Microbial Assessment of Herbal Products in Ota And Its Environs

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Abstract: This study was carried out to assess the microbial quality of herbal medicinal products being sold in **Ota** and its environs. A total of 24 bacterial isolates were identified from 32 herbal products purchased from 8 sellers in different parts of Ota. The total plate count (TPC) for bacteria in the herbal samples analysed ranged from 1.1 x 10 **to** 9.3 x 1OCFU/ml; while the total fungal count ranged from 1.2 x 10 to 9.8 x lOCFU/ml. The bacterial and fungal counts of the herbal products increased with the period of storage (24, 48, 96 hours). Bacteria! isolates obtained from this study belong to four genera; *Escherichia coli, Vibrio cholerae, Salmonella typhi and Staphylococcus aureus.* Sixty percent of the herbal products analysed were contaminated with *Escherichia coli,* 50% by *Vibrio cholerae,* 30% of *Staphylococcus aureus,* and 40% with *Salmonella typhi.* The antibiotic resistance and susceptibility patterns of the isolates showed 45% and 36% of *Escherichia coli* were resistant to ofloxacin and ciprofloxacin respectively; **all** the *Staphylococcus aureus* were susceptible to ofloxacin and ciprofloxacin; 80% of the *Vibrio cholerae* were resistant to ofloxacin; and 50% of the *Salmonella typhi* isolates were resistant to ofloxacin and ciprofloxacin. All the bacterial isolates recovered from herbal products in this study were resistant to cefuroxime, ampicillin, and ceftazidime. The result of this study revealed the need for adequate quality control measure to be put in place for herbal preparations used for commercial purpose in order to safeguard the health of the public.

Keywords: Microbial, herbal products, isolates, storage, Ota.

Introduction

In view of unabated increase in microbial resistance to available antibiotics, many people have found succour in herbal preparations as the solution to their various ailments. However, many of these herbal preparations are not adequately prepared in order to avoid microbial contamination.

Herbal medicine also called phytomedicine refers to using a plants seed, berries, roots, leaves, bark and flowers for medicinal purposes (Izzo and Ernst, 2009).They are crude preparations of various kinds of medicinal plants or any part such as: leaf, stem, root, flower, or seed, they are promoted as natural and safe and are therefore the preferred choice (Adewunmi and Ojewole, 2004). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento *et al.*, 2000).

In Nigeria, there appear to be an overwhelming increase in the public awareness and usage of herbal medicine products in the treatments and or prevention of diseases (Okunlola *et al.*, 2007). According to a world health organization (WHO) survey, about 70-80 percent of the world's populations, particularly in the developing countries, rely on non-conventional medicines, mainly from herbal sources, for their primary healthcare (Akerele, 1988).

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Herbal products are prone to microbial contamination if not properly produced due to their exposure to various environmental factors. The objectives of this study were to determine the microbial quality and safety of medicinal herbal products and to ascertain the antimicrobial susceptibility of the isolates.

Materials and Methods

Study Site

The study was carried out in Ota, the town is an urban setting and mostly inhabited by the Yoruba speaking indigenes and people from other parts of Nigeria and West Africa.

Sample Collection

The herbal drug samples were collected from herbal drug hawkers and sellers at Iyana-Iyesi, Sango and Ota. A total of 8 herbal sellers were selected for the study, two from Iyana-Iyesi (IB, IY) four from Ota (OR, OS, OJ, OT) and 2 from Sango (SA, IJ). The same herbal products were purchased from all sellers with the same method of extraction. The 32 herbal products were collected in a polythene bag and transferred to the Microbiology Laboratory of Bells University of Technology, Ota for microbiological analysis.

Determination of microbial loads of the herbal samples

One millimeter of each of the herbal samples was transferred into pre-labelled Petri dishes and then mixed with 20ml of sterile molten Nutrient agar and Potato Dextrose agar media which had been cooled to 45°C for bacteria and fungi respectively. The plates were allowed to set and incubated at 37°C for 24 hours and 28°C for 24-72 hours for bacterial and fungal counts respectively.

Isolation and identification of potential pathogens in the herbal samples

The pour plate method was used and the media used were MacConkey agar, Eosin Methylene Blue, Mannitol Salt agar, *Salmonella-Shigella* agar, and Thiosulphate Citrate Bile Salts Sucrose agar (TCBS) agar. Exactly 1ml of the herbal sample was transferred unto the sterile Petri dish and 20ml of these media which had been cooled to 45°C was poured and mixed thoroughly. The plates were incubated at 37°C for 24 hours.

Pure cultures were obtained from the plates and stored on agar slants and kept in the refrigerator at 4°C until used. Cellular characteristics of the isolates were observed using Gram staining procedure. Then, the following biochemical tests - Kligler iron agar test, citrate utilization test, sulphideindole motility test, coagulase, catalase and oxidase tests were conducted on the isolates.

Antibiotic susceptibility testing

The antibiotic susceptibility test was performed on Mueller Hinton agar (Oxoid) plate. The bacterial isolates from the samples were reactivated by subculturing from agar slant unto Nutrient agar plate and was incubated for 18-24hours.

A colony was picked and dispensed in McCartney bottle containing 9mls of distilled water which had been previously autoclaved at 121°C for 15minutes. The test organisms were dissolved in the distilled water till the density was equivalent to the turbidity of 0.5 Mcfarlandstandard.The standardized inocula were swabbed unto Mueller Hinton agar plate. The Multiple Antibiotic disc was used containing the following: Ceftazidime (30µg), Cefuroxime (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Ofloxacin (5µg), Augmentin (50µg), Nitrofurantoin (300µg), and Ampicillin (10µg).

The antibiotic disc were placed on the inoculated plates and pressed firmly onto the agar for complete contact using the forceps. The plate were inverted and left on the work table for diffusion of antibiotics into the agar for 30minutes and the plates were incubated at 37°C for 18-24 hours. The susceptibility of each isolate to the antibiotic disc was shown by a clear zone of inhibition and this was measured using a meter rule in millimetres. The diameter of the zone of inhibition was interpreted using standard chart.

Results

In this study, a total of 24 bacterial isolates were identified from different 32 herbal products purchased from 8 sellers in different parts of Ota. These bacterial isolates were identified on the basis of their morphological, cultural and biochemical characteristics on various selective media.

The total heterotrophic bacterial count of herbal samples ranges between 1.1 x 10 and 9.3 x 10 CFU/ml as shown in Table 1. Table 2 shows fungal population count of the herbal samples with the count ranges between 1.0 x 10and 9.8 x 10CFU/ml.

Figures 1 - 4 show the antibiotic resistance and susceptibility profiles of bacterial isolates recovered from herbal products. These include *Escherichia coli*, *Staphylococcus aureus*, *Vibriocholerae* and *Salmonella typhi*.

Table 1: Bacterial count of herbal samples (CFU/ml)

Herbal Sample	0 hours	48 hours	96 hours
IBA	4.0×10^2	1.0×10^2	4.2×10^2
IBB	NG	5.3 x 10	4.7×10^2
IBC	1.1×10^2	1.3×10^2	5.0×10^2
IBD	4.8 x 10	1.7×10^2	5.2×10^2
OSA	2.3×10^2	9.3 x 10	4.2×10^2
OSB	NG	2.1×10^2	5.0×10^2
OSC	2.7×10^2	4.9×10^2	5.0×10^2
OSD	2.6×10^2	5.0×10^2	5.4×10^2
OJA	7.3 x 10	TNTC	TNTC
OJB	3.0×10^2	4.0×10^2	5.0×10^2
OJC	3.2×10^2	3.9×10^2	5.2×10^2
OJD	3.5×10^2	4.2×10^2	5.1×10^2
SAA	3.6 x 10	9.3 x 10	4.1×10^2
SAB	2.0 x 10	4.2×10^2	5.3×10^2
SAC	1.0×10^2	1.8×10^2	5.2×10^2
SAD	2.5×10^2	2.9×10^2	5.0×10^2
OTA	1.6×10^2	3.6×10^2	5.0×10^2
OTB	3.0 x 10	3.8×10^2	4.1×10^2
OTC	4.2 x 10	6.3 x 10	1.8×10^2
OTD	1.3×10^2	1.9×10^2	3.2×10^2
IJA	1.6 x 10	1.6×10^2	4.7×10^2

IJB	2.0 x 10	3.2 x 10	5.0×10^2
IJC	1.1 x 10	5.5 x 10	4.1×10^2
IJD	4.0 x 10	4.8 x 10	3.1×10^2
ORA	1.9 x 10	7.5 x 10	1.0×10^2
ORB	4.1×10^2	5.8×10^2	5.9×10^2
ORC	3.0×10^2	4.0×10^2	4.2×10^2
ORD	1.9×10^2	2.3×10^2	2.3×10^2
IYA	1.3×10^2	4.0×10^2	4.1×10^2
IYB	1.9×10^2	4.3×10^2	4.5×10^2
IYC	3.1×10^2	4.5×10^2	4.9×10^2
IYD	1.0×10^2	2.9×10^2	3.3×10^2

KEY: NG- No Growth; TNTC- Too Numerous To Count

Table 2: Fungal population count of the herbal samples (CFU/ml)

Herbal Sample	0 hour	48 hours	96 hours
IBA	2.2×10^2	4.5×10^2	4.9×10^2
IBB	1.8×10^2	1.8×10^2	3.5×10^2
IBC	2.5×10^2	1.9×10^2	TNTC
IBD	3.0 x 10	7.3 x 10	TNTC
OSA	1.7×10^2	3.5×10^2	4.5×10^2
OSB	NG	8.3 x 10	1.0×10^2
OSC	1.5×10^2	1.6×10^2	1.7×10^2
OSD	4.7×10^2	NG	4.9×10^2
OJA	4.1×10^2	TNTC	TNTC
OJB	3.8×10^2	3.9×10^2	4.3×10^2
OJC	2.0×10^2	3.6×10^2	4.5×10^2
OJD	3.1×10^2	4.0×10^2	4.4×10^2
SAA	2.0 x 10	5.5 x 10	2.0×10^2
SAB	2.0 x 10	4.8×10^2	3.0×10^2
SAC	4.0×10^2	1.3×10^2	4.8×10^2
SAD	8.9 x 10	1.8×10^2	5.6×10^2
OTA	3.2×10^2	5.0×10^2	5.5×10^2
OTB	1.0 x 10	5.8 x 10	2.3×10^2
OTC	6.2 x 10	9.8 x 10	2.5×10^2
OTD	1.9×10^2	2.0×10^2	4.0×10^2
IJA	1.6×10^2	1.6×10^2	2.0×10^2
IJB	3.2 x 10	1.3×10^2	4.5×10^2
IJC	1.2 x 10	5.5 x 10	3.2×10^2
IJD	2.0×10^2	4.8 x 10	3.1×10^2
ORA	8.2 x 10	3.9×10^2	4.2×10^2
ORB	6.8 x 10	3.0×10^2	3.4×10^2
ORC	3.2×10^2	5.3×10^2	5.9×10^2
ORD	4.9×10^2	5.8×10^2	5.9×10^2
IYA	2.0 x 10	3.6×10^2	4.2×10^2
IYB	1.7 x 10	4.9 x 10	2.3×10^2
IYC	5.0 x 10	4.3 x 10	1.7×10^2
IYD	1.8 x 10	7.8 x 10	3.3×10^2

KEY: NG- No Growth; TNTC- Too Numerous To Count

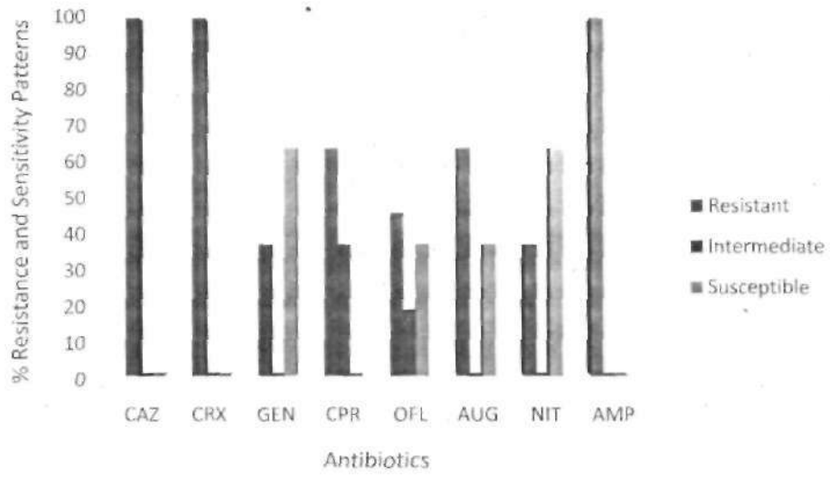

Figure 1: Percentage antibiotic resistance and susceptibility of *Escherichia coli*isolated from herbal samples

