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Bacteriological Quality of Some Local Herbal Drugs Sold In Port Harcourt, Nigeria and Antibacterial Susceptibility of Isolates Compared with Clinical Isolates

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Abstract

The world is witnessing an unprecedented rise in the use of herbal medicines. In Nigeria some individuals solely believe in the curative ability of local herbal drugs for the treatment of different forms of ailment. Most local herbal drugs are advertized to cure more than an aliment such as infections and hepatitis, diabetes and gastroenteritis, malaria, general body pain and weakness etc. The preparation and packaging of these local herbal medications are not carried out under appropriate hygienic conditions and they could be prone to contamination by potential pathogenic bacteria.

Aim: The aim of this study was to determine the bacteriological quality of some local herbal drugs marketed in some parts of Port Harcourt Rivers State Nigeria and their antimicrobial activity compared to some orthodox antimicrobial agents.

Materials and Methods: Ten (10) different local herbal drugs were purchased from hawkers, examined for bacteriological quality and antibacterial activities. Each of the sample for bacteriological examination were diluted serially in sterile peptone water by pipeting 1mL of the neat to 9ml of sterile peptone water in a test tube and subsequently diluted to 10^5 and 0.01mL of the last dilution 10^5 were cultured on Nutrient and MacConkey agar plates. Susceptibility testing were carried out with the isolated bacteria. The susceptibility was compared with that of already identified clinical isolates of *E. coli* and *S. aureus*.

Results: The results obtained showed *E. coli* (69.9%), *S. aureus* (18.9%), *Klebsiella sp.* (3.7%) and *Bacillus sp.* (7.5%) respectively. Antimicrobial susceptibility testing of the isolated bacteria from the local herbal drugs were 100% resistant to the orthodox drugs tested against them, whereas the clinical isolates tested were 50% susceptible to the orthodox drugs. The bacteria isolated from herbal drugs and the isolates from clinical specimens were 100% resistant to the local herbal drugs.

Conclusion: The bacteriological qualities of the local herbal drugs marketed in some parts of Port Harcourt were poor due to improper hygiene during processing and packaging. Bacterial isolates from the local herbal drugs were resistant to

the local herbal drugs and the orthodox drugs. Contaminated local herbal drugs might serve as means of transmitting infections and medium for exchanging resistant genes by the contaminating bacteria. The antibacterial property of herbal drugs may have been altered by the activities of bacteria

Keywords: Antibacterial; Bacterial Quality; Herbal Drugs; Marketed

Introduction

Herbal medicines also known as botanical medicine or phytomedicines are herbal materials, herbal preparations and finished herbal products that contain parts of plant materials as active ingredients [1]. The plant material includes seeds, berries, roots, leaves, barks or flowers [2]. The use of herbal medicine had been part of human culture because some possess important therapeutic properties for human cure and even animal diseases [3]. Plants have been shown to posses healing potentials. The use of plant, plant extracts or chemical derived from plants to treat diseases and other therapeutic modalities is not disputable [4]. More than 75 pure compounds derived from higher plants were in use in herbal medicine, although some have been synthesized in the laboratory [5]. For a plant or plant extract to be used as herbal drug it must contain therapeutic substances of health benefit and it was estimated that half of all synthetic drugs have a natural origin [6]. Many studies have shown the antimicrobial efficiency and or properties of herbal medications [7]. Some microbes may develop resistance due to superbug that renders antimicrobial therapy infective and this may lead to persistence of infection and the risk of spreading the resistant gene to other microbes [8].

The rapid and sudden increase in microbial resistance globally is a threat to human life and has dammed the effective treatment of infectious diseases [9]. Factors that have enhance the spread of antimicrobial resistant microbes were, over use of antimicrobial agent, failure of pharmaceutical companies formulating new antimicrobial agents and the use of sub-inhibitory concentration of prescribed antimicrobial agents. People have turned away from conventional medicines, with the faith that natural substances such as herbs are safer than synthetic substances. Herbal drugs are usually not evaluated for purity and consistency of the active compounds and they sometimes contain contaminants [10]. Inappropriate uses of antimicrobial agents have contributed to resistance of microbes to antimicrobial agents.

