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BACTERIOLOGICAL QUALITY OF SOME HERBAL MEDICINES COMMONLY SOLD IN DUTSE METROPOLIS, JIGAWA STATE

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Abstract

Herbal medicine as the oldest form of health care system known to mankind have been used for centuries. Although it is still effective in treating a vast range of diseases, but there are some concerns on the mode of preparations and the possibility of microbial contamination. A study to determine the safety and assessment of some herbal medicines commonly sold in Dutse metropolis, Jigawa state was conducted in May, 2017. A total of thirty(30) herbal medicinal samples comprising of ten (10) liquids, ten(10) powders and ten (10) teas were purchased from some herbal shops and retail outlets in different parts of Dutse metropolis and were analyzed for bacterial loads using standards pour plate method.Total Aerobic Bacterial Count (TABC) of the herbal samples obtained ranged from 1.5 x 10^7 CFU/ml to 4.9 x 10^7 CFU/ml for liquid medicinal samples and 1.0 x $10^7$to 2.8 x $10^7$for solid medicines, while four (4) of the samples (13.3%) were Too Numerous To Count (TNTC).Escherichia coli and Salmonella spp were isolated from all the samples while Shigellaspp were only isolated from 8 (26.67%) of the samples. Due to high degree of bacterial contamination of these herbal medicines and the pathogenic potentials of the isolates, it is necessary that herbalists should be enlightened about Good Manufacturing Practice (GMP). Regular bacteriological monitoring of herbal preparations marketed in Dutse metropolis should be encouraged.

Keywords: Bacteria, Dutse, Herbal medicine, Pathogenic, and Quality

INTRODUCTION

Herbal medicines also called botanical medicines or phytomedicines are those exclusively made from different parts of plants and also their extracts (Oreagba*et al*, 2011). They have been used in the maintenance of health as well as in prevention, improvement or treatment of physical and mental illnesses. These medicines have been delivered raw, in tea and tinctures, as topical application, in liquid forms, pills and capsules.

The indigenous practice of medicine has existed for centuries and is known to be the oldest health care system (Yadav and Dixit, 2008). Ancient Egyptian papyrus writings described medicinal uses for plants as early as 3000 B.C. The synthetic drugs has been reported to have high incidence of adverse drug reactions and these have motivated mankind to go back to nature for safer remedies.

The general perception that herbal remedies or drugs are very safe and devoid of adverse effects is untrue and misleading. Herbs have been shown to be capable of producing a wide range of undesirable or adverse reactions when contaminated, some of which are capable of causing serious injuries, life-threatening conditions, and even death (Ekor, 2014).

The therapeutic efficacy of plants depends on their active constituents which are effective against the specific ailments. In the case of high microbial load in plants it may be possible that active constituents of the plants may deteriorate. The contamination of herbal drugs by microbes is a very common phenomenon as many reports are available on the occurrence of microbial contamination in herbal drugs (*Manisha et al*.,2014).

In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. The plant materials used in herbal drugs preparations are organic in nature, it provides nutrition and supports the growth of bacteria and facilitates the multiplication of bacteria which lead to contamination, biodeterioration and variation in chemical composition of the herbal medicine .This gives rise to inferior quality of herbal product with little or no therapeutic efficacy .The quality of herbal drug also depends on many factors like environment, collection method, cultivation, harvest, post- harvest processing, transport and storage practices *(*Khurana *et al*., 2011*).*

A World Health Organization (WHO, 2007) survey indicated that about 70-80% of the world population particularly in the developing countries rely on non-conventional medicines mainly of herbal sources, so there is need to put in place an effective quality assurance system such as bacteriological analysis of herbal medicine in order to ascertain the quality and safety of herbal preparations.

The increase in the consumption and popularity of natural drugs made their use a Public Health problem due to the lack of effective surveillance of the use, efficacy, toxicity and quality of these natural products, (Rajapandiya*et al.*, 2013*).*

In Nigeria, herbal medicines and related products are introduced into the market without any mandatory safety or toxicological evaluation and also lack effective machinery to regulate manufacturing practices and quality standards. These herbal products are continuously made available to consumers without prescription in most cases. (Ekor, 2014).
Plant materials used in herbal medicine preparations are organic in nature, providing nutrition and support microbial growth which could lead to contamination when proliferated *(*Khurana *et al.*, 2011*).*

This research work was aimed to assess the bacteriological quality of some herbal medicines commonly sold in Dutse metropolis which could serve as a wake-up call to the regulatory bodies or agencies on the need for adequate monitoring of herbal medicines.

