

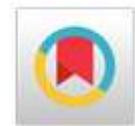


## Antimicrobial activity of *Ricinus comunis*, *Swietenia mahogani* and *Crusentia cujete* ethanol extracts against multidrug resistant pathogens, recovered from a hospital environment

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### Abstract

Medicinal plants have been used as effective approaches to manage multidrug resistant pathogens, including infectious agents that cause nosocomial infections. This study aimed to investigate the antimicrobial potentials of ethanolic extracts of *Ricinus comunis*, *Swietenia mahogani* and *Crusentia cujete* against five multidrug resistant nosocomial pathogens namely; *Staphylococcus aureus*, *Pseudomonas* sp., *Klebsiella* sp., *Escherichia coli* and *Candida* sp., which were isolated from hospital fomites. Using standard microbiological methods, fomite swab samples from ward beddings and door handles from the casualty, women, and children ward of Dalhatu-Araf Specialist Hospital, Lafia, Nigeria, were assessed. A total of 251 microbial isolates, consisting of 8 bacterial and 6 fungal genera were recovered. The highest frequency of microbial pathogens was recorded in the casualty unit (98[39%]), followed by the women's unit (90[36%]), while the children's ward (63[25%]) was the least contaminated. *S. aureus* (25[42%]) and *Aspergillus* sp. (43[72%]) were the most isolated bacteria and fungi; respectively, while *Salmonella* sp. (7[12%]) and *Trichoderma* sp. (9[15%]) were the least isolated. However, there were no significantly statistical differences across wards and microbial isolates. The five selected isolates were tested for *in vitro* susceptibility against several standard antibiotics to check their multiple drug resistance. The tested microorganisms exhibited various levels of multidrug resistance patterns except for *Candida* sp. which was resistant to two classes of antibiotics (azole group and griseofulvin). On the other hand, *Klebsiella* sp. was resistant to eight antibiotics of four classes. The ethanolic leaf extract of *C. cujete* was more effective against all the selected microbial pathogens, while the bark extract of *S. mahogani* was substantially effective. *R. comunis* exhibited no inhibitory potential against any of the tested pathogens. All the plant extracts were not as effective against the tested microorganisms as the conventional antibiotics that were used as positive controls. Results obtained indicate the risk of nosocomial infections caused by multidrug resistant pathogens originating from the hospital environment. Good hygienic practices, public awareness on nosocomial infections and further research into ethnomedicine are hereby recommended.

**Keywords:** Fomites, Multidrug-resistant microorganisms, Nosocomial infection, Plant extracts, Antimicrobials

## 1. Introduction

Hospital environment and fomites including; doorknobs, rails, beds, among others, serve as reservoirs of bacterial pathogens causing nosocomial infections in patients attending to the hospitals for treatment (Suleyman *et al.*, 2018). A recent study of Peters *et al.*, (2019) revealed that the constant increase in cases of nosocomial infections is in line with the increase in bacterial resistance to antibiotics, and constitutes a considerable risk to the human health. Hence, it is vital to search for future strategies to combat these microbial infections and antibiotic resistance.

A previous study conducted by Iwu *et al.*, (2009) revealed that in developing countries, infectious diseases have accounted for approximately one-half of all deaths. In addition, incidence of epidemics in the industrialized nations due to multidrug-resistant microorganisms, poses enormous public health concerns. Accordingly, upon increase of the problem of antibiotic resistance, ethnomedicine is gaining popularity as an alternative to orthodox medicine.

In 2003, the World Health Organization (WHO) observed that up to 80% of the rural areas in the developing countries depend on herbal medicine, and requested the member countries to explore safe indigenous medicine for their national health care (Ogbonnia *et al.*, 2008). The high cost of synthetic drugs has made herbs the most common means for treatment of diseases in rural areas. A high percentage of the population in developing countries, including Nigeria, do not have adequate means to purchase synthetic drugs; thus, herbs become an alternative sources for treatment, since they are readily available and abundant.

Several recent studies conducted by Abdul *et al.*, (2018); Olaniyi *et al.*, (2018) reported that, in many African countries; extracts from plants are currently used for treatment of several infectious diseases,

varying from minor skin infections to gastrointestinal disturbances, in addition to serious infectious diseases such as typhoid fever. A previous study of Ogbonnia *et al.*, (2008) highlighted that antimicrobial compounds from plants may inhibit bacterial infections by a mechanism that differs from that of the used antibiotics, and may have clinical values in treatment of the antibiotic resistant bacterial strains.

Therefore, it is important to ascertain the antimicrobial efficacies of more herbs, as they could provide cost-effective drug delivery strategies, to combat the multidrug resistant microorganisms. The objective of this study was to investigate the antimicrobial potentialities of *R. comunis*, *S. mahogani* and *C. cujete* ethanolic extracts against selected multidrug resistant nosocomial pathogens isolated from hospital fomites.

## 2. Material and methods

### 2.1. Collection and ethanolic extraction of plant materials

Fresh leaves and fruits of *Crusentia cujete* and *Ricinus comunis*, and fresh leaves and stem bark of *Swietenia mahogani* were collected from different sites in Lafia Local Government, Nasarawa State, Nigeria. The plants were properly air dried at room temperature under shade. Ethanolic extraction of plant samples was carried out according to Akinyemi *et al.*, (2005). Plant materials were shredded and ground into fine powder. About 100 g of each powdered plant material was transferred into Erlenmeyer flasks containing 1000 ml of 80 % ethanol and then soaked for 48 h. The mixture was separated by filtration using Whatman no. 1 filter paper; the resulting filtrate was retained and then air-dried to remove excess ethanol, in reference to Doughari *et al.*, (2012). Air-dried extracts were collected in air-tight containers and stored at 4 °C till further analysis.

## 2.2. Microbial samples collection and processing of hospital fomites

Using sterile swab sticks, a total of 60 samples were collected from ward beddings and door handles of the children ward, women ward, and casualty unit of Dalhatu-Araf Specialist Hospital Lafia, Nasarawa state, and then transported immediately to the Microbiology laboratory, for analysis. The door handles were those found on the entrance doors of the wards and the lavatories.

