currencies [9-14], there is still paucity of information about the probable antimicrobial effect of volatile oils on microbes isolated from the Nigerian currencies. Therefore, this study was carried out to isolate, identify and determine the susceptibilities of the microbes isolated from Nigerian currency notes, circulating within selected markets in Ilorin to standard antibiotics and selected volatile oils.

EXPERIMENTAL METHODS

Collection and extraction of volatile oils from plant materials. Fresh leaves of *Ocimum gratissimum* were purchased from Uselu market, Benin City while leaves of *Cymbopogon citratus* and *Eucalyptus citriodora* were collected at the University of Benin Ugbowo Campus, Benin City. The identities of the plants were authenticated by Prof. Henry Akininibosun, a Taxonomist of the Department of Plant Biology and Biotechnology, University of Benin Herbarium where the plants were assigned the following voucher numbers: *Ocimum gratissimum*, UBH 0341, *Cymbopogon citratus*, UBH 0712 and *Eucalyptus citriodora*, UBH 0911. The volatile oil of each plant was extracted by hydro-distillation Clevenger apparatus method [15] for 3 to 4 h. At the end of the distillation, the volatile oils obtained were separately collected into sample bottles, measured and kept in refrigerators maintained at 4^{o}C.The percentage yield of volatile oils produced was calculated using:

$$\%\ yield = \frac{\text{volume of extracted oil (mL)}}{\text{dry weight of sample (g)}}\ x\ 100$$

Collection of Naira notes. A total of fifteen (15) Nigerian currency notes of different denominations were obtained from fish sellers, meat sellers, pepper sellers, and pepper millers of two (2) major markets, Ipata and Oja Oba markets in Ilorin, Kwara State, Nigeria. The notes were collected into sterile polythene bags and transported to the Pharmaceutical Microbiology and Biotechnology laboratory for immediate processing.

Isolation of microorganisms. This was performed using the aerobic plate culture method [13] with slight modification. The notes were aseptically soaked differently in sterile Petri dishes containing normal saline for about thirty (30) minutes after which they were removed and dried. The resulting normal saline served as a stock sample for each note. Loopful of the stock samples were inoculated onto prepared agar plates of Mannitol Salt Agar (MSA), Eosin methylene blue (EMB), MacConkey agar (MCA) and potato dextrose agar (PDA) (Oxoid, Basingstoke, UK). All the plates were incubated at 37°C for 18 hours while PDA plates were incubated at 25°C for 5 days. Post incubation, colonial characteristics were read and repeated sub-culturing was performed to obtain pure colonies maintained on Nutrient Agar (NA) slants and stored at 4°C until required.

Identification of isolates. The isolates obtained were identified using Gram reactions and standard biochemical test [16]. Further identification of isolates was done using an analytical profile index test kit (API 20 E test, BioMérieux), a rapid identification kit as described by the manufacturer and confirmed on the API Identification software [17]. The fungi were identified using the appropriate growth media, morphological and microscopic characteristics using lactose phenol cotton blue stain [18].

Antibiotic susceptibility testing (AST) using standard antibiotics. Antibiotic susceptibility profiles of the bacterial isolates were evaluated using modified Kirby-Bauer disc diffusion technique [19]. Ten (10) antibiotic discs (Oxoid, Basingstoke, UK) of Ciprofloxacin (CIP) 5 µg, Imipenem (IPM) 10 µg, sulfamethoxazole/trimethoprim (SXT) 1.25/23.75 µg, gentamicin (CN) 10 µg, ceftazidime (CAZ) 30 µg, Amoxicillin-clavulanate (AMC) 20/10 µg, erythromycin (E) 15 µg, Ampicillin (AMP) 10 µg, Oxacillin (OX) 5 µg (Detection of methicillin resistant *S. aureus* MRSA), vancomycin 5 µg/mL and Fluconazole (50 mg/mL) were used.

Freshly sub cultured isolates were standardized to 0.5 McFarland turbidity (1.5 x 10^8 CFU/mL) and inoculated aseptically onto MHA and the antibiotic discs were placed at equidistant. The MHA plates were then incubated at 37◦C for 24 h. After 24 h incubation, the inhibition zones were measured in millimeter and interpreted based on the recommendations of the Clinical and Laboratory Standards Institute [20] and interpreted as Sensitive (S), Intermediate (I) and Resistant (R).

