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MICROBIOLOGICAL QUALITY OF HERBAL FORMULATION USED FOR THE TREATMENT OF TYPHOID FEVER SOLD IN MAKURDI METROPOLIS, CENTRAL NIGERIA.

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ABSTRACT

Herbal formulation has been used in recent years to treat various diseases including malaria, typhoid, dysentery and cholera. To investigate the microbiological quality of herbal formulations. Herbal formulations were purchased from four different markets (Wadata market, Wurukum market, Modern market, and North bank market) in Makurdi metropolis Microbiological analysis was carried out using pour plate isolation method. Identification of isolated microorganisms was based on their cultural, morphological, biochemical characteristics using standard microbiological procedures. Microbiological analyses showed that the total bacterial counts (TBC) of all the test herbal samples obtained from the various markets ranged from 1.8 $x10^3$ to 9.3$x10^3$ cfu/ml and the total fungal count in the herbal mixture had a range of 1.0$x10^3$ to 2.5$x10^3$, Four bacterial species were identified and they include; B*acillus* spp, *Escherichia coli, Staphylococcus aureus* and *Enterobacter* spp. The least occurring bacterial isolates was *Bacillus* spp (12.5%),while the highest occurring was *Staphylococcus aureus* (37.5%).Four fungal isolates were identified and they include, *Aspergillus niger,Penicillium*spp, *Scedosporium*spp and *Phialophoraparasiticum*. *Aspergillus niger* and *Phialophoraparasiticum* were the least occurring fungal isolate (12.5%) while *Scedosporium*spp and *Phialophoraparasiticum* were the most occurring fungal isolate (37.5%). Since applications of herbal medicines for curative purposes is on the increase, there is a need for risk assessment of microbial load of the medicinal plants at critical control points during processing.Furthermore, the danger associated with the potential toxicity of herbal therapies employed over a long period

of time demand that the practitioners be kept abreast of the reported incidence of renal and hepatic toxicity resulting from the ingestion of medicinal herbs.

KEY WORDS: Herbal formulation; typhoid fever; Bacterial counts; *Bacillus* spp; Toxicity

INTRODUCTION

It is estimated that approximately80% of the population in developing countries uses herbal medicine as part of their primary health care. The use of herbs dates back to the time of the early man, who had crudest tools as his implements and used stones to start his fire (Kafani, 1994). The medicinal science came into existence through a Greek man called Hippocrates and as such he earned his reputation as father of medicine (Heber, 2008). Most savage people have believed that disease was due to the presence of evil spirits in the body and could be driven out only by the use of poisonous and disagreeable substances calculated to make the body an unpleasant place to remain. The knowledge regarding source and use of the various products suitable for the purpose was usually restricted to medicine men of the tribe (Sharma, 2006)

Contamination of herbal medicine product is defined as the "undesired introductionof impurities of a chemical or microbial nature or of foreign matter, into or onto a starting material, intermediate product or finished product or finished flavored product throughout production, sampling, packaging, or repackaging, storage or transport"

The introduction of micro-organisms in the herbal medicinal products can alter the physicochemical characteristics of the products, which may lead to harmful effects to the quality of the herbal medicine products. The organisms he said were *Staphylococcus aureus, Eschericha coli, Rhyzopusstolonifer, Candida* species,etc(Adeleye*et al*., 2005).Bacteria are ubiquitous in nature, therefore because of the ubiquitous nature of bacteria, they contaminate almost every material, herbal juice no exception (Ochei.,2007).

Among the main safety risks related to medicine is contamination by micro-organisms of various kinds that may be adherent in leaves, stems, flowers, seeds, and roots from which herbal medicines are prepared. Alternatively, microorganisms can be introduced during harvesting, handling, open air drying, preserving, and manufacturing.