The swab sticks were dipped and gently vortexed in 1 ml 0.85% physiological saline. An aliquot of 0.1 ml of stock solution was streaked on blood agar, MacConkey agar, Mannitol salt agar and Eosin methylene blue agar media for bacterial isolation, and Potato dextrose agar (PDA) for fungal isolation. Bacterial plates were incubated at 37 °C for 24 h while fungal plates were incubated for 72 h at 27± 2 °C. After incubation, the bacterial isolates were sub-cultured on nutrient agar to obtain pure distinct colonies ([Cheesbrough, 2006](#); [Nwankwo, 2012](#)). The assay plates were used in triplicates for each culture medium.

## 2.3. Biochemical identification of the microbial isolates

The bacterial and fungal isolates were identified as described by [Cheesbrough, \(2006\)](#); [Crous \*et al.\*, \(2009\)](#), respectively. In addition to Gram staining, the used biochemical assays included; catalase test, indole test, coagulase test and citrate utilization test, to identify the bacterial isolates ([Lincy \*et al.\*, 2016](#)). On the other hand, the fungal isolates were identified through staining the cultures with lactophenol blue, and then examining the spores' arrangement under a light microscope.

## 2.4. Antibiotics susceptibility pattern of the isolates

Using the Kirby-Bauer disk diffusion method described by [Nwankwo \(2012\)](#); [Adikwu \*et al.\*, \(2016\)](#), the 5 selected microbial isolates mainly; *S. aureus*, *Pseudomonas* sp., *E. coli*, *Klebsiella* sp. and *Candida*

sp. were tested in duplicates for their sensitivity or resistance to a panel of antibiotics including: ofloxacin (20 µg), reflacin (5 µg), cefrozolin (25 µg), augmentin (10 µg), gentamicin (5 µg), nalidixic acid (10 µg), trimethoprim (20 µg), penicillin (10 µg), ampicillin (10 µg), ciprofloxacin (5 µg), norfloxacin (15 µg), amoxicillin (15 µg), streptomycin (30 µg), erythromycin (15 µg), rifampicin (20 µg), ampiclox (10 µg), levofloxacin (5 µg), fluconazole (10 µg), ketoconazole (10 µg), terbinafine (5 µg), miconazole (15µg), itraconazole (10 µg), griseofulvin (5 µg), voriconazole (5 µg) and econazole (5 µg). These microorganisms were selected based on previous reports of their involvement in nosocomial infections in the hospital environment ([Beck-Sague and Jarvis, 1993](#); [WHO, 2002](#); [Bereket \*et al.\*, 2012](#)). Overnight grown broth cultures of the bacterial isolates and 5 days old culture of *Candida* sp. were standardized to approximately 0.5 McFarland standard, and then 0.1 ml aliquots were spread using sterile swab sticks on Mueller-Hinton agar plates, to form a lawn and allowed to stand for 5 min. After incubation for 24 h (for bacteria) and 5 d (for *Candida* sp.), zones of inhibition was measured and recorded in mm using a calibrated ruler. Results were interpreted in accordance with Clinical Laboratory Standards Institute guidelines ([CLSI, 2017](#)).

## 2.5. Determination of antimicrobial potency of the plants extracts

Antimicrobial efficacy of the plants extracts against the multidrug resistant microbial isolates was tested using the agar well diffusion method ([Debalke \*et al.\*, 2018](#)). A 0.1 ml aliquot of 24 h old culture of each bacterial and fungal isolate was spread on plates containing 20 ml of Muller Hinton agar using a sterile glass spreader. About 6 mm well was made individually in each of the seeded media using sterile cork borer, and then 20 µl of each ethanolic plant extract was dispensed separately into each well. The plates were allowed to stand for 1 h and subsequently incubated at 37°C for 24 h. The antimicrobial potential was assayed by measuring the diameters (in mm) of the zones of inhibition formed around each well using

a calibrated ruler, and results were interpreted using [CLSI. \(2017\)](#) guidelines. Ciprofloxacin disc was used as a positive control for Gram-negative bacteria, Levofloxacin for Gram-positive bacteria and Fluconazole for fungi, based on [CLSI. \(2017\)](#) recommendations.

## 2.6. Statistical analysis

Data were analyzed using one-way Analysis of variance (ANOVA) to calculate the significant difference in the frequency of occurrence of microbial species in each ward and across the wards. Statistical significance differences in antimicrobial activities of the plants extracts were also analyzed. The Statistical Package for Social Sciences (SPSS) version 22 was used for the analysis.

## 3. Results

### 3.1. Prevalence of nosocomial pathogens isolated from hospital fomites

A total of 251 microbial contaminants, consisting of 8 bacterial and 6 fungal genera, were isolated from the 60 collected samples, as shown in Table (1). The casualty unit has the highest prevalence of contaminants (98[39%]), followed by the women's ward (90[36%]), while the children's ward has the least microbial contaminants (63[25%]). *Aspergillus* sp. (43[72%]) is the most prevalent fungus, while *S. aureus* (25[42%]) is the most predominant bacterial strain. *Salmonella* sp. (7[12%]) and *Trichordema* sp. (9[15%]) are the least isolated bacteria and fungi, respectively. Statistically, *Aspergillus* sp. is significantly different ( $p \leq 0.05$ ) from all the other microbial isolates. Across the three wards, the recovery of *Candida* sp. in the women's ward is significantly different from the children and casualty wards.

Results presented in Table (2) shows the distribution of the 60 bacterial isolates recovered from ward beddings. The casualty unit and women's ward have the highest frequency of recorded bacteria

(21[35%]), while the least frequency of (18[30%]) is observed in the children's ward. *S. aureus* is the predominant bacteria with a frequency of 13[43%] from beddings, and *Shigella* sp. is the least observed bedding bacterial contaminant with a frequency of 2[7%]. Statistically, there is no significant difference ( $p \leq 0.05$ ) among all the isolates recorded in the various wards.