The screening of *Staphylococcus aureus* isolates for vancomycin resistance was done using the Tenover method [21]. Overnight cultures of *S. aureus* were adjusted to 0.5 McFarland and 10µL was inoculated on MHA agar containing 5µg/mL of vancomycin. The plates were incubated at 37°C for 24 - 48 h and observed at interval of 24 h.

Screening for resistance to fluconazole was performed using the protocol described by [20] with slight modifications. With the aid of a sterile scalpel blade, conidia from fresh and actively growing fungi culture on PDA were carefully transferred into a sterile tube containing 5 mL of sterile water, supplemented with 0.1% Tween 20. The suspension was vortexed for 30 seconds and finally adjusted to a turbidity of 0.5 McFarland containing 2×10^5 spore/mL. Prepared PDA plates were inoculated with 100 µL of standardized fungus suspension and allowed to dry for 10 minutes. With the aid of a sterile 6 mm cork-borer, four wells were made at equidistant and aliquot of 100 µL of 50 mg/mL prepared using sterile distilled water, was carefully introduced into the well and the plate was incubated aerobically for 3 days at 25˚C. Post incubation, the zones of inhibition corresponding to the antimicrobial activity of the tested agents were measured in millimeter (mm) and interpreted according to European Committee on Antimicrobial Susceptibility Testing criteria [23].

Antimicrobial susceptibility assay of selected volatile oils. The inhibitory effect of volatile oils of *Ocimum gratissimum, Cymbopogon citratus* and *Eucalyptus citriodora* for different concentrations (12.5, 25, 50 and 100) mg/mL prepared using 10% v/v Tween – 80 were carried out using the well diffusion method [15]. Prepared MHA and PDA plates were inoculated with previously prepared to 0.5 McFarland turbidity of 1.5 x 10^8 CFU/mL and 2×10^5 spore/mL for both bacterial and fungal isolates after boring wells using 6 mm cork borer. Hundred (100) µL of each of the different concentrations were introduced into wells and the plates were allowed to pre-diffuse for 45 minutes. Ciprofloxacin (10 mg/mL), fluconazole (25 mg/mL) and 10% Tween – 80 were used as positive and negative controls respectively. The plates were all incubated for 24 and 48 h at 37˚C and 25˚C respectively. This was performed in triplicates and zones of inhibition were measured in millimeter (mm).

Determination of Minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC). In order to evaluate the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the volatile oils of *Cymbopogon citratus* and *Ocimum gratissimum* against the test bacteria, broth dilution method [24]. Starting

concentration of 12.5 mg/mL for volatile oils of *Cymbopogon citratus* and *Ocimum gratissimum* were prepared using 10% v/v Tween-80 and dilutions were prepared to yield concentration ranges of 6.25 – 0.20 mg/mL for both volatile oils. Fifty (50) microliters of 0.5 McFarland turbidity standardized bacterial inocula (approximately 10^8 CFU/mL) was then introduced into tubes and all tubes were then incubated at 37◦C for 24 hours. Control tubes (organism viability, broth sterility), ciprofloxacin (10 mg/mL), fluconazole (25mg/mL) and 10% Tween–80 were used as positive and negative controls respectively. Twenty-four (24 hours) post incubation, the tubes were examined for growth by observing for the presence or absence of turbidity. The tube with the lowest concentration of volatile oil at which no visible turbidity was regarded as the MIC. The MIC tubes and tubes with concentrations higher than the MIC were vortexed for 30 seconds and sub cultured on MHA plates. The plates were incubated at 37◦C for 24 hours and then observed for any growth of colonies. MBC was regarded as the higher concentration above the MIC of essential oil at which no growth occurred following the sub culturing onto MHA plates.

Data analysis. Obtained data were analyzed using Microsoft Excel version 2013, organized into percentage frequency, tables, bar charts and zones of inhibition given as mean values of triplicates experiments ± Standard Deviation (SD).

RESULTS

Percentage yield of volatile oils. Each of the volatile oil containing plants produced almost similar quantities of the oil within the period of extraction with the hydro-distillation method as indicated in Table 1. *Eucalyptus citriodora* had the highest volatile oil yield of 1.2% from 800g plant sample while *Ocimum gratissimum* and *Cymbopogon citratus* had 1.1% yield from 1000 g plant samples each.

