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Bacteriological Quality Of Some Liquid Herbal Preparations Sold Within Maiduguri, Borno State

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Abstract: ***Herbal medicines are commonly used for medicinal purposes in Nigeria. Despite their wide use, there is insufficient information on the safety of the plants used and the associated risk of microbial contamination associated with different liquid herbal medicines consumed at state levels in Nigeria. Thus, this study was based on six (6) most famously consumed liquid herbal mixtures in Maiduguri, Borno state. A total of 60 liquid herbal medicine samples were collected within the Metropolis in Maiduguri. Bacterial isolates were obtained by serial dilution and plating of the samples onto nutrient agar plates. The bacteria isolates were identified using a combination of gram staining and biochemical tests. A total of 56 isolates were screened and were identified as Staphylococcus aureus, Escherichia coli, Bacillus spp and Salmonella spp. These isolates were subjected to antibiotic susceptibility tests where some of these isolates were susceptible to antibiotics and some were resistant. The results suggest that the herbal medicine samples sold in Maiduguri, Nigeria, are contaminated with bacteria and could potentially pose a risk to human health. This study provides important information on the microbial quality of traditional medicine samples from Maiduguri, Nigeria, which may be useful in assessing the safety of these products.***

I. INTRODUCTION

Herbal medicines are one of the constantly consumed forms of medicines for the purposes of healing sicknesses and illnesses (Petrović *et al.*, 2022). These drugs have gained wide acceptance for use and is consumed by up to three quarter of people around the globe with a significant increase in this trend as the year goes(Bello and Isah, 2015). Herbal medicines which are also called traditional medicines or phytomedicines are drugs made from a mixture of herbal materials, solutions or mixtures of plant materials (Liu, 2011). The plant materials used are vegetative and reproductive parts of the plants such as the seeds, back, stem, roots or flowers (Omara *et al.*, 2020). There are different types of herbal medicines in Nigeria; some are common across the country while some are common to certain parts of the country and are prepared with different plant parts. Examples of herbal medicines include agbo Jedi, Maganin karfi, agbo igba etc. Although liquid herbal medicines are believed to be the best by consumers, herbal medicines may contain some toxic substances (Wanwimolruk and Prachayasittikul, 2014) and some contaminants (Chemat *et al.*, 2019) which can be a serious health concern. The presence of pathogenic microorganisms can contribute to the growing concern of antibiotic resistance. Antibiotic resistance is a continuous health and therapeutic challenge (Shaikh *et al.*, 2015). However, there are challenges of quality assurance of liquid herbal medicines due to inadequate quality control in processes of production like handling raw materials, production processes and exposure during consumption or day to day activities (de Freitas Araújo and Bauab, 2012; Ekor, 2014). This research was aimed at isolating bacterial

contaminants present in herbal medicines to determine their antibiotic resistance.

II. MATERIAL AND METHODS

STUDY AREA

This research work was conducted in Maiduguri Metropolitan Council Area of Borno State from February, 2022 to December, 2022 in Borno state, Nigeria. All laboratory experimentation was carried out at the Department of Microbiology, Faculty of Science, University of Maiduguri, Maiduguri Nigeria.

SAMPLE COLLECTION

A total of sixty samples were purchased from traditional medicine sellers within Borno Metropolis. Ten samples of each of the following herbal medicines were collected: Maganin Basir, Maganin Gonorrhea, Maganin Rana, Maganin Sanyi, Maganin shawara and ‘’Maganin karfin maza”. All samples were collected aseptically in sterilized bottles and immediately transported to the Department of Microbiology Laboratory for analysis.

Standard Plate Count: The conventional method of serial dilution was used to determine the standard plate count of each sample as described by Ma and Kim (2018). 0.1mL of the highest dilution was plated in triplicates on Plate Count Agar. All inoculated plates and control plate were incubated at 37^0C and was observed for growth after 24 hours. The colonies were counted and recorded as Colony Forming Units per Mililitre (CFU/ML). Different distinct colonies were picked, isolated and subcultured severally till pure cultures were obtained. Isolates were grown on MacConkey Agar, Plate Count Agar, Nutrient agar, Salmonella- Shigella Agar.

IDENTIFICATION OF ISOLATES

All isolates were identified morphologically by subjecting the isolates to Gram Staining and biochemical tests as described by Cheesbrough, 2002.

ANTIBIOTIC SUSCEPTIBILITY TEST

The standard disc diffusion method was used as described by NCCLS (2012). Identified isolates were freshly grown on nutrient agar overnight and was inoculated into a known volume of isotonic solution to be standardized to an equivalent of 0.5 McFarland standard. Different sterile cotton swab was dipped into the standardized isotonic solution for different isolates and was spread on the surface of Mueller Hinton Agar Plates. Sterile forceps were used to place sterile antibiotic discs aseptically on the surface of Mueller Hinton agar plates. All plates were incubated at 37^0C for 24 hours.

Antibiotic discs were placed aseptically on the surface of the agar plates and all plates were incubated at 37^0C for 24hrs.

Oxoid antibiotic discs set were used to determine the antibacterial susceptibility of bacteria isolates. The discs used include; Amoxicillin/Clavulanate (AMC) 30μg, Ofloxacin (OFL) 5µg, Gentamycin (GEN) 10µg, Amoxicillin (AMX) 25µg, Peflacin(PEF) 10μg, Ciproflox (CIP) 30µg, Streptomycin (S) 30μg, Ceftriaxone (CRO) 30μg, Cefuroxime (CXM) 30μg and Cefotaxime (CTX) 30μg, Erythromycin (ERY) 5μg, Cloxacillin (CXC) 5µg, Chloramphenicol (CH) 30μg and Streptomycin (S) 30μg. The Zone of inhibition was measured in millimeters using calibrated ruler. The diameter of each zone was measured to the nearest 0.1mm. This was done by viewing the plate illuminated from below against a dark background.

Determination of Multiple Antibiotic Resistance (MAR) Index

The Multiple antibiotic resistance index was calculated using the formula; MAR = a/(b.c). Where, (a) represents the aggregate resistance of antibiotics to all isolates, while (b) represents the total number of antibiotics and (c) stands for the number of isolates from the specimen site (Krumperman, 1983; Sandhu *et al.*, 2016)

III. RESULTS

Herbal preparation	Number of factors	Number of Colonies	Mean bacterial count Cfu/ml
Maganin Shawara	10^4	120	3.6×10^7
	10^5	60	
Maganin Gonorrhea	10^4	253	1.4×10^8
	10^5	112	
Maganin Karfin Maza	10^4	112	3.7×10^7
	10^5	63	
Maganin Sanyi	10^4	130	4.2×10^7
	10^5	70	
Maganin Basir	10^5	65	4.0×10^7
	10^4	149	
Maganin Rana	10^4	176	5.5×10^7
	10^5	91	

Table 4.1: Standard Plate Count of the herbal preparation

Herbal Medicine	Number of Samples	Mean Coliform Count
Maganin Shawara	10	190
Maganin Gonorrhea	10	250
Maganin Karfin Maza	10	213
Maganin Sanyi	10	124
Maganin Basir	10	119
Maganin Rana	10	170

Table 4.2: Coliform counts of the herbal preparations

Sample	Number of screened samples	*Escherichia Coli*	*Bacillus Species*	*Staphylococcus aureus*	*Salmonella Spp*	Total Isolates
Maganin Shawara	10	5	3	1	3	12
Maganin Gonorrhea	10	5	3	1	5	14
Maganin Karfin maza	10	2	0	6	3	11
Maganin	10	4	0	2	2	8

Sanyi						
Maganin Basir	10	1	3	1	1	5
Maganin Rana	10	1	2	2	1	6
Total	**60**	**18**	**10**	**13**	**15**	**56**

Table 4.3: Frequency of Occurrence of the Bacterial Isolates in the Liquid Herbal Preparation

Antibiotic Susceptibility of Isolates

Isolates	Resistivity	Sensitivity	Zone of Inhibition
Escherichia coli	Ampicilin	Streptomycin Pefloxacin Gentamycin Erythromycin Zithromax Rifampicin Sulfamethazole	14mm 23mm 20mm 14mm 14mm 20mm
Staphylococcus aureus	Sulfamethazole	Ampicilin Ciprofloxacin Pefloxacin Gentamycin Erythromycin Zithromax Amoxicilin Sulfamethazole Streptomycin	24mm 21mm 23mm 18mm 24mm 22mm 17mm 12mm 22mm
Bacillus species	Ciprofloxacin Streptomycin Pefloxacin Sulfamethazole	Ampicilin Zithromax Amoxicilin Rifampicin Erythromycin Gentamycin	18mm 15mm 24mm 20mm 24mm
Salmonella species	Ampicilin Zithromax Amoxicilin Sulfamethazole Erythromycin Gentamycin	Rifampicin Ciprofloxacin Streptomycin Pefloxacin	22mm 24mm 24mm 17mm

RESULTS

Table 4.1 shows the liquid herbal medicine with the highest mean bacterial load is Maganin Gonorrhea and was recorded to have 1.4×10^{8} CFU/mL while the least was Maganin Shawara with 3.6×10^{7} CFU/mL

Maganin Gonorrhea still had the highest amount of mean coliform count of 250 while the least was Maganin Basir with mean coliform count of 119 as seen on Table 4.2

As seen in table 4.3, a total of 56 bacterial isolates were identified from the total samples used for this research. Maganin Gonorrhea had the highest total number of bacterial isolates (14 isolates) while maganin basir had the least total number of bacterial isolates (5 isolates) closely followed by maganin rana (6 isolates). *Escherichia coli* had the highest number of total isolates from the total sample (18 isolates) while *Bacillus* species had the least total number of isolates (10 isolates). However, *Bacillus* species were not isolated from maganin sanyi and maganin karfin maza.

Out of the identified isolates isolated from the samples, *salmonella species* were resistant to six antibiotics. However, *Escherichia coli* was resistant to only ampicillin and *Staphylococcus aureus* was resistant to only sulfamethazole.

IV. DISCUSSION

The outcome of this research shows that the bacteriological quality of liquid herbal mixtures sold in Maiduguri, Borno State is low and does not meet the standard set by the National Agency for Food and Drugs Administration and Control (NAFDAC), Nigeria which states that there should be complete absence of pathogenic bacteria from herbal preparations made for consumption (NAFDAC SOP, 2000). The presence of pathogenic bacteria in these liquid herbal medicines implies that the water supply, poor hygiene, poorly washed raw materials may have been major sources of contamination during the process of production or packaging as discussed by Mahboob et al., (2004). The bacterial isolates found in these herbal mixtures pose a health risk to the consumers of these mixtures and as such is a serious health concern to avoid outbreaks of different diseases. There is currently a lot of research going on antibiotic resistance

Similar research in Jos Metropolis Nigeria has shown high bacterial contamination in three liquid herbal medicines (maganin shawara, maganin basir, and maganin susan ciki). These medicines had bacterial contaminants such as *Escherichia coli, Salmonella species, Staphylococcus aureus* and *Proteus Vulgaris* (Dashen et al., 2020).

Although herbal medicines are still being used without control in some environments, it is very important to monitor the consumptions and preparations of this herbal medicines to reduce a possible deterioration in the health of the population and also, to avoid an outbreak.

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