Science World Journal Vol. 15(No 2) 2020
www.scienceworldjournal.org
ISSN 1597-6343
Published by Faculty of Science, Kaduna State University

Full Length Research Article

BACTERIOLOGICAL QUALITY OF SOME LIQUID HERBAL PREPARATIONS SOLD WITHIN JOS METROPOLIS, NIGERIA AND ANTIBIOTIC SUSCEPTIBILITY OF THE ISOLATES

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ABSTRACT
A total of 45 samples of herbal preparation consisting of 15 each of "Maganin Shawara," Maganin tsusan ciki and "Maganin Basir" claimed to cure typhoid fever, intestinal parasites and pile respectively were analyzed. The isolation, identification and antibiotic susceptibility pattern of bacterial contaminants of the herbal products were carried out using standard procedures. The study was aimed at determining the bacteriological quality of some liquid herbal preparations sold within Jos metropolis, Nigeria and the antibiotic susceptibility of the isolates. The results showed that out of the 45 liquid herbal samples analyzed, 21 (46.67%) were contaminated with *Esherichia coli,* 8 (17.78%) with *Staphylococcus aureus,* 6 (13.33%) with *Salmonella* sp, 7(15.56%) and 3(6.67%) were contaminated with *Bacillus* sp and *Proteus vulgaris* respectively. Antibiotic susceptibility tests showed that all the isolates have high level of resistance to the antibiotics used in this study. Findings of this study imply that the herbal preparations are of poor bacteriological quality and may be potential source for the dissemination of multi-drug resistant microorganisms.

Keywords: Bacteriological quality, Antibiotic susceptibility, Isolation, Identification, Bacterial contaminants, Liquid herbal preparations

INTRODUCTION

The leaves, flowers, stems, roots and other components derived from plants have been reported to be effective antibacterial agents. Traditional herbalists in Nigeria use herbal preparations to treat various types of ailments, including diarrhea, urinary tract infections (UTIs), typhoid fever and skin diseases (Sofowora, 1993). The World Health Organization (WHO, 1998) survey indicated that about 70 – 80% of the world's population particularly in developed countries rely on non-conventional medicines mainly of herbal origins for their primary health care. This is because herbal medicines are accessible and cheap (Sofowora, 1993). With the increased usage of herbal preparations in Nigeria, the safety, efficacy and quality of these medicines have been an important concern for health authorities and health professionals (Lau *et al.,* 2003; Adeleye, *et al.,* 2005).
Antibiotic resistant bacteria have been a source of an ever increasing therapeutic problem (Sheikh *et al.,* 2003). Drug resistant infectious microorganisms have become an important public health concern (NIAID, 2011). Aside from the public health threat drug resistant microorganisms pose, research into newer antibiotics to overcome resistant microbes is usually very expensive and contributes to the higher costs of health care (NIAID, 2011).
Resistant bacteria strains may develop almost anywhere particularly in a pressurized environment containing previously non-resistant bacteria strains as contaminants. One of such environments can be herbal medicinal products (HMPs). Herbal medicinal products have been previously implicated as a pool for such contaminations (Cotton *et al*., 1987; Sliman *et al*., 1987; Esimone *et al*., 2007). It is of utmost importance to monitor and ascertain the microbial purity of HMPs given the huge medical and economic implications of any such microbial contamination especially with multiple drug resistant strains. Such surveillance will help to identify microbial contamination of herbal products and slow down or prevent the emergence of drug-resistant strains (Odimegwu *et al.,* 2011).
This was study aimed at evaluating the potential hazards associated with the consumption of liquid herbal preparations and susceptibility profile of pathogenic bacteria isolated from such products sourced from traditional medicine sellers and hawkers within Jos and Bukuru metropolis.

MATERIALS AND METHODS

Sample collection

A total of forty-five (45) samples were purchased from traditional medicine sellers and hawkers within Jos and Bukuru metropolis. Fifteen (15) samples each of ''Maganin Basil'', ''Maganin Shawara'' and ''Maganin Susan Ciki''. All samples collected from the sites were analyzed in the laboratory of the Department of Microbiology, Faculty of Natural Sciences, University of Jos.

Aerobic plate counts of the herbal preparations

Each sample was serially diluted and aliquots of 0.1ml of the last two dilutions inoculated on Plate Count Agar (PCA) plates in duplicates. All the plates were incubated at 37^{o}C for 24 h. Colonies on the plates were counted and results expressed as Colony Forming Unit per mililitre (CFU/mL).