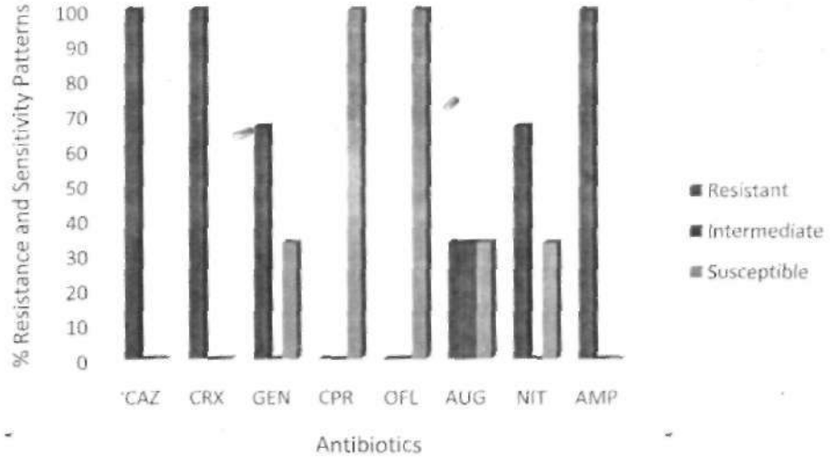

Figure 2: Percentage antibiotic resistance and sensitivity of *Staphylococcus aureus*isolated from herbal samples

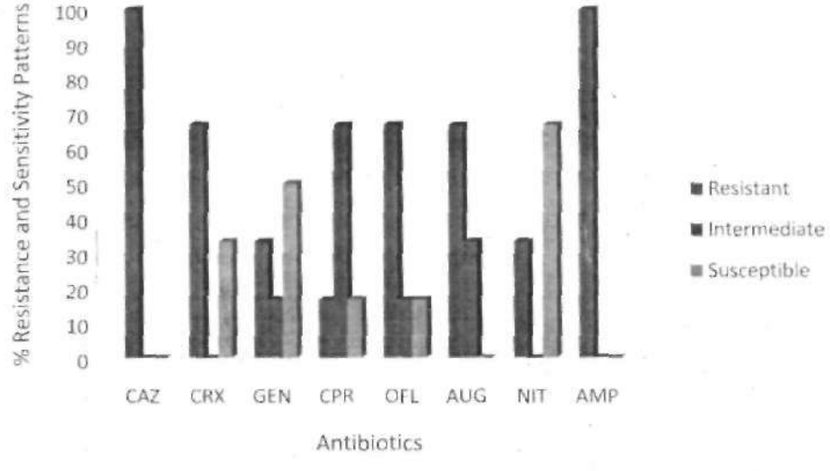

Figure 3: Percentage antibiotic resistance and sensitivity of *Vibrio cholerae* isolated from herbal samples

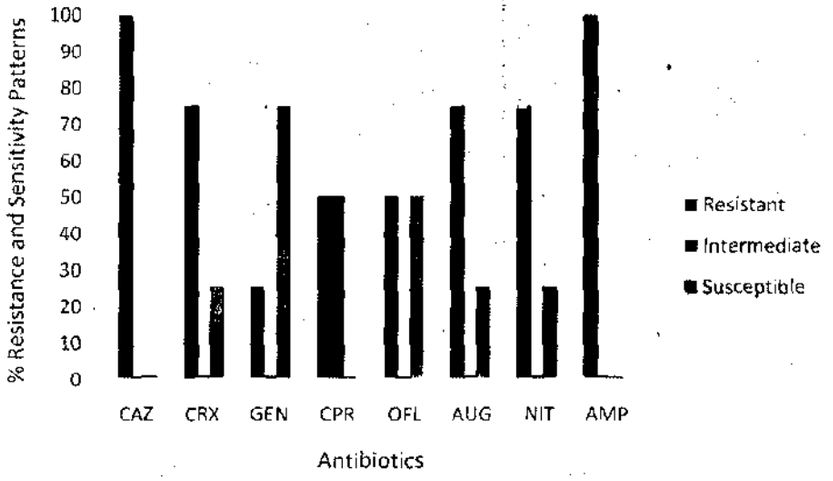

Figure 4: Percentage antibiotic resistance and sensitivity of *Salmonella typhi*isolated from herbal samples
Key:CAZ-Ceftazidime, CRX-Cefuroxime, GEN-Gentamicin, CPR-Ciprofloxacin, OFL-Ofloxacin, AUG-Augmentin, NIT-Nitrofurantoin and AMP-Ampicillin

Discussion

In this study the microbial quality of different herbal products being sold in the various markets in Ota was investigated. The total plate count (TPC) for bacteria in the herbal samples analysed ranges between 1.1 x 10 and 9.3 x 10 CFU/ml; while the total fungal count ranges between 1.0 x 10and 9.8 x 10 CFU/ml. Herbal medicines analysed in this study were oral remedies prepared using water. The limits for microbial contamination by the BP for aerobic microbial and fungi counts for herbal products to which boiling water is added before use are not more than 10^7 and 10^5 CFU/g respectively (Onyambu *et al.*, 2013) and also based on US Pharmacopoeia (USP 30), the total aerobic microbial count of herbal medicine must not be more than 10^5 CFU/g (Rajapandian *et al.*, 2013). Based on total viable count, the examined herbal products in this study were below the maximum permissible count.

From the result of this study, bacterial and fungal counts of the herbal products increased with the period of storage (24, 48, 96 hours). The presence of the following bacterial isolates were discovered in the herbal products purchased from the markets in Ota that is *Escherichia coli* which is an intestinal bacterium and an indication of faecal contamination, *Vibrio cholerae* which is a bacterium that causes cholera with presence of vomiting and stooling, *Staphylococcus aureus* which is associated with a number of complications especially to immune compromised individuals. It can produce proteins that disable the immune system and damage tissues in the body, it may also release exotoxins that causes gastroenteritis (Lowy, 1998), *Salmonellatyphi* which is a major problem globally and has been known to cause typhoid and food poisoning. *Salmonella* can infect plant cells and successfully evade all the defense mechanisms of the plants and multiply inside the cells of the plant thereby making washing not sufficient enough to remove the pathogen (WHO, 2003).

The high levels of microbial contamination observed in this study may be attributed to the methods of preparation. Sellers and hawkers could also introduce microorganism to the herbal product during handling and packaging of the products. Abba *et al.* (2009) also suggested that unhygienic equipment and materials could also be a source of contaminants.

The antibiotic resistance and susceptibility patterns of the isolates were also determined. 45% and 36% of the isolates of *Escherichia coli* were resistant to ofloxacin and ciprofloxacin respectively; all the *Staphylococcus aureus* identified were sensitive to ofloxacin and ciprofloxacin; 80% of the *Vibrio cholerae* isolates were resistant to ofloxacin; and 50% of the *Salmonella typhi* isolates identified were resistant to ofloxacin and ciprofloxacin.

All the bacterial isolates recovered from herbal products in this study were resistant to cefuroxime, ampicillin, and ceftazidime. The pathogens isolated from herbal products in this study is in agreement with previous studies by Varnam and Evans (1991), Rowe-Taitt*et al.* (2004) and Prescott *et al.* (2005).

In view of this multidrug resistance by these bacterial isolates most especially to ofloxacin and ciprofloxacin which are members of the quinolones that are effective against a wide range of organisms, the appropriate regulatory authority should take drastic action in controlling proliferation of "herbal poison" called herbal products in this area.

All the pathogens which were isolated from the marketed herbal product samples in this study have been implicated in previous studies on gastroenteritis and other transmissible diseases (Okeke and Nataro, 2001; CDC 2002; Ogunshe, 2004). Taking into consideration the above facts and increased use of herbal drugs in the society along with poor quality control measures taken by the manufactures and vendors leave a great question mark on the safety of consumers' health.

Conclusion

This study has shown that herbal medicinal products marketed in Ota are highly contaminated with microorganisms, some of which are pathogenic. These products can serve as a means of disseminating infections among the populace. In view of the findings of this study, adequate monitoring of the producers of these products should be adopted by various regulatory agencies such as NAFDAC. Any herbal product that failed to meet minimum microbiological standard should not be sold to the public.

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