MATERIALS AND METHODS

Area of study

The study was conducted in Dutse metropolis, Jigawa State with the geographical coordinate between latitude 11˚ 42ˈ 04ˈˈ North and longitude 9˚ 20ˈ 31ˈˈ east of the equator. Dutse had an estimated population of 153,000 (World Gazetteer, 2007).

Collection of samples

A total of 30 different packaged herbal samples comprising of 10 herbal solutions (liquid),10 herbal powders and 10 herbal teas were purchased randomly from identified herbal shops and retail outlets in different parts of Dutse metropolis which were collected in May, 2017, while those that were not packaged (such as herbal preparations sold by local herbalist) were collected in sterile polythene bags. All the collected samples were conveyed to the Microbiology laboratory of the Department of Microbiology and Biotechnology, Federal University Dutse, Jigawa state for further analyses.

Microbiological analyses

For solid samples(teas and powders), a stock solution of the sample was prepared by weighing one gram (1g) of the sample into 9ml of distilled water and shaken thoroughly, A ten-fold Serial dilutions were made until 10^{-7} dilution was achieved, 1ml aliquot of the 10^{-7} dilution was transfered onto the Petri-dish and viability assessed using the pour plate method. The plates were incubated at 37^{o}C for 24h after which the plate was placed on a colony counter and the

number of colonies were enumerated and expressed in colony forming units per grams (CFU/g).

For the herbal liquid formulations, the sample was shaken vigorously after which 1ml was dissolved in 9ml of sterile distilled water. A ten-fold serial dilution was made until 10^{-7} dilution was achieved, 1ml was then pipetted from the 10^{-7} dilution onto the Petri-dish and viability assessed using the pour plate method. The plate was incubated at 37^{o}C for 24h. The plate was placed on a colony counter to determine the number of colonies and expressed in CFU/ml (technologyinscience.blogspot.com, Retrieved 28-08-2017).

Isolation and identification of the selected bacteria

Enrichment of the bacteria was achieved by adding one gram (1g) of each herbal samples into 9 ml sterile water aseptically in a test tube and the content mixed thoroughly. One ml of each of these diluents was pippetted into a sterile petri dish and mixed with molten autoclaved nutrient agar at about 45° C that was poured aseptically into the petri dishes, each colony growing on the nutrient agar was carefully picked and then streaked onto the surface of an Eosin methylene blue (EMB) agar and incubated for 24 hr at 44°C. Colonies with green metallic sheen indicated the presence of lactose fermenters and were confirmed to be *Escherichia coli* by some biochemical tests such as indole test, methyl red test and vogesproskauer test. (Odedara and Memuletiwon, 2014).

Other colonies growing on the nutrient agar were carefully picked and then streaked onto the surface of salmonella-shigella agar and incubated for 24h at 37°C. Dark colonies indicated the presence of lactose fermenters and were confirmed to be *salmonella spices* using some biochemical tests such as indole, methyl red and vogesproskauer. Non lactose fermenters that grew as colourless colonies were identified as *Shigellaspp* with suitable biochemical test such as indole, methyl and vogesproskauer.

Each of the bacterial isolate were identified by their Gram's reaction using the Gram's staining technique.

RESULTS

The results of TABC and isolation of *E .coli, Salmonella spp* and *Shigellaspp*from the samples of herbal medicine is shown in Table 1, which includes the colony forming unit per millilitre (CFU/ml) and the occurrence (presence or absence) of *E .coli, Salmonella spp* and *Shigellaspp*in each and every herbal medicine samples under study. It was observed from the results obtained that the total aerobic count ranges from 1.1 x $10^{7}$to 4.9 x 10^{7} .The highest count (4.9 x 10^{7} CFU/ml) was obtained in one of the liquid herbal medicines, while the lowest count (1 x 10^{7}CFU/g) was obtained in one of the tea herbal medicines. Four of the samples {3 liquid medicines and 1 powder medicine (13.3%)} were too numerous to count. *Salmonella spp* and

Escherichia coli were isolated from all the samples while *Shigellaspp* wereislolated from only eight (8) of the samples (26.67%).

