Microbiological Quality Evaluation of Typhoid and Diarrheal Herbal Formulations Sold in Yola and Environs

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Abstract

The study was aimed at evaluating the microbiological quality of herbal formulations sold in Yola and environs. Herbal formulations were collected from three different area namely; Yola, Jimeta and Girei local government areas. The assessment of the microbiological quality of the herbal formulations was carried out using standard procedures. For antimicrobial susceptibility testing, minimum inhibitory concentration (MIC) and the minimum bactericidal concentrations (MBC) were determined. The herbal formulations have an inhibitory effect on the test isolate at 12.5mg/mL to 25mg/mL. However, when the concentration of the herbal formulations was increase to 50mg/mL it inhibited all the test organisms. Typhoid formulations collected from Girei have the highest viable count with 1.85×10^6 CFU/mL and the count is lowest in samples collected from Yola with 3.1×10^5 CFU/mL. Viable counts were also found to be highest in diarrhea formulations collected from Yola with 9.17×10^5 CFU/mL and lowest in Jimeta with 3.7×10^5 CFU/mL. Fungal count was found to be highest in samples collected from Girei with 1.85×10^6 CFU/mL and lowest in Yola with 3.1×10^5 CFU/mL. It is highest in samples collected from Yola with 9.1×10^5 CFU/mL and lowest in those collected from Jimeta. The results showed that all the samples collected from Girei were found to be contaminated with *E. coli* and *Salmonella typhi*. Those collected from Jimeta were contaminated with *S. aureus*. While the samples collected from Yola were found to be contaminated with *E. coli, S. aureus* and *Bacillus* spp. However, all the samples collected from the three different study area were found to be contaminated with fungi mainly *Aspergillus* spp. and *Penicillium* spp.

Kevword: Microbiological. Ouality. Evaluation. Herbal. Formulations. Yola.

INTRODUCTION

The medicinal value of plants lies in their chemical constituents that produce a definite physiological action on the human body (Edeoga *et al.,* 2005). The most important of these bioactive compounds of plants are alkaloids, tannins and phenolic compounds (Edeoga *et al.,* 2005). Rural communities depend on plants resources for herbal medicines, foods, forage, construction of dwellings, household implements, sleeping mats and for fire and shade (Sandhu and Heinrich, 2005). The use of medicinal plants as traditional medicine is well known in rural areas of many developing countries (Sandhu and Heinrich, 2005). Traditional herbalists in Nigeria use various herbal preparations to treat various types of ailments, including diarrhoea, urinary tract infections, typhoid fever and skin diseases (Sofowora, 1994). Most of the herbal preparations are used in different forms and may carry a large number of various kinds of microbes originating from soil usually adhering to leaves, stems, flowers, seed and root of the herbs (Okunlola *et al.,* 2007). World Health Organization (WHO, 1998) described traditional medicine as one of the surest means to achieve total health care coverage of the world's population. In pursuance of its goal of providing accessible and culturally acceptable health care for the global population, World Health Organization (WHO) has encourage herbal medicines by member states and has developed technical guidelines for the assessment of herbal medicine (WHO, 1998; WHO, 2000).

The leaves, stems, bark and root of different plants have been used by the local populace and people with less income for incurring different types of ailment because of the inadequate medical facilities across the nook and cranny of these countries (Gupta *et al.,* 2005). In developing countries, low income people such as farmers, use folk medicines for the treatment of common infections (Rojas *et al.,* 2006). With the prevalence in the Nigerian market of these products, it would be of great interest to evaluate the pharmaceutical qualities of these herbal medicinal products irrespective of the medicinal contents and therapeutic claim (Adenike *et al.,* 2007). The widespread use of herbal compounds in the prevention and treatment of many illnesses such as sepsis and septic shock, remain in its infancy (Joseph-Varon 2009).