Coliform Count of the herbal preparations

The membrane filtration technique was used to determine the coliform counts. One hundred milliliters (100ml) of each sample was filtered through a membrane filter of pore size 0.45µm and

diameter 25mm aided by suction pressure. Each membrane filter was placed on Eosin Methylene Blue Agar (EMB) plates and incubated at 37°C for 24 h. Characteristics colonies of coliform bacteria were counted and results expressed as colony forming units for 100ml (CFU/100mL).

Isolation of Salmonella sp

The stock solutions of each sample were first subcultured in Tetrathionate broth for 18 hours before inoculation on Salmonella Shigella agar (SSA) plates followed by incubation of all the inoculated plates at 37°C for 24 h.

Identification of Isolates

All isolates on PCA, MSA, EMB and SSA plates were identified based on their Gram reactions and biochemical tests as described by Cheesbrough, 2002 and Goldman and Green, 2009.

Antibiotic Susceptibility Test

Susceptibility tests were performed based on disc diffusion method recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2012) using nutrient agar. Isolates were grown overnight on nutrient agar and suspended in sterile physiological saline to obtain turbidity equivalent of 0.5 McFarland standards. A sterile, non-toxic cotton swab was dipped into the standardized inoculum and used to spread the entire surface of Mueller Hinton agar plates (NCCLS, 2002). Antibiotic discs were placed aseptically on the surface of the agar plates and all plates were incubated at 37°C for 24hrs. The antibiotics screened include the following: cloxacillin (CXC), 5µg; tetracycline (TET), 30µg; erythromycin (ERY), 5µg; ciprofloxacin (CPX), 10µg; chloramphenicol (CHI), 10µg; gentamycin (GEN), 10µg; ampicillin (AMP), 10µg and amoxicillin (AMC), 20µg .

RESULTS

"Maganin Basir" had the highest mean bacterial load of 1.01 x 10^7 cfu/ml followed by "Maganin Shawara" with a mean bacterial load of 8.53 x 10^6 CFU/ml and ''Maganin Tsutsan Ciki" had the least mean bacterial load of 6.24 x 10^7 CFU/ml.

Table 2 shows the coliform counts, "Maganin Basir" had the highest mean coliform count of 247 CFU/100ml, followed by "Maganin Shawara" and "Maganin Tsutsan Ciki" with mean coliform counts of 238 CFU/100ml and 144 CFU/100ml respectively.

The bacteria species isolated from the liquid herbal preparations were *Esherichia coli, Staphylococcus aureus, Salmonella* sp, *Bacillus* sp and *Proteus* sp. Table 3 shows the frequency of occurrence of these isolates with *Escherichia coli* having the highest frequency of occurrence of 21 (52.26%), followed by *Staphylococcus aureus* with 8 (21.05%), *Salmonella sp* with 6 (15.79%) and *Proteus vulgaris* having the least frequency of occurrence of 3 (7.89%). "Maganin Basir" had the highest number of bacteria isolates of 21 (55.26%), followed by "Maganin Shawara" with total bacterial isolates of 12 (31.57%) and "Maganin Tsutsan Ciki" with the least total bacterial isolates of 5 (13.16%).

The antibiotic susceptibility pattern of the isolates is as shown in Table 4. *Escherichia coli* isolates have intermediate sensitivity to gentamicin and chloramphenicol but resistant to ampicillin, cloxacillin, erythromycin, tetracycline, amoxicillin and ciprofloxacin. *Staphylococcus aureus* isolates have intermediate sensitivity to only ciprofloxacin, but resistant to the remaining antibiotics. *Proteus vulgaris* show intermediate sensitivity to only ciprofloxacin and also resistant to the remaining antibiotics. *Salmonella sp* isolates have intermediate sensitivity to ciprofloxacin and amoxicillin but resistant to all the other antibiotics.

Table 1: Aerobic plate counts of the herbal preparations

Herbal preparation	No. of Samples Analysed	Range of bacterial count (CFU/mL)	Mean bacteria count (CFU/mL)
Maganin Shawara	15	$1.0x10^6$-$1.4x10^7$	$8.5 x 10^6$
Maganin susan ciki	15	$1.0x10^6$ – $9.6x10^6$	$6.2 x10^6$
Maganin Basir	15	$6.0x10^6$- $1.6x10^7$	$1.0 x 10^7$

Table 2: Coliform counts of the herbal preparations

Herbal preparation	No. of samples analysed	Mean coliform count/100ml
Maganin Shawara	15	238
Maganin susan ciki	15	144
Maganin Basir	15	247
Total	45	

Table 3: Frequency of Occurrence of the Bacterial Isolates in the Liquid Herbal Preparations

(%)