Distribution and sources of Naira notes collected within selected markets in Ilorin
The fifteen (15) different naira note denominations collected from two of the markets comprised of ₦50, ₦100, ₦200 and ₦500 obtained from different sellers within the market as shown in Table 2 below with their respective codes.

Distribution of microbial isolates. A total of 36 organisms were isolated from the 15 different currency notes comprising of Gram-positive, Gram-negative bacteria and fungi (Figure 1). *S. aureus* was the most predominant at 42% (15/36), followed by *Serratia odorifera* 11.1% (4/26), *Enterobacter cloacae* 8.3% (3/36), *Aeromonas hydrophilia* 5.5% (2/36), *Raoutella ornithinolytica* 5.5% (2/36) and others at lower frequencies including *Aspergillus niger* (Table 3). Microbial contamination of collected naira notes showed that *S. aureus* was isolated from all currency notes, four bacteria were isolated from A2, three from B4 and *A. niger* was isolated only from B7 (Table 4).

Antibiotic susceptibility testing (AST) using standard antibiotic discs. All the organisms obtained from the naira notes exhibited varying resistance and susceptibility patterns to the antibiotics used. Bacterial isolates exhibited a 100% resistance to amoxicillin/clavulanate and oxacillin. Other notable resistance include; erythromycin and ampicillin (80%), ceftazidime (57%) and sulphamethoxazole trimethoprim (43%). All the isolates were sensitive to gentamicin, all *S. aureus* isolates were vancomycin-susceptible (VSSA) and *A. niger* was also sensitive to fluconazole (Figure 2).

Antibacterial susceptibility assay of the volatile oils. All three volatile oils generally showed significant levels of growth inhibitions against *S. aureus* with *C. citratus* exhibiting the highest level of mean zones of inhibition of 20.00 ± 0.00 mm, followed by *O. gratissimum* (19.17 ± 0.76) mm and *E. citriodora* (9.67 ±

0.58) mm (Table 5). Against the Gram-negative bacteria (*Serratia odorifera 1*), *C. citratus* exhibited the highest level of growth inhibition with mean zones of inhibition of 40.33 ± 0.58 mm while *O. gratissimum* had 19.50 ± 0.50 mm for *E. aerogenes*. The volatile oil of *E. citriodora* showed no activity (Table 6).

Antifungal susceptibility assay of the volatile oils. The volatile oils of the plants produced varying levels of growth inhibition on the fungus (Table 7). *Cymbopogon citratus* exhibited activity at all the concentrations ranging from 29.00 ± 1.00 (12.5mg/mL) to 39.67 ± 0.58 mm at 50mg/mL while *O. gratissimum* had the highest activity of 20.5 ± 0.50 mm at 100mg/mL and *E. citriodora* exhibited no activity at all the concentrations.

Table 1: Percentage yields of volatile oils

S/N	Plant source	Weight of plant material (g)	Extractive value of the volatile oils (%)	Amount extracted (mL)
1	*Ocimum gratissimum*	1000	1.1	10
2	*cymbopogon citratus*	1000	1.1	10
3	*Eucalyptus citriodora*	800	1.2	10

Table 2: Distribution and sources of Naira notes collected within selected market in Ilorin

S/N	Location	Currency denomination (₦)	Sources (Traders)	Sample code
1	Oja Oba market	50	locust beans seller	A1
2	Oja Oba market	200	fish seller	A2
3	Oja Oba market	500	meat seller	A3
4	Oja Oba market	200	meat seller	A4
5	Oja Oba market	500	pepper seller	A5
6	Oja Oba market	200	pepper seller	A6
7	Oja Oba market	100	meat seller	A7
8	Ipata market	100	Pepper	B1
9	Ipata market	500	Pepper	B2
10	Ipata market	500	fish seller	B3
11	Ipata market	200	meat seller	B4
12	Ipata market	50	pepper miller	B5
13	Ipata market	500	meat seller	B6
14	Ipata market	200	locust beans seller	B7
15	Ipata market	100	meat seller	B8