MATERIALS AND METHODS

Sample Collection and Processing

This study was carried out in three different areas, Yola, Jimeta and Girei local government of Adamawa state. Four samples of herbal formulations were collected from each study area which gives a total of twelve (12) samples. From each study area, two samples of typhoid formulations for the treatment of typhoid fever and two samples of diarrhoea formulations used for the treatment of diarrhoea were collected. However, from each two samples, one liquid formulation of 30mL volume and one powdered of 30g were collected.

Sample Preparation and Extraction

For each of the powdered samples collected, 10g of it was added to 100mL of distilled water and 70% w/v ethanol to obtain water and ethanolic extract (100mg/mL) respectively. This crude extraction was done at room temperature (25^{0}C) for 2weeks. Muslin cloth was then used to filter the residues and the filtrate was further purified by filtration through a Whatman filter paper (Atata *et al.*, 2003).

The extract was then sterilized by filtration through membrane 0.45µm pore size (Ronald, 1995). The extract was then stored in sterile screw capped bottles that were autoclaved and refrigerated at 4°C until when required for use.

Inoculum Preparation

The test organisms for this study were collected from the Department of Microbiology, Modibbo Adama University of Technology Yola. Three to five colonies of the test organisms were transferred into sterile test tubes containing sterile nutrients broth and it was incubated at 37°C for 24hours. The turbidity produced by the organisms was adjusted to match the turbidity standard of 0.5 McFarland standards using normal saline as described by Cheesbrough (2006).

Determination of the Sterility of the Sample

The extract was sterilized by filtration through a millipore membrane filter of 0.45µm pore size (Ronald, 1995). It was tested for sterility after Millipore filtration by introducing 2ml of the extract each of typhoid and diarrhoea formulations into 10mL of sterile nutrient broth and incubated for 24hours at 37°C. A sterile extract was indicated by absence of turbidity or cloudiness of the broth after inoculation and incubation (Ronald, 1995).

Determination of the MIC of the Extracts

The initial concentration of the extract (100mg/mL) was diluted by using a double fold serial dilution by transferring 5mL of the sterile extract (stock solution) into 5mL of sterile nutrients broth and 50mg/mL concentration was obtained. The above procedure was repeated several times to obtain other dilutions of 25mg/mL, 12.5mg/mL, 6.25mg/mL and 3.125mg/mL (Ibekwe *et al.*, 2001). Each concentration was inoculated with 0.1mL of the inoculum containing the test organisms and incubated at 37°C for 24hours. The lowest concentration of the extracts that inhibits the growth of the test organisms were taken as the minimum inhibitory concentration (MIC) (Cheesbrough, 2006).

Determination of the Minimum Bactericidal Concentration (MBC) of the Herbal Formulations

The Minimum Bactericidal Concentration (MBC) was determined by sub-culturing from the tubes with minimum inhibitory concentration (MIC) onto a fresh extract-free solid medium and it was incubated further at 37^{0}C for 24hours. The highest dilution that yields no single bacterial colony on the solid media was taken as the MBC (Cheesbrough, 2006).

Determination of Microbial Contamination

i. Total Viable Counts

The total viable count in the typhoid and diarrhea formulations was assessed by using the pour plate method using nutrient agar medium. The fresh samples of typhoid and diarrhea formulations were serially diluted and 1mL was transferred to a duplicate petri dishes followed by 25mL of molten nutrient agar. The plates were mixed by swirling and allowed to cool. The plates were incubated at 37°C for 24hours. Sabouraud Dextrose Agar was used for the cultivation of Fungi in the typhoid and diarrhoea formulations. The plates were placed on the colony counter and the numbers of colonies were counted and recorded in colony forming unit per mL of the sample (Cheesbrough, 2006).

ii. Isolation and Identification of Microbial Contaminants

S. aureus, E. coli, Bacillus spp, *Aspergillus* spp and *Penicillium* spp isolated from both typhoid and diarrhoaea formulations were characterized and identified on the basis of their cultural, morphological, physiological and biochemical properties as described by Cheesbrough (2006); Arora and Arora (2012).