Although the medicinal plants with their chemical constituents carry huge applications in the treatment or prevention of various diseases. The plant materials are exposed to various

contaminants like toxic elements, pesticides, residues etc. but the chief contaminants mainly responsible for the deterioration of herbal products are the microbe. Several studies showed that herbal plants are associated with broad variety of microbial contaminants (kneifel*et al*., 2002).When herbal products contaminated with these micro-organisms are consumed by people, they can cause serious health issues, one of the most serious issues surrounding the safety of herbal formulations concerns regulating the level of mycotoxins found in herbal preparations. Mycotoxins are a group around 400 toxic secondary metabolites produced by fungi such as *Aspergillus, penicillium, Fusarium, Claviceps and Alternaria*Recently Nyarko*et a*l.(2007) showed that 22% of herbal plant samples studied failed to comply with the quality requirement for traditional medicine.

Herbal medicine is most often polyherbal, being prepared from mixtures of different plant parts obtained from various plant species and families and may contain multiple bioactive constituents that could be difficult to characterize (Ogbonnia*et al.,* 2010). The bioactive principles in most herbal preparations are not always known and there could be possibilities of interaction with each other in solution. The quality as well as the safety criteria for herbal drugs may be based, therefore, on a clear scientific definition of the raw materials used for such preparations. Also herbal medicine may have multiple physiological activities and could be used in the treatment of a variety of disease conditions. (Yidana and Bayorbor, 2002) It could be administered in most disease states over a long period of time without proper dosage monitoring and consideration of toxic effects that might result from such prolonged usage (Ogbonnia*et al*., 2010).

The usage of herbs as medicines is determined mostly by the community and environment in which one grows up. Addo (2007) carried out a study to determine the socio-demographic characteristics and pattern of use of herbal medicines by women admitted to the Obstetrics and Gynaecology Department in the Komfo Anokye Teaching Hospital (KATH), a teaching hospital serving the Northern part of Ghana and made the following observations: More than fifty percent (50%) of patients used herbal medicines which were mostly unknown to the attending health workers. The less educated as well as the unskilled/ semi-skilled used herbal medicines more frequently compared to their more skilled and educated counterparts. Herbal medicine use is thus more prevalent in the groups who usually have poor socio-economic facilities and carry most of the burden of social deprivation. It is possible that their disease conditions may be adversely affected.

To achieve the desired benefit from herbal preparations, an individual must take the required dose over a certain length of time. Although it is generally believed that most herbal preparations are safe for consumption, some herbs like most biologically active substances could be toxic with undesirable side effects (Bisset, 1994).

Materials and method

Study area

The study was carried out in Makurdi, Benue State. It has a population of 226,198 (1991 census) with a landmass of 16km radius. It has major market like North Bank market, Wurukum market, High level market, Modern market and Wadata market.

Sample collected

Commercially sold herbal formulation was purchased from four different markets(North bank market, Wurukum market, Modern market, and Wadata market). Two samples were purchased from each market and were immediately transported to the Department of Microbiology laboratory of the Federal University of Agriculture, Makurdi for microbiological analysis.

Preparation of culture media

Nutrient agar

This is an all-purpose medium, very rich for the support of bacterial growth, 28 g of Nutrient agar was weighted and dissolved in 1000 ml of de-ionized water and autoclaved, it was allowed to cool before it was aseptically poured into sterile Petri dishes.

Potatoe dextrose agar (PDA)

65g of potatoe dextrose agar was dissolved in 1000 ml of de-ionized water, the powder was be allowed to dissolve and the tube was plugged with cotton wool, covered with aluminium foil and

autoclaved at 121°C for 15 minutes. It was allowed to cool aseptically and poured into a sterile plate. The surface was flamed to remove air bubbles. The plates was allowed to solidify then dried in the hot air dryer 10% lactic acid (0.5ml) was incorporated to prevent the growth of bacteria and other organisms apart from fungi.

Isolation of Microorganisms

One millilitre (1ml) of each sample was measured into sterile test tube containing 9ml of sterilized distilled water. The 10^{-1}suspension was serially diluted using tenfold serial dilution up to 10^{-4}. Aliquot of 1ml of the appropriate dilution was plated in nutrient agar for isolation of bacteria while Potato dextrose agar was used for fungi isolation. The inoculated nutrient agar plates were incubated at 37^{o}C for 24-48 hours, while PDA plates were incubated at room temperature (28^{0}C) for 3-5 days. After incubation, the number of discrete colonies was counted in terms of colony forming units. The viable counts were obtained by reference to the serial dilution used.