Table 3: Percentage of isolates obtained from collected naira notes

S/N		Isolates	Frequency (F)	Percentage (%)
1	Gram-Positive Bacteria	*Staphylococcus aureus*	15	42
2	Gram-Negative Bacteria	*Serratia odorifera 1*	4	11.1
3		*Aeromonas hydrophilia*	2	5.5
4		*Raoutella ornithinolytica*	2	5.5
5		*Klebsiella oxytoca*	1	2.7
6		Cronobacter spp	1	2.7
7		*Enterobacter aerogenes*	1	2.7
8		*Klebsiella pneumoniae 1*	1	2.7
9		*Klebsiella pneumoniae 2*	1	2.7
10		*Enterobacter cloacae*	3	8.3
11		*Serratia liquefaciens*	1	2.7
12		*Serratia marcescens*	1	2.7
13		*Salmonella enterica*	1	2.7
14		Salmonella spp	1	2.7
15	Fungi	*Aspergillus niger*	1	2.7
		TOTAL	36	100

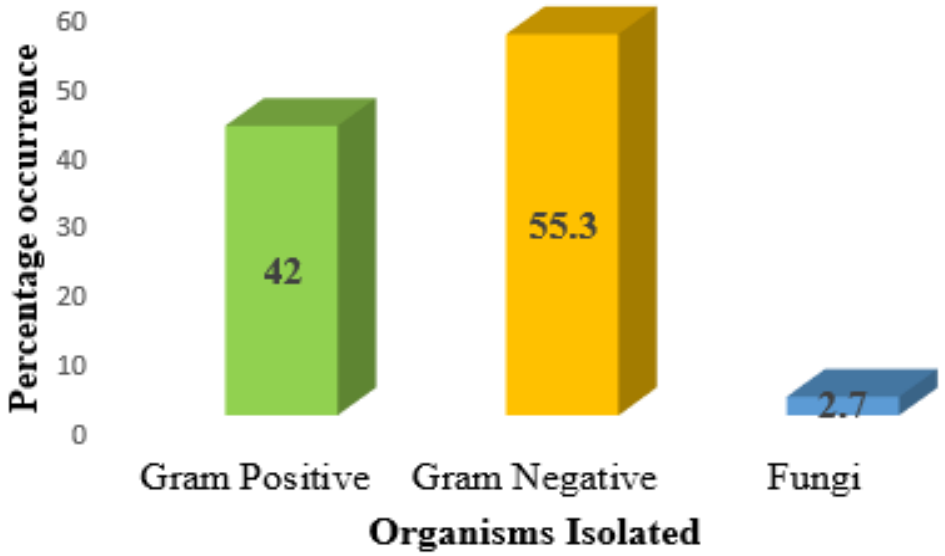

Figure 1: Percentage occurrence of organisms isolated from collected Naira notes

Table 4: Identities of microorganisms obtained from the different denomination of Naira notes

S/No.	Code	Organisms Isolated	
		Gram-positive	Gram-negative
1	A1	*Staphylococcus aureus*	*Serratia odorifera 1*
2	A2	*S. aureus*	*S. odorifera 1, Aeromonas hydrophilia Rautella ornithiolytica, Enterobacter cloacae*
3	A3	*S. aureus*	*Klebsiella oxytoca*
4	A4	*S. aureus*	*S. liquefaciens*
5	A4	*S. aureus*	*Klebsiella pneumonia 1*
6	A6	*S. aureus*	*E. cloacae*
7	A7	*S. aureus*	*Serratia marcescens*
8	B1	*S. aureus*	Cronobacter spp
9	B2	*S. aureus*	*E. aerogenes*
10	B3	*S. aureus*	*S. odorifera 1, S. odorifera 1, R. ornithiolytica*
11	B4	*S. aureus*	*E. cloacae*
12	B5	*S. aureus*	*Klebsiella pneumonia 2*
13	B6	*S. aureus*	*Salmonella enterica*
14	B7	*S. aureus*	*A. hydrophilia, Aspergillus niger*
15	B8	*S. aureus*	Salmonella spp