The Gram's staining and biochemical test results of the isolated bacteria are shown in Table 2. They were all Gram negative rods, positive for methyl red test, and negative for Vogesproskauer test. The indole test was negative for *Salmonella spp* but positive for both *E. coli* and *Shigella spp*.

Table 1: Total Aerobic Count and Bacterial Isolationfrom the Herbal Medicines.

Samples	Dosage Form	TBC (CFU/ml/g)x 10^7	*Salmonella spp*	*Shigellaspp*	*Escherichia coli*	Inference
A	Liquid	4.2	+	_	+	Unacceptable
B	Liquid	3.8	+	_	+	Unacceptable
C	Liquid	4.9	+	_	+	Unacceptable
D	Liquid	1.5	+	+	+	Unacceptable
E	Liquid	2.7	+	_	+	Unacceptable
F	Liquid	2	+	_	+	Unacceptable
G	Liquid	TNTC	+	_	+	Unacceptable
H	Liquid	1.8	+	_	+	Unacceptable
I	Liquid	2.3	+	+	+	Unacceptable
J	Liquid	TNTC	+	+	+	Unacceptable
K	Powder	1.7	+	_	+	Unacceptable
L	Powder	2.3	+	_	+	Unacceptable
M	Powder	2.7	+	+	+	Unacceptable
N	Powder	1.1	+	_	+	Unacceptable
O	Powder	TNTC	+	_	+	Unacceptable
P	Powder	2.2	+	+	+	Unacceptable
Q	Powder	4	+	_	+	Unacceptable
R	Powder	TNTC	+	_	+	Unacceptable
S	Powder	2.8	+	_	+	Unacceptable
T	Powder	2.2	+	+	+	Unacceptable
U	Tea	1	+	_	+	Unacceptable
V	Tea	1.5	+	_	+	Unacceptable
W	Tea	1.8	+	_	+	Unacceptable
X	Tea	1.7	+	+	+	Unacceptable
Y	Tea	1.9	+	_	+	Unacceptable
Z	Tea	1.5	+	_	+	Unacceptable
A2	Tea	2	+	_	+	Unacceptable
B2	Tea	1.2	+	+	+	Unacceptable
C2	Tea	1.1	+	_	+	Unacceptable
D2	Tea	2.1	+	_	+	Unacceptable

TBC: Total bacterial count, -: Absent, +: Present, TNTC: Too Numerous to Count

Table 2: Biochemical and Gram's Staining Test Results

BACTERIA	GR	SHAPE	MR	MOT	VP	CIT	I
Salmonella spp	_	Rod	+	+	-	-	-
Escherichia coli	_	Rod	+	+	-	-	+
Shigellaspp	_	Rod	+	-	-	-	+/-

GR: Gram's reaction, MR: Methyl red, MOT: Motility, VP: Vogesproskauer, CIT: Citrate I: Indole

DISCUSSION

The results of this study have shown that majority of the herbal preparations examined were grossly contaminated by bacteria and the mean bacterial counts of the preparations were generally higher than the accepted value (10^5 CFU/ml) of herbal medicine specification (WHO, 2007).

The highest TABC and TNTC results obtained were not in agreement to those of Atanassova *et al*. 2011 in Bulgaria probably due to strict adherence to GMP. However, they are in agreement with those of Danladi *et al*. 2009 (5 x 10^7 CFU/ml), Noor *et al*. 2013 (1.2 x 10^5CFU/ml) in Bangladesh, and Archibong *et al*. 2017 (2.1 x 10^6 CFU/ml) in Awka, Anambra state, indicating non-compliance of most herbal medicines with the given standard.

The higher bacterial counts obtained during this research (1.1 x 10^7 to4.9 x 10^7) and TNTC might be associated with preparations of the medicines under poor hygienic conditions. For instance, the water used for dilutions might not be free from contaminants, the equipment and utensils used during processing, holding, transferring and packaging might not be clean and sanitized enough. More so, since the preparations are locally made, it might be devoid of any serious supervision from various public or private organizations meant for this purpose. The environment and equipment used during the medicinal preparations could be below rules of hygienic. Other possible sources of contaminants may include the personnel(s) that could introduce bacteria when handling the raw materials and during processing (Kosalec*et al*., 2009). Therefore, the process of harvesting, drying, storage, handling and the soil influence the bacteriological quality of raw material which in turns affects the entire quality of the herbal preparation.