Table (3) shows the distribution and frequency of bacteria isolated from the door handles. About 51 bacterial isolates are recovered from the door handles, of which 23[45%] are from the casualty unit (the highest), while 15[29%] and 13[26%] are from the women's and children's (lowest) wards, respectively. *Staphylococcus* sp. and *Streptococcus* sp. predominantly recorded the highest frequency of 12[40%] from the sampled door handles, while *Klebsiella* sp. is the least recorded bacterial sp. (2[7%]). However, there is no significant statistical difference ( $p \leq 0.05$ ) observed in all the bacterial isolates across the wards tested for door handles.

Results presented in Table (4) demonstrate the frequency distribution of 60 fungal isolates recovered from ward beddings. *Aspergillus* 917[57%]) is the most frequent fungal isolate, while *Trichoderma* (3[10%]) has the least frequency. The ward beddings from the women's ward have the highest fungal isolates (26[43%]), whereas beddings from the children's ward have the least fungal frequency (14[23%]). All the fungal isolates are not significantly different ( $p \leq 0.05$ ) across all the examined wards.

As shown in Table (5), a total of 80 fungal isolates were recovered from door handles. *Aspergillus* (26[87%]) is the most predominant, whereas *Trichoderma* (6[20%]) is the least prevalent. Children's ward has the least fungal isolates (18[23%]), followed by the women's ward (28[35%]), and the casualty unit (34[43%]) has the highest recorded fungal isolates. In the children's ward, *Aspergillus* sp. is significantly different ( $p \leq 0.05$ ) from *Trichoderma*, *Candida* and *Fusarium* spp.

**Table 1:** Frequency of occurrence of bacterial and fungal isolates recovered from 60 samples of a hospital environment

Microbial isolates	Wards			Cumulative frequency (n=60) (%)
	Children (n=20) (%)	Women (n=20) (%)	Casualty (n=20) (%)	
<i>Pseudomonas</i> sp.	2 (10)	3 (15)	7 (35)	12 (20)
<i>S. aureus</i>	7 (35)	8 (40)	10 (50)	25 (42)
<i>Streptococcus</i> sp.	8 (40)	7 (35)	8 (40)	23 (38)
<i>Klebsiella</i> sp.	2 (10)	2 (10)	5 (25)	9 (15)
<i>Proteus</i> sp.	0 (0)	3 (15)	7 (35)	10 (17)
<i>E. coli</i>	5 (25)	7 (35)	2 (10)	14 (23)
<i>Shigella</i> sp.	3 (15)	3 (15)	5 (25)	11 (18)
<i>Salmonella</i> sp.	4 (20)	3 (15)	0 (0)	7 (12)
<i>Aspergillus</i> sp.	14 (70)	15 (75)	14 (70)	43 (72)
<i>Penicillium</i> sp.	11 (55)	12 (60)	8 (40)	31 (52)
<i>Trichoderma</i> sp.	0 (0)	0 (0)	9 (45)	9 (15)
<i>Candida</i> sp.	0 (0)	11 (55)	7 (35)	18 (30)
<i>Rhizopus</i> sp.	7 (35)	6 (30)	8 (40)	21 (35)
<i>Fusarium</i> sp.	0 (0)	10 (50)	8 (40)	18 (30)
<b>Total (%)</b>	<b>63 (25)</b>	<b>90 (36)</b>	<b>98 (39)</b>	<b>251</b>

Where; the isolates were tested at  $p \leq 0.05$ .

**Table 2:** Distribution of bacterial isolates recovered from ward beddings

Bacterial isolates	Wards			Cumulative frequency (n=30) (%)
	Children (n=10) (%)	Women (n=10) (%)	Casualty (n=10) (%)	
<i>Pseudomonas</i> sp.	2 (20)	3 (30)	4 (40)	9 (30)
<i>S. aureus</i>	4 (40)	4 (40)	5 (50)	13 (43)
<i>Streptococcus</i> sp.	4 (40)	4 (40)	3 (30)	11 (37)
<i>Klebsiella</i> sp.	2 (20)	2 (20)	3 (30)	7 (23)
<i>Proteus</i> sp.,	0 (0)	3 (30)	4 (40)	7 (23)
<i>E. coli</i>	3 (30)	4 (40)	0 (0)	7 (23)
<i>Shigella</i> sp.	0 (0)	0 (0)	2 (20)	2 (7)
<i>Salmonella</i> sp.	3 (30)	1 (10)	0 (0)	4 (13)
<b>Total (%)</b>	<b>18 (30)</b>	<b>21 (35)</b>	<b>21 (35)</b>	<b>60</b>

Where; the isolates were tested at  $p \leq 0.05$

**Table 3:** Distribution of bacterial isolates recovered from the door handles

Bacterial isolates	Ward			Cumulative Frequency (N=30) (%)
	Children (n=10) (%)	Women (n=10) (%)	Casualty (n=10) (%)	
<i>Pseudomonas</i> sp.	0 (0)	0 (0)	3 (30)	3 (10)
<i>S. aureus</i>	3 (30)	4 (40)	5 (50)	12 (40)
<i>Streptococcus</i> sp.	4 (40)	3 (30)	5 (50)	12 (40)
<i>Klebsiella</i> sp.	0 (0)	0 (0)	2 (20)	2 (7)
<i>Proteus</i> sp.	0 (0)	0 (0)	3 (30)	3 (10)
<i>E. coli</i>	2 (20)	3 (30)	2 (20)	7 (23)
<i>Shigella</i> sp.	3 (30)	3 (30)	3 (30)	9 (30)
<i>Salmonella</i> sp.	1 (10)	2 (20)	0 (0)	3 (10)
<b>Total (%)</b>	<b>13 (26)</b>	<b>15 (29)</b>	<b>23 (45)</b>	<b>51</b>