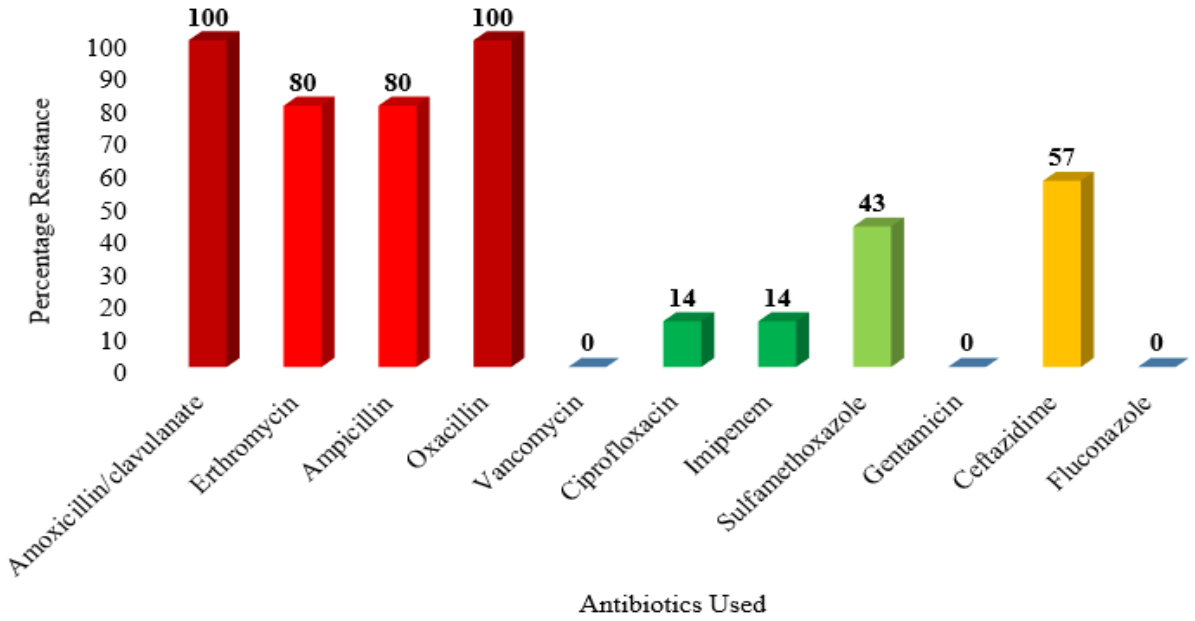

Figure 2: Percentage resistance of selected antibiotics against isolates obtained from naira notes

Table 5: Diameter of zones of inhibition (mm) of volatile oils against *S. aureus* at 12.5 mg/mL concentration

	Zones of Inhibition (mm) at 12.5 mg/mL		
Code	*O. gratissimum*	*C. citratus*	*E. citriodora*
A1	13.67 ± 0.51	18.17 ± 0.29	9.67 ± 0.58
A2	14.67 ± 0.58	19.50 ± 0.50	8.00 ± 0.00
A3	15.00 ± 0.00	19.00 ± 0.00	0 ± 0.00
A4	14.00 ± 0.00	19.00 ± 0.00	0 ± 0.00
A5	14.33 ± 0.58	17.33 ± 0.58	0 ± 0.00
A6	15.00 ± 0.50	20.50 ± 0.50	0 ± 0.00
A7	15.33 ± 0.58	19.00 ± 1.00	0 ± 0.00
B1	18.00 ± 0.50	19.50 ± 0.50	8.50 ± 0.00
B2	13.00 ± 1.00	18.00 ± 0.00	0 ± 0.00
B3	15.50 ± 0.50	19.17 ± 0.29	0 ± 0.00
B4	15.17 ± 0.29	19.50 ± 0.29	0 ± 0.00
B5	14.50 ± 0.50	20.00 ± 0.00	0 ± 0.00
B6	15.33 ± 0.29	20.00 ± 0.00	0 ± 0.00
B7	19.17 ± 0.76	20.00 ± 0.76	0 ± 0.00
B8	15.50 ± 0.50	20.00 ± 0.58	0 ± 0.00

Values of zones of inhibition are presented as mean ± SD from triplicate investigations (n = 3)

Minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC) of volatile oils. Tables 8 and 9 show the MIC and MBC values of *O. gratissimum* and *C. citratus* volatile oils. *O. gratissimum* and *C. citratus* had MIC ranging from 1.56 – 0.39 and 0.78 – 0.20 mg/mL among the Gram-positive and negative bacteria respectively. The volatile oils also had MBC ranging from 3.13 – 0.39 and 1.56 – 0.39 mg/mL among the Gram-positive and negative bacteria respectively with some isolates exhibiting both MIC and MBC at the same concentrations.

