



(RESEARCH ARTICLE)



Microbial quality and antimicrobial evaluation of some herbal concoctions in a Rural Town in Nigeria

Ayantade Dayo Victor Ayansina * and Aderonke Oluwatomisin Akinsola

Department of Biological Sciences, Bowen University, Iwo, Osun State, Nigeria.

Publication history: Received on 21 November 2020; revised on 30 November 2020; accepted on 02 December 2020

Article DOI: <https://doi.org/10.30574/wjbphs.2020.4.3.0098>

Abstract

The use of local herbal concoctions has been on the increase for the cure of various illnesses in Iwo community and Nigeria generally. Experiment aimed at isolating major microorganisms and carrying out antibiotic resistant test on the bacterial isolates was carried out. Bacteria isolated were identified through biochemical tests and molecular characterization. Susceptibility levels of these isolates was evaluated using antibiotic rapid test multidisc containing eight different antibiotics and resistance genes were amplified from some of the isolates. Five fungal species were isolated from the samples and these included *Aspergillus niger* and *Mucor* sp which occurred the most (33.3%), while *Rhizopus stolonifera* and *Aspergillus nidulans* had the lowest occurrence (6.7%). Twenty- four bacterial species belonging to fifteen (15) genera were identified. These includes *Providencia* sp 17(19%) recorded the highest occurrence, followed by *Pantoea* sp 13(14.4%) and *Citrobacter* sp 10(11.1%) while *Salmonella* sp, *Cedecea* sp, *Pseudomonas* sp and *Yersinia* sp had the lowest occurrence (2.2%) each. All the isolates showed resistance to three or more antibiotics used, especially the cephalosporins. The genes found to be responsible for the cephalosporin resistance in some of the isolates were TEM-972 and CTX-M-200. Presence of the multi-antibiotic resistant microorganisms in herbal concoctions poses a serious public health threat.

Keywords: Antibiotics; Resistance; Herbal; Concoction; Microorganisms

1. Introduction

The first line of therapy known to man in the past was medicinal crops, traditional medicinal exercise continues to be a significant component of the main care scheme in most emerging countries [1]. Approximately 70-80 per cent of the world's inhabitants lives in developing nations and depends on herbal concoctions due to their simple availability and cheapness [2,3]. Recently used synthetic or standard drugs have been obtained from herbs such as *Cinchona* sp from where the antimalarial medicine quinine is obtained [4]. In the US, about 25% of prescribed medicinal products have a minimum of one active component extracted from crop products [5]. The majority of herbal preparations are in various forms. The existence of plant secondary metabolites has been ascribed to most plants' medicinal activity [3, 4]. In general, crops represent a significant source of orthodox drugs. In Nigeria, "public awareness and use of herbal medicines in the treatment and/or prevention of diseases" appear to be increasing significantly. However, it is important that herbal preparations are of high quality and safety. WHO [6] said the quality is the foundation of herbal pharmaceutical reproductive efficacy and safety, and that the quality of the plant products or preparations is of great significance in order to guarantee that study standards in herbal medicines are maintained. The quality requirements for medicinal medicines have been shown by [7] based on a definite science concept of raw materials.

Herbal medicine has also been defined by the World Health Organization as complete label medicines containing active constituent's plants or other herbal materials or combinations in crude form, or in plant preparations [8]. Plant products include fatty oils, gums, juices and other such ingredients. Using herbal medication has always been

* Corresponding author: Ayantade Dayo Victor Ayansina
Department of Biological Sciences, Bowen University, Iwo, Osun State, Nigeria.

component of human society, as some crops have significant medicinal characteristics that can be used to heal human and other animal illnesses [9]. The primary health care recognized to humans is herbal medicine, practiced throughout history by all societies, it has become an essential component of the growth of contemporary civilization [10].

Herbs and herbal products have long been commercialized and are currently being used for a variety of applications [11]. Herbal medicine has also been defined by the World Health Organization (WHO) as a fresh or dry, fractioned or pulverized plant material that can be utilized in its raw form or subsequently processed into something more refined. Herbal products such as resins, essential oils, fixed oils, gums, infusions, decoctions, tinctures and juices can be produced by subjecting the herb to processes as baking or sweetening with honey, alcohol extraction, infusing in water, decocting, roasting, steaming or even squeezing. The resulting mixture can then be used as medical supplements or as food stuffs and starting material for further processing [12]. The products may be subjected to a number of biological, chemical or physical processes, including fermentation, concentration, purification, fractionation, expression, distillation, extraction or pulverization, depending on how sophisticated the herbal preparation is. The finished product can be formulated by blending one or more plant preparations with animal products or minerals and components isolated from herbal or synthetic materials [13].

Antibiotics or antimicrobials are drugs or chemicals used to combat infections caused by bacteria [14]. They constitute one of the world's most effective types of treatment, but a growing amount of antibiotic-resistant pathogens compromise their efficacy [15]. Almost all major bacterial diseases today in the globe are caused by antibiotic resistance; it is one of the most urgent public health issues in the globe with enhanced mortality and morbidity, and increased expenses for therapy [16,17]. In this work we investigated the microbial quality of some local herbal concoctions and antibacterial sensitivity reactions of isolated bacteria.

2. Material and methods

2.1. Sample collection

Twenty-one different samples of locally prepared herbal concoctions were bought from vendors in Iwo town and designated as A to U. The concoctions were classified based on diseases they cure as claimed by the vendors. These diseases include worm, malaria, constipation, stool control, rheumatism, diabetes, typhoid fever and pile. The concoctions were collected and transferred to the laboratory for assessment.

2.2. Isolation of bacterial isolates

Isolation of bacteria was carried out by inoculating nutrient agar to aid the total viable bacterial count, Eosine methylene blue (EMB) agar was used for selective bacterial count. Routine serial dilutions of the herbal concoctions were carried out up to dilution 10^{-5} . One millilitre was taken at different dilutions. Routine bacterial identification steps included Gram's staining, catalase test, MR-VP test, indole test, sugar fermentation test etc.

2.3. Antibiotic Sensitivity Test

Antibiotic sensitivity test was carried out using Mueller Hinton agar. The inoculum was prepared using the 0.5 McFarland standard. A sterile inoculating loop was used to pick the test organism and suspended into 2ml of sterile normal saline. The absorbance of this suspension was measured using the spectrophotometer (Jenway 6305) at a wavelength of 625nm. The turbidity of the suspension was adjusted by adding more organisms when the suspension was found to be too light, and diluting with sterile normal saline when the suspension was too turbid. A sterile swab stick was dipped into the inoculum suspension and allowed to soak. The dried surface of the agar was then inoculated by streaking the entire surface uniformly with the swab stick. Using sterile forceps, a Gram negative antibiotic multi-disc containing 8 antibiotics was placed aseptically on the plate and pressed firmly onto the surface of the agar to allow proper diffusion of the antibiotics on the agar. The antibiotics on the disc are ceftazidime (30µg), cefuroxime (30µg), gentamicin (10 µg), cefixime (5µg), ofloxacin (5µg), augmentin (30µg), nitrofurantoin (300µg) and ciprofloxacin (5µg). This process was repeated for all test organisms, and the plates were incubated at 37°C for 24 hours. After incubation, the zones of inhibition were measured using a transparent ruler and were recorded in millimeter [18].

2.4. Extraction of genomic DNA

The boiling technique was used to extract DNA from each organism. Cells of each organism was harvested into 1000µL of sterile water, and were vortexed until completely dissolved. Centrifugation was carried out at 10000 rpm for 5 minutes. The supernatant was discarded, and another 1000µL of sterile water was added, after which the mixture was vortexed and centrifuged again. The supernatant was discarded and 200µL of sterile water was added. The mixture was

vortexed again until thoroughly mixed. The mixture was boiled at 100°C after which it was cooled immediately on ice. The mixture was again vortexed and centrifuge at 10000rpm for five minutes. The supernatant was transferred into fresh Eppendorf tubes, and the pellets were discarded. The DNA was then stored at 4°C.

2.5. ESBL Multiplex PCR amplification

The PCR reaction was carried out using the Solis Biodyne 5X HOT FIREPol Blend Master mix. PCR was performed in 20 µl of the reaction mixture, and the reaction concentration was brought down from 5x concentration to 1X concentration. The concentration contains 1X Blend Master mix buffer Buffer (Solis Biodyne), 1.5 mM MgCl₂, 200µM of each deoxynucleoside triphosphates (dNTP)(Solis Biodyne), 20pMol of each primer (Jena Bioscience, Germany), 2 unit of Hot FIREPol DNA polymerase (Solis Biodyne), Proofreading Enzyme, 5µl of the extracted DNA, and sterile distilled water which was used to make up the reaction mixture. Thermal cycling was conducted in an Pielter thermal cycler (MJ Research Series) for an initial denaturation of 95°C for 5 minutes followed by 30 amplification cycles of 95°C for 30 seconds at; 56°C for 1 minute and 72°C for 1 minute 30 Seconds. This was followed by a final extension step of 72°C for 10 minutes. The amplification product was separated on a 1.5% agarose gel and electrophoresis was carried out at 80V for 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by ethidium bromide staining and 100bp DNA ladder (Solis Biodyne) was used as DNA molecular weight marker [19].

3. Results

Twenty-four bacterial isolates were obtained, with fifteen (15) different genera identified during the study. Table 1 shows the distribution of all isolates from the different herbal samples. *Providencia* sp with 17 isolates (19%) recorded the highest occurrence, followed by *Pantoea* sp with 13 isolates (14.4%), *Citrobacter* sp with 10 isolates (11.1%), *Serratia* sp, *Proteus* sp and *Klebsiella* sp with 7 isolates each (7.8%), *Kluyvera* sp and *Enterobacter* sp with 5 isolates each (5.6%), *Brenneria* sp and *Escherichia coli* with 4 isolates each (4.4%), *Edwardsiella* sp with 3 isolates (3.3%), *Salmonella* sp, *Cedecea* sp, *Pseudomonas* sp and *Yersinia* sp with 2 isolates each (2.2%).

Table 1 Frequency of occurrence of bacteria isolated from different herbal concoctions

Probable organisms	Frequency of Isolates	Percentage (%)
<i>Providencia</i> sp	17	19
<i>Pantoea</i> sp	13	14.4
<i>Citrobacter</i> sp	10	11.1
<i>Serratia</i> sp	7	7.8
<i>Proteus</i> sp	7	7.8
<i>Klebsiella</i> sp	7	7.8
<i>Kluyvera</i> sp	5	5.6
<i>Enterobacter</i> sp	5	5.6
<i>Brenneria</i> sp	4	4.4
<i>Escherichia coli</i>	4	4.4
<i>Edwardsiella</i> sp	3	3.3
<i>Salmonella</i> sp	2	2.2
<i>Cedecea</i> sp	2	2.2
<i>Pseudomonas</i> sp	2	2.2
<i>Yersinia</i> sp	2	2.2
TOTAL	90	100

Figure 1 shows the distribution of the isolated bacteria genera according to solvents used for extraction in the concoctions. *Pantoea* sp was the highest isolate in water based herbal concoctions, while *Salmonella* sp was the lowest. Alcohol based concoctions had *Providencia* sp as the highest occurring isolate while *Serratia* sp, *Klebsiella* sp, *Enterobacter* sp, *Kluyvera* sp and *Yersinia* sp were found to be the lowest. The 'omikan' (fermented-steep water) based concoctions had *Pantoea* as the highest, while *Providencia* sp and *Yersinia* sp were the lowest. *Providencia* sp was the

highest while *Proteus* sp, *Edwardsiella* sp and *Salmonella* sp were the lowest in the Seven-up (soft drink) based concoctions.

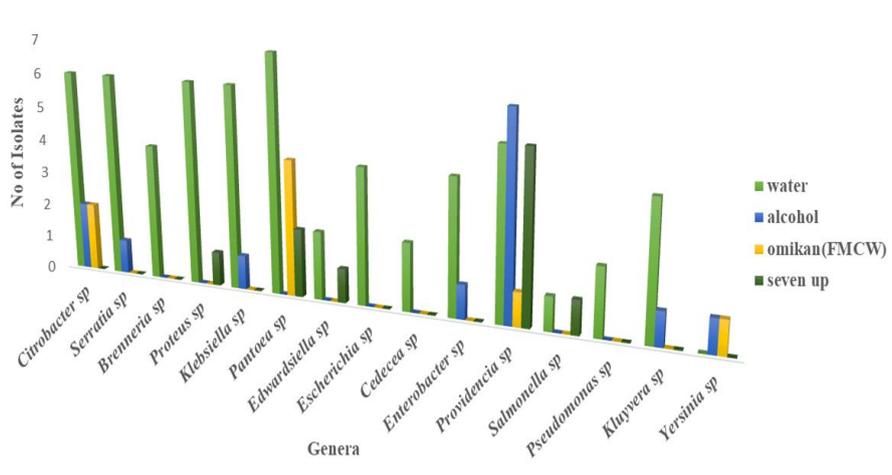


Figure 1 Distribution of isolated bacterial genera based on solvents used for the herbal concoctions

Table 2 shows differences observed in the antibiotic resistance patterns of all isolates. All isolates (100%) were sensitive to nitrofurantoin and none was resistant. Of the 90 isolates, 74 organisms (82%) were resistant to ceftazidime, 85 (94%) were resistant to cefixime, 3 (3%) were resistant to ofloxacin, 41 (46%) were resistant to augmentin, 10 (35%) were resistant to nitrofurantoin, and 3 (3%) were resistant to ciprofloxacin. 4 (4%) were resistant to gentamicin. 68 (76%) were resistant to cefuroxime. All isolates were found to be multi-antibiotic resistant (MAR), forming resistance to three or more antibiotics.

Table 2 Resistance patterns of different bacterial genera to various antibiotics

Antibiotics	Gentamicin (10µg)	Cefixime (5µg)	Ofloxacin (5µg)	Augmentin (30µg)	Nitrofurantoin (30µg)	Ciprofloxacin (5µg)	Ceftazidime (30µg)	Cefuroxime (30µg)
<i>Providencia</i> (n=17)	0(0)	17(100)*	0(0)	9(53)	0(0)	0(0)	17(100)	16(94.2)
<i>Pantoea</i> (n=13)	0(0)	13(100)	0(0)	8(61.5)	0(0)	0(0)	12(92.4)	12(92.4)
<i>Citrobacter</i> (n=10)	4(40)	9(90)	4(40)	6(60)	0(0)	5(50)	10(100)	9(90)
<i>Serratia</i> (n=7)	1(14.3)	6(85.7)	0(0)	3(42.9)	0(0)	1(14.3)	6(85.7)	6(85.7)
<i>Proteus</i> (n=7)	0(0)	7(100)	1(14.3)	5(71.4)	0(0)	2(28.6)	5(71.4)	6(85.7)
<i>Klebsiella</i> (n=7)	0(0)	7(100)	0(0)	5(71.4)	1(14.3)	0(0)	7(100)	7(100)
<i>Kluyvera</i> (n=5)	0(0)	5(100)	0(0)	3(60)	0(0)	0(0)	4(80)	4(80)
<i>Enterobacter</i> (n=5)	1(20)	5(100)	1(20)	3(60)	1(20)	1(20)	5(100)	5(100)
<i>Brenneria</i> (n=4)	0(0)	4(100)	0(0)	0(0)	0(0)	0(0)	2(50)	2(50)
<i>Escherichia coli</i> (n=4)	0(0%)	4(100%)	0(0%)	2(50%)	0(0%)	1(25%)	4(100%)	4(100%)
<i>Edwardsiella</i> (n=3)	0(0%)	3(100%)	0(0%)	2(66.7%)	0(0%)	0(0%)	3(100%)	2(66.7%)
<i>Salmonella</i> (n=2)	0(0%)	2(100%)	0(0%)	1(50%)	0(0%)	1(50%)	2(100%)	2(100%)
<i>Cedecea</i> (n=2)	0(0%)	2(100%)	0(0%)	2(100%)	0(0%)	0(0%)	2(100%)	2(100%)
<i>Pseudomonas</i> (n=2)	0(0%)	2(100%)	0(0%)	2(100%)	0(0%)	0(0%)	2(100%)	2(100%)
<i>Yersinia</i> (n=2)	0(0%)	2(100%)	0(0%)	1(50%)	0(0%)	0(0%)	1(50%)	1(50%)

KEY: n= the actual number of each genera

*= number of isolates tested; figures in parenthesis represent percentage (%) of resistance

Providencia was found to be 100% resistant to cefixime and ceftazidime but 100% susceptible to gentamicin, ofloxacin, nitrofurantoin and ciprofloxacin, it was also found to be quite susceptible to cefuroxime with 94%. *Pantoea* was found to be highly resistant to the cephalosporins but 100% susceptible to gentamicin, ofloxacin, nitrofurantoin and ciprofloxacin. *Pseudomonas*, *Edwardsiella*, *Salmonella*, *Escherichia coli*, *Klebsiella* and *Cedecea* were found to be 100% resistant to the cephalosporins but were highly susceptible to gentamicin, ofloxacin, nitrofurantoin and ciprofloxacin and slightly susceptible to augmentin. A further description of antibiotic resistance pattern in bacterial isolates and resistance genes is presented in table 3.