Where; the isolates were tested at  $p \leq 0.05$

**Table 4:** Distribution of fungal isolates recovered from ward beddings

Fungal isolates	Ward			Cumulative Frequency (N=30) (%)
	Children (n=10) (%)	Women (n=10) (%)	Casualty (n=10) (%)	
<i>Aspergillus sp.</i>	5 (50)	7 (70)	5 (50)	17 (57)
<i>Penicillium sp.</i>	6 (60)	7 (70)	2 (20)	15 (50)
<i>Trichoderma sp.</i>	0 (0)	0 (0)	3 (30)	3 (10)
<i>Candida sp.</i>	0 (0)	5 (50)	3 (30)	8 (27)
<i>Rhizopus sp.</i>	3 (30)	3 (30)	3 (30)	9 (30)
<i>Fusarium sp.</i>	0 (0)	4 (40)	4 (40)	8 (27)
<b>Total</b>	<b>14 (23)</b>	<b>26 (43)</b>	<b>20 (33)</b>	<b>60</b>

Where; the isolates were tested at  $p \leq 0.05$

**Table 5:** Distribution of fungi isolates recovered from door handles

Fungal isolates	Ward			Cumulative Frequency (N=30) (%)
	Children (n=10) (%)	Women (n=10) (%)	Casualty (n=10) (%)	
<i>Aspergillus sp.</i>	9 (90)	8 (80)	9 (90)	26 (87)
<i>Penicillium. sp.</i>	5 (50)	5 (50)	6 (60)	16 (53)
<i>Trichoderma sp.</i>	0 (0)	0 (0)	6 (60)	6 (20)
<i>Candida sp.</i>	0 (0)	6 (60)	4 (40)	10 (33)
<i>Rhizopus sp.</i>	4 (40)	3 (30)	5 (50)	12 (40)
<i>Fusarium sp.</i>	0 (0)	6 (60)	4 (40)	10 (33)
<b>Total</b>	<b>18 (23)</b>	<b>28 (35)</b>	<b>34 (43)</b>	<b>80</b>

Where; the isolates were tested at  $p \leq 0.05$

### 3.2. Antibiotic susceptibility patterns of the microbial species

Five microbial species including; *Pseudomonas sp.*, *E. coli*, *Klebsiella sp.*, *S. aureus* and *Candida sp.*, were tested for their susceptibility against a panel of antibiotics. Results of the antibiotic susceptibility of the bacterial and fungal spp. are presented in Table (6). These microorganisms were selected based on previous reports of their involvement in nosocomial infections in the hospital environment. Multidrug

resistance is considered as resistance to a single antibiotic in three or more antibiotic groups. *Klebsiella sp.*, *E. coli* and *Pseudomonas sp.* showed resistance to 8, 7 and 6 antibiotics, respectively. *Klebsiella sp.* demonstrated resistance to all of the tested antibiotics, except for cefprozil and gentamicin. *Pseudomonas sp.*, and *E. coli* expressed resistance to several antibiotics in four classes of antibiotics including; Quinolones (ofloxacin, reflacin, ciprofloxacin and nalidixic acid), Penicillin (augmentin, streptomycin), Aminoglycosides (gentamicin, streptomycin), and

Trimethoprim. *S. aureus* is resistant to 5 antibiotics in four classes of antibiotics including; Fluoroquinolones (norfloxacin), Aminopenicillins (amoxicillin), Macrolides (erythromycin) and Penicillin (ampicillin). On the other hand, *Candida* sp. is resistant to 3

antibiotics in the Azole group (miconazole, itraconazole and voriconazole) and to griseofulvin. It showed intermediate reaction to ketoconazole and econazole; however, is susceptible to fluconazole and terbinafine.

**Table 6.** Antibiotic susceptibility patterns of the selected microbial isolates

Gram-negative bacteria	Antibiotics									
	OFX	PEF	CPR	AU	CN	S	CPX	NA	SXT	PN
<i>Pseudomonas</i> sp.	+	+	/	-	+	-	-	-	-	-
<i>E. coli</i>	+	-	+	-	+	-	-	-	-	-
<i>Klebsiella</i> sp.	-	-	+	-	+	-	-	-	-	-
Gram-positive bacteria	CPX	NB	CN	AMX	S	RD	E	CH	APX	LEV
<i>S. aureus</i>	+	-	+	-	+	+	-	+	-	+
Fungi	FLU	KET	TBF	MCL	ITR	GF	V	E		
<i>Candida</i> sp.	+	/	+	-	-	-	-	/		

The assay tests were carried out in duplicates and repeated twice for reproducibility. Where; + = Susceptible, - = Resistant, / = Intermediate. OFX= Oxacilin; CPR= Cefprozil; PEF= Reflacin; CPX= Ciprofloxacin; AU= Augmentin; CN= Gentamycin; S= Streptomycin; NA= Nalidixic acid; SXT= Trimethoprim; PN= Amplicin; NB= Norflox; AML= Amoxil; RD= Rifampin; E= Erythromycin; APX= Ampiclox; CH= Chloramphenicol; LEV= Levofloxacin; FLU= Fluconazole; KET= Ketoconazole; TBF= Terbinafine; MCL= Miconazole; ITR= Itraconazole; GF= Griseofulvin; V= Variconazole; E= Econazole

### 3.3. Antimicrobial potential of the plants extracts against the antibiotic-resistant microbial species

The antimicrobial efficacy of the ethanolic extracts of *R. comunis*, *C. kujete* and *S. mahogani* plants tested against the antibiotic-resistant bacterial and fungal spp. are presented in Table (7). Both the beans and leaf extracts of *R. comunis*, and leaf extract of *S. mahogani* showed no inhibitory efficacy against all the tested isolates. Conversely, the leaf extract of *C.*

*cujete* expressed significant inhibitory potential against all the selected microbial isolates. The fruit extract of *C. kujete* exhibited no inhibitory activity against all the Gram-negative bacteria, but is active against *S. aureus* and *Candida* sp., giving inhibition zone diameters of  $2.00 \pm 2.82$  and  $6.00 \pm 2.82$  mm, respectively. The stem bark extract of *S. mahogani* showed inhibitory effect on *Pseudomonas* sp. ( $7.00 \pm 1.41$ ) mm, *S. aureus* ( $6.00 \pm 2.82$ ) mm, *E. coli* ( $7.00 \pm 1.41$ ) mm, *Klebsiella* sp. ( $8.00 \pm 2.82$ ) mm and *Candida* sp. ( $13.00 \pm 4.24$ )

mm. However, the inhibitory efficacy of all the tested plant extracts against the selected microbial isolates is

low, compared to the conventional antibiotics used as positive controls.