A major problem of local herbal drugs is the contamination with potential pathogenic bacteria. The first case of drug induced infection was when bubonic plaque vaccine was contaminated with bacilli in 1907. Another was *Salmonella* infection associated with tyroidine tablets and pacreatine powder, *Pseudomonas capacia* present in iodated provdone and oncular infection caused by *Pseudomonas aeruginosa* in hydrocortisone ointment [11-13]. In Kenya the bacteria isolated from unregulated medicinal products were, *Escherichia coli, Klebsiella pneumonia, Enterobacter aerogenes, Staphylococcus aureus, Salmonella* sp. and *Shigella* sp. [14]. Examining the quality of herbal drugs in Thailand, yeasts and molds were found in 10 samples, *E. coli* in 11 samples, *S. aureus* in 45 samples, *Salmonella* in 12 samples and *Clostridium* in 18 samples [15]. In assessments of the microbiological quality of some marketed herbal drugs from public market in the city of Trichy India the marketed herbal drugs had total aerobic counts above WHO bacteriological limits. The bacteria isolated were, *S. aureus, E. coli, Pseudomonas aeruginosa*, *Shegella* sp. and *Salmonella* sp. [16]. The aim of this research was to determine the bacteriological quality of some local herbal drug marketed in Port Harcourt, Nigeria and the antimicrobial susceptibility of isolated bacteria to the herbal drugs, as compared to conventional antimicrobial agents and isolates form clinical sources.

Materials and Methods

- **Collection of Samples:** Ten different local herbal drugs commonly marketed were purchased from the local herbal drug hawkers in Diobu area of Port Harcourt, Rivers State. The herbal drugs were taken immediately to the Microbiology Laboratory Department of Medical Laboratory Science, Rivers State University Port Harcourt for examination.
- **Preparation of Media:** Nutrient, MacConkey and Muller Honton agar were reconstituted and sterilized according to the manufacturer's instructions at 121^0C for 15 minutes and stored in the refrigerator for subsequent uses.

Determination of the Microbial Quality of Herbal Drugs

Each of the herbal drugs were mixed by inverting the bottles gently several times. Then 1ml of the local herbal drug was pipetted and added to 9ml of sterile peptone water. Subsequent dilutions were made by adding 1mLof the previous dilution to 9 mL of sterile peptone water in tests tube up to 10^3 and 0.1ml of the last dilution (10^3) was placed on already prepared dried agar plates in duplicate. This was spread evenly with the aid of a sterile glass rod (sterilized by dipping in absolute alcohol and in Bunsen flame). The plates were allowed to dry and incubated in incubator at 37°C for 18-24 hours and examined for growth.

Antimicrobial Susceptibility Testing using known Isolates of *E. coli* and *S. aureus*

Agar well Diffusion Test: Two (2) plates of Muller Horton agar were inoculated with 24 hours culture of *E. coli* and *S. aureus* respectively. Well were made on the agar and 50µl of each local herbal drug were added to each well. The plates were incubated at 37°C for 18-24 hours. The antibacterial activities of the herbal drugs were taken by measuring the diameter of the zone of inhibition around the well [17].

Disk Diffusion Method: Two (2) plates each of Muller Honton were seeded with 24 hour cultures of *E. coli* and *S. aureus*. Antimicrobial disks (commercial) were placed on the surface of the inoculated agar plates and incubated for 18-24 hours at 37°C. The zones of inhibition were read by measuring the diameter of the zone of inhibition formed around the disk [17].

Results

Classification of Herbal Drugs

The classification of the local herbal drugs examined, their compositions, colour, dosage and solvents used for extraction of active ingredients were as shown in table 1 below.

Code	Local Name and Cure	Composition	Colour	Dosage: A glass of 100ml per day	Solvent
A	Atosiatogedigedi (stooling/vomiting)	Root/leaves	Brown	One	Water
B	Atosi-atarayunyun (infection/rashes)	Root/leaves	Dark brown	One	Water
C	Atosi akopenyl (infection/itching)	Root/leaves	Green	One	Water
D	Atosi (general infection)	Root	Brown	One	Water
E	Ibatara bigbonor (typhoid)	Root	Brown	One	Water
F	Atosi akokpeyinyien (infection, lower abdominal pain)	Root/leaves	Dark brown	One	Alcohol
G	Titabandum-norni (diabetes)	Root	Brown	One	Water
H	Arariro-cheyindundan (waste pain)	Root/leaves	Black	One	Water
I	Ibara-bigbonorwo (typhoid/malaria)	Root/leaves	Black	One	Water
J	Ararinor (general body pain)	Root	Orange	One	Water

Table 1: List of Local Herbal Drugs Examined, Composition and Dosage.