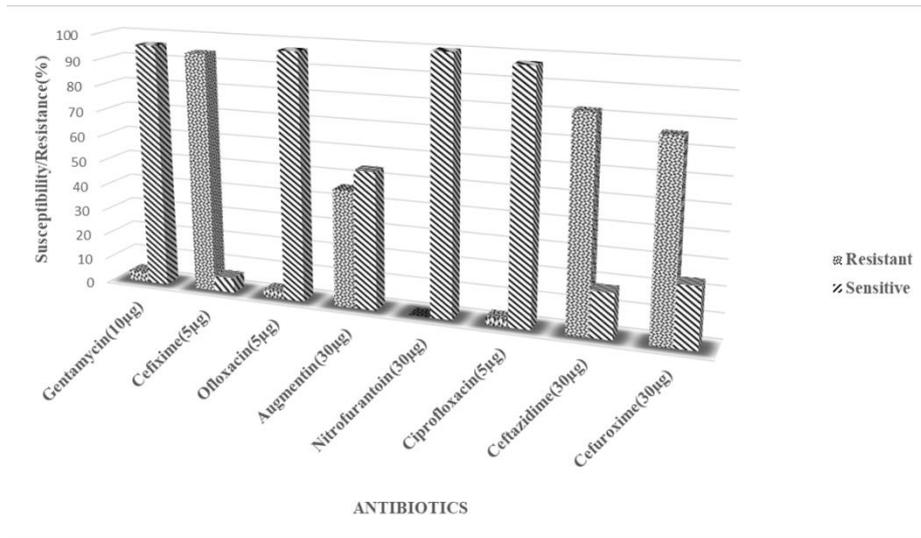


Figure 2 Cumulative susceptibility and resistance of bacterial isolates to antibiotics tested

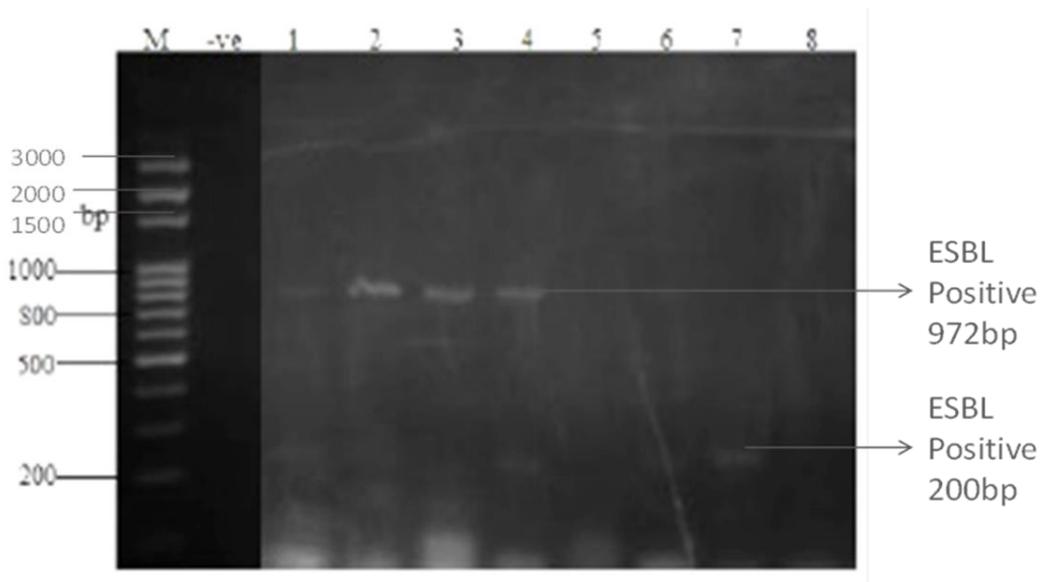


Plate 1 Agarose gel electrophoresis of PCR-amplified CTX-M, SHV and TEM genes from cephalosporin resistant organisms

Lane 1: Marker, Lane 2: Negative control, Lane 3: *Pantoea* sp, Lane 4: *Citrobacter* sp, Lane 5: *Citrobacter* sp, Lane 6: *Pantoea* sp, Lane 7: *Citrobacter* sp, Lane 8: *Klebsiella* sp, Lane 9: *Enterobacter* sp, Lane 10: *Klebsiella* sp.

Table 3 Occurrence of amplified resistance genes in antibiotic resistant isolates

S/N	Organism	Lane	GENES		
			Bla _{TEM}	Bla _{CTX-M}	Bla _{SHV}
1	<i>Pantoea</i> sp	3	-	-	-
2	<i>Citrobacter</i> sp	4	+	-	-
3	<i>Citrobacter</i> sp	5	+	-	-
4	<i>Pantoea</i> sp	6	+	-	-
5	<i>Citrobacter</i> sp	7	-	-	-
6	<i>Klebsiella</i> sp	8	-	-	-
7	<i>Enterobacter</i> sp	9	-	+	-
8	<i>Klebsiella</i> sp	10	-	-	-

A total of fifteen (15) different fungal species were isolated from some of the herbal concoctions. The cultural and morphological characteristics of the fungal isolates which included; colony colour, colony texture, hyphae type and spore types were used as basis of identification.

Table 4 shows the frequency of the isolated species. *Aspergillus niger* and *Mucour* sp were five (5) isolates each. *Rhizopus* sp and *Aspergillus nidulans* occurred once, while *Candida albicans* occurred thrice.

Total 4 Frequency of fungal isolates from the herbal concoctions.

Isolates	Number of Isolates	Percentage (%)
<i>Aspergillus niger</i>	5	33.3%
<i>Mucour</i> sp	5	33.3%
<i>Rhizopus stolonifer</i>	1	6.7%
<i>Candida stolonifer</i>	3	20%
<i>Aspergillus nidulans</i>	1	6.7%
Total	15	100%

4. Discussion

Herbal concoctions used in the present study are orally taken in the form of water extracted remedies, fermented-steeped milled corn water ('omikan'), soft drink or alcoholic spirits. More than 60% of the herbal specimens used in this research, however, were water-based. Food and drinks based study were not carried out by producers on any of the analyzed herbal samples which most likely accounted for the elevated rates of regeneration of herbal coliforms. Coliforms are members of the Enterobacteriaceae family and are the most reliable indicators of faecal pollution in drinks. The test of their presence is therefore a pollution level index that can indicate potential presence of hazardous disease causing organisms [20,21,22].

The predominant fungal isolates obtained in the present study are the members of *Aspergillus* sp. The presence of these fungi in herbal medicines has been previously reported by [23,24,25]. The predominance of *Aspergillus* sp in herbal drugs have been reported by Elshafie et al. [26] and Mandeel [27]. The contamination of herbal preparations by fungi has been attributed to neutral extraneous contamination by dust following storage in humid conditions [28]. Some of the identified contaminants have been reported to have the ability to produce mycotoxins [29].

The microbiological status of herbal mixtures relies on many environmental variables and this could have major effects on the general quality of herbal products [30]. Results from this study have shown that the herbal concoctions being

consumed by some or most residents of the Iwo Local Government Area of Osun State, Nigeria are contaminated with microorganisms (both bacterial and fungal), which might have detrimental health implications on consumers.

In order to enhance consumer acceptability, most herbalists in Nigeria have adopted the use of bottles and plastic containers as packaging vessels for their preparations. Unfortunately, these vessels are not subjected to any form of sterilization after mere rinsing them. Contamination of the preparations coupled with the humid tropical environments may result in the proliferation of microbial contaminants in the herbal concoctions; this probably explains the high microbial counts recorded in this study. Such elevated concentrations of microbial contamination have proven to spoil and degrade products or pose a health risk to the consumer [31,32,33]. Most herbal preparations are made up of different components of various plant species and these preparations are not standardized with respect to colour, taste, consistency etc. Unlike the orthodox drugs, changes in the appearance, odour, taste etc of herbal concoctions due to spoilage are hardly readily detected by the patients.

Some of the bacteria isolated in this research were *Proteus* sp, *Pseudomonas* sp, *Salmonella* sp which were in agreement with studies by Kolajo [34] and Mukhtar et al. [35]; all of which are naturally occurring in soil and water habitats. Their occurrence, well stated in the results, was probably due to their presence on plant parts and in the soil as their natural habitat. Kolajo [34] had reported that the presence of these microorganisms in the herbal mixtures signifies the substandard processing steps in the production of the herbs. Some other bacterial isolates like *Providencia* sp, *Citrobacter* sp, *Klebsiella* sp and *Escherichia coli* in this study were also reported in the work of Esimone et al. [36]. Poor hygiene conditions during the production of the herbal concoctions could introduce these microorganisms into the preparations. Bacterial contaminants could also be associated with bad quality of water used for the processing of these herbal concentrations, as reported by Mukhtar et al. [35].

Some of the genera that cause enterotoxigenic gastroenteritis such as *Pseudomonas* sp, *Enterobacter* sp, *Klebsiella* sp, *Proteus* sp [37] isolated from herbal concoctions in this research were also earlier associated with infant gastroenteritis by Back et al. [38] and Jiva et al. [39]. In infantile gastroenteritis transmitted by water and food, *Pseudomonas aeruginosa* was likewise implicated [40]. Klipstein and Engert [41] also identified *Enterobacter* sp as an opportunistic pathogen in children's diarrhoea-related extra-intestinal diseases. Also acknowledged as opportunistic pathogens, *Klebsiella* sp, has become increasingly important. *Klebsiella pneumoniae*, was also involved as a cause of diarrhoea [42], while *Citrobacter* sp. was likewise implicated as an opportunistic pathogen [43]. Guarino et al. [44] proposed its role in diarrhoeal disease. It was also revealed that *Proteus mirabilis* was connected with diarrhoeal illness [45].

Some fungal isolates were also obtained from some of the herbal concoctions. The fungal strains were distributed into the following genera: *Mucor* sp, *Penicillium* sp, *Aspergillus* sp, *Candida* sp which were also in agreement with the work of Esimone et al. [36]. The presence of yeasts, *Aspergillus* spp, *Rhizopus* spp, *Mucor* spp, and *Penicillium* spp, were not so surprising as they are part of the mycoflora of plants and the soil and they are common spoilage organisms of carbohydrate containing foods [46]. The isolation of *Aspergillus niger* was similar to the results obtained in the isolation of *Aspergillus* sp. by Odedara and Muletiwon [47]. The isolation of *Candida* sp. corroborated the results of Adeleye et al. [24] and Anyawu [48], who in their studies isolated *Candida* sp. from various Nigerian herbal preparations.

Fungal species such as *Rhizopus*, *Mucor*, *Penicillium* and *Aspergillus* may endanger consumer health as they are involved in human pathogenicity. They produce potent mycotoxins that are involved in cancer, dermatitis, hepatotoxicity and nephrotoxicity [21,49,50].