**Table 7.** Mean zones of inhibition (mm) of the plants extracts against the selected multiple drug-resistant bacterial and fungal spp.

Extracts	<i>Pseudomonas</i> sp.	<i>E. coli</i>	<i>Klebsiella</i> sp.	<i>S. aureus</i>	<i>Candida</i> sp.
<i>R. comunis</i> beans	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
<i>R. comunis</i> leaves	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
<i>C. cujete</i> leaves	7.00 ±1.41*	17.00 ±1.41*	17.00 ±1.41*	11.00 ±1.41*	18.00 ±2.82*
<i>C. cujete</i> fruit	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	2.00 ±2.82	6.00 ± 2.82
<i>S. mahogani</i> leaves	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
<i>S. mahogani</i> bark	7.00 ±1.41*	7.00 ±1.41*	8.00 ±2.82*	6.00 ±2.82	13.00 ±4.24*
Positive controls	22.50 ±3.53	23.00 ±4.24	21.00 ±0.70	21.00 ±1.41	22.00 ±1.41

Values are expressed as Mean ±SD (n=2). Where; \* demonstrates a significant difference in the inhibitory potential among the tested plant extracts against the selected microbial isolates at  $p < 0.05$ : Positive control: Ciprofloxacin for Gram-negative bacteria, Rifampicin for Gram-positive bacteria, Fluconazole for fungal species. SD = standard deviation

#### 4. Discussion

In the current study, the most frequently isolated microbial pathogens included; *S. aureus*, *Streptococcus* sp., *E. coli*, *Pseudomonas* sp., *Aspergillus* sp., *Penicillium* sp., *Rhizopus* and *Candida* spp. The highest microbial count recorded in the casualty unit was not surprising, as the unit is frequented with accident emergencies, a high population flow, and high patient turnover, compared to the other wards. Similarly, a recent study conducted by [Awanye and Amrasawore, \(2020\)](#) reported the high prevalence of microbial pathogens including; *S. aureus*, *Pseudomonas* sp., *Klebsiella* sp., *Salmonella* sp. and *Candida* sp. from fomites in the Accident and Emergency Unit, and Plastic and Burn Unit of the

University of Port Harcourt Teaching Hospital, Nigeria.

Recovery of microbial pathogens from fomites supports the earlier reports of isolating similar microbes from toilet door handles in various settings including; hospitals in India ([Lincy et al., 2016](#)), beds and door handles in Kiwoko Hospital in Uganda ([Segujja et al., 2016](#)) and hospital surface samples in Ethiopia ([Getachew et al., 2018](#)). Recovery of methicillin-resistant *S. aureus* from different surfaces including; glass, vinyl floor tile, countertop and stethoscopes have been documented by [Williams and Davis, \(2009\)](#). In Mexico, *Klebsiella* sp., *E. coli*, *Enterobacter* sp., *Pseudomonas* sp. and *Aspergillus* sp. were isolated from the hospital environment, as

documented by [Garcia-Cruz \*et al.\*, \(2012\)](#). The risk of infection is markedly exhibited in these hospital wards sampled in this study, as these microorganisms are known to cause various forms of infections.

The highest bacterial isolates recovered from the casualty unit and children's ward was *S. aureus*, in accordance with [Saka \*et al.\*, \(2016\)](#), who reported the predominance of *S. aureus* from the pediatric ward of the University of Ilorin Teaching Hospital, Nigeria. A previous study of [Bereket \*et al.\*, \(2012\)](#) also documented that *S. aureus* is the foremost source of host infections, including surgical sites infections and lower respiratory site infections.

*Aspergillus* sp. was the predominant fungus recovered from fomites in all the wards, and showed statistically significant difference from all other isolates across all the wards. A total of 80 isolates of *Aspergillus* spp. were recovered from hospital wards (bone marrow transplant, hematology-oncology wards) in a hospital in Lisbon, Portugal ([Sabino \*et al.\*, \(2014\)](#)).

The beddings were more contaminated than the door handles. The level of contamination in the ward beddings causes a concern to the patients and medical practitioners, as it increases the possibility of transfer of infection and prolonged stay in the hospital.

Multidrug resistance is a leading factor that predisposes the patients to nosocomial infections. *S. aureus* was resistant to four classes of antibiotics and is known to be resistant to  $\beta$ -lactamases. The resistance to  $\beta$ -lactams, including ampicillin is due to the production of the enzyme carbapenemase, as reported by [Iroha \*et al.\*, \(2009\)](#). As such, the presence and high frequency of *S. aureus* in the casualty and children's ward exposes the patient to high risk of infections.

*Klebsiella* sp. exhibited the highest multidrug resistance to 80 % of all the tested antibiotics. This agrees with the recent study conducted by [Sserwadda \*et al.\*, \(2018\)](#), which reported the prevalence of high multidrug resistance in *K. pneumonia* recovered from fomites and work surfaces in a general hospital in

Uganda. Moreover, *K. pneumonia* has been implicated in urinary and respiratory tract infections as reported by [Chung, \(2016\)](#), and its presence is indicative of poor hygienic practices and fecal contamination ([Sserwadda \*et al.\*, \(2018\)](#)).