The highest bacterial counts obtained in liquid preparations could be as a result of hydrated nature which is known to favour microbial growth.

Detection of*Salmonella spp, Shigellaspp*and *E. coli*in the herbal medicines render them unacceptable based on WHO specification. The presence of these bacteria including S. aureus and other coliforms were also recorded in previous related researches (Danladi*et al*., 2009; Noor *et al*., 2013; Oluwatoyin and Adelayo,2016; Archibong*et al*., 2017). These organisms have been reported as contaminants presenting serious health hazards (Erich *et al*., 2001; Okunlola*et al*., 2007). However, no *Salmonella spp* and *Shigellaspp* were recorded in Noor *et al*. 2013 hence, complied with given standard. The absence of salmonellae, *E. coli* and Gram-negative bacterial species have been used as indicators of microbiological quality. Gram negative bacteria such as *Salmonella spp*, *Shigellaspp* and *E.coli* should be absent per gram/ml of the herbal medicine.
All medicinal herbs must be clear of bacterial pathogens such as *Salmonella spp*.
Coliforms such as *E. coli* are the most reliable indicators of faecal contamination, thus the test for their presence is an index of the degree of faecal contamination, which may indicate a possible presence of harmful disease-causing organisms. These isolated bacteria constitute the intestinal flora of humans and other animals, and are therefore used as indicator organisms and as an index of possible contamination by human pathogens(Forest, 2004). Therefore, the high recovery rates of these suspected perilous bacteria from indigenous herbal medications could be of clinical relevance.

It has been shown that Shigella among others grows on herbal materials and produces endotoxin which when liberated, could lead to secondary infection in the consumers (Arani*et al*., 2014; Https://www.merriam-webster.com retrieved, 08-03-2017)
It was observed from the result obtained that there were no difference between the package herbal medication and non-package herbal medication in terms of the degree of bacterial contamination.
The presence of bacteria in herbal medicine do not only make them hazardous from the infectious stand point, but may also change the physical, chemical, and also change the natural properties of the herbal medicine, alter the contents of active ingredients, or convert them to toxic products. Product quality is obviously the major criteria that could affect not only the efficacy but also the safety of patients or consumers (Mullika*et al*., 2003).
The presence of microbes in herbal medicine may also change the physical, chemical and natural properties of the herbal medicines such as altering the composition of the active ingredients, or convert them to toxic products thereby, affecting the consumers negatively, that is, complicating their health challenges rather than improving.
Due to the increase use of herbal medicine worldwide, the quality and safety of medicinal plant and finished herbal products has become a major concern for health authorities.

CONCLUSION

Bacterial contamination of samples of herbal drugs commonly sold in Dutse metropolis, Jigawa state is confirmed in this study. The contamination was routed to sources including the environment, raw materials, hands of the producers, poor microbiological quality of water used and generally non-adherence to Good Manufacturing Practice.

The excessive bacteriological contamination of the drugs might increase health hazard to consumers rather than curing illnesses.

The advantages associated with using herbal medicine to treat diseases or infections cannot be over emphasized and should be complemented with sound bacteriological quality in order to ensure safety.

RECOMMENDATIONS

It is recommended that microbiological safety of herbal medicine preparations be promoted and sustained by collective effort from the workers, quality control officers and quality assurance officers.

A number of critical control points including the source of the herbal substance, the manufacturing processes and any decontamination procedure used, bacteriological purity of excipients, the protective capacity of the packaging material chosen and preventive measures are to be preferred rather than interventions for decreasing the contamination.

Good manufacturing practises throughout the entire manufacturing process of herbal substance to the finished product is crucial in order to ensure acceptable bacteriological quality.

Proper ventilation through HEPA filters and cleaning at regular intervals would improve the quality of the environment. The water used for preparation of herbal medicines should be subjected to treatment in order to reduce the bacterial counts. Containers should be sterilized before use for packaging any preparations.

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