Most of the bacteria isolated were mostly resistant to ceftazidime, cefuroxime, and cefixime which belong to the class of beta-lactam antibiotics known as cephalosporin, used to treat most bacterial infections [51].

As earlier mentioned contamination could get into the concoctions through the type of water used as solvent. According to Samie et al. [52] who studied the diversity of bacteria isolated from samples of household drinking water (which happens to be the solvent used for most of the herbal concoctions), ciprofloxacin is one of the most active antibiotics used, and is proving to be more effective than the cephalosporins due to the plasmid-mediated genes, and indiscriminate use of the cephalosporins. In a similar study by Ben-Ami et al. [53], enterobacteria have been found to increasingly produce extended- spectrum beta- lactamase, thereby increasing their resistance to the beta-lactam antibiotics such as the cephalosporins.

Among the antibiotics studied in this work, the third class cephalosporins such as cefuroxime, ceftazidime and cefixime were found to be the least active agents against all the organisms isolated, while gentamicin was one of the most active agent. Moosavian & Deiham [54] corroborated this finding in their study, indicating the increasing resistance of Gram negative bacteria to antibiotics, especially the third class cephalosporins. Dou et al. [55] and Ee et al. [56] suggested that

the use of cefuroxime, ceftazidime and other cephalosporins should be used cautiously in treating infections to counter the related increase in resistance levels. Most of the isolates were found to be resistant to cefixime, cefuroxime and ceftazidime which are members of the class of cephalosporins.

Molecular analysis was carried out on few of the isolates which included DNA Extraction, ESBL Multiplex PCR analysis and amplification of three genes, the TEM, CTX-M and SHV genes. TEM gene was detected in three isolates and CTX-M gene was detected in only one isolate. However, SHV gene was not detected in any of the isolates. The TEM gene are found to be among those found responsible for the extended-spectrum beta-lactamase (ESBL) phenomenon [57]. While the CTX-M genes are usually the most frequent ESBL-producing genes for Gram negative bacteria as reported by Ben-Ami et al. [53], it was found in only one isolate in this study, and this corroborated the result of Champs et al. [58] who showed that among *Enterobacteriaceae* isolates, CTX-M genes were the least isolated. Extended spectrum beta-lactamase (ESBL) is a class of beta-lactamase enzyme responsible for the hydrolysis and inactivation of a wide range of beta-lactam antibiotics [59]. The emergence of the TEM gene could be due to selective pressure from the incorrect use of the cephalosporin class of antibiotics. The transfer of resistant genes by plasmids and extra chromosomal elements may also be responsible for the resistance of isolates who were previously susceptible to the beta-lactam antibiotics, according to Moosavian & Deiham [54].

5. Conclusion

In conclusion in spite of the benefits of using traditional medicine (e.g. herbal concoctions). The results of the present study reveal that the herbal concoctions were all contaminated by potentially pathogenic microbes to an unacceptable level, thereby making them un-safe for consumption. The herbal concoctions sold in Iwo town were, therefore, not sterile and could be a vehicle for transmission of pathogens to the end users. The ability of most of the isolated bacteria to resist more than one antibiotic which leads to multi-drug and multi- antibiotic resistance is of public health concern because the consumers of these concoctions run a risk of getting infected while trying to cure other illnesses, hence, most of the herbal concoctions used in this study cannot be recommended for use as drugs. It is therefore necessary to monitor and regulate the quality of herbal medicines sold in local communities continuously.

Compliance with ethical standards

Acknowledgments

We appreciate the technical assistance of Mrs. Atobatele, B. and Mrs. Akinola, O. who are both laboratory staff in the Dept. of Biological Sciences, Bowen University, Iwo, Osun State, Nigeria.

Disclosure of conflict of interest

The authors declare no conflict of interest.

Authors Contributions

We declare that this work was done by Ayansina, A.D.V. and Akinsola, O.A. and all liabilities pertaining to claims relating to the contents of this article will be borne by the authors. Ayansina, A.D.V. supervised the work and provided necessary literature while Akinsola, O.A. did most of the laboratory work.

References

- [1] Akerele O. Medicinal Plants and primary healthcare: Agenda for action. *Fitoterapia*. 1998, 59: 353-363.
- [2] World Health Organization. Quality Control Methods for Medicinal Plant Materials. Published by WHO, Geneva. 1998.
- [3] Sofowora A. Medicinal Plants and Traditional Medicine in Africa. Spectrum books limited, Ibadan. 2008; 84-85.
- [4] Lamikanra A. Essential Microbiology for Students and Practitioner of Pharmacy, Medicine and Microbiology. 2nd edition. Amkra Books, Lagos. 1994; 30.
- [5] Tapsell LC, Hemphill, I, Cobiac L, Patch CS, Sullivan DR, Fenech M, Roodnrys S, Keogh JB, Clifton PM, Williams PG, Fazio VA, Inge KE. Health benefits of herbs and species: The past, The present and The future. *Nat. Lib. Med.* 2006; 185 (4): 24-29.