*Escherichia coli* showed the second-highest resistance to five classes of antibiotics in this study. This supports the findings of [Basak \*et al.\*, \(2016\)](#), which reported that the most common multidrug-resistant microorganism isolated from the clinical specimens was *E. coli*. An early study of [Bereket \*et al.\*, \(2012\)](#) revealed that *E. coli* carries a large number of virulence factors and is common in wound infections, urinary tract infections and causes pneumonia in immunocompromised patients. Recently, [Mojica \*et al.\*, \(2020\)](#) highlighted that *Pseudomonas*, *Klebsiella* and *E. coli* are common microorganisms exhibiting multidrug resistance, due to production of  $\beta$ -lactamases, including extended  $\beta$ -lactamases (ESBLs).

*Candida* sp. was resistant to drugs in the azole group and griseofulvin. The Triazole drugs include fluconazoles, which are the first line of antibiotics used for treatment of fungal infections, while voriconazole is a second-generation drug known for its strong activity against *Candida* spp. ([Maroszynska \*et al.\*, \(2013\)](#); [Mandras \*et al.\*, \(2009\)](#)). However, resistance of *Candida* spp. to voriconazole ([Maroszynska \*et al.\*, \(2013\)](#)) and itraconazole ([Zaidi \*et al.\*, \(2018\)](#)) has been reported. Moreover, resistance of *Candida* spp. including *C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis* to several triazole drugs have also been reported by several previous studies conducted by [Maroszynska \*et al.\*, \(2013\)](#); [Li \*et al.\*, \(2013\)](#); [Bhattacharjee, \(2016\)](#). Sensitivity to fluconazole in this study contrasts the report of [Mahmoudabadi \*et al.\*, \(2013\)](#) and [Li \*et al.\*, \(2013\)](#), where *Candida* spp. were resistant to fluconazole. Sensitivity to fluconazole has been reported to vary among the *Candida* spp. as revealed by [Maroszynska \*et al.\*, \(2013\)](#); [Dagi \*et al.\*, \(2016\)](#). Previous studies of [Dagi \*et al.\*, \(2016\)](#); [Tercas \*et al.\*, \(2017\)](#) have indicated the variability in response of *Candida* spp. to the antifungal agents; however, this

was not reflected in the current study, as we did not delineate *Candida* spp. into several species, or test their response to the different antifungal drugs. Intermediate reaction of *Candida* sp. to econazole has been recorded in our study, which contradicts the sensitivity of *Candida* sp. to econazole reported in the early study of [Mahmoudabadi \*et al.\*, \(2013\)](#). The ethanolic seed and leaves extracts of *R. comunis* demonstrated no activity against all the tested microorganisms. This agrees with the study conducted by [Oloyede, \(2012\)](#), but contrasts with the report of [Jeyaseelan and Jashothan, \(2012\)](#), which found that ethanolic extracts of *R. comunis* were the most effective against the various microbial pathogens. The study of [Jeyaseelan and Jashothan, \(2012\)](#) demonstrated the highest activity of ethanolic extract of *R. comunis* against *S. aureus* with an MIC of 5 mg/ml, and was highly effective against *C. albicans*, *A. niger* and *A. fumigatus*. The lack of inhibitory effects of the seed and leaf extracts of *R. comunis* may be attributed to using ethanol as an extracting solvent. [Nour \*et al.\*, \(2012\)](#) reported that methanol is a better extractant than ethanol in the extraction of phytochemicals from plants materials. The leaf extract of *C. cujete* had the highest inhibitory activity against all the microbial isolates tested, in accordance with the report of [Khandaker \*et al.\*, \(2011\)](#). On the other hand, the fruit extract of *C. cujete* demonstrated no inhibitory potency against the Gram-negative bacteria which contradicts the results of [Khandaker \*et al.\*, \(2011\)](#). The effectiveness of the leaf extract of *C. cujete* against the microbial isolates may be attributed to the high phytochemical residues present in its leaves.

Overall, the activities of all the plant extracts tested in this study were low, compared to the activities of the conventional antibiotics. Various reasons may account for these results, including the use of crude forms of these extracts.

## Conclusion

This study evaluated the antimicrobial potential of ethanolic extracts of some plants against multidrug

resistant nosocomial bacterial and fungal pathogens. The casualty unit and the children ward had the highest and lowest frequency of these pathogens across the ward beddings and door handles, respectively. The presence of multidrug resistant microorganisms responsible for nosocomial infections especially in the casualty ward is worrisome as it poses a health risk to the patients on admission to the hospital. The antimicrobial potency of the crude plants extracts tested in this study was not as effective against the microbial isolates as the conventional antibiotics. It is therefore recommended to carry out further research using different plant parts and different extractors/extraction methods. In addition, the bioactive compounds should be further isolated and then examined for their antimicrobial potential.

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## Conflict of interest

The authors declare that there's no conflict of interests.

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## Ethical approval

The ethical approval was obtained from the Ethics Committee at the Dalhatu-Araf specialist hospital (DASH) Lafia, Nasarawa state, Nigeria.

## 5. References

Abdul, W.M.; Hajrah, N.H; Sabir J.S.; Al-Garni, S.M.; Sabir, M.J.; Kabli, S.A.; Saini, K.S. and Bora. R.S. (2018). Therapeutic role of *Ricinus communis* L. and its bioactive compounds in disease

prevention and treatment. *Asian Pacific Journal of Tropical Medicine*. 11: 177-85.

**Adikwu, P.; Umeh, E.U.; Azua, E.T. and Godwin, A.O. (2016).** Prevalence and Antimicrobial Susceptibility of *Listeria monocytogenes* Isolated from Beef, Pork and Chicken Sold in Makurdi Metropolis. *British Microbiology Research Journal*. 14(5): 1-7.