Microbiological evaluation of sample

Culture of samples;

Each of the herbal formulation was shaken vigorously and inoculated into the two different media: Nutrient agar for the cultivation of microbes, PDA for isolation and cultivation of pathogenic fungi and yeast.

The liquid herbal formulation was diluted by dissolving 1g of the sample in 9 ml of normal saline, the aliquots (0.1ml) of the herbal preparations was inoculated into plates containing different culture agar and incubated at 37ºC for 24-48 hrs for visible colonies. The plates containing potatoe dextrose agar were incubated at room temperature for 5 days. After incubation, visible colonies that developed were enumerated and recorded as colony forming units/ml (cfu/ml) of cfu/g.

Identification of colonies on the different media

The following parameters that will be used to identify colonies on Nutrient Agar and potatoes dextrose Agar. The different parameters used include; Colour, Elevation, opacity, size, and surface.

Subculture

This was done in order to obtain a pure culture for the identification of organisms, A discrete colony of the organism to be identified was collected from cultured plates, using sterile wire loop and subcultured on mannitol salt agar, salmonella shigella agar, EMBA, using streaking method and incubated for 24 hrs at 37°C for bacteria.

Again a discrete colony from fungi culture was subcultured into another PDA and allowed to grow for 72 hours, after which identification of organism was done.

Characterization of isolates

Gram staining

This is a differential staining which classifies bacteria into gram positive or negative. Thin smears of fresh, pure bacteria cultures were made on a clean grease free glass slides. The slides were allowed to air dry and heat fixed by passing it over a Bunsen burner flame. The slides were placed on a rack over a sink and flooded with crystal violet for 60 seconds, the slides were rinsed with clean running tap water and flooded with iodine for 60 seconds, the slides were rinsed with water and decolorized with 95% alcohol for 30 seconds, the slides were counter stain with safranin 60 seconds, they were rinsed with clean running water and allowed to air dry after which they are viewed under the microscope using oil immersion lens (100x).Gram positive bacteria stained purple while gram negative bacteria stained red.

Biochemical test for identification of bacteria

Indole test

This test is carried out to test the ability of an organism to break down tryptophan, an amino acid to pyruvate and indole. The test organism is inoculated into 5 ml of sterile peptone water and incubated aerobically at 37°C for 24 hrs. The production of indole was tested by adding 0.5 ml of kovac's reagent to the broth culture and shaken gently. A positive result shows a red colour in the alcohol layer which indicates that indole was produced while a negative result shows absence of red colour.

Catalase Test

The test demonstrates the presence of catalase which is an enzyme that catalyses the release of oxygen from hydrogen peroxide (H_2O_2). A colony of 24 hours old culture was picked using a sterile loop and then emulsified in a few drops of hydrogen peroxide on a clean slide. Presence of effervescence indicated catalase positive reaction whereas negative reaction showed no effervescence

Coagulase Test

The slide method described by Cheesbrough (2005) was used for the test. A drop of normal saline was placed on each end of a slide. An 18-24 hours old culture of test organism was emulsified in each of the drops to make two thick suspensions. Thereafter, a drop of human plasma was added to one of the suspensions. The mixture was stirred for about 5 seconds. Clumping of the organism within 10 seconds is a positive test. No plasma was added to the second suspension which is the control to differentiate any granular appearance of the organism from true coagulase clumping.

Urease test

Isolates were inoculated into liquid urea agar supplemented with urea and aseptically dispensed into sterile test tubes, and slanted to solidify. They were incubated at 37^0C for 24-48 hours. Development of bright pink or red color indicates positive urea reaction (Cheesbrough, 2005)

Citrate Utilization Test

Simmon‟s citrate agar was prepared based on manufacturer's instruction, sterilized, poured and allowed to solidify at slant angle of 45^0C. The test organism was streaked on the surface of the agar slant and then incubated at 37^0C for 48 hours. A change in the color of agar medium from green to blue following growth of the organism on the slant indicated a positive test, while no color change indicated a negative test.