Samples	No. of samples analysed	*Escherichia coli*	*S. aureus*	*Salmonella* sp.	*Protues vulgaris*	Total
''Maganin shawara''	15	6 (40.00)	3 (20.00)	3 (20.00)	0 (0.00)	12(31.57)
''Maganin susan chiki''	15	3 (20.00)	0 (0.00)	1 (6.67)	1 (6.67)	5(13.16)
''Maganin basil''	15	12 (80.00)	5 (33.33)	2 (13.33)	2(13.33)	21(55.26)
Total	45	21 (55.26%)	8 (21.05)	6 (15.79)	3 (7.89)	38

Table 4: Antibiotic susceptibility of the organisms

ORGANISMS	RESISTIVITY	SENSITIVITY	ZONES OF INHIBITION
Esherichia coli	Ampicillin Cloxacillin Erythromycin Tetracycline	Gentamicin Streptomycin Chloramphenicol	18mm 17mm 10mm
Staphylococcus aureus	Erythromycin Ampicillin Amoxicillin Chloramphenicol Tetracycline Streptomycin	Ciprofloxacin Gentamycin	16mm 19mm
Proteus vulgaris	Erythromycin Tetracycline Ampicillin Amoxicillin Streptomycin Gentamycin	Ciprofloxacin Chloramphenicol	14mm 10mm
Salmonella sp	Ampicillin Tetracycline Erythromycin Streptomycin Gentamycin	Ciprofloxacin Chloramphenicol Amoxicillin	18mm 16mm 10mm

DISCUSSION

Findings from this work imply that all the herbal preparations are of poor bacteriological quality. According to the European pharmacopoeia (2007), no *Salmonella* spp *or Escherichia coli* strain should be present in oral medicines and total aerobic bacteria should be ≤10^5cfu/ml. National Agency for Food and Drugs Administration and Control (NAFDAC), Nigeria recommended the total absence of pathogenic bacteria from the herbal preparations (NAFDAC SOP, 2000).

The high coliform count is an indication of the use of contaminated water in the preparation of these herbal medicines (Oli *et al.,* 2013). The bacterial load of the herbal preparations agrees with the findings of Abba *et al.* (2009) and Okukpe *et al.* (2013) who also reported bacterial load and coliform counts that are above acceptable limits. *Escherichia coli* was the most predominant contaminant. The presence of *Escherichia coli* indicates feacal contamination. It is also an indication of poor hygiene practices and lack of adequate handling of the products (Oli *et al.,* 2013). The predominance of *Escherichia coli* may be because they are widely distributed in the soil, dust, air and because they are resistant to environmental destructive factors. Several other works reported the presence of pathogenic bacteria similar to the ones reported in this work (Lamikanra *et al*. 1992; Mendie *et al.* 1993;Erich *et al.,* 2001, Esimone *et al.,* 2007, Okunlola *et al.,* 2007, Abba *et al.,* 2009, Muhammad, 2011, Odimegwu *et al.,* 2011, Okukpe *et al.,* 2013, Oli *et al.,* 2013).

Most of the isolates are resident in the soil, water, air and vegetations, and their public health implications had been reported. *Staphylococcus aureus* produce potent enterotoxins associated with food borne intoxication, toxic shock syndrome and staphylococcal scalded skin syndrome. The presence of these contaminating microorganisms could constitute a source of infection and serious health risk to the consumers of the herbal preparations who were probably already overwhelmed by the medical conditions for which the herbal drugs were initially indicated (Mangram *et al.*, 1999; Bowler *et al*., 2001).

All the isolates exhibited a high degree of antibiotic resistance. *Staphylococcus aureus* and *Proteus vulgaris* were sensitive to only one (ciprofloxacin) of the eight antibiotics used, while *Escherichia coli* and *Salmonella* sp were sensitive to only two of the eight antibiotics. The high resistance displayed by *S. aureus* agrees with the findings of other researchers involving clinical strains of *S. aureus* who also reported multi-antibiotic resistance of this organism (Richard, 2007; Oyetayo, 2008). The high degree of antibiotic resistance observed in this study for all the isolates may be due to the transfer of drug resistance plasmids among the isolates. Intergeneric transfer of drug resistance among different genera with *Staphylococci spp* playing a prominent donor role has been reported (Odimegwu *et al.,* 2011). The high level of resistance to many antimicrobial agents shown by the two gram negative rods *Proteus vulgaris* and *Escherichia coli* may also be due to mutation in addition to plasmids acquisition (Esimone *et al.,* 2007).

Conclusion

The poor bacteriological quality of the herbal preparations implies that they may serve as vehicles for the transmission of pathogenic bacteria to their consumers. Findings of the study also imply that herbal preparations are a potential source of dissemination of multi-drug resistant microorganisms.

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