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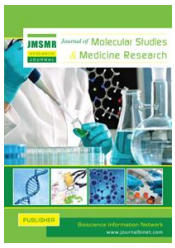

Contamination of locally produced herbal medicine sold in Gombe main market with selected pathogenic bacteria

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ABSTRACT

The study aimed to evaluate the bacterial contamination of herbal medicinal preparations sourced from identified herbal retail outlets in different parts of Gombe main Market. The assessments of the contamination of the herbal products were carried out using Total Aerobic Bacterial Plate Count, Isolation and Characterization of Selected Bacterial pathogens. The results showed that samples were contaminated with Staphylococcus aureus and Escherichia coli. The Total Aerobic Bacterial Plate Count results showed that the highest average count of > 12.4×10 cfu/ml were samples A to E of the preparations, while least average plate count was samples F to H. Also, Salmonella typhi and Shigella dyseteriae were absent in all the samples. Most traditionally prepared herbal medications in Gombe main market are likely to be contaminated with a wide variety of potentially pathogenic bacteria. The quality assurance of these products should be thoroughly enforced and monitored in the production and distribution of herbal preparations.

Key Words: *Bacterial contamination, Bacterial pathogens and Aerobic bacterial plate count*

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I. Introduction

The history of using herbs is inextricably intertwined with that of modern medicine. Many synthetic drugs listed as conventional medication were originally derived from plant, for example the antimalarial drug quinine from Cinchona species (Janetzang, 1994). Traditional herbalists in Nigeria use various herbal preparations to treat various types of ailments, including diarrhea, urinary tract infections, typhoid fever and skin diseases (Sofowora, 1993). Most of the herbal preparations are used in different forms and may carry many various kinds of microbes originating from soil and the environment usually adhering to leaves, stems, flowers, seed and root of the herbs (Adeleye et al., 2005). The World Health Organization (WHO, 1998) survey indicated that about 70-80 % of the world population particularly in developing 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). Therefore, the quality and safety of herbal preparations are also of great concern. The WHO (1993) explained that

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quality is the basis of reproducible efficacy and safety of herbal drugs, and to ensure the standard of research on herbal medicines, the quality of the plant materials or preparations is of most importance. Bauer (1998) showed that the criteria for quality herbal drugs are based on a clear scientific definition of the raw materials. It is difficult to establish comprehensive quality criteria for herbal drugs due to 'professional secrecy' of herbalists, but to improve the purity and safety of the products, observation of basic hygiene during preparation, standardization of some physical characteristic such as moisture content, pH, temperature, and microbiological contamination levels are desirable. Previous studies have confirmed the presence of potential contaminants in herbal preparations (De-Smet, 1999). The contaminants that present serious health hazard are pathogenic bacteria, such *Salmonella, Esherichia coli, Staphalococcus aureus, and Shigella spp* (Arias et al., 1999; Erich et al., 2001; Wolfgang et al., 2002; Adeleye et al., 2005; Okunlola et al., 2007).

Plant have been used for thousands of years in the treatment of human diseases and the selection of plant for uses as medicine was probably made as result of quassipharma-cological studies. The plant part and dose level which were used properly arose from empherical studies culminating in the cure of patients' ailments. More so, the absence of galenical (liquid extract, tincture, decoction, dry extract, etc.) studies in herbal preparation have been cited as reasons for doubting the validity of herbal remedies (Dewar et al., 1968).

In many Nigerian and African homes, teeth cleaning in the morning is done by chewing the root or slim stem of certain plants (Said et al., 1970). The fibrous end of the chewed stem is then used to brush the teeth thoroughly. These chewing sticks impart varying taste sensation; such as peppery taste, numbness is provided by *Zanthoxylum zanthaxyloides* and *Fagara xanthoxyloides* root. A strong bitter taste and is experienced from *Masularia ocuminata.* These chewing sticks, in addition to providing mechanical stimulation of gums, also destroy microbes present in the mouth. Chewing sticks were used before the advance of tooth paste in Africa.