Fungal identification

The fungal isolates were identified microscopically using lactophenol cotton blue test. The identification was achieved by placing a drop of the stain on clean slide with the aid of a wire loop. A small portion of mycelium from fungal culture was removed and placed in a drop of lactophenol. The mycelium was spread on a slide with the aid of the wire loop. A cover slip was

gently applied with little pressure to eliminate air bubbles. The slide was then mounted and observed with x10 and x40 objective lenses respectively.

RESULTS

Table 1 Presents the Morphological and bacterial counts of each sample in 4 different markets in Makurdi metropolis. It shows that MM1 (Modern market 1) had the highest counts of $12.5x10^3$cfu/ml, while WD2 (Wadata 2) had the least count of $1.7x10^{3.}$

Table 1: Morphological and colony count of bacteria isolated from herbal formulation from 4 different markets in Makurdi metropolis.

Sample source	Markets	Counts (cfu/ml)

Herbal formulation	NB1	$9.3X10^3$
Herbal formulation	NB2	$3.4X10^3$
Herbal formulation	WD1	$4.2X10^3$
Herbal formulation	WD2	$1.7X10^3$
Herbal formulation	WK1	$2.5X10^3$
Herbal formulation	WK2	$1.8X10^3$
Herbal formulation	MM1	$12.5X10^3$
Herbal formulation	MM2	$1.9X10^3$

Table 2 Shows the average counts of fungal isolates which shows that NB1 (North bank 1) had the highest count of $2.5x10^3$ cfu/ml, while the least count was obtained from WD1 (Wadata 1) with $1.0x10^3$ cfu/ml.

Table 2: Average colony count of fungal isolates from herbal formulation in 4 different markets in makurdi metropolis

Sample source	**Market**	**colony count(cfu/ml)**

Herbal formulation	NB1	2.5 $X10^3$
Herbal formulation	NB2	2.0 $X10^3$
Herbal formulation	WD1	1.0 $X10^3$
Herbal formulation	WD2	2.0 $X10^3$
Herbal formulation	WK1	2.3 $X10^3$
Herbal formulation	WK2	1.4 $X10^3$
Herbal formulation	MM1	1.5 $X10^3$
Herbal formulation	MM2	1.8 $X10^3$

Table 3 and 4 shows the occurrence of bacterial isolates from the sample collected from four different market. *Staphylococcus aureus* had the highest frequency of 37.5% of the isolates in the sample. *Bacillus* spp had the lowest frequency of 12.5% of the bacterial isolates in the sample. (North Bank 1) and WK2 (Wurukum 2) had all the bacterial isolates recorded in this study from herbal formulation, while WD1 (Wadata 1) had only 1 of bacterial isolate.

Table 3: Occurrence of bacterial isolates from herbal formulation in 4 different markets in makurdi metropolis

Isolates	Frequency	Percentage
Staphylococcus aureus	3	37.5%
*Entermoeba*spp	2	25%
Escherichia coli	2	25%
Bacillus spp	1	12.5%

Table 4: Occurrence of the bacterial isolates from the different markets

Sample Source	Markets	*Staphylococcus aureus*	*Enterobacter* spp	*Escherichia coli*	*Bacillus* spp
Herbal Formulation	**NB1**	+	+	+	+
	NB2	+	+	-	-
	WD1	+	-	-	-
	WD2	-	+	+	+
	WK1	+	-	-	+
	WK2	+	+	+	+
	MM1	+	-	+	+
	MM2	+	-	+	+

- = Absent
+ = Present

The percentage occurrence of fungal isolates from the herbal formulation, shows that *Scedoporium*sppand *Penicillium*spp both had the same frequencies of occurrence representing 37.5% of the total fungal isolates. While *Aspergillus* spp, had a percentage frequency 12.5% of the total fungal identified. (Table 5)

Table 5: Percentage occurrence of fungal isolates from herbal formulation in 4 different markets in Makurdi metropolis

Isolates	Occurrence	Percentage
*Scedosporium*spp	3	37.5%
*Penicillium*spp	3	37.5%
Aspergillus niger	1	12.5%
Phialophoraparasiticum	1	12.5%

On table 6the occurrence in percentage of fungal isolates in four different markets is presented. The study shows that WK2 (Wururkum 2) had all the fungal isolates in the herbal formulation identified representing the highest frequency of occurence, while MM1,(Modern market 1) had had the least fungal isolates.

