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MICROBIAL QUALITY AND ANTIBACTERIAL ACTIVITY OF WATER- PROCESSED HERBAL CONCOCTIONS SOLD WITHIN KADUNA METROPOLIS AGAINST URINARY TRACT INFECTION ISOLATES

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ABSTRACT

The use of herbal concoction in the treatment of diseases has been in existence from time immemorial. Many of these herbal mixtures are not produced under hygienic conditions and safety issues associated with them may have an exacerbated impact in immunocompromised and elderly individuals. This study assessed the microbial loads of locally- prepared water-processed herbal concoctions sold within Kaduna metropolis and their activity on urinary tract clinical isolates. In triplicates, eight (8) water - processed herbal concoctions sold in Kaduna metropolis, Nigeria purchased randomly from different locations were evaluated for the presence of microorganisms. The mean total viable bacterial count was determined by the plate count method. Bacteria were identified upon growth on culture media and using conventional biochemical tests. The antibiotics susceptibility profile of the isolates from the herbal concoctions as well as the susceptibility of urinary tract infection isolates to the herbal formulations were determined using Kirby-Bauer disk diffusion technique and analyzed using chi-square statistic. Bacteria isolated from the herbal samples had mean bacterial load that exceeded the safety limit set by the World Health Organization (WHO). They include *Staphylococcus aureus* (25%), *Bacillus* specie (16.67%), *S. typhi* (16.67%), *Pseudomonas* specie (12.50%), *E. coli* (8.33%), *Enterococcus* specie (8.33%), *Streptococcus* specie (8.33%) and *Klebsiella* specie (4.17%). The water- processed herbal concoctions showed poor antibacterial activity against the clinical isolates. The isolates from the herbal concoctions showed more resistance to standard antibiotics than the clinical isolates. The study thus shows the presence of microbial contaminants, which exceeded the safety limits of 105 CFU/ml or g according to World Health Organization for herbal preparation. The use of locally prepared water- processed herbal medicine sold in Kaduna could thus, pose a major health risk due to lack of microbial quality control.

Keywords: Water processed herbal concoction, microbial quality, antimicrobial resistance, UTI, Kaduna, phytochemical screening, minimum inhibitory concentration

INTRODUCTION

Urinary tract infections (UTIs) are infections that can occur in the urethra (urethritis), bladder (cystitis), or kidneys (pyelonephritis) (1). They are one of the most prevalent infectious diseases globally, affecting 150 million people annually, among all age range and gender, with significant morbidity and high medical costs (2). Several pathogens belonging to Gram-positive and Gram-negative bacteria classes are regarded as primary cause of UTI;

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the most common pathogens include *Escherichia coli*, *Klebsiella pneumonia*, *Enterococcus* spp., *Staphylococcus saprophyticus*, *Staphylococcus aureus*, group B *Streptococcus*, *Proteus mirabilis* etc (3). Among all bacterial species, *E. coli* is known to be the most common in complicated and uncomplicated UTIs (4). Because of the rapid increase in the rate of infections, coupled with antibiotic resistance in microorganisms, and due to side effects of synthetic antibiotics, medicinal plants are gaining popularity as sources of anti-infectives (5). The increasing occurrence of multidrug-resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics further raised the danger of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting strategies (6).

The use of plants as source of medicine, due to the abundant resource of biologically beneficial compounds dates back to several years (7). According to Umair *et al*. (8), medicinal plants are rich sources of antimicrobial agents. Herbal concoctions made from these medicinal plants are widely used in many cultures as a traditional means of treating diseases including bacterial diseases. Considering the fact that herbal concoctions commonly contain many biologically beneficial compounds from different plant sources, using them in the treatment and/or management of urinary tract infection is attractive. In Africa, including Nigeria generally, herbal concoctions are prepared using water or alcohol. This process and solvent used in the preparation of drugs could influence or encourage microbial growth, which will in turn affect the bioactivity and efficacy of the herbal concoction. Herbal remedies are often perceived as natural and safe alternatives to conventional medications. However, there is a risk of microbial contaminations in these preparations, which can lead to adverse health effects, especially in individuals with compromised immune systems or underlying health conditions. More so, while herbal concoctions are commonly claimed to be used for treating UTIs, their efficacy in combating bacterial infections may vary. Understanding the microbial load present in these preparations can help assess their effectiveness in treating UTIs and provide insights into potential factors influencing their therapeutic outcomes. Hence, this study aimed at determining the efficacy of water -processed herbal concoctions used in the treatment of uropathogens within Kaduna metropolis and their microbial load.