RESULTS

Minimum Inhibitory Concentration

The results of the study revealed that the minimum inhibitory concentration of the typhoid formulations ranged from 12.5mg/mL to 50mg/mL for both water and ethanolic extract with ethanolic extract having higher activity at 12.5mg/mL than the water extract.

The result also showed that the water extract of typhoid formulations collected from Girei and Jimeta inhibited the growth of the test organism (*Salmonella typhi* and *E. coli*) at a concentration of 25mg/mL while the water extract of the sample collected from Yola inhibited the test organisms at 50mg/mL but the ethanolic extract from Girei and Jimeta inhibited the growth of the test organism at a concentration of 12.5mg/mL and those collected from Yola inhibited the growth at concentration of 25mg/mL. The MIC of the Diarrheal formulations ranged from 12.5mg/mL to 50mg/mL for the water and ethanolic extracts. The Minimum Inhibitory Concentration of the water extract of diarrhea formulation collected from all the study areas inhibited the growth of the test organism at 50mg/mL. However, the ethanolic extract collected from Girei and Jimeta inhibited the growth of the test organisms at concentrations of 12.5mg/mL and those collected from Yola inhibited the growth of the test organism at concentration of 25mg/mL (Table 1).

Table 1: The Minimum Inhibitory Concentration (MIC) (mg/mL) of the Typhoid and Diarrhoea Formulations against *Salmonella typhi* and *E. coli*

Sampling Sites	Test Organism	Water Extract	Ethanolic Extract
Girei	*Salmonella typhi*	25	12.5
	E. coli	50	12.5
Jimeta	*Salmonella typhi*	25	12.5
	E. coli	50	12.5
Yola	*Salmonella typhi*	50	25
	E. coli	50	12.5

Minimum Bactericidal Concentration of the Typhoid and Diarrhoea Formulations against *Salmonella typhi* and *E. coli*
The ethanolic extract of the typhoid formulations collected from different study areas showed bactericidal activity against *Salmonella typhi* at concentration of 25mg/mL while the ethanolic extract of diarrhea formulations collected from those areas has a bactericidal activity against *E. coli* at a concentration ranged from 25mg/mL to 50mg/mL (Table 2).

Table 2: Minimum Bactericidal Concentration (MBC) (mg/ml) of the Ethanolic Extracts Typhoid and Diarrhoea Formulations

Locations	Test organisms	TP-Formulations	DR-Formulations
Girei	*E. coli*	-	50
	Salmonella typhi	25	-
Jimeta	*E. coli*	-	25
	Salmonella typhi	-	-
Yola	*E. coli*	-	50
	Salmonella typhi	25	-

Key: TP – Typhoid
        DR – Diarrhea
         – Mean Not Tested

Total Viable Counts obtained from the Herbal Formulations
The result of viable count revealed that typhoid formulations collected from Girei has the highest number of viable count with 1.85×10^6CFU/mL and the viable count is lowest in the samples collected from Yola with viable count of 3.1×10^5CFU/mL. The diarrheal formulations collected from Yola has the highest number of viable bacteria with a count of 9.1×10^5CFU/mL while samples collected from Jimeta has the lowest count with 3.7×10^5CFU/mL (Table 3).

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Table 3: Mean Viable Count of the Typhoid and Diarrhoea formulations expressed in CFU/mL

Location	Typhoid Formulations	Diarrhoea Formulations
Girei	1.85×10^6	3.9×10^5
Jimeta	3.6×10^5	3.7×10^5
Yola	3.1×10^5	9.1×10^5

Identified Microbial Contaminants from Herbal Preparations

The microbial contaminants in the typhoid and diarrhoea formulations varied considerably and different types of micro organisms contaminated both typhoid and diarrhoea formulations. Five (41.7%) of the typhoid and diarrhoea formulations were contaminated by *E. coli*. Four (33.3%) were contaminated with *Salmonella typhi*. Six (50%) of the products were contaminated by *Staphylococcus aureus* and nine (75%) of the samples were contaminated by fungi (Table 4). However, all the samples collected from Girei were found to be contaminated with *E. coli* and *Salmonella typhi*. Those collected from Jimeta were found to be contaminated with *S. aureus*. While the samples collected from Yola were found to be contaminated with *E. coli, S. aureus* and *Bacillus* spp. Furthermore all the samples collected from different sources were found to be contaminated by fungi mainly *Aspergillus* spp. and *Penicillium* spp.

Table 4: Percentage Occurrence of Microbial Contaminants in the Typhoid and Diarrhoea Formulations　　Expressed in %

Organisms	No of samples contaminated	Percentage (%)
S. aureus	4	33.3%
E. coli	5	41.7%
S. aureus and E. coli	6	50.0%
Fungi	9	75.0%

DISCUSSION

The result of this study revealed that the ethanolic extracts of the herbal formulations showed high antimicrobial activity against the test organisms than the water extract. Although the mechanism of action of these formulations is yet to be known; however, it is clear that effectiveness of the extract depend on the extraction solvent used (Ibekwe *et al.,* 2001). The results of the study showed that the different herbal formulations used for the treatment of typhoid and diarrhea has inhibitory effect against the *Salmonella typhi and E. coli* respectively at varying concentrations ranging from concentration of 12.5mg/mL to 50mg/mL. The results of the study revealed that the water extract of the typhoid and diarrhoea formulations has less inhibitory effect against *Salmonella typhi and E. coli* respectively than the ethanolic extract of those formulations. Researchers in the past have also showed that the ethanolic extracts were more effective than the water extracts (Dutta, 1993; Ibekwe *et al.,* 2001). They have attributed these observations to the high polarity of ethanol which tends to extract more active compounds from the sample than water. Hence, this study followed similar trends. In all cases were possible, the ethanolic extract of these herbal formulations should be used at a concentration up to 50mg/mL so as to give a better treatment margin than the maximum 25mg/mL obtained in the MIC. Also the water extract should be used at a concentration up to 100mg/mL than the maximum 50mg/mL obtained in the MIC (Ibekwe *et al.,* 2001).

Herbal medicinal products usually contain bacteria and moulds from soil and atmosphere. The limits of microbial contamination are: total aerobic bacteria 10^5cfu/mL, yeasts and moulds 10^3cfu/mL, *Enterobacteria* and other Gram negative organism's 10^3cfu/mL and *E. coli* and *Salmonella* should be absent (EMEA, 2003).. The samples were contaminated to varying degrees with bacteria and Fungi. Of concern is the level of contamination of the products by Gram negative organisms which are considered pathogenic. This result is in line with ten (47.6%) for *E.coli* and seven (33.0%) of *Salmonella* obtained by Okunlola *et al.,* (2007) in their work. The presence of microbial contaminant in non sterile pharmaceutical products can reduce or even inactivate the therapeutic activity of the products and has the potential to adversely affect patients taking the medicines. The possible causes of these contaminants could be either from the initial raw materials such as plant roots, stems, barks, leaves or during the cause of drying the materials (Nakajima *et al.,* 2005).

However, typhoid formulations collected from Girei contain high number of viable bacteria contaminants than Jimeta and Yola; this could be as a result of improper handling, processing, and storage condition as well as contaminated raw materials (WHO 1998). Also the diarrheal formulations collected from Yola contain high number of microbial contaminants than those collected from Girei and Jimeta. Moreover, contamination can occur during the preparations of the formulations. Soil, harvesting, drying, storage conditions and improper handling influence the microbiological quality of herbal drugs (WHO 1998).

Conclusion
The number of total viable count of the formulations was found to be within the acceptable limit of 10^5cfu/mL with only typhoid formulations collected from Girei having 10^6cfu/mL which is slightly above the standard limit. However, ethanolic extract have higher antimicrobial activity than the water extract because ethanolic extract release more active compound from the formulations than the water extract. Hence ethanol should be use during extraction in order to release large number of active compound in the formulations. Also care should be taking during processing of the herbal formulations to avoid contamination of the products.

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