- [6] World Health Organization (WHO). Guidelines for assessing quality of herbal medicines with reference to contaminants and residues. 2007.
- [7] Bauer R. Quality criteria and standardization of phytopharmaceuticals: Can acceptable drug standard be achieved? *Drugs Information Journal*. 1998; 32: 101-110.
- [8] WHO. Guidelines for the Assessment of Herbal Medicines. WHO Technical Report Series, World Health Organization, Geneva. 1996; 863.
- [9] Oyetayo VO. Microbial load and antimicrobial property of two Nigerian herbal remedies. *African Journal of Traditional Complementary and Alternative Medicine*. 2008; 5: 74 – 76.
- [10] Girish D, Shridhar D. History of Medicine: Sushruta – the Clinician – Teacher par Excellence. National Informatics Centre. 2007.
- [11] 11. Mathur SC. Senior Scientist Indian Pharmacopoeia Commission, Ghaziabad Brief about the herbs and herbal products in Indian Pharmacopoeia, 2007, Herbal Pharmacopeia.
- [12] WHO Media Centre. Traditional Medicine. Fact sheet N°134. 2008.
- [13] Elvin-Lewis M. Safety issues associated with herbal ingredients. *Advances in food and Nutrition Research*, Elsevier. 2005; 50: 222-313.
- [14] World Health Organization WHO. Programmes and Projects: Traditional Medicine. 2011.
- [15] Lin J, Nishino K, Roberts MC, Tolmasky M, Amino RI, Zhang L. Mechanisms of antibiotic resistance. *Front. Microbiol*. 2015, 6: 34-38.
- [16] Davies J, Davies D. Origins and evolution of antibiotic resistance. *Microbiology and Molecular Reviews*. 2010; 74(3): 417-433.
- [17] Nosanchuk JD, Lin J, Hunter RP, Aminov RI. Low-dose antibiotics: current status and outlook for the future. *Front. Microbiol*. 2014; (4): 208-211.
- [18] CLSI- Clinical Laboratory Standards Institute. Performance Standards for Antimicrobial Disc Susceptibility Test: approved Standards- 13th ed., 2018, CLSI Standards M02. Wayne, PA: Clinical Laboratory Standard Institute.
- [19] Ghasemi Y, Talieh A, Mohammed K, Milad M. A simple multiplex PCR for assessing prevalence of extended spectrum β -lactamases producing *Klebsiella pneumoniae* in Intensive Care Unit of a referral hospital in Shiraz, Iran. *Asian Specific Journal of Tropical Medicine*. 2013; 6(9): 703-708.
- [20] APHA. Standard Methods for The Examination of Water and Wastewater, 18th Ed., 1992, p. 9-66. American Public Health Association, Washington D.C.
- [21] Pelczar MJ, Chan ECS. Laboratory Exercise in Microbiology. Bank Pot. Inc, New York, USA. 1977.
- [22] Jay JM. Modern Food Microbiology. Fifth Edition, 2012, Aspen Publishers, Inc, Maryland. 2012; 633.
- [23] Pitt JI, Samson RA, Frivad JC. List of accepted species and their telemorphs in the family Trichocomaceae. In: Integration of Modern Taxonomic Methods for Penicillin and Aspergillus. Samson R A, Pitt J I, eds. 2000, Amsterdam: Harwood Academic Publishers. 2000; 9-47.
- [24] Adeleye IA, Okoji G, Ojo EO. Microbial Contamination of Herbal Preparations in Lagos, Nigeria. *Journal of Health Population Nutrition*. 2005; 23: 296-297.
- [25] Okunlola A, Adewoyin BA, Odeku OA. Evaluation of pharmaceutical and microbial qualities of some herbal medicinal products in South Western Nigeria. *Tropical Journal of Pharmaceutical Research*. 2007; 6: 661–670.
- [26] Elshafie A F, Al-Rashdi TA, Al-Bahry SN, Bakheil CS. Fungi and aflatoxins associated with spices in Sultanatic of Oman. *Mycopathologia*. 2002; 155: 155 – 160.
- [27] Mandeel QA. Fungal contamination of some imported spices. *Mycopathologia*. 2005;159: 291-298.
- [28] Domsch KH, Gams W, Anderson TH. Compendium of Soil Fungi, 1 & 2. Academic Press, London. 1998.
- [29] Bugno A, Almodovar AAB, Pereira TC, Pinto TD, Sabino M. Occurrences of toxigenic fungi in herbal drugs. *Brazilian Journal of Microbiology*. 2006; 37: 47-51.
- [30] Akerele O. Summary of World Health Organization guidelines for the assessment of herbal medicine. *Herbal Gram*. 1993; 28: 13-19.