**Akinyemi, K.O.; Oladapo, O.; Okwara, C.E.; Ibe, C.C. and Fasure; K.A. (2005).** Screening of Crude Extracts of Six Medicinal Plants Used in South-West Nigerian Unorthodox Medicine for Anti-Meticillin resistant *Staphylococcus aureus* activity. *African Journal of Traditional, Complement Alternative Medicine*. 5: 6-13

**Awanye, A.M. and Amrasawore, B. (2020).** Microbiological assessment of environmental surfaces in a healthcare facility. *GSC Biological and Pharmaceutical Sciences*. 12 (02): 046-055. DOI: <https://doi.org/10.30574/gscbps.2020.12.2.0236>

**Basak, S.; Singh, P. and Rajurkar, M. (2016).** Multidrug resistant and extensively drug resistant bacteria: A study. *Journal of Pathogens*. 2016: 1-5. <http://dx.doi.org/10.1155/2016/4065603>

**Beck-Sagué, C. and Jarvis, W.R. (1993).** Secular trends in the epidemiology of nosocomial fungal infections in the United States, 1980-1990. National Nosocomial Infections Surveillance System. *Journal of Infectious Diseases*. 167(5): 1247-51. <https://doi.org/10.1093/infdis/167.5.1247>

**Bereket, W.; Hemalatha, K.; Getenet, B.; Wondwossen, T.; Solomon, A.; Zetnudin, A. and Kannan, S. (2012).** Update on bacterial nosocomial infections. *European Review for Medical and Pharmaceutical Sciences*. 16: 1039-1044.

**Bhattacharjee, P. (2016).** Epidemiology and antifungal susceptibility of *Candida* species in a tertiary care hospital, Kolkata, India. *India. Current Medical Mycology*. 2 (2): 20- 27. <https://doi.org/10.18869/acadpub.cmm.2.2.5>

**Cheesbrough, M. (2006).** District Laboratory in Tropical Countries. Part 2, 2<sup>nd</sup> Edition. Cambridge University Press. pp. 145-149. <https://doi.org/10.1017/CBO9780511543470>

**Chung, P.Y. (2016).** The emerging problems of *Klebsiella pneumoniae* infections: carbapenem resistance and biofilm formation. *FEMS Microbiology Letters*. 363(20): fnw219. <https://doi.org/10.1093/femsle/fnw219>

**Clinical and Laboratory Standard Institute (CLSI). (2017).** Performance standards for antimicrobial susceptibility testing; CLSI document M100-S27. Clinical Laboratory Standards Institute (CLSI), Wayne, PA.

**Crous, P.W.; Verkley, G.J.M.; Groenewald, J.Z. and Samson, R.A. (2009).** CBS laboratory Manual Series: Fungal Biodiversity. CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands.

**Dagi, H.T.; Findik, D.; Senkeles, C. and Arslan, U. (2016).** Identification and antifungal susceptibility of *Candida* species isolated from bloodstream infections in Konya, Turkey. *Annals of Clinical Microbiology and Antimicrobials*. 15: 36.

**Debalke, D.; Birhan, M.; Kinubeh, A. and Yayeh, M. (2018).** Assessments of Antibacterial Effects of Aqueous-Ethanol Extracts of *Sida rhombifolia*'s Aerial Part. *The Scientific World Journal*. 2018: 1-8. <https://doi.org/10.1155/2018/8429809>

**Doughari, J.H.; Ndakidemi, P.A.; Human, I.S. and Benade, S. (2012).** Antioxidant, antimicrobial and antiverotoxic potentials of extracts of *Curtisia dentata*. *Journal of Ethnopharmacology*. 141:1041-1050.

**Garcia-Cruz, C.P.; Aguilar, M.J.N. and Helguera, O.E.A. (2012).** Fungal and Bacterial Contamination on indoor surfaces of a hospital in Mexico. *Jundishapur Journal of Microbiology*. 5(3): 460-464.

**Getachew, H.; Derbie, A. and Mekonnen, D. (2018).** Surfaces and Air Bacteriology of Selected Wards at a Referral hospital, northwest Ethiopia: A cross-

sectional study. International Journal of Microbiology. 2018: 1-7. <https://doi.org/10.1155/2018/6413179>

**Iroha, I.R.; Adikwu, M.U.; Esimone, C.O.; Aibinu, I. and Amadi, E.S. (2009).** Extended spectrum beta lactamase (ESBL) in *E. coli* isolated from a tertiary hospital in Enugu State, Nigeria. Pakistan Journal of Medical Sciences. 25(2): 279-282

**Iwu, M.W.; Duncan, A.R. and Okunji, C.O. (2009).** New antimicrobials of plant origin. In: Perspective on New crops and new uses. ASHS Press, Alexandria, VA. 45(16): 457-462.

**Jeyaseelan, E.C. and Jashothan, P.J. (2012).** *In vitro* control of *Staphylococcus aureus* (NCTC 6571) and *Escherichia coli* (ATCC 25922) by *Ricinus communis* L. Asian Pacific Journal of Tropical Biomedicine. 2(9):717-721. [https://doi.org/10.1016/S2221-1691\(12\)60216-0](https://doi.org/10.1016/S2221-1691(12)60216-0)

**Khandaker, R.M.; Mojibul-Hoq, Md.; Monzur, M.A. and Animesh, S. (2011).** *In vitro* antibacterial activity of *Crescentia cujete* and *Moringa oleifera*. Bangladesh Research Publications Journal. 5(4): 337-343.

**Li, F.; Wu, L.; Cao, B.; Zhang, Y.; Li X. and Liu, Y. (2013).** Surveillance of the prevalence, antibiotic susceptibility, and genotypic characterization of invasive candidiasis in a teaching hospital in China between 2006 to 2011. BMC Infectious Diseases. 13: 353.

**Lincy, M.; Arunadevi, V.; Ali, P.S.S. and John, J. (2016).** Determination of bacterial and fungal loads and antibiotic susceptibility testing of bacteria isolated from public toilet door handles in Vellore district, Tamilnadu, India. International Journal of Research in Bioscience. 5(4): 69 -78.

**Mahmoudabadi, A.Z.; Zarrin, M. and Fard, M.B. (2013).** Antifungal susceptibility of *Candida* species isolated from Candiduria. Jundishapur Journal of Microbiology. 6 (1): 24 -28.