Ocimum gratissimum leaf and the whole herb are popular for the treatment for diarrhoea (Dalziel, 1956). The plant is rich in volatile oil which contains up to 75 percent of thymol. The antimicrobial activity of the water saturated oil has been shown to be proportional to the thymol content. In the preparations where *Ocimu gratissimum* is used as a cold infusion, therefore the antimicrobial effect of the extracted *thymol* is probably sufficient explanation for the anti-diarrhoea effect. However, in certain other preparation *Ocimu gratissimum* is boiled with water to form a decoction which contains little of the steam – volatile thymol. Although such aqueous decoctions were shown to be devoid of antimicrobial activity, they do relax the guinea pig ileum and rat jejunum in vitro. Such decoctions when cold, therefore, is still be effective in calming the gut, thus curing diarrhoea.

Okapanyi and Ezenkwu (1981) have demonstrated that an extract of neem leaves and bark shows anti-pyretic effect, thus providing some justification for its use in fevers. Various skin diseases due to infection with bacterial or fungal origin are treated successfully with herbal remedies. Limikanra et al. (1982) have made extensive studies on the antibacterial activities of Nigerian soft soaps. Another remedy popular for treating skin disease in Nigeria is the leaf of *Acalypha wikesianan* for which scientific evidence in support of its use has been documented (Alade and Irobi, 1993).

Medicinal plants continue to be of use for the treatment of diseases on a worldwide-basic plant are logical source for new drug discover as and currently many thousands are being screened for biological activities to develop new drug entities. In recent novel anticancer and antimalarial drugs, have been developed from plant sources. Although many potent and specific drug are available today for treatment of diseases there is a public swing to alternative/complimentary medicine in developed countries. The sale of herbal product in Europe during 1992 costed $1. Billon. Most herbal product on sale are not licensed as medicines. This is a matter of concern to both consumers and health –care professionals. Many of the world's population cannot afford modern medicines and rely on tradition medicine which are mainly plant based. These medicinal plants require investigation in collaborative research programmers between scientists in developing countries.

The menace of herbal contamination has become a growing concern worldwide, particularly in West Africa. Many people depend on local herbs for medicinal purposes. The way these herbal preparations

are handled may constitute some contamination which may cause some diseases. Therefore, there is need to undertake a research to find out any possible contamination which can be utilised to improve quality of the locally produced herbs used and consumed by people of Gombe State.

Therefore, the study aimed to determine the possible presence of bacterial contamination, in locally prepared herbal medicine sold in and around the Gombe main market by ascertaining the type and level of bacterial contamination.

II. Materials and Methods

Sample collection: A total of 8 different herbal preparations samples were purchased and labeled A, B, C, D, E, F, G and H randomly from identified herbal shops and retail outlets in different parts of Gombe main market. The samples contained plant parts of Neem (*Azadirachta indica*), whole lemon (*Citrus spp*), Mango leaves (*Mangifera indica*), Black locust beans (*Parkia biglobosa*). All the samples were mostly used for the treatment of malaria and typhoid fever. The herbal samples were collected in sterile flasks that contain ice, and the samples were transported to microbiology laboratory for further investigations.

Processing of samples: The samples were kept in freezer and 10-fold serial dilution were been carried out for each sample using distilled water. 1 ml of each sample was dispensed in 9 ml of distilled water in a test tube using syringe and were mixed to obtain 1:10 dilution. 1ml of the mixture was transfer to 10 test tubes which have already contained 9mls of water before inoculating.

Media preparation: All the media is used in this study were from *0xoid*. They were prepared according to the manufacturers' instructions since they were in a dehydrated form. MacConkey agar and Nutrient agar were used for bacterial isolation. The agar mediums that usually used by other authors (Sultana et al. 2010; Siddique et al. 2006) were not tried for this experiment.

Inoculation of samples on media: Inoculation of samples was done by using pour plate method. 1ml of each of the diluted sample was transferred into sterile petri dish and swirled and each prepared media (Nutrient agar) was then poured into sterile petri dish that contained the samples at 40 C – 45 C and swirled. The media was allowed to solidify and later was incubated at 37 ^{0}C for 24 hours.