Table 6: Diameter of zones of inhibition (mm) of volatile oils against Gram-negative bacteria at 12.5 mg/mL

Code	Organism	Zones of inhibition (mm) at 12.5mg/mL		
		O. gratissimum	*C. citratus*	*E. citriodora*
A1	*Serratia odorifera 1*	14.00 ± 0.00	40.33 ± 0.58	0 ± 0.00
A2	*Serratia odorifera 1*	0 ± 0.00	0 ± 0.00	0 ± 0.00
	Aeromonas hydrophilia	18.33 ± 0.29	29.50 ± 0.50	0 ± 0.00
	Rautella ornithiolytica	17.67 ± 0.58	24.83 ± 0.76	0 ± 0.00
	Enterobacter cloacae	0 ± 0.00	27.33 ± 0.58	0 ± 0.00
A3	*Klebsiella oxytoca*	14.83 ± 0.76	0 ± 0.00	0 ± 0.00
A4	*S. liquefaciens*	0 ± 0.00	0 ± 0.00	0 ± 0.00
A5	*Klebsiella pneumoniae 1*	0 ± 0.00	0 ± 0.00	0 ± 0.00
A6	*E. cloacae*	15.33 ± 0.58	0 ± 0.00	0 ± 0.00
A7	*Serratia marcescens*	0 ± 0.00	0 ± 0.00	0 ± 0.00
B1	*Cronobacter* species	0 ± 0.00	0 ± 0.00	0 ± 0.00
B2	*E. aerogenes*	19.50 ± 0.50	0 ± 0.00	0 ± 0.00
B3	*S. odorifera 1*	18.00 ± 1.00	0 ± 0.00	0 ± 0.00
B4	*S. odorifera 1*	0 ± 0.00	0 ± 0.00	0 ± 0.00
	R. ornithiolytica	16.33 ± 0.58	0 ± 0.00	0 ± 0.00
	E. cloacae	0 ± 0.00	22.17 ± 0.29	0 ± 0.00
B5	*Klebsiella pneumoniae 2*	15.83 ± 0.76	40.00 ± 0.00	0 ± 0.00
B6	*Salmonella enterica*	14.00 ± 0.50	0 ± 0.00	0 ± 0.00
B7	*A. hydrophilia*	15.00 ± 0.00	0 ± 0.00	0 ± 0.00
B8	*Salmonella* species	14.83 ± 0.29	37.83 ± 0.29	0 ± 0.00

Values of zones of Inhibition are presented as mean ± SD from triplicate investigations (n = 3)

Table 7: Diameter of zones of inhibition (mm) of volatile oils against *A. niger* at different concentrations

Fungi	Concn. (mg/mL)	Zones of inhibition (mm)		
		Ocimum gratissimum	*Cymbopogon citratus*	*Eucalyptus citriodora*
Aspergillus niger	12.5	0.00 ± 0.00	29.00 ± 1.00	0.00 ± 0.00
	25	12.00 ± 0.00	39.67 ± 0.58	0.00 ± 0.00
	50	16.67 ± 0.58	32.33 ± 0.58	0.00 ± 0.00
	100	20.5 ± 0.50	27.83 ± 0.29	0.00 ± 0.00
Fluconazole	50	24.00 ± 0.00	27.00 ± 0.00	22.00 ± 0.00

Values of zones of Inhibition are presented as mean ± SD from triplicate investigations (n = 3)

Table 8: Minimum inhibitory concentrations (MIC) of two volatile oils against isolated bacteria (mg/ml)

		O. gratissimum			*C. citratus*	
Code	MIC (*S. aureus*)	G –ve Isolates	MIC	MIC (*S. aureus*)	G –ve Isolates	MIC
A1	1.56	*S. odorifera 1*	0.78	0.78	*S. odorifera 1*	0.39
A2	1.56	*A. hydrophilia*	0.78	0.78	*A. hydrophilia*	0.39
		R. ornithiolytica	0.78		*R. ornithiolytica*	0.39
					E. cloacae	0.39
A3	0.78	*K. oxytoca*	0.78	0.39		-
A4	1.56		-	0.39		-
A5	0.78		-	0.78		-
A6	0.78	*E. cloacae*	0.39	0.78		-
A7	0.78		-	0.39		-
B1	0.78		-	0.20		-
B2	0.78	*E. aerogenes*	0.78	0.78		-
B3	0.78	*S. odorifera 1*	0.78	0.20		-
B4	0.78	*R. ornithiolytica*	1.56	0.39	*E. cloacae*	0.78
B5	1.56	*K. pneumonia 2*	0.39	0.78	*K. pneumonia 2*	0.39
B6	0.78	*Salmonella enterica*	0.39	0.78		-
B7	1.56	*A. hydrophilia*	0.78	0.39		-
B8	0.39	Salmonella spp	0.78	0.78	Salmonella spp	0.39