- [31] Morse LJ, Williams HI, Grenn FP, Eldrige EF, Rotta JR. *N. Engl. J. Med.* 1976; 277: 472 - 473.
- [32] Ringertz O, Ringertz SH. The clinical significance of microbial contamination in pharmaceutical products. *Adv. in Pharm. Sci.* 1982; 5: 201 – 225.
- [33] De Freitas MG, Bauab TM. Microbial quality of medicinal plant materials. 2012.
- [34] Kolajo TT. The Study of ecology and microbial flora of some locally prepared herbal medicines in Abeokuta. Unpublished National Diploma Project. Moshood Abiola Polytechnic, Abeokuta, Nigeria. 2000.
- [35] Mukhtar MD, Shamsuddeen U, Rakiya A. A survey on the microbiological quality of traditional medicine vended in Kano state, Nigeria. *Journal of Medicinal and Pharmaceutical Sci.* 2008; 4(1): 30-35.
- [36] Esimone CO, Chah KF, Ikejide SC. Microbiological quality of herbal preparations marketed in South east Nigeria. *Journal of Natural Remedies.* 2001; 2(1): 42-48.
- [37] Salyers AA, Whitt DD. *Bacterial Pathogenesis: A Molecular Approach.* 1984, ASM Press, Washington DC. 1984.
- [38] Back E, Molby R, Kaijer B. Enterotoxigenic *Escherichia coli* and other Gram-negative bacteria if infantile diarrhea surface antigens, haemagglutinins, colonization factor antigens and loss of enterogenicity. *Journal of Infection and Diseases.* 1980; 142: 318-327.
- [39] Jiva SFH, Krovacek K, Wadstom T. Enterotoxigenic bacteria in food and water from an Ethiopian community. *Applied and Environmental Microbiology.* 1998; 41: 1010-1019.
- [40] Thom AR, Cole AP, Watrasiewicz K. *Pseudomonas aeruginosa* infection in a neonatal nursery, possibly transmitted by a breast-milk pump. *Lancet.* 1970; 1: 560-561.
- [41] Klipstein FA, Engert RA. Partial purification of *Enterobacter cloacae* heat-stable enterotoxin. *Infection and Immunity.* 1976; 13: 1307-1314.
- [42] Klipstein FA, Engert RA, Short HB. Relative enterotoxigenicity of coliform bacteria. *Journal of Infection and Diseases.* 1977; 136: 205-215.
- [43] Sakazaki R. *Citrobacter.* In: *Bergey's Manual of Systematic Bacteriology.* Vol. 1. Krieg NR and Holt JG (Eds.). Williams and Wilkins, Baltimore and London. 1984.
- [44] Guarino A, Capano G, Malamisura B. Production of *Escherichia coli* STa-like heat stable enterotoxin by *Citrobacter freundii* isolated from humans. *Journal of Clinical Microbiology.* 1987; 25: 110-114.
- [45] Back E, Molby R, Kaijser B. Enterotoxigenic *Escherichia coli* and other Gram-negative bacteria of infantile diarrhoea surface antigens, haemagglutinins, colonization factor antigen and loss of enteroxigenicity. *Journal of Infection and Diseases.* 1980; 142: 318-327.
- [46] Shamsuddeen U, Mukhtar MD, Rakiya A. A survey on the microbiological quality of traditional Medicine herbs concoctions vended in Kano, Nigeria. *Journal of Medicinal and Pharmaceutical Sciences.* 2008; 4 (1): 34-38.
- [47] Odedara OO, Memuletiwon EJ. Microbial Quality of Some Locally Consumed Herbal Concoctions in Abeokuta Metropolis, Nigeria. *Journal of Natural Science, Engineering and Technology.* 2014; 13: 58-66.
- [48] Anyanwu CU. Fungal contaminants of powdered herbal drugs sold in parts of Enugu State, South-east, Nigeria. *Plant Product Research Journal.* 2010; 14: 46-50.
- [49] Frazier WF, Westhoff PC. *Food Microbiology,* 3rd Edition, 1978, Tata McGraw Hill Publishing Co-Limited, New Delhi, India. 1978; 456: 17 – 64.
- [50] Braide W, Sokari TG, Nwaoguikie RN, Okorondu SI. Microbes from Soil Associated with Metamorphosing Moth Larvae. *Current Trends in Microbiology.* 2008;4: 11 – 14.
- [51] Ehlers M, Veldsman C, Makgotlho E, Dove M, Hoosen A, Kock M. Detection of bla-SHV, bla-TEM and bla-CTX-M antibiotic resistance genes in randomly selected bacterial pathogens from the Steve Biko Academic Hospital. *FEMS Immunol. Med. Microbiol.* 2009; (56):191-196.
- [52] Samie A, Mashao M, Bessong P, Momba M, Obi C. Diversity and antibiograms of bacterial organisms isolated from samples of household drinking water consumed by HIV positive individuals in rural settings, South Africa. *J. Health Popul. Nutr.* 2012; 30(3): 241-249.
- [53] Ben-Ami R, Rodriguez-Bano J, Arslan H, Pitout J, Quentin C, Calbo E, Azap O, Arpin C, Pascual A, Livermore D, Garcu J, Calbo Y. A multi-national survey of risk factors for infection with extended-spectrum beta-lactamase producing *Enterobacteriaceae* in non-hospitalized patients. *Clin. Infect. Dis.* 2009; 49: 682-690.

- [54] Moosavian M, Deiham B. Distribution of TEM, SHV and CTX-M genes among ESBL-producing Enterobacteriaceae isolates in Iran. *Afr. J. Microbiol. Res.* 2012; 6(26): 433-5439.
- [55] Dou Y, Huan J, Guo F, Zhou Z, Shi Y. Pseudomonas aeruginosa prevalence, antibiotic resistance and antimicrobial use in Chinese burn wards from 2007 to 2014. *Journal of International Medical Research.* 2017; 45(3): 1124-1137.
- [56] Ee R, Madhaiyan M, Ji L, Li Y, Nor N, Tee K, Chen J, Yin W. Multi-resistant genes and N-acyl-homoserine-lactone-producing bacterium in the family Enterobacteriaceae isolated from landfill site soil. *Int. J. Syst. Evol. Microbiol.* 2016; 66: 297-304.
- [57] Ahmed D, Saman Z, Hira B. *In Vitro* Analysis of Antioxidant Activities of Oxalis Corniculata Linn. Fractions in Various Solvents. *Afr. J. Tradit. Complement Altern. Med.* 2013; 10 (1): 158-165.
- [58] Champs C, Sirol D, Chanal C, Bonnet R, Sirotet J. A 1998 study of ESBL in Enterobacteriaceae in France. *Antimicrob. Agents Chemother.* 2000; 44(11): 3177-3179.
- [59] Sheikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA. Antibiotic Resistance and Extended spectrum β -lactamases: Types, Epidemiology and Treatment. *Saudi J. Biol. Sci.* 2015; 22 (1): 90-101.
- [60] Tapsell LC, Hemphill I, Cobiac L, Patch CS, Sullivan DR, Fenech M, Roodenrys S, Keogh JB, Clifton PM, Williams PG, Fazio VA, Inge KE. Health benefits of herbs and spices: the past, the present, the future. 2006; 185(4): 24.