Total plate count: Colonies on each of the plates were counted at the end of the incubation period by using the Stuart Digital colony counter.

Isolation and identification of bacteria: After the incubation period elapsed, colonies formed were sub cultured on the MacConkey agar plate to get pure culture for identification. Growth obtained were used for the following.

Microscopy (gram staining): Biochemical tests, such as catalase test, coagulase test, oxidase test and indole test.

Microscopy: This test is carried out to differentiate between gram positive and gram negative bacteria. One colony of the culture was transferred on the slide by the used of sterile wired loop. The culture was fixed by heating slide over Bunsen Burner. A few drops of crystal violet stain were dropped onto the fixed culture, it stood for a minute and was rinsed off with the distilled water. Few drops of iodine solution were added on the smear and it was also allowed to stand for a minute before it is rinsed off with distilled water. Few drops of decolorize was added and was left to stand for 30 minutes before being rinsed off. Few drops of basics counter stain were added and was left for a minute and the solution was washing thoroughly. A drop of oil immersion was added covered with the cover slide. The slide was viewed under electric microscope.

Biochemical tests

Catalase test: Two to three drops of hydrogen peroxide were disposed on a clean grease free slide. The isolated was added to the hydrogen peroxide by using sterile applicator stick to emulsify. Organisms

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that produce the enzyme catalase oxidized the hydrogen peroxide to water and oxygen. The result was observed by rapid appearance of bubbles as described by (Cheesbrough, 2003).

Coagulase test: This test is done to identify presence of *Staphylococcus* species that produce the enzyme coagulase (*Staphylococcus aureus*). Human plasma was used after being allowed to warm at room temperature. A drop of distilled water was placed at both ends of grease slide and a colony of each test organism was emulsified at both ends on each slide. Then a drop of plasma was added to one of the slides while other was serve as control. The slide was then rocked for 10 seconds. A positive test showed by the presence of clumping while negative test showed no clumping (Cheesbrough, 2003).

Oxidase test: This test is used to identify bacterial species that produce oxidase enzyme. Oxidase reagent is used (phenylenediamine solution). Two to three drops of oxidase reagent were place on filter paper with the aid of sterile pepped pure culture. The colony was collected with the aid of sterile wire loop and smeared on filter paper contained the drops of the reagent. A development of blue – purple color from oxidation of phenylenediamine within ten 10 second confirms the test positive and lack of coloration signifies negative result (Cheesbrough, 2003).

In dole test: Colonies were picked and inoculated into the test tube containing the indole medium are finally incubated at 37c for 48hours at 37c may be required. 0.5ml of Kovac's reagent was added drop wise to the test tubes and was shake gently. This production of indole is confirmed by the formation of red ring coloration on the surface of the medium, which indicated positive reaction while; in negative reaction, red coloration is not produced (Cheesbrough, 2000).

III. Results and Discussion

The results of eight (8) samples of the bacteriological analyses of the herbal medicinal preparations obtained are shown in Table 01. Samples A-E contained highly average aerobic bacterial, count of about 12.4×10^{-1} to $10.0\text{x}10^{-9}$ cfu/ml while samples F to H showed least bacterial counts about 9.5×10^{-1} to 4.0×10^{-9} cfu/ml. The most frequently occurring member of the selected pathogenic bacteria screened was *Staphylococcus aureus* (Table 02). They appeared in all samples (A, B, C, D, E, F, G and H). *E. coli*, was the least frequently isolated, and was noticed to present in sample A, B, and C, while *Salmonella typhi* and *Shigella spp* were not observed in all the samples (Table 02).

The results of the herbal content showed that there was remarkable variation among the different herbal samples. European Agency for the Evaluation of Medicinal products (1998) suggested that water content should be included in the list of comprehensive specifications for herbal medicinal products. The limits of bacterial contamination given in European Pharmacopoeia as reported by Okunlola et al. (2007) are: high total aerobic bacteria count (10^5 cfu/ml), *Enterobacteria* and other gram negative organisms. *Escherichia coli and Salmonella* were absent.