G –ve = Gram-negative

Table 9: Minimum bactericidal concentrations of two volatile oils against isolated bacteria (mg/ml)

	O. gratissimum			*C. citratus*		
Code	MBC (*S. aureus*)	Gram –ve Isolates	MBC	MBC (*S. aureus*)	Gram –ve Isolates	MBC
A1	3.13	*S. odorifera 1*	1.56	1.56	*S. odorifera 1*	0.78
A2	3.13	*A. hydrophilia*	1.56	1.56	*S. odorifera 1*	0.78
		R. ornithiolytica			*A. hydrophilia*	0.78
					E. cloacae	
A3	1.56	*K. oxytoca*	0.78^	0.78		-
A4	3.13		-	0.78		-
A5	0.56		-	1.56		-
A6	0.56	*E. cloacae*	0.39^	1.56		-
A7	0.56		-	0.78		-
B1	0.56		-	0.39		-
B2	0.56	*E. aerogenes*	1.56	1.56		-
B3	0.56	*S. odorifera 1*	1.56	0.39		-
B4	0.56	*R. ornithiolytica*	1.56^	0.78	*E. cloacae*	0.78^
B5	3.13	*K. pneumonia 2*	0.78	1.56	*K. pneumonia 2*	0.78
B6	1.56	*Salmonella enterica*	0.78	1.56		-
B7	1.56^	*A. hydrophilia*	1.56	0.78		-
B8	0.78	Salmonella spp	1.56	1.56	Salmonella spp	0.78

Gram –ve = Gram-negative; ^ = Isolates exhibiting both MIC and MBC at the same concentration

DISCUSSION

Currency notes serve as medium of transaction, exchange of goods and remuneration of services. The pieces remain in circulation and continue this mobility until completely old and worn out, becoming unworthy of presentation. In the course of this mobility, hand to hand transfer particularly in areas where the currencies are not well kept and suffer exposure to moist handling and storage, it is bound to harbor microorganisms. In this study, all the Naira notes obtained from the markets had microbial contaminations and the most predominant was *S. aureus* (42%). The dominance of *S. aureus* is similar to the reports by Bhat *et al.* [25] and Usman *et al.* [26] but with a higher rate of 100% each among Indian and Nigerian currencies respectively. In the same manner, lower rates of *S. aureus* (8.1%, 22.5% and 25%) contamination of Nigerian and Sudanese currency notes have been reported [8,27,28]. Both bacteria and fungi were isolated from naira notes collected from different markets. This concurs with the findings [13,26] which reported the isolation of both bacteria and fungi from Indian and Nigerian currency notes while other researchers documented only bacterial contamination of currency notes [8,28]. This suggest that currency notes serve and provide ideal conditions for the growth and multiplication of microbes and could serve as vehicles for the transmission of potential pathogenic resistant microorganisms.

The findings in this study further revealed a 55.3% occurrence of Gram-negative bacteria. This is similar to the report of Gram-negative bacteria dominance [29, 26], however with lower and higher rates of 40% and 70% respectively which differ from other reports [27,28] which documented varying prevalence of Gram-positive bacteria. The results suggest a probable poor level of hygiene practices within markets and dwellers. As the currencies are in perpetual motion through changing of hands, there is high tendency of contracting the organisms. Gram-negative bacteria are of global health threats significance due to their high resistance to antibiotics and easy transfer resistant genes leading to the emergence of resistant strains [30].

The isolation of *S. aureus* from all the sampled notes implies the ubiquitous nature of the *S. aureus*. It is a normal flora of the skin, nose, mouth and soil. The handling, counting and storage of naira notes as well as transfer

from person to person of different hygiene backgrounds suggest possible routes of cross contamination with foodstuffs. This bacterium is of medical importance causing food poisoning and its ability to produce toxins leading to toxic shock [31].

Although several reports demonstrated the isolation of high microorganisms from lower currency such as ₦10, ₦20, ₦50 and ₦100 denominations [12,32] due to their constant use for exchange and settlement of values, this study reported the isolation of higher bacterial isolates from ₦200 notes collected from fish and meat seller within the markets. This is in concordance with the publication [33] which reported higher contamination rates among denominations of ₦500, ₦200, and ₦100 notes, with the exception of ₦1,000 notes. This suggests the possibility that bacteria can grow and proliferate on currency notes with the availability of blood, moisture and other nutrients providing conducive environmental and climatic conditions. This may also explain the multiple nature of the organisms obtained from each marketer, indicating high mobility of the organisms from one person to another.