Percentage Occurrences of Bacterial Isolated from Herbal Drugs

The percentage occurrences of bacteria isolated from the herbal drugs examined were A – *E. coli* 300(81.1%), *Klebsiella sp.* 50(14%), *Staphylococcus sp.* 20(5%); B – *E. coli* 310(86%), *Klebsiella sp.* 50(14%); C – *E. coli* 200(82%), *Klebsiella sp.* 5(2%), *S. aureus* 40(16%); D – *E. coli* 36(69%), *S. aureua* 16(31%); E – *E. coli* 200(84%), *Klebsiella sp.* 17(7%) and *S. aureus* 20(8%); F – *E. coli* 150(69%), *S. aureus* 75(33%); G – *E. coli* 150(70%), *Klebsiella sp.* 17(10%); H – *E. coli* 100(30%), *Staphylococci sp.* 160(48%), *Bacillus sp.* 72(22%); I - *E. coli* 37(29%), *Klebsiella sp.* 10(8%), *Bacillus sp.* 80(63%); J – *E. coli* 60(68%), *S. aureus* 18(20%) and *Bacillus sp.* 10(11%) respectively.

Herbal drug code	*E. coli*	*S. aureus*	*Klebsiella sp.*	*Bacillus sp.*	Total (cfu/ml)/%
A	300 (81)	20 (5)	50 (14)	NIL	370 (16.7)
B	310 (86)	50 (14)	NIL	NIL	360 (16.3)
C	200 (82)	40 (16)	5 (2)	NIL	245 (11.1)
D	36 (69)	16 (31)	NIL	NIL	52 (2.4)
E	200 (84)	20 (8)	17 (7)	NIL	237 (10.7)
F	150 (67)	75 (33)	NIL	NIL	225 (10.2)
G	150 (90)	17 (10)	NIL	NIL	167 (7.6)
H	100 (30)	160 (48)	NIL	75 (22)	335 (15.2)
I	37 (29)	NIL	10 (8)	80 (63)	127 (5.7)
J	60 (68)	18 (20)	NIL	10 (11)	88 (3.9)
Total	**1543 (69.9)**	**416 (18.9)**	**82 (3.7)**	**165 (7.5)**	**2206**

Numbers in parenthesis = percentages

Table 2: Percentage Occurrences of Bacterial Isolated from Herbal Drugs.

Antibiotic Susceptibility of Some Isolated Bacteria from the Herbal Drugs to Conventional Antibiotics

The antibiotic susceptibility testing showed all the bacterial isolated from the herbal drugs were resistant to the conventional antibiotics tested against them.

	SS	SXT	PEF	CN	APX	Z	AM	CPX	Resistance
E. coli	R	R	R	R	R	R	R	R	100
S. aureus	R	R	R	R	R	R	R	R	100
Klebsiella sp.	R	R	R	R	R	R	R	R	100
Bacillus sp.	R	R	R	R	R	R	R	R	100
Percentages	100	100	100	100	100	100	100	100	

Table 3: Antibiotic Susceptibility of Isolated Bacteria from Herbal drugs to conventional antibiotics.

R = Resistance, S = sensitive, M = moderately sensitive.

SS = Streptomycin (10µg), SXT = Septrin (10µg), PEF = Perflaxacine (10µg), CN = Gentamycin (10µg), AM = Amoxicillin (10µg), Z = Zinnacef (20µg), APX = Ampiclox (30µg), CPX = Ciprofloxacin (10µg).

Antibiotic Susceptibility of Clinical Isolates to Conventional Antibiotics

Antibiotic sensitivity of *S. aureus* and *E. coli* isolated from clinical specimens showed that *S. aureus* was sensitive to streptomycin, ciprofloxacin, gentamicin, perfloxacin and septrin; while *E. coli* was sensitive to streptomycin and ciprofloxacin, perfloxacin and septrin.

Antibiotic sensitivity of standard clinical isolates to conventional drugs.

	SS	SXT	PEF	CN	APX	Z	CPX	AM
S. aureus	S	S	S	S	M	M	S	M
E. coli	S	S	S	M	R	M	S	M
Percentage Resistant	0(0.00)	0(0.00)	0(0.00)	1(50.0)	2(100)	2(100)	0(0.00)	2(100)

Table 4: Antibiotic sensitivity of standard clinical isolates to conventional drugs.

R = Resistant, S = sensitive, M = moderately sensitive.