The herbal products under study did not meet these specifications in most cases. The samples were contaminated to varying degrees with pathogenic bacteria. The bacterial counts observed in samples F to H showed least bacterial counts in the result range between 9.5×10^{-9} to 4.0×10^{-1} cfu/m; while samples A-E has high bacterial count between 12.4×10^{-1}to10^{-9}.0 ×10 cfu/ml. The bacterial counts in general, ranged between 1.0×10^7 and 1.8×10^8 cfu/ml, of concern. Also, the level of contamination of herbal medicinal preparations by pathogenic Gram negative and gram positive bacteria of the samples A-C were contaminated by *E. coli*, which is an intestinal bacterium and is an indicator for faecal contamination, while all samples A-E were contaminated with *Staphylococcus aureus*, which reflects easy entrance of the organism into the processed product since, it is a normal flora of the skin, easily contaminating product during handling.

The presence of large numbers of selected pathogenic bacteria in the analysed herbal medicinal preparations in this study may be due to the methods of their preparation or the equipment and materials used in preparing the herbal medicines. Other possible sources of contaminants are the personnel(s) that could introduce the bacteria when handling the raw materials during processing. 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. Thus, manufacturers should ensure the highest possible level of hygiene during manufacturing as well as the

lowest possible level of pathogenic organisms in their herbal products to maintain correct quality, safety and efficacy of the final herbal preparations. In the present study, the herbal medicinal preparations contained high levels of bacteria, and the counts were beyond the European Pharmacopoeia stated limit, pathogenic Gram negative and gram positive bacteria, such as *E. coli* and *Staphylococcus aureus* that are expected to be absent were present. *E. coli* are the most reliable indicators of faecal contamination, which may indicate a possible presence of harmful organisms in the water. The significance of pathogenic bacteria such as E coli in water is that, other harmful microorganisms may also be present, such as *Salmonella* (Forest, 2004). Therefore, the high recovery rates of these suspected perilous bacteria from indigenous orally consumed herbal medications could be of clinical relevance.

Table 01. Presence of colonies count after culturing of the samples

Sample	No of colonies	Result	Result in STD	STD coliform of water
A	120×10^{-1}	12.0	$12. \times 10^{1}$	1.0×10^{7} and 1.8×10^{8} cfu/ml
	110×10^{-3}	11.0	11.0×10^{2}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	108×10^{-6}	10.8	10.8×10^{5}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	100×10^{-9}	10.0	10.0×10^{8}	1.0×10^{7} and 1.8×10^{8} cfu/ml
B	124×10^{-1}	12.4	12.4×10^{1}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	118×10^{-3}	11.8	11.8×10^{2}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	110×10^{-8}	11.0	11.0×10^{7}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	105×10^{-9}	10.5	10.0×10^{8}	1.0×10^{7} and 1.8×10^{8} cfu/ml
C	120×10^{-1}	12.0	12.0×10^{1}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	112×10^{-3}	11.2	11.2×10^{2}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	109×10^{-8}	10.9	10.9×10^{7}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	100×10^{-9}	10.0	10.0×10^{8}	1.0×10^{7} and 1.8×10^{8} cfu/ml
D	120×10^{-1}	12.0	12.0×10^{1}	1.0×10 and 1.8×10^{8} cfu/ml
	111×10^{-3}	11.1	11.1×10^{2}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	108×10^{-6}	10.8	10.8×10^{5}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	101×10^{-9}	10.1	10.1×10^{8}	1.0×10^{7} and 1.8×10^{8} cfu/ml
E	124×10^{-1}	12.4	12.4×10^{1}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	110×10^{-3}	11.0	11.0×10^{2}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	108×10^{-7}	10.8	10.8×10^{7}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	100×10^{-9}	10.0	10.0×10^{8}	1.0×10^{7} and 1.8×10^{8} cfu/ml
F	95×10^{-1}	9.5	9.5×10^{1}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	86×10^{-3}	8.6	8.6×10^{2}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	70×10^{-6}	7.0	7.0×10^{5}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	50×10^{-9}	5.0	5.0×10^{8}	1.0×10^{7} and 1.8×10^{8} cfu/ml
G	60×10^{-1}	6.0	6.0×10^{1}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	63×10^{-3}	6.3	6.3×10^{2}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	45×10^{-7}	4.5	4.0×10^{7}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	40×10^{-9}	4.0	4.0×10^{8}	1.0×10^{7} and 1.8×10^{8} cfu/ml
H	95×10^{-1}	9.5	9.5×10^{1}	1.0×10^{7} and 1.8×10^{8} cfu /ml
	72×10^{-3}	7.2	7.2×10^{2}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	70×10^{-6}	7.0	7.0×10^{5}	1.0×10^{7} and 1.8×10^{8} cfu/ml
	50×10^{-9}	5.0	5.0×10^{8}	1.0×10^{7} and 1.8×10^{8} cfu/ml