**Mandras, N.; Tullio, V.; Allizond, V.; Scalas, D.; Banche, G.; Roana, J.; Robbiano, F.; Fucale, G.; Malabaila, A.; Cuffini, A.M. and Carlone, N. (2009).** *In vitro* activities of fluconazole and voriconazole against clinical isolates of *Candida* spp. determined by disk diffusion testing in Turin, Italy. Antimicrobial agents and Chemotherapy. 53 (4): 1657-1659.

**Maroszynska, M.; Kunicka-Styczynska, A.; Rajkowska, K. and Maroszynska, I.A. (2013).** Antibiotics sensitivity of *Candida* clinical and food-borne isolates. Acta Biochemica Polonica. 60 (4): 719 -724.

**Mojica, M.F.; De La Cadena, E.; Correa, A.; Appel, T.; Pallares, C. and Villegas, M. (2020).** Evaluation of Allplex™ Entero-DR assay for detection of antimicrobial resistance determinants from bacterial cultures. BMC Research Notes. 13(1): 154. <https://doi.org/10.1186/s13104-020-04997-4>

**Nour, A.H.; Nour, Ab.H.; Sandanasamy, J.A.P. and Yusoff, M.M. (2012).** Antibacterial Activity of Different Extracts of *Swietenia Macrophylla* King. Proceedings of the 13<sup>th</sup> Seminar on Medicinal and Aromatic Plants. pp. 17-18.

**Nwankwo, E. (2012).** Isolation of pathogenic bacteria from fomites in the operating rooms of a specialist hospital in Kano, North-western Nigeria. The Pan African Medical Journal. 12: 90. PMID: 23077711; PMCID: PMC3473976.

**Ogbonnia, S.O.; Enwuru, N.V.; Onyemenem, E.U.; Oyedele, G.A. and Enwuru, C.A. (2008).** Phytochemical Evaluation and Antibacterial Profile of *Treculia africana* Decne bark extract on Gastro-intestinal Bacterial Pathogens. African Journal of Biotechnology. 7(10): 1385-1389.

**Olaniyi, M.B.; Lawal, I.O. and Olaniyi, A.A. (2018).** Proximate, phytochemical screening and mineral analysis of *Crescentia cujete* L. leaves. Journal of Medicinal Plants for Economic Development. 2: a28. <https://doi.org/10.4102/jomped.v2i1.28>.

- Oloyede, G.K. (2012).** Antioxidant activities of Methyl Ricinoleate and Ricinoleic Acid Dominated *Ricinus communis* seeds Extract Using Lipid Peroxidation and Free Radical Scavenging Methods. *Research Journal of Medicinal Plant*. 6: 511-520.
- Peters, L.; Olson, L.; Khu, D.T.K.; Linnros, S.; Le, N.K.; Hanberger, H.; Hoang, N.T.B.; Tran, D.M. and Larsson, M. (2019).** Multiple antibiotic resistance as a risk factor for mortality and prolonged hospital stay: A cohort study among neonatal intensive care patients with hospital acquired infections caused by gram-negative bacteria in Vietnam. *PLoS ONE*. 14(5): 1-18. <https://doi.org/10.1371/journal.pone.0215666>
- Sabino, R.; Verissimo, C.; Parada, H.; Brandao, J.; Viegas, C.; Carolino, E.; Clemons, K.V. and Stevens, D.A. (2014).** Molecular screening of 246 Portuguese *Aspergillus* isolates among different clinical and environmental sources. *Medical Mycology*. 52: 517-527.
- Saka, K.H.; Akanbi II, A.A.; Obasa, T.O.; Raheem, R.A.; Oshodi, A.J. and Kalgo, Z.M. (2016).** Pathogenic Aerobic Bacterial contaminants on non-Critical Hospital surfaces within paediatric ward of a Nigerian hospital. *Journal of Medical Microbiology and Diagnosis*. 5(3): 241. <https://doi.org/10.4172/2161-0703.1000241>
- Segujja, F.; Mwambi, B.; Drago, C.K.; Lubowa, N.M.; Mugambwa, J. and Wabuyi, P. (2016).** Characterization and antimicrobial susceptibility patterns of isolates from ward fomites. *British Biotechnology Journal*. 14(4): 1-16.
- Sserwadda, I.; Lukenge, M.; Mwambi, B.; Mboowa, G.; Walusimbi, A. and Segujja, F. (2018).** Microbial contaminants isolated from items and work surfaces in the post-operative ward at Kawolo general hospital, Uganda. *BMC Infectious Diseases*. 18: 68.
- Suleyman, G.; Alangaden, G. and Bardossy, A.C. (2018).** The Role of Environmental Contamination in the Transmission of Nosocomial Pathogens and Healthcare-Associated Infections. *Current Infectious Disease Reports*. 20: 12. <https://doi.org/10.1007/s11908-018-0620-2>
- Tercas, A.G.; Marques, S.G.; Moffa, E.B.; Alves, M.B.; de Azevedo, C.M.P.S.; Siqueria, W.L. and Monteiro, C.A. (2017).** Antifungal drug susceptibility of *Candida* species isolated from HIV-Positive patients recruited at public hospital in Sao Luis, Maranhao, Brazil. *Frontiers in Microbiology*. 8: 298. <https://doi.org/10.3389/fmicb.2017.00298>
- Williams, C. and Davis, D.L. (2009).** Methicillin-resistant *Staphylococcus aureus* fomite survival. *Clinical Laboratory Science Winter*. 22(1): 34-8.
- World Health Organization (WHO) (2002).** Prevention of hospital-acquired infections. A practical guide 2<sup>nd</sup> Edition: WHO/CDS/CSR/EPH/2002.12. WHO, Geneva.
- Zaidi, K.U.; Mani, A.; Parmar, R. and Thawani, V. (2018).** Antifungal susceptibility patterns of *Candida albicans* in human infections. *Open Biological Sciences Journal*. 4: 1- 6.