Table 6: Occurrence of fungal isolates from herbal formulation in 4 different markets in Makurdi metropolis

Sample Source	Market	*Scedosporium* spp	*Penicillium* spp	*Aspergillus* spp	*Phialophora* spp
Herbal Formulation	NB1	+	-	-	+
	NB2	-	+	+	+
	WD1	-	-	-	+
	WD2	+	+	+	-
	WK1	+	-	+	+
	WK2	+	+	+	+
	MM1	-	+	-	-
	MM2	+	+	+	-

- = Absent
+ = Present

DISCUSSION

This study has shown that there are varieties of microorganism present in our various herbal formulation which could have resulted from contaminated soils, plants and its products, preparation processes, quality of water, containers and processing equipment. However, these microorganisms exhibit multi-resistance to many antibiotics. Since herbal formulations are mainly prepared for human consumption, there is a very high chance of passing the antibiotics resistant microorganisms into the human ecosystem. This poses a great danger to human health. Since herbal concoctions are prepared using varieties of medicinal plants which contain active constituents that are cheap and effective against common bacterial infections.

A number of sources of contamination of herbal preparation especially during preparation have been identified. The microflora of the final product may represent contaminants from the raw materials, equipment, water, and atmosphere and from personnel. Microorganisms such as *Escherichia coli,* and *Scedosporium* spp reported in this study are generally known to proliferate in potable, distilled and de-ionized water while *Bacillus* spp, *Staphylococcus,Aspergillus* and *penicillin* are commonly isolated from air. The most common source of post preparation herbal

contamination is the packaging vessels. In order to enhance consumer acceptability, most herbalists in Nigeria have adopted the use of bottles and plastic containers as packaging vessels for their preparations. Unfortunately these vessels are not subjected to any form of sterilization after washing them. Contamination of the preparation coupled with the humid tropical environments may result in the proliferation of microbial contaminants in the herbal remedies (WHO, 2007); this probably explains the high microbial counts recorded in this study .Such high levels of microbial contamination have been shown to result in spoilage and degradation of the products or may constitute a health hazard to the user. (Akande*et a*l., 2013) Most herbal preparations are made up of different components of various plant species and these preparations are not standardized with respect to color, taste, consistency etc. Unlike the orthodox drugs, changes in the appearance, odor, taste etc of herbal formulations due to spoilage are hardly readily detected by the patients. (Acharya *et a*l., 2008) Among the microorganisms isolated from the Herbal Medicine, *Bacillus, Staphylococcus, E. coli* and *penicillin* were the major contaminants. Although the pathogenicity of these organisms was not assessed, species of these agents have been incriminated in serious human infections. *Bacillus* spp are widely distributed in the soil, dust, air and water and they are resistant to environment destructive factors. Apart from the unacceptable microbial loads observed in the samples, the presence of contaminants considered to be completely unacceptable in herbal preparations was demonstrated. The most common isolates in the tested samples were Gram-positive organisms belonging to the genera *Bacillus* and *Staphylococcus aureus* are normal commensals of the mammalian skin, hands and mucous membranes. Upon the consideration of the extent of human contact involved in the preparation of herbal medicinal samples, it is most likely that sources of the contaminating *Staphylococcus* spp. are the producers of the herbal formulations this suggests that the level of hygiene of persons involved in the preparation of the tested samples may be low. Similar studies carried out on herbal samples include work by Akande*et al.* (2013); Alakali*et a*l. (2016); Oluyege*et al.(*2010); Okeniyi*et al*. (2005); Oreagba*et al.* (2011); