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Antimicrobial Activity of Selected Medicinal Plants, Nano-Based Plant Extracts and Honey on Some Uropathogens

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Authors' contributions

This work was carried out in collaboration among all authors. Author BRE designed and implemented of the research. Author OMK managed the analyses of the work. Author ATT provided critical feedback and helped shape the research, analysis and manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Uropathogens are the causative agents of urinary tract infections (UTIs). Although, UTI can be treated with antibiotics but, the increasing prevalence of antibiotic-resistant uropathogens poses a major setback to the treatment of UTIs hence the need to search for alternative therapies. This study is therefore aimed to analyze the antimicrobial properties of the extract of some medicinal

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plants such as *Zingiber officinale, Hibiscus sabdariffa, Justicia carnea*, their nanoparticles and honey on common uropathogens are; *Staphylococcus aureus, S. saprophyticus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli* and *Candida* sp. The antibiogram profile of the test uropathogens was determined using standard assay prior the plant extract and nanosynthesized plant extract assays. The plant extracts were prepared using ethanol, chloroform and hot water as extraction solvents while the nanosynthesis of the plant extracts was performed using standard methods. The antibiogram of the test uropathogens showed that almost all the tested urobacteria were resistant to all the antibiotics used with multiple antibiotics resistance index ranged from 0.3-0.9, *E. faecalis* having the highest MARI (0.9). On the other hand, all the *Candida* strains used were resistant to the tested antifungal agents. Out of all the prepared extracts, the ethanol calyx extract of *H. sabdariffa* was the most effective on the test urobacteria. The nanosynthesized plant extracts however, was effective against all the uropathogens with the 1M nanobased hot water calyx extract of *H. sabdariffa* displaying the highest growth inhibitory activity on all the test uropathogens. This work revealed that the ethanol calyx extract of *H. sabdariffa* and nanobased hot water calyx extract of *H. sabdariffa* can be explored for the development of more effective drugs against MARI uropathogens.

Keywords: Uropathogens; multiple antibiotics resistance index; nanoparticles; medicinal plants.

1. INTRODUCTION

Uropathogens are microorganisms found to cause urinary tract infection in women and men. This infection can be caused by both bacteria and fungi when these organisms enter into the urinary tract and establish their stay to the extent that they can be recovered in quantities more than 10^5 colony-forming unit per ml (CFU/ml). They can cause infections like cystitis, urethritis and also candidiasis. Although urinary tract infections (UTIs) can be treated using antibiotics there is however an alarming rate of bacterial resistance to most antibiotics treatment of UTI which has resulted in the need for more effective drugs. The use of plants as alternative medicine in bridging the gap of multidrug-resistance antimicrobial infections, is becoming an important area in public health researches. [1] reported the use of plants as an antibacterial agent with the properties of managing some disease states. Studies have also shown that the presence of some bioactive components in these plants is possible reasons for their effective usefulness [2,3,4,5]. More so, the introduction of nanotechnology into medicine is meant to improve the mechanism of effectiveness that plants possess. Silver nanoparticles have been reported to enhance high antimicrobial properties and also used as anticancer agents among other modern applications. The combination of nanotechnology and traditional methods of treating infections is therefore a novel area of interest [6]. *Hibiscus sabdariffa* L, also known as roselle, has been studied for its anti-obesity properties and its role in traditional Indian medicine [7]. The calyces of *H. sabdariffa* have been used in various food products due to their exceptional flavor and potential health benefits [8]. Additionally, *H. sabdariffa* has been investigated for its antioxidant properties and antihypertensive effects [9]. *Zingiber officinale* L*,* also known as ginger, is a plant with a great history of medicinal use. Studies have highlighted its metabolic, analgesic, and anti-inflammatory effects [10], as well as possibilities of alleviating liver pro-inflammatory reactions [11]. It has also been explored for its antioxidant activities [12] and also for controlling diabetes [13]. Furthermore, *Z. officinale* have been reported to have the potential of averting myelotoxicity and hepatotoxicity caused by certain compounds [14]. *Justicia carnea* L, another medicinal plant is known for its potential therapeutic properties. It is used as blood tonic. Many studies have found out that *J. carnea* contains quite a lot of properties such as: management of inflammation, respiratory tract infection, arthritis, antimicrobial, antitumoral and antiviral [15]. Honey, on the other hand is both a natural product and sweetener produced by bees. It has been recognized for having potential health benefits as its antimicrobial property have often been employed as alternative therapy in treating various infections caused by fungi [16]. It contains various phenolic and flavonoid compounds which plays a crucial role in antimicrobial activity and inhibit bacterial growth [17,18]. Hence the objective of this study is to investigate the antimicrobial potentials of these medicinal plants and nano-based extracts of these plants on uropathogens.

2. MATERIALS AND METHODS

2.1 Synthesis of Silver Nanoparticles

The synthesis of silver nanoparticles from *Zingiber officinale, Hibiscus sabdariffa* and *Justicia carnea* was performed using the methods of Vanlalveni et al. [19] and [20] respectively. Ten (10 mls) of the extracts was added to 90 mls of 1mM aqueous silver nitrate solution and then the mixture was heated at 60˚C for 2 hours and stirred consistently. The formation of silver nanoparticles (AgNPs) was noticed with a change in the colour of each plant extract to brown or orange-brown color depending on the plant extract.

2.2 Source of Uropathogens

The uropathogens examined in this study was grouped into Gram-positive, Gram-negative and fungal isolates after using specific and specialized selective media. They were isolated from patients attending some hospitals in the western part of Nigeria, specifically in Akure, Ondo-State. The study was undertaken in the Microbiology laboratory of the Federal University of Technology, Akure.

2.3 Collection and Preparation of Plant Materials

Plants used for this work were obtained from the Oba market area of Akure (*Zingiber officinale* and *Hibiscus sabdariffa*) while *Justicia carnea* was harvested from a farm land in Ijoka -Akure, Nigeria. The plants were pulverized and extracted using ethanol, chloroform and hot water. After these processes, the plants were preserved in the refrgerator for further use. Plant extracts from selected ethnomedicinal plants were obtained following standard extraction procedures. The extracts were dissolved in 30% DMSO to prepare stock solutions of 200 mg/ml.

2.4 Antibiotic Susceptibility Test

Antibiotic susceptibility test of the bacterial isolates were evaluated using disc diffusion assay. Ceftazidime (CAZ) 30 µg, Gentamicin (GN) 10 µg, Ciprofloxacin (CPX) 10 μ g, Nitrofurantoin (NIT) 300 µg, Cefuroxime (CRX) 30 µg, Ceftriaxone (CTR) 30 µg, Cloxacillin (CXC) 1 µg, Ofloxacin (OFL) 5 µg, Cefixime (CXM) 5 µg and Augmentin (AU) 30 μg. The plates were then incubated at 37˚C for 24 hours. After overnight incubation, zones of inhibition and resistance were recorded and compared with Committee for Clinical Laboratory Standards Interpretative Chart, [21]. The isolates that were found resistant to more than three antibiotics were termed multiple drugs resistant and were subjected to the (MARI) multiple drug resistance index [22].

$$\text{MARI} = \frac{\text{No. of antibiotics to which organism is resistant}}{\text{Total number of antibiotics}}$$

2.5 Antifungal Susceptibility Test

For the fungal isolate potato dextrose agar was prepared and the following anti-fungi disc were placed on the solid medium of the potatoes dextrose agar (PDA); Fluconazole 50 µ g, Itraconzole 10 µg, and Ketoconazole 10 µg, after the inoculum had been spread evenly the plates were then incubated at normal room temperature and results were read after 24 hours [23].

2.6 Antimicrobial Assay

The antimicrobial activity of the plant extracts was carried out on the bacterial isolates using agar well diffusion method. The bacterial isolates were cultured for 16 – 18 hours in Muller Hinton broth at 37℃. The broth cultures were diluted with sterile distilled water corresponding to 0.5 Mac Farland standard solutions which is equivalent to about 1.5 x 10^8cfu/ml. Potato Dextrose Agar (PDA) was used for yeast. This was assayed in triplicate and all plates were incubated at 37℃ for 24 hours. It was observed that the yeast were also able to produce discrete colonies within 24 hours, thus all the plates was read by measuring the diameter of each of the clear zones (area without growth) of inhibition around the wells containing plant extracts. No measurement was recorded where there were no clear zones of inhibition [24].

2.7 Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were carried out using agar well diffusion method [21]. These were done at different concentrations of the extracts (100 mg/ml, 50 mg/ml and 25 mg/ml) which were reconstituted using 30% DMSO. Sterile agar plates of Muller Hinton agar and potato dextrose agar were seeded aseptically

with 0.5 ml of the standardized test organisms [24]. The seeded broth was incubated at 37°C for 24 hours [21].

2.8 Determination of Minimum Inhibitory Concentration

The least concentration of the plant extracts that induced inhibitory effect on the microbial growth was assayed using standard microbiological methods. This was conducted using serial dilution of the plant extracts (*H. sabdariffa, J. carnea* and *Z. officinale*) were prepared in broth. Standard inoculums of the cells were added to each of the wells and the plates were incubated at 37˚C in the laboratory incubator (Model No. 9092A GALLENKOMP). The minimum inhibitory concentration was recorded as the lowest concentration in respect to each extract of the plants, which inhibited the noticeable growth of the uropathogens.

2.9 Determination of the Minimum Fungicidal Concentration

Determination of the minimum fungicidal concentration (MFC) was done by sub-culturing the aliquots from wells that did not grow in the MIC. This was performed on freshly prepared agar plates of Muller Hinton agar (MHA) and incubated at 37˚C and readings were taken after 24 hours.

3. RESULTS

Out of all the antibiotics used, gentamicin (GEN), ofloxacin (OFL) and ciprofloxacin were the most potent agents against the uropathogens. All the other antibiotics; CXC, CAZ and CRX were not 100% effective on any of the Gram-positive uropathogens. Augumentin (AUG) was only effective on a strain of *S. aureus* with 11.00±0.0 mm zone of inhibition. Only *S. aureus* strains were susceptible to CTR with zones 13.00±0.0 mm and 19.00±0.0 mm while, other Gram-positive uropathogens were resistant. One strain each from *S. aureus* and *S. saprophyticus* was inhibited by the antibiotics with 13.50±0.0 and 11.50±0.50 mm zones respectively for Erythromycin (ERY). The highest multiple antibiotic resistance of (0.9) MARI was recorded against *E. feacalis* while its lesser value of (0.4) was found with *S. aureus* as shown in Table 1.

Klebsiella pneumoniae was susceptible to CPR with highest zone 30.05±0.50 mm zone of inhibition while, *E. coli* had the least zones of 9.00±0.0 mm. Out of all the tested conventional Gram-negative agents, only OFL and CPR were most effective. In this study, AUG was not effective on any of the tested Gram-negative bacteria. CAZ, CXM and CRX were effective on only a strain of *P. aeruginosa* with 18.50±0.50 mm, 14.50±0.50 mm and 18.50±0.50 mm respectively while, the rest of the test uropathogens had no zone of inhibition. MARI shows *E. coli* to be most resistant to the conventional agent with 8 (0.9) while, *P. aeruginosa* had least resistant 2 (0.2) to the conventional agents as shown in Table 2.

Table 3, shows the susceptibility of fungi isolates to conventional antifungal agents. The fungal strains have over 70% resistance to the drugs. The antifungal drugs exhibited inhibition zones ranging from 22.00±1.15 mm – 7.33±0.33 mm. *Candida* strain III had the highest and only zone of inhibition of 22.00±1.15 mm to itraconazole (ITR). For fluconazole (FLU), *Candida* strain VIII had the highest 13.00±1.53 mm zone of inhibition while, *Candida* strain X had 9.67±0.33 mm zone. For ketoconazole (KET); only three of the strains were susceptible to the agents. *Candida* strain VIII had the highest zone of 12.33±0.33 mm inhibition while, *Candida* strain X had the least zone of inhibition.

The antibacterial properties of extracts of *H. sabdariffa, J. carnea, Z. officinale* at 200 mg/ml and honey shows several potency zone of inhibition of the test urobacteria as shown in Figs. 1, 2 and 3.

Z. officinale extracts at 200 mg/ml on the fungal strains shows that, hot water or aqueous extract was most effective. *Candida* IX had the highest 11.33±0.67 mm zone of inhibition while, *Candida* VI had the least zone of 5.00±0.00 mm. Chloroform extract was not effective on any of the fungal strains. The antifungal activity of hot water extract was very notable on *Candida* I with 18.67±0.33 mm and least with *Candida* VII 11.33±0.33 zone of inhibition. Honey sample was most effective with these set of *Candida* strains at 14.33±0.33 mm and least at 8.67±0.88 mm zones of inhibition (Table 4).

Table 5; shows the antifungal activity of *J. carnea* ethanol, chloroform and hot water extract alongside positive and negative controls. *Candida* IV had the highest inhibition zone of 23.00±0.58 mm while, *Candida* I had the least zone of 10.33±0.33 mm. Chloroform extract was not effective at all on any of the fungal strains.

Hot water extract was most effective on *Candida* V and *Candida* VII with the highest zone 29.00±0.58 mm of inhibition, and least with 14.67±0.33 mm with *Candida* IV. Honey was effective on *Candida* VI, *Candida* VIII and *Candida* IX with the same zone of inhibition and least against *Candida* I with 11.00±0.58 mm zone of inhibition.

Table 6; shows the antifungal efficacy of *H. sabdariffa* calyx at 200 mg/ml and honey on *Candida* strains. Ethanol extract was most effective on all the tested fungal strains in this study. *Candida* IX had the highest 29.00±0.58 mm zone of inhibition while *Candida* IV had the lowest zone of 15.00±0.58 mm inhibition. Chloroform extract also did not have any inhibitory effect on any of the tested strains. Hot water extract of *H. sabdariffa* had the highest zone of inhibition against *Candida* I 18.00±0.00 mm while, *Candida* IX had the lowest 10.33±0.33 mm zone of inhibition. At this phase, honey had the highest zone of 20.00±0.00 mm inhibition against *Candida* I while, the lowest zone of inhibition was recorded against *Candida* IX with 11.00±0.58 mm.

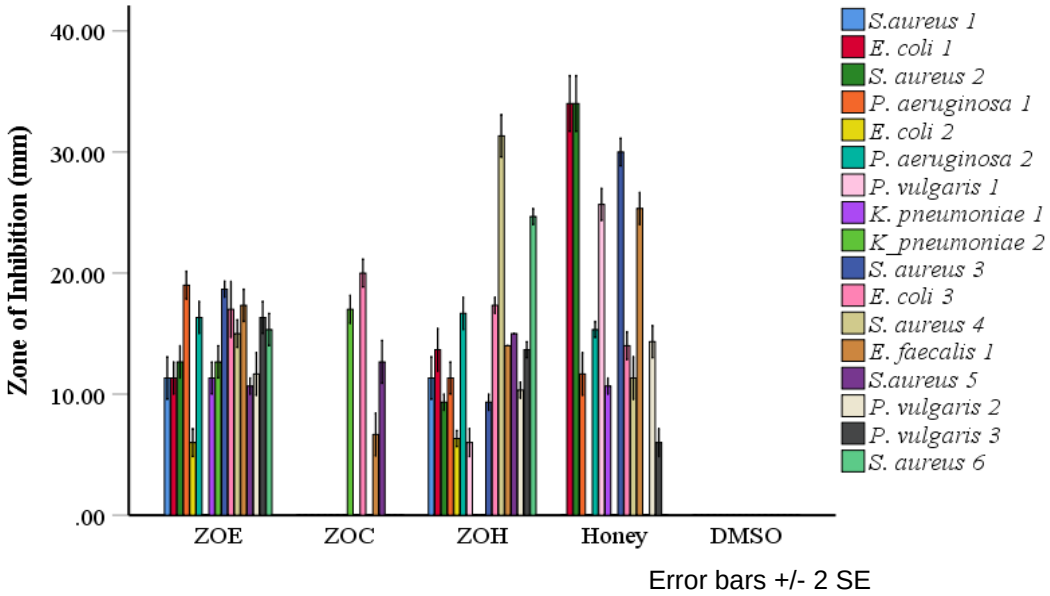

Fig. 1. Antibacterial activity of *Zingiber officinale* at 200mg/ml on test urobacteria

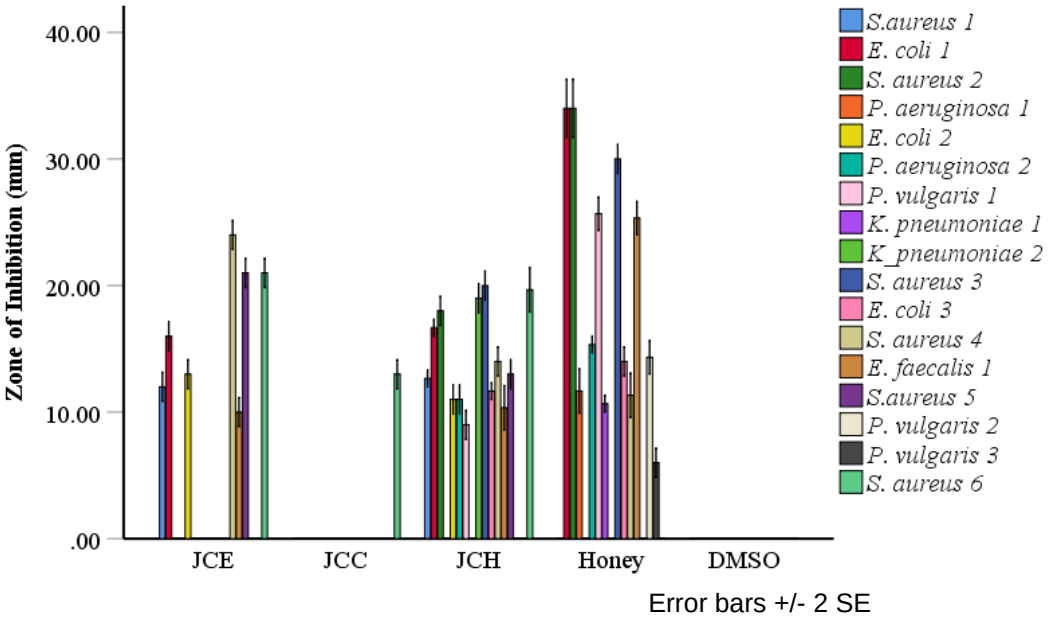

Fig. 2. Antibacterial activity of *Justicia carnea* leaves extracts at 200mg/ml on test urobacteria

Table 1. Antibacterial susceptibility pattern of the gram-positive urobacteria examined

Isolate NO.	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	MARI
E. fa1	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	8 (0.9)
E. fa2	0.00±0.00^{a}	0.00±0.00^{a}	21.00±0.00^{c}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	11.00±0.00^{b}	0.00±0.00^{a}	6 (0.8)
S. au1	0.00±0.00^{a}	0.00±0.00^{a}	29.50±0.50^{d}	13.00±0.00^{c}	13.50±0.00^{c}	0.00±0.00^{a}	28.50±0.00^{d}	11.00±0.00^{b}	3 (0.4)
S. au2	0.00±0.00^{a}	0.00±0.00^{a}	14.50±0.50^{b}	19.00±0.00^{c}	0.00±0.00^{a}	0.00±0.00^{a}	26.50±0.50^{d}	0.00±0.00^{a}	5 (0.6)
S. sap1	0.00±0.00^{a}	0.00±0.00^{a}	9.50±0.50^{b}	0.00±0.00^{a}	11.50±0.50^{c}	0.00±0.00^{a}	25.50±0.00^{d}	0.00±0.00^{a}	6 (0.8)
S. sap2	0.00±0.00^{a}	0.00±0.00^{a}	18.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	22.50±0.50^{c}	0.00±0.00^{a}	6 (0.8)

Values with the same superscript alphabet along the same column are not significantly different (P>0.05) according to Tukey's Honestly Difference

Key: *CAZ= Ceftazidime, CRX= Ceftriaxone, GEN= Gentamicin, CTR= Cefotaxime, ERY= Erythromycin, CXC= Cloxacillin, OFL= Ofloxacin, AUG= Amoxicillin-Clavulanate, E. fa= E. feacalis, S. au= S. aureus, S. sap= S. saprophyticus*

Table 2. Antibiotic susceptibility pattern of the gram-negative urobacteria examined

Isolates NO.	CAZ	CRX	GEN	CXM	OFL	AUG	NIT	CPR	MARI
E. co1	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	24.50±0.50^{b}	0.00±0.00^{a}	0.00±0.00^{a}	24.50±0.50^{b}	6 (0.8)
E. co2	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.0`0^{a}	9.00±0.00^{b}	8 (0.9)
K. pn1	0.00±0.00^{a}	0.00±0.00^{a}	21.50±0.50^{c}	0.00±0.00^{a}	23.50±0.50^{d}	0.00±0.00^{a}	18.50±0.50^{b}	24.50±0.50^{d}	4 (0.5)
K. pn2	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	23.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}	23.50±0.50^{b}	6 (0.8)
P. vu1	0.00±0.00^{a}	0.00±0.00^{a}	16.50±0.50^{c}	0.00±0.00^{a}	22.50±0.50^{d}	0.00±0.00^{a}	11.50±0.50^{b}	23.50±0.50^{d}	4 (0.5)
P. vu2	0.00±0.00^{a}	0.00±0.00^{a}	18.50±0.50^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	21.50±0.50^{c}	6 (0.8)
P. ae1	0.00±0.00^{a}	0.00±0.00^{a}	11.50±0.50^{b}	0.00±0.00^{a}	20.50±0.50^{d}	0.00±0.00^{a}	17.50±0.50^{c}	19.00±0.00^{cd}	4 (0.5)
P. ae2	18.00±0.00^{c}	14.50±0.50^{b}	20.50±0.50^{d}	18.50±0.50^{cd}	25.50±0.50^{d}	0.00±0.00^{a}	0.00±0.00^{a}	25.50±0.50^{d}	2 (0.3)

Values with the same superscript alphabet along the same column are not significantly different (P>0.05) according to Tukey's Honestly Difference

KEY: CAZ= Ceftazidime, CRX= Ceftriaxone, GEN= Gentamicin, CXM= Cefuroxime, OFL= Ofloxacin, AUG= Amoxicillin-Clavulanate, NIT= Nitrofurantoin, CPR= Ciprofloxacin, E. co= E. coli, K. pn= K. pneumoniae, P. vul= P. vulgaris, P. ae= P. aeruginosa,

Table 3. Antibiogram of fungi isolates to conventional antifungal agents

Isolates	Zones(mm)	ITR (10 µg)	Zones (mm)	FLU (25 µg)	Zones (mm)	KET (10 µg)
		S I R		S I R		S I R
		≥ 15, 10-14, ≤ 9		≥ 19, 12-19, ≤ 11		≥ 28, 21-17, ≤ 20
Candida I	0.00±0.00[a]	R	0.00±0.00[a]	R	0.00±0.00[a]	R
Candida II	0.00±0.00[a]	R	11.33±0.88[b]	R	0.00±0.00[a]	R
Candida III	22.00±1.15[b]	S	0.00±0.00[a]	R	0.00±0.00[a]	R
Candida IV	0.00±0.00[a]	R	0.00±0.00[a]	R	0.00±0.00[a]	R
Candida V	0.00±0.00[a]	R	0.00±0.00[a]	R	0.00±0.00[a]	R
Candida VI	0.00±0.00[a]	R	0.00±0.00[a]	R	0.00±0.00[a]	R
Candida VII	0.00±0.00[a]	R	0.00±0.0 [a]	R	0.00±0.00[a]	R
Candida VIII	0.00±0.00[a]	R	0.00±0.00[a]	I	12.33±0.33[b]	R
Candida IX	0.00±0.00[a]	R	0.00±0.00[a]	R	0.00±0.00[a]	R
Candida X	0.00±0.00[a]	R	6.67±0.88[b]	R	7.33±0.33[c]	R

Key: R=Resistance, I= Intermediate, S= Susceptible

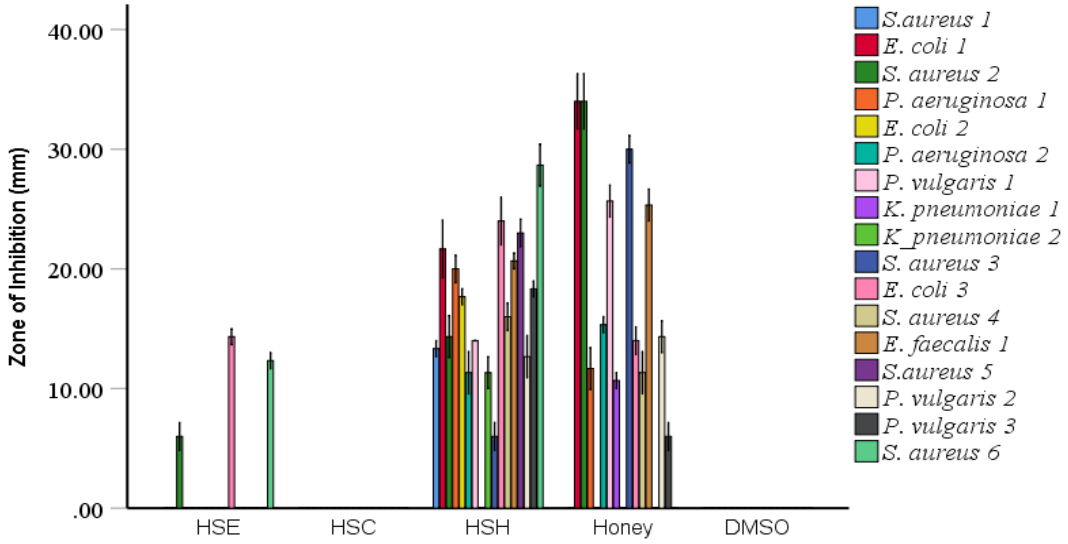

Fig. 3. Antibacterial activity of *Hibiscus sabdariffa* calyx extracts at 200mg/ml on test urobacteria

Table 4. Antifungal Activity of *Zingiber officinale* at 200mg/ml on Test Fungal Strains

Fungal Strains	ZOE	ZOC	ZOH	Honey	Keto	DMSO
Candida I	11.00±0.58^{b}	0.00±0.00^{a}	18.67±0.33^{c}	10.33±0.33^{b}	0.00±0.00^{a}	0.00±0.00^{a}
Candida IV	0.00±0.00^{a}	0.00±0.00^{a}	17.00±0.58^{c}	10.67±0.33^{b}	0.00±0.00^{a}	0.00±0.00^{a}
Candida VI	5.00±0.00^{b}	0.00±0.00^{a}	15.00±0.58^{c}	13.33±0.88^{c}	0.00±0.00^{a}	0.00±0.00^{a}
Candida VII	0.00±0.00^{a}	0.00±0.00^{a}	11.33±0.33^{b}	14.33±0.33^{c}	0.00±0.00^{a}	0.00±0.00^{a}
Candida 1X	11.33±0.67^{bc}	0.00±0.00^{a}	13.00±1.00^{c}	8.67±0.88^{b}	0.00±0.00^{a}	0.00±0.00^{a}

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same rows are not significantly different (P >0.05) according to Tukey's Honestly Significant Difference
***Key:** ZOE- Z. officinale ethanol, ZOC- Z. officinale chloroform, ZOH- Z. officinale hot water, Keto- Ketoconazole*

Table 5. Antifungal Activity of *Justicia carnea* at 200mg/ml on some Isolates

Fungal Strains	JCE	JCC	JCH	Honey	Keto	DMSO
Candida I	10.33±0.33^{b}	0.00±0.00^{a}	18.67±0.67^{c}	11.00±0.58^{b}	0.00±0.00^{a}	0.00±0.00^{a}
Candida IV	23.00±0.58^{d}	0.00±0.00^{a}	14.67±0.33^{c}	12.00±0.58^{b}	0.00±0.00^{a}	0.00±0.00^{a}
Candida VI	0.00±0.00a	5.00±0.00^{b}	29.00±0.58^{d}	13.67±0.88^{c}	0.00±0.00^{a}	0.00±0.00^{a}
Candida VII	0.00±0.00^{a}	0.00±0.00^{a}	29.00±0.58^{c}	13.67±0.88^{b}	0.00±0.00^{a}	0.00±0.00^{a}
Candida 1X	0.00±0.00^{a}	0.00±0.00^{a}	23.00±0.58	13.67±0.33^{b}	0.00±0.00^{a}	0.00±0.00^{a}

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same rows are not significantly different (P >0.05) according to Tukey's Honestly Significant Difference
***KEY:** JCE- J. carnea ethanol, JCC- J. carnea chloroform, JCH- J. carnea hot water, Keto- Ketoconazole*

Tables 7, 8, 9; show the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the microbial strains when tested against *H. sabdariffa*, *J. carnea* and *H. sabdariffa* ethanol and hot water extracts at concentrations of 100 mg/ml, 50 mg/ml and 25 mg/ml.

Table 10, shows the antimicrobial activity o varying concentrations of nanosynthesized hot water extract of *H. sabdariffa* on some uropathogens. This assay was varied 1 M, 50 mM, 25 mM, AgNO3 was the negative control, 1M showed the greatest zones of inhibition compared to 50 mM and 25 mM contraction while AgNO3 had no zone of inhibition.

Table 6. Antifungal Activity of *Hibiscus sabdariffa* Calyx at 200mg/ml on Test Uropathogens

Fungal Strains	HSE	HSC	HSH	Honey	Keto	DMSO
Candida I	21.67±0.33^{d}	0.00±0.00^{a}	18.00±0.00^{b}	20.00±0.00^{c}	0.00±0.00^{a}	0.00±0.00^{a}
Candida IV	15.00±0.58^{b}	0.00±0.00^{a}	15.67±0.33^{c}	13.67±0.67^{bc}	11.67±0.88^{b}	0.00±0.00^{a}
Candida VI	23.67±0.33^{c}	0.00±0.00^{a}	14.00±0.58^{b}	14.00±0.58^{b}	0.00±0.00^{a}	0.00±0.00^{a}
Candida VII	20.67±0.33^{c}	0.00±0.00^{a}	15.67±0.33^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Candida 1X	29.00±0.58^{c}	0.00±0.00^{a}	10.33±0.33^{b}	11.00±0.58^{b}	0.00±0.00^{a}	0.00±0.00^{a}

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same rows are not significantly different (P >0.05) according to Tukey's Honestly Significant Difference

KEY: *HSE- H. sabdariffa ethanol, HSC- H. sabdariffa chloroform, HSH- H. sabdariffa hot water, Keto- Ketoconazole*

Table 7. Comparative Minimum Inhibitory Concentration MIC (mg/mL) of the Different Extracts on Test Uropathogens

Microorganisms	A	B	1	2	I	II
Proteus vulgaris	ND	50	200	50	100	200
P. aeruginosa	100	50	25	12.5	25	25
S. aureus	200	100	50	12.5	ND	50
E. faecalis	200	50	100	25	50	50
E. coli	200	100	200	25	ND	12.5
Candida I	200	200	200	200	200	200
Candida IV	ND	100	200	200	200	200
Candida VI	ND	200	200	200	200	200
Candida VII	ND	200	200	200	ND	200
Candida IX	200	200	200	100	ND	200

Key: A=Ethanol extract of Zingiber officinale, B=Aqueous extract of Zingiber officinale, 1=Ethanol extract of Hibiscus sabdariffa, 2=Aqueous extract of Hibiscus sabdariffa, I= Ethanol extract of Justicia carnea, II= Aqueous extract of Justicia carnea, ND=Not detectable

Table 8. Comparative Minimum Bactericidal Concentration MBC (mg/mL) of the Different Extracts on Test Urobacteria

Microorganisms	A	B	1	2	I	II
Proteus vulgaris	ND	100	200	100	200	200
P. aeruginosa	100	100	50	25	50	25
S. aureus	200	100	50	12.5	ND	100
E. faecalis	200	50	100	25	100	100
E. coli	200	100	200	25	ND	25

Key: A=Ethanol extract of Zingiber officinale, B=Aqueous extract of Zingiber officinale, 1=Ethanol extract of Hibiscus sabdariffa, 2=Aqueous extract of Hibiscus sabdariffa, I= Ethanol extract of Justicia carnea, II= Aqueous extract of Justicia carnea, ND=Not detectable

Table 9. Comparative Minimum Fungicidal Concentration MFC (mg/mL) of the Different Extracts on Test Uropathogens

Microorganisms	A	B	1	2	I	II
Candida I	ND	100	ND	100	ND	ND
Candida IV	ND	100	100	ND	ND	ND
Candida VI	100	100	ND	100	ND	100
Candida VII	ND	100	200	100	ND	100
Candida IX	200	100	100	100	ND	100

Key: A=Ethanol extract of Zingiber officinale, B=Aqueous extract of Zingiber officinale, 1=Ethanol extract of Hibiscus sabdariffa, 2=Aqueous extract of Hibiscus sabdariffa, I= Ethanol extract of Justicia carnea, II= Aqueous extract of Justicia carnea, ND=Not detectable

Table 10. Antibacterial Activity of Varying Concentrations of Synthesized Nanoparticles on Test Uropathogens

Isolates	1M	50mM	25mM	AgNO3	Anti	HSH
P. vulgaris	15.67±0.33^{d}	13.67±0.33^{c}	13.67±0.67^{c}	0.00±0.00^{a}	9.67±0.33^{b}	9.67±0.33^{b}
P. aeruginosa	26.00±1.15^{d}	22.67±0.67^{d}	17.67±0.33^{b}	0.00±0.00^{a}	22.67±1.20^{b}	19.67±1.45^{bc}
S. aureus	23.67±0.33^{cd}	19.67±0.33^{bc}	18.33±0.33^{b}	0.00±0.00^{a}	28.00±1.52^{d}	18.33±1.76b
E. faecalis	20.67±0.33^{c}	21.00±0.58^{c}	14.67±0.33^{b}	0.00±0.00^{a}	13.33±0.88^{b}	11.67±1.20^{b}
E. coli	23.00±0.58^{d}	16.00±0.58^{bc}	13.00±0.00^{b}	0.00±0.00^{a}	17.00±1.15^{c}	15.00±1.15^{bc}
Candida I	19.33±0.33^{d}	15.00±1.15^{c}	15.33±0.33^{c}	0.00±0.00^{a}	9.67±0.33^{b}	10.00±0.57^{b}
Candida IV	24.00±0.58^{d}	21.00±0.58^{d}	17.00±0.58^{c}	0.00±0.00^{a}	10.33±0.33^{b}	14.00±1.15^{c}
Candida VI	19.00±0.58^{d}	16.00±1.15^{cd}	12.67±0.33^{bc}	0.00±0.00^{a}	10.33±0.33^{b}	14.00±1.15^{c}

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same rows are not significantly different (P>0.05) according to Tukey's Honestly Significant Difference

Key: Anti= Ofloxacin for bacteria and Ketoconazole for fungi, HSH= H. sabdariffa hot water extract

4. DISCUSSION

Antibiotic resistance is fast becoming a thing of concern with several uropathogens displaying resistance to both antibacterial and antifungal agents [25]. The conventional antibiotics used in this study were greatly resisted by the urobacteria. This could be as a result of production of β-lactameses by the uropathogens. Some uropathogens possess enzymes that breakdown antibiotics such as cephalosporins. Findings have reported that some of these bacteria move around the host body with proteins that expel antibiotics from the bacterial cell, while some cause alteration in the protein of the bacterial that is targeted by antibiotics thereby rendering the drugs not to be effective [26,27,28]. Some of these uropathogens such as *E. coli* could also form biofilm which complicates treatment with antibiotics. The biofilm shields the bacteria from the antibiotics and the immune system of the host, making infection to persist and thereby increasing the rate of microbial resistance [27]. Findings from this study however has shown that hot water extract of the calyx of *H. sabdariffa* was the most effective antimicrobial agents at different concentration on the uropathogens. This could have resulted from the chemical composition of the plants.

The ability of these chemical compounds to be soluble varies owning to the solvent used during extraction process. Hot water extraction may dissolve certain bioactive components that are inherent in the plants which are good antimicrobial agents. These compounds can disrupt microbial cell membrane, they could also inhibit microbial enzyme activity and interface with their growth process compared to chloroform and ethanol, making hot water extract *H. sabdariffa* most efficient in this study. This observation corroborates with the report of Jabeur *et al.* [29].

Some bioactive compounds are sensitive to heat, which can facilitate the release of more active compounds from the *Z. officinale* and *J.carnea* [30]. This can result in the higher concentration of antimicrobial components in the hot water extract. This finding did not also rule out the fact that hot water could also distort the production and effectiveness of some other compounds that could have been good candidate for other activity [30].

Chloroform extract was found to be ineffective in the three plants used. This could be as a result of the fewer bioactive compounds that contains antimicrobial properties due to differences in the rate of solubility. In addition, some findings have revealed that the use of chloroform could even alter the chemical composition of the extracts thereby reducing their antimicrobial properties [29].

Multidrug resistance by uropathogens could be due to the fact that the organisms resistant genes, aided them to withstand the antimicrobial agents. From time to time bacterial strategizes technique to pump the antibiotic agent out of the cell thereby rendering the drugs useless. Hence, when an individual carries bacteria that produce Extended spectrum beta lactamase (ESBL) most of the administered antibiotics to be abortive [31,32,33].

The antimicrobial effect of honey across the microbial cells corroborates with the findings of Anyanwu [34,35,18] who revealed that honey contains high level of sugar content resulting in osmotic pressure that expels water from the microbial cells thereby leading to cell dehydration and death. Honey was also reported to contain peptide which possess antimicrobial properties. The reduced pH of honey creates an acidic environment that became unfavorable for the proliferation of uropathogens [36,24]. The result of this study is in agreement with the report of Cianciosi *et al.* [37] who stated that the possible reasons for the therapeutic and antimicrobial properties of honey could be owning to its ability to produce hydrogen peroxide, low pH and the phenols level [38].

The antimicrobial properties of silver nanoparticles from the results showed effectiveness compared to all plant extracts. This could be due to the ability of the nanoparticles (NPs) to attach to the cell membrane and its penetrating power into the microbial cell. When plant-based silver nanoparticles gains access into a cell, there is reduction in the molecular weight of the microbial cells [39,40,41].

5. CONCLUSION

The findings from this study show that plant-based nanoparticles were effective against the tested uropathogens by inhibiting their rate of growth and proliferation. It is concluded that these nanoparticles may be used in the production of novel drugs for the treatment of urinary tract infections caused by MARI Uropathogens.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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ETHICAL APPROVAL

Ethical approval (OSHREC 23/9/2021/385) has been taken from Health Research Ethics Committee (NHREC/18/08/2016) to carried out the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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