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Table 02. Test results of some micro organisms

Samples	Gram stain	CT	GT	OX	Indo	Expected organism
A1	+ve	+	+		+	*S. aureus*
A3	-ve	+	-	+		*S. dysenteria Absent*
A6	-ve	+		-	+	*E. coli*
A9	-ve	+		-	+	*S. typhi, Absent*
B1	+ve	+	+		+	*S. aureus*
B3	-ve	+			+	*E. coli*
B8	-ve	+	+	+		*S. dysenteria Absent*
B9	-ve	+		-	+	*E. coli*
C1	-ve	+	-	+		*S. dysenteria Absent*
C3	+ve	+	+		+	*S. aureus*
C8	-ve	+		-	+	*E. coli*
C9	-ve	+		-		*S. typhi, Absent*
D1	-ve	+	-	+		*S. dysenteria Absent*
D3	+ve	+	+		+	*S. aureus*
D6	-ve	+		-	+	*E. coli*
D9	-ve	+		-		*S. typhi, Absent*
E1	-ve	+	-	+		*S. dysenteria Absent*
E3	+ve	+	+		+	*S. aureus*
E7	-ve	+		-	+	*E. coli*
E9	-ve	+		-		*S. typhi, Absent*
F1	+ve	+	+		+	*S. aureus*
F3	+ve	+	+			*S. aureus*
F6	+ve	+	+		+	*S. aureus*
F9	+ve	+	+		+	*S. aureus*
G1	+ve	+	+		+	*S. aureus*
G3	+ve	+	+			*S. aureus*
G4	+ve	+	+		+	*S. aureus*
G9	+ve	+	+		+	*S. aureus*
H1	+ve	+	+		+	*S. aureus*
H3	+ve	+	+			*S. aureus*
H6	+ve	+	+		+	*S. aureus*
H9	+ve	+	+		+	*S. aureus*

CT= Catalase test, GT= Coagulase test, OX= oxidase, Indo = Indole.

IV. Conclusion

It has been concluded that from this study the most traditionally prepared herbal medications in Gombe main market are likely to be contaminated with a wide variety of potentially pathogenic bacteria, and the quality assurance should be thoroughly enforced and monitored in the production and distribution of herbal preparations from the beginning to the final products. It was recommended that, though herbal medicine can be used as an effective means for treatment of various diseases, methods of preparation and handling should be adequately checked by considering undertaking bacteriological analysis to avoid contamination of these herbal medicinal plants during preparation. The quality assurance of these products should be thoroughly enforced and monitored in the production and distribution of herbal preparations.

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Author contributions

A. U. collected the samples from the collection sites. A. A. Z and K. M. T. developed and proof read through the manuscript. U. A. conducted the experiments in the laboratory.

Competing interest

Authors have declared that no competing interests exist.

Ethical considerations

None of the collected samples were tested on human but enquiries were made from residence on modes of consumption, purchase and preparations.

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APA (American Psychological Association)

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MLA (Modern Language Association)

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