Aspergillus niger, an opportunistic and unusual cause of pulmonary aspergillosis especially among immune compromised or suppressed individuals, was isolated from ₦200 notes collected from locust bean seller, similar to the reported isolation of *A. niger* from currency notes collected within Bauchi metropolis in Nigeria [26]. Locust bean is a fermented and highly nutritional condiment [34] which may likely provide all necessary growing requirements for the organism. Incidentally, it was obtained from this singular source.

The antibiotic resistance profile of Gram-negative isolates from currency notes showed 100% susceptibility to gentamicin and high resistance to amoxicillin clavulanate, ampicillin and erythromycin. This is similar to the report in Sudan [27], Pakistan [35] and Ghana [36] among bacteria isolates from paper currency. Another notable resistance was observed with ceftazidime and sulphamethoxazole + trimethoprim. A hundred percent (100%) oxacillin resistance was recorded. This is a clear indication of high prevalence of methicillin resistant *S. aureus* (MRSA) which has also been documented among *S. aureus* isolated from Pakistani currency with 33.3% [35] resistance rate and all *S. aureus* isolates were vancomycin susceptible. *A. niger* was fluconazole susceptible. Generally, this high level of resistance is due to their readily available nature, ease of administration and their cheap nature. However, high resistance to penicillin and ceftazidime (cephalosporin) probably suggests beta-lactamases production [26] highlighting the urgent need for an alternative search for antibiotics from varying sources [37] and can guide the health providers on the appropriate antibiotics for empirical treatment of infections.

The activities of the volatile oils are due to the constituents they contain. The volatile oil of *O. gratissimum* has been reported to contain eugenol (61.9%) as the major constituent while geranial (40.9 %) and neral (29.7 %) were reported to be major constituents of *C. citratus* [38,39]. These major constituents of the volatile oils must have contributed to the growth inhibitory effects observed on the microorganisms. The variations in the constituents can also explain the differences observed in the activities.

The volatile oils used in this study showed varying activities against isolated organisms in the order *C. citratus* > *O. gratissimum* > *E. citriodora*. This is in concordance with several reports which demonstrated antimicrobial activities for different volatile oils [2, 5 and 15]. *C. citratus* and *O. gratissimum* exhibited good antimicrobial activities with the least MIC of 0.20 and 0.39 mg/mL respectively for Gram-positive and negative bacteria. This is can be

compared with reports [40,41]. However, *E. citriodora* demonstrated minimal against *S. aureus* isolates with no activity against Gram-negative and fungi isolates. This anti-staphylococci activity of *E. citriodora* is comparable to a reported work [42] but its poor activity contradicts other reports [42, 43]. This could be due to the lower concentration of 12.5 mg/mL used in this study and the fact that all *S. aureus* isolated in the study were MRSA. Generally, Gram-positive bacteria were more susceptible compared to the Gram-negative bacteria with both direct vapor absorption and indirect diffusion via the media contributing to the antimicrobial activity of volatile compounds [44]. Reduced susceptibility of volatile oils to Gram-negative bacteria could be due to the outer membrane surrounding the cell membrane preventing adequate diffusion of hydrophobic compounds through the lipopolysaccharide layer [45].

Conclusion

The study shows that Naira notes circulating in selected markets within the Ilorin metropolis are heavily contaminated with pathogenic organisms predominantly the drug resistant Gram-negative bacteria and *S. aureus* which are methicillin resistant. These contaminated notes are capable of circulating highly resistant isolates posing serious public health challenges thereby increasing human suffering and economic burden. Volatile oils of *O. gratissimum*, *C. citratus* and *E. citriodora* exhibited varying antimicrobial activity against the isolates obtained from Naira notes with the volatile oil of *E. citriodora* exhibiting poor antimicrobial activity**.**

Because of the valid role of Naira notes in a Nation's economy and transmission of resistant pathogenic organisms, urgent advocacy and awareness should be created to improve proper currency handling and hygiene practices in the study area. Also, the decontamination of Naira notes using volatile oils in the banks and markets could help to reduce the transmission of infectious pathogens.

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