SS = Streptomycin (10µg), SXT = Septrin (10µg), PEF = Perflaxacine (10µg), CN = Gentamycin (10µg), AM = Amoxicillin (10µg), Z = Zinnacef (20µg), APX = Ampiclox (30µg), CPX = Ciprofloxacin (10µg).

Susceptibility of Clinical Isolates to Herbal Drugs

The result showed that all the clinical isolates, *S. aureus* and *E. coli* were resistant to the local herbal drugs

claimed to possess antimicrobial activities as shown in table.

Herbal drugs	A	B	C	D	F	I
S. aureus	R	R	R	R	R	R
E. coli	R	R	R	R	R	R

Table 5: Susceptibility of clinical isolates to herbal drugs.

Discussion

The ten (10) local herbal drugs examined, 90% used water as solvent, while 10% used alcohol as solvent. Out of these, 60% of the herbal drugs were combination of root and leaves, while 40% were roots only. The herbalist might be acquainted with the trees from which they obtain the roots and leaves but it may be difficult to quantify the quantity or quantities of the active ingredient (phytochemical constituents) of the roots and leaves responsible for the medicinal values. The type of soil a plants is grown (rich or poor) might play a major role in determining the amount of the active ingredients accumulated by the plant (phytochemicals) and extracts from the tree by the solvent for therapeutic use.

The bacteria isolated from herbal drugs in this study were *E. coli* (69.9%), *Staphylococci* (18.9%), *Klebsiella sp.* (3.7%) and *Bacillus sp.* (7.5%) respectively. In a study on microbiological contamination of liquid herbal medicinal products the bacteria isolated were *Klebsiella pneumonia* 31(34%), *Enterobacter aerogenes* 26(29.2%), *Enterobacter cloacae* 17(19.1%), *E. coli* 11(12.4%) and some unidentified GMB 4(4.5%). The contaminations were attributed to low education, lack of formal training in the process and poor hygienic level in processing and packaging [9]. Previous report had also shown that *Salmonella sp., E. coli, S. aureus, Shigella sp.* and other Gram negative and Gram positive bacteria were associated with the contamination herbal drug [18]. The microbial contamination of herbal drugs might cause bio-deterioration and reduction and the efficacy of the herbal drugs. Bacteria might also produce toxin that may render herbal drugs unsafe for human consumption [19,20].

In another study on the microbial quality of herbal drugs, in India the bacteria isolated were *S. aureus, E. coli, Pseudomonas aeruginosa, shigella sp. Salmonella sp*. and *S. aureus* [16]. *S. aureus* was believed to contaminate during plant harvesting, manure and unhygienic handling by manufacturers and users [20]. In Kenya, the bacteria isolated were *E. coli, S. aureus, Salmonella* sp., *Klebsiella* sp., *Enterobacter* sp. and *Shigella* sp. respectively. The researchers noted that the herbal drugs have poor microbial quality and were contaminated with pathogenic microbes. They called for the need to regulate herbal drug production to enhance microbial quality and safety [14]. In Thailand workers investigating the microbial quality of herbal products isolated yeasts and molds in 10 samples, *E. coli* in 11 samples, *S. aureus* in 45 samples, *Salmonella* sp. in 12 samples and *Clostridium* sp. in 8 samples. It was concluded that contamination with enterobacteria might originate from faecal-oral route, *Clostridium* sp. from soil and intestinal tract of animals; most of the herbal drugs marketed do not conform to standards [15]. Herbal product in Nigeria requires urgent and serious focus in production processes to provide better quality for consumer health and to compete favourably in international market. Unsterile containers (recycled water bottles) and water used as solvents might aid contamination. Hawkers have a 100ml glass cup used to dispense for customers which may be a medium of transferring bacteria from one source to another. There is need for global harmonization of WHO specific guidelines for the assessment of safety, efficacy, quality and purity throughout all phases of the cycle [21].

The bacteria isolated from the examined local herbal drugs were resistant to all the conventional antibiotics tested against them but the clinical isolates were susceptible to about 50% of the antibiotics. The activities of contaminating bacteria may have been associated with the bio-deterioration and reduced potency of the herbal drugs. The bacteria may as well produce toxin which may results to it's flabby for human consumption [19]. Multidrug resistant bacteria are responsible for serious infections in humans and the rate is on increase globally. Bacterial resistant to multiple antibiotics may use activation of efflux pump, ABC-transport genes or specific antibiotic activating genes. The genes responsible could be transferred from one bacterium to another in the liquid local herbal products.

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