MATERIALS AND METHODS

Collection of Herbal Concoction Samples

Eight (8) herbal concoctions were randomly purchased from vendors located at different selling points within Kaduna metropolis and placed in sterile containers. The collected herbal concoctions were labeled, transported to the laboratory and stored at -20^{O}C for further analysis.

Total Bacterial Count of herbal samples

For each sample, serial dilution was undertaken by introducing 1.0 mL of herbal concoction into a test tube containing 9.0 mL of sterilized distilled water to form a stock solution. Ten-fold serial dilutions labeled 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and $l0^{-5}$ were made. Thereafter, 0.1 mL of each of the dilutions was spread on already prepared sterile nutrient agar using the pour plate technique and incubated at 37^{o}C for 24 h for bacterial growth. Colonies, which developed on the plates were counted and recorded as colony forming units per millilitre (CFU/mL) of the sample (9).

Purification of Isolates from Herbal Concoction.

Each colony from the total bacteria count was isolated in a pure form by subculturing in a fresh nutrient agar plate and pure cultures were preserved on nutrient agar slants for further

identification. An overnight activated culture was then used to determine Gram staining and biochemical behaviors.

Identification of Bacteria Isolates from Herbal Concoction

Gram Staining

Each single colony of the culture was mixed with a drop of water placed on a microscope slide and was allowed to dry to prevent distortion of the cells. The film formed on the slide was then fixed by passing the slide, with the side containing the bacteria facing down, through a Bunsen flame, 2 or 3 times. The film was flooded with crystal violet stain for 1-2 mins and the stain was poured off. The remainder was washed off with Lugol iodine and flooded with fresh iodine for 30 secs – 1 min. The iodine was then washed off with absolute alcohol and was continuously treated with fresh alcohol until all the colour was off. It was then washed with distilled water, drained and counter - stained with Safranin for 30 secs. The slide was further washed again with distilled water, drained, carefully blotted dry with bibulous paper and the film was viewed using a light-microscope under oil-immersion. The bacteria were said to be Gram-positive if the cells are not decolourized by the decolorizing agent and thus retained the violet colour even after the counter stain whereas Gram-negative bacteria are decolourized and absorb the counter stain (pink/red) (10).

Growth on Selective Media

The following 4 media: mannitol salt agar, cetrimide, MacConkey and eosin methylene blue agar were prepared according to the manufacturers' instruction. Overnight culture of the isolates from the herbal concoction samples were streaked on the selective media plates and incubated at 37°C for 24 hours. Any sample with golden yellow growth on mannitol salt agar was suspected as positive for *S. aureus*; growth with green yellow colour on cetrimide agar to dark blue-green was suspected as *Pseudomonas* spp.; growth on MacConkey agar with pink or red color indicates a lactose fermenter positive Enterobacteriacea; greenish metallic sheen growth on eosin methylene suggests *E. coli*.

Biochemical Tests of Herbal Concoction Isolates

The following biochemical tests as described by Cheesbrough, (9) were adopted: coagulase test, methyl red test, Voges -Proskauer test, citrate utilization test, catalase test, indole test, oxidase test, triple sugar iron test and urease test.

Collection of Clinical Uropathogens

The antibacterial potency of each herbal concoction was evaluated using nine bacterial strains causing urinary tract infection. They are: *E. coli, Bacillus* spp., *S. aureus, Streptococcus* spp., *Enterococcus* spp., *Salmonella* spp., *Klebsiella* spp., *P. aeruginosa,* and *Shigella* spp. The already identified clinical isolates were collected from Barau Dikko Teaching Hospital, Kaduna, transferred into transport media and reconfirmed in our laboratory for further use.

Phytochemical analysis of water- processed herbal concoctions samples

The confirmatory qualitative phytochemical screening of herbal concoctions was performed to identify the main classes of bioactive compounds (tannins, saponins, flavonoids, alkaloids, phenols, glycosides, steroids, and terpenoids) present in the herbal concoction samples following standard protocols (11).

a. Test for Tannins

About 200 mg of the herbal concoction was boiled with 10 mL of distilled water; and 0.1% Ferric chloride was added to the mixture; which was then observed for blue-black coloration indicating the presence of tannins.

b. Test for Alkaloids

The herbal concoction was dissolved in 100 mL of water, filtered, and heated in steam with 2 mL of the filtrate and three drops of 1% HCl. Then, 1 mL of the heated mixture was combined with 6 mL of the Mayer-Wagner reagent. The appearance of a cream or brown-red colored precipitate indicated the presence of alkaloids.

c. Test for Saponins

About 0.5 milliliters of the herbal concoction sample and 5 mL of distilled water were combined and agitated. The formation of foam confirms the presence of saponins.

d. Test for Flavonoids and Glycosides

A 200 mg quantity of the herbal concoction was mixed with 10 mL of ethanol and filtered. Two mL of the filtrate, concentrated HCl, and magnesium ribbon were mixed. The formation of a pink or red color indicates the presence of flavonoids. Adding 1 mL of distilled water and NaOH to 0.5 mL of herbal concoction, the formation of a yellowish color indicated the presence of glycosides.

e. Test for Steroids

About 1 mL of the herbal concoction was combined with 10 mL of chloroform and 10 mL of sulfuric acid, and the formation of a bilayer (red top layer and greenish bottom layer) reveals the presence of steroids.

f. Test for Terpenoids

The presence of terpenoids was determined by the formation of a reddish-brown color in the test for terpenoids, which included mixing of 0.5 mL of herbal concoction with 2 mL of chloroform and 3 mL of sulfuric acid.

g. Test for Phenols

About 1 mL of the herbal concoction was combined with three drops of $FeCl_3$, and 1 mL of K_2Fe (CN_6). The formation of greenish-blue colour confirmed the presence of phenols.

Antibacterial Activity of the Herbal Concoctions against Clinical Uropathogenic Isolates

The antibacterial study of the herbal concoctions followed the method described in Oyeleke and Manga, (12). From an overnight culture of the bacteria at $35^{O}C$ on Mueller-Hilton agar, 2 to 3 colonies of the bacterial growth were harvested into a 5 ml sterile saline water, its absorbance was adjusted at 580 nm and diluted to attain viable cell count of 10^7 CFU/ml using spectrophotometer. Ten ml of Mueller-Hilton agar was melted and poured into sterile Petri dishes (as a basal layer) followed with 15 ml of seeded medium previously inoculated with 1 ml of bacterial suspension of already prepared inoculum. The cork-borer of 6 mm radius was then sterilized by flame, cooled, and pressed on the top of seeded Muller Hinton agar to make 6 wells at a distance of about 15 mm apart. Each well was filled with 0.1 ml herbal concoction. The plates were kept in the refrigerator at 5 ^0C for 2 h to permit herbal concoction mixture diffusion, and then incubated at 35 ^0C for 24 h. Two replicas of each plate were prepared, and the diameter of the inhibition zone was recorded from the edge of the well.

Antibiotics Susceptibility Tests of Bacteria Isolates from the Herbal Concoction and Clinical Isolates

Each bacterial strain was subcultured overnight at $35^{O}C$ in Mueller-Hilton agar slants. The bacterial growth was then harvested using 5 ml of sterile saline water, its absorbance was adjusted at 580 mm and diluted to attain viable cell count of 10^7 CFU/ml using a spectrophotometer. A sterile swab was then dipped into the standard culture of the isolate, and squeezed gently against the inside of the tube in order to remove excess fluid in the swab. The swab with the test organism was then streaked on a sterile Mueller -Hinton agar plate to form a lawn. Antibiotics discs were placed on the surface of the streaked plate using sterilized forceps and gently pressed. The plates were then allowed to stand for 15 minutes pre-diffusion time. The inoculated plates were inverted carefully and incubated for

24 hours at 37°C. After incubation the mean zones of inhibition were recorded.

RESULTS

Phytochemical Analysis

The preliminary phytochemical screening carried out on herbal concoctions showed the presence of saponins, tannins, flavonoids, alkaloids, anthraquinone, carbohydrates and cardiac glycosides as presented in Table 1.

Microbial Quality of Water Processed Herbal Concoctions sold in Kaduna Metropolis

Microbial evaluation (Total aerobic count) of the 8 herbal concoction samples was carried out in triplicates and their mean was taken as shown in Table 2. Sample HC4 recorded the highest bacteria count (4.96 X 10^8 CFU/ml), followed by sample HC7 (3.84X10^8 CFU/ml), while the samples with the least bacterial count were sample HC5 (1.27 X 10^8 CFU/ml) and Sample HC8 (1.12 X10^8 CFU/ml). All the samples analyzed had more than the WHO recommended 10^5 CFU/ml for herbal samples. According to the European Pharmacopeia, the acceptable limit of total bacteria is as follows: 10^3 CFU/g or ml for non-aqueous oral preparations, 10^2 CFU/g or ml for aqueous oral preparations, 10^2 CFU/g or ml for remedies used for cutaneous treatments and vaginal treatments, and 10^7 CFU/g or ml for herbal drugs.

This study isolated bacteria of public health importance which have been implicated in most diseases. *Staphylococcus aureus* (25%) had the highest percentage occurrence, followed by *Salmonella typhi* (16.67%) and *Bacillus* specie (16.67%), then *Pseudomonas aeruginosa* (12.50%), *E. coli* (8.33%), *Enterococcus specie* (8.33%) and *Streptococcus* specie (8.33%), while the least in occurrence is *Klebsiella* specie (4.17%).

Antibacterial Activity of Herbal Concoctions against Some Clinical Isolates

Table 3 shows the antibacterial activity of herbal concoctions against the UTI pathogens. *Escherichia coli* had the highest susceptibility (20 mm), followed by *Bacillus species* (12.5 mm), *Staphylococcus aureus* (10.5 mm), *Streptococcus* species (8.5 mm), *Enterococcus* species (6.0 mm), *Salmonella* species (8.0 mm), *Klebsiella* species (6.0 mm) and *Pseudomonas* species (7.0 mm). Samples HC4 and HC6 had no zone of inhibition on *Salmonella typhi* and *Bacillus* species. Samples HC2, HC4 and HC5 showed no zone of inhibition on *Streptococcus* species, while samples HC5 and HC8 showed no zone of inhibition on *Enterococcus* species. Sample HC7 showed no zone of inhibition on *Klebsiella species*. Samples HC4, HC6 and HC7 also showed no zone of inhibition on *Pseudomonas aeruginosa*.

Table 4 shows that all the bacteria (100%) isolated from the water- processed herbal concoction samples were found to be multidrug resistant i.e. resistant to more than 3 classes of the antibiotics tested.

Analyzing the antibiotics susceptibility profile of isolates from clinical samples showed that 77.8% of the bacteria tested were not multidrug resistant, while 22.2%, which were *Streptococcus* species and *Salmonella* species exhibited MDR activities (Table 5).

Comparative assessment of the antibiotics susceptibility profile of herbal concoctions isolates and clinical isolates, showed that the clinical isolates were resistant to the herbal preparation. Further analysis showed that bacteria isolated from the herbal preparations were more resistant to commonly prescribed antibiotics impregnated on disc while clinical isolates were susceptible. This is shown in Figure 2.

Table 1: Phytochemical Constituents of Water - Processed Herbal Concoction Samples

Phytochemicals	Herbal Concoction (HC) samples							
	HC1	HC2	HC3	HC4	HC5	HC6	HC7	HC8
Saponins	++	++	++	++	++	++	++	+
Tanins	++	++	++	++	++	+	++	++
Flavonoid	++	+	++	+	++	++	++	+
Alkaloid	++	+	+	+	++	+	++	+
Anthraquinone	+	++	++	++	++	++	++	++
Carbohydrate	++	++	++	++	++	++	++	++
Cardiac glycoside	++	++	+	+	++	++	++	+

Key: Present (+), Intense (++)

Table 2: Mean of Total Aerobic Count of Herbal Concoction Samples

Sample	Bacterial Count (CFU/mL)
HC1	$3.58x10^8$
HC2	$3.63x10^8$
HC3	$2.52x10^8$
HC4	$4.96x10^8$
HC5	$1.27x10^8$
HC6	$2.41x10^8$
HC7	$3.84x10^8$
HC8	$1.12x10^8$

Key: HC= Herbal Concoction

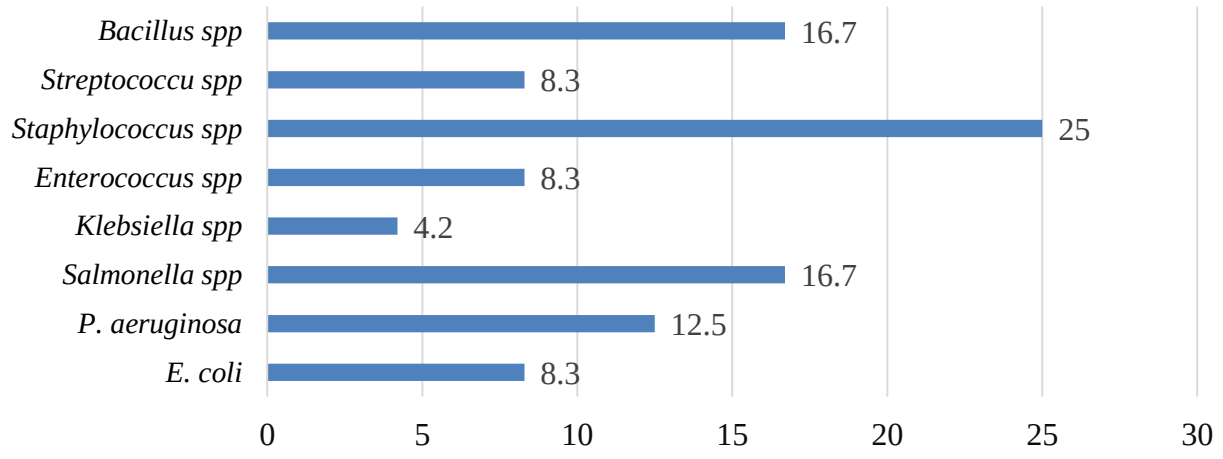

Figure 1: Percentage occurrence of Bacteria Isolated from Water - Processed Herbal Concoctions

Table 3: Antibacterial Activity of Herbal Concoctions against Some Clinical Isolates

Bacteria	Mean Zone of Inhibition (mm) of Herbal Concoction Samples							
	HC1	HC2	HC3	HC4	HC5	HC6	HC7	HC8
Escherichia coli	20.0	11.5	5.0	7.0	2.0	3.0	5.0	2.0
Bacillus species	12.5	7.5	3.0	0.0	6.5	0.0	2.0	5.0
Staphylococcus aureus	10.5	3.0	2.0	4.0	2.0	2.0	2.5	4.0
Streptococcus species	8.5	0.0	2.0	0.0	0.0	3.0	7.0	2.0
Enterococcus species	6.0	8.5	5.0	2.5	0.0	5.0	5.0	0.0
Salmonella species	8.0	6.5	4.0	0.0	2.0	0.0	2.0	4.0
Klebsiella species	6.0	4.5	3.0	3.0	5.0	3.5	0.0	2.5
Pseudomonas aeruginosa	7.0	9.5	4.0	0.0	5.0	0.0	0.0	3.0
Shigella specie	8.0	6.0	0.0	2.0	0.0	6.5	4.0	0.0

Key: HC=Herbal Concoction

Table 4: Antibiotics Susceptibility to the Herbal Concoction Isolates

Bacteria	**Mean of Zone of inhibition (mm) of Antibiotic on Herbal Concoction Isolates**								**Resistance Profile**	**CoR**
	CRO	AML	IMI	CIP	SXT	CN	S	E		
Escherichia coli	R	R	R	R	R	S	R	R	CRO,IMI, CIP, SXT, S	MDR
Bacillus species	S	R	R	R	R	S	R	R	AMI,S, IMI, CRO	MDR
Staphylococcus aureus	I	R	R	S	R	S	R	R	AMI, IMI, S, E	MDR
Streptococcus species	R	R	R	R	R	S	S	I	IMI, SXT, S	MDR
Enterococcus species	R	R	R	I	R	S	R	R	CRO,AMI, IMI, SXT, S, E	MDR
Salmonella species	I	I	R	I	S	S	R	R	IMI, S, E	MDR
Klebsiella species	I	R	I	R	R	S	S	I	AMI, SXT, E	MDR
Pseudomonas aeruginosa	R	I	R	I	S	S	R	R	CRO, IMI, S, E	MDR

Key: CRO=Ceftriaxone 30 µg, AML=Amoxicillin 30 µg, IMI= Imipenem 10µg, CIP = Ciprofloxacin 5 µg, SXT = Trimethoprim Sulfamethoxazole 25 µg, CN = Gentamicin 30 µg, S=Streptomycin 10 µg, E=Erythromycin 15 µg, CoR = Classification of resistance

Table 5: Antibiotics Susceptibility of the Clinical Isolates

Bacteria	Mean of zone of inhibition (mm) of antibiotics on Clinical Isolates (mm)								Resistant Profile	CoR
	CRO	AMI	IMI	CIP	SXT	CN	S	E		
Escherichia coli	R	S	I	S	R	S	R	R	SXT, E	NMDR
Bacillus species	R	S	S	R	S	S	R	S	CRO, S	NMDR
Staphylococcus aureus	S	S	R	R	S	S	S	S	-	NMDR
Streptococcus species	R	R	R	S	S	S	S	S	CRO, AMI, IMI	MDR
Enterococcus species	R	S	S	S	R	S	R	S	SXT, S	NMDR
Salmonella species	I	R	R	S	R	S	R	R	AMI, IMI, SXT,S, E	MDR
Klebsiella species	S	R	I	R	S	S	I	S	AMI	NMDR
Pseudomonas aeruginosa	R	I	R	I	R	S	S	S	CRO, IMI,	NMDR
Shigella species	S	R	R	R	R	S	R	S	IMI	NMDR

Keys: CRO = Ceftriaxone 30 µg, AML = Amoxicillin 30 µg, IMI = Imipenem 10 µg, CIP = Ciprofloxacin 5 µg, SXT = Trimethoprim-Sulfamethoxazole 25 µg, CN = Gentamicin 30 µg, S = Streptomycin 10 µg, E = Erythromycin 15 µg, CoR = Classification of resistance

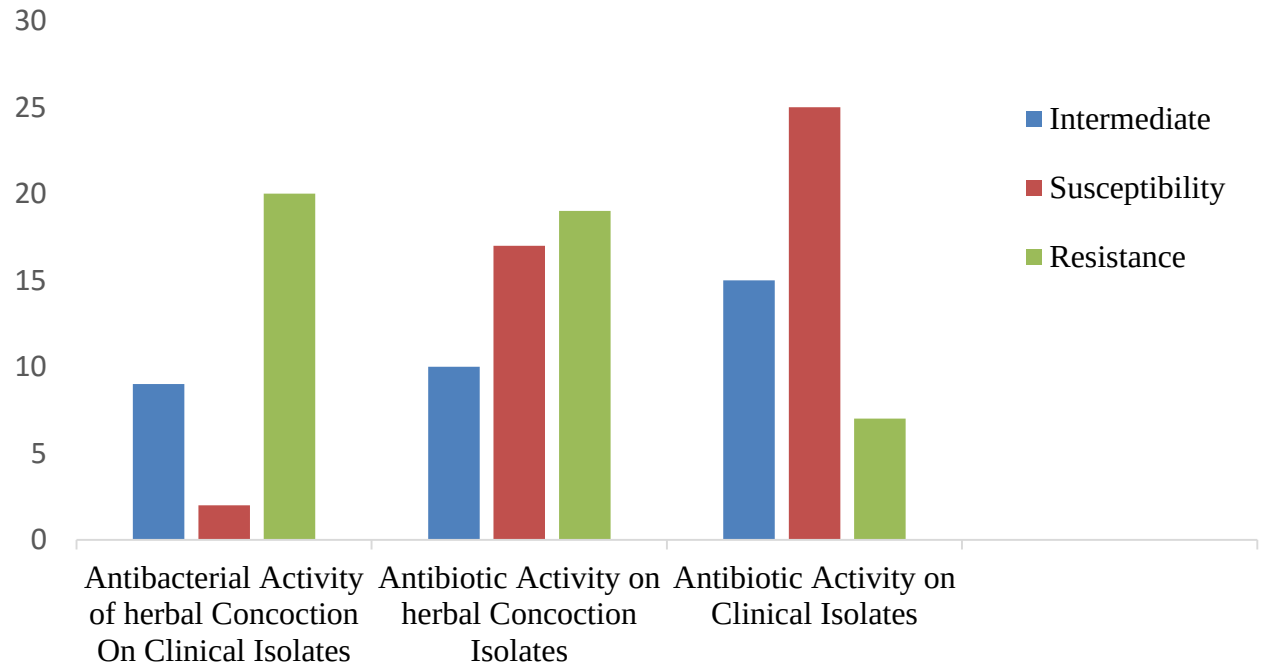

Figure 2: Comparative Resistance Profile Analysis of Bacteria Isolated from Herbal Concoctions and Clinical Isolates

DISCUSSION

Studies have shown that antimicrobial resistance has rendered many currently in-use medications ineffective (13). This challenge has further reinforced the use of herbal concoctions especially among rural dwellers. This study evaluated the antimicrobial potentials of eight herbal water- processed concoctions sold within Kaduna metropolis on uropathogens. The majority of the investigated herbal concoctions were found to contain different bioactive phytochemicals including flavonoids, alkaloids, glycosides, and steroids. These bioactive compounds have been reported in several studies to contribute to the bioactivity exhibited by herbal preparations. However, the variation in the bactericidal effect of different herbal concoctions may arise from variation in their chemical constituents and volatile nature of their constituents. . Flavonoids are reported to be more effective against different microbial strains than conventional medications (11). Some studies have also revealed that hydroxyl groups at specific positions on the aromatic rings of alkaloids improve antibacterial activity (14, 15). There was a limited antibacterial activity of the water- processed herbal concoctions sold in Kaduna, Nigeria. This suggests that there may not be a strong justification for the traditional uses of the herbal concoctions for the treatment of urinary tract infectious diseases for which they are being used. There is also the possibility that the limited antibacterial potency of the herbal concoctions may be due to the method of preparation, interaction between the compounds from various admixtures prepared, and the source of water used to prepare the concoctions (16, 17). Another reason that may be adduced for the differences in the antibacterial effects of the herbal concoction includes the season of plant collection, and/or environmental variations (11).

The mean total aerobic count of the evaluated herbal concoctions showed that these samples contained pathogenic contaminants that exceed WHO recommended CFU/ml, which could have various health implications on the users of the products. These contaminants could have been acquired from use of water of poor quality for the preparation of the products and rinsing of containers and dirty environment (17). This finding has also been reported by Olaniran *et al*., (18), suggesting that consuming these products could serve as route for exposure to the pathogenic microorganisms and could have gross public health implications, if not curtailed. Therefore, recommendations abound from studies that stringent quality control and good manufacturing procedures of such products are important. The review conducted by Opuni *et al*. (19), which analyzed manuscripts published for 35 years from different published articles from 28 countries further supports the finding of this study that most herbal medicinal products sold in the market have significant contaminants, mainly metals, followed by microorganisms, then mycotoxin. The most prevalent bacteria were *E. coli* and *Salmonella* spp. in the said review by Opuni *et al*. (19). However, our study showed that *Staphylococcus aureus* and *Salmonella typhi* were the most isolated. This finding corroborated the results of the studies by Olaniran *et al*., (18) and Korir *et al*., (20), which noted that most herbal concoctions in Shagamu and Nairobi were contaminated by bacteria (76% and 84.8% respectively) mostly *S. aureus* and 50% and by fungi (44.2%).

The slight bioactivity exhibited by some of the water - processed herbal concoctions might be attributed to the fact that such concoctions contain one or more secondary metabolites. The variations in the biological activity profiles could summarily be adduced to either the individual classes of compounds present in the herbal concoctions or the synergistic effect of a combination of classes (21).

The assessment of the antibiotic susceptibility profile of the isolates from both the herbal concoctions and clinical isolates reveals that the clinical isolates were more susceptible to most of the conventional/commonly prescribed antibiotics than the herbal concoction isolates. This could be due to the fact that the herbal concoction isolates had been pre-exposed to lower doses of bioactive compounds and subsequent mutation in their genetic constituents, influenced by adaptation to other environmental conditions, making them develop strong resistance to conventional antibiotics and environment, for survival. These in turn could cause resistance to most of the antibiotics used on them. Therefore consuming these products will result in bacteria overload of the system with no systemic resistance, which is highly dangerous for immunocompromised and elderly individuals. Our finding is further supported by the study carried out by Walusansa *et al*., (22), which showed that extended beta lactamases genes (TEM, CTX-M and SHV) known to exhibit resistance to multiple drugs have been isolated from bacteria obtained from different herbal preparations sold in open markets. This submission possibly informed why 100% of the bacteria isolated from the water - processed herbal concoctions sampled in Kaduna were multidrug resistant i.e. resistant to more than 3 classes of the antibiotics tested. This finding also concurs with the report by Ejimofor *et al*. (23) in Onitsha, South east Nigeria, who also observed that isolates from herbal preparations were highly multidrug resistant to commonly prescribed drugs.

Also, on testing urinary tract infection pathogens from the clinic against the herbal preparation, it was observed that the clinical isolates were resistant to the herbal concoctions. This is contrary to the result of other studies that showed that herbal concoctions were effective in managing uropathogenic infections and for short-term prophylaxis (24). In addition to contaminated water sources, other possible sources of contamination of the herbal concoctions might be, the hygiene level of the personnel preparing the concoction and method of preparation of the herbal concoction. Therefore, stringent approaches and regulation policies should be put in place to reduce the public health implications of water- processed herbal concoction sold in the open market.

CONCLUSION AND RECOMMENDATIONS

This study showed high microbial contamination above WHO and Europian Pharmacopoeia recommendations, in water processed herbal concoctions sold in Kaduna metropolis. The bacteria isolated were also observed to be multidrug resistant and not susceptible to conventional antibiotics compared to clinical isolates. The products were also found not to have significant therapeutic effect on purified clinical isolates from UTI patients although there appeared to be minimal antibacterial effects with all eight herbal concoctions against the nine (9) bacterial strains tested; however the effects varied but were not comparable to those by conventional drugs. The slight *in vitro* activity against some of the bacteria species, might not translate to any substantial activity, *in vivo*. Further investigations are necessary to evaluate the minimum inhibitory concentration and minimum bactericidal concentration of the water- processed herbal concoctions sold in open markets in Kaduna, Nigeria and also to determine their toxicological properties, *in vivo* potencies, sources of contamination and mechanism of action against urinary tract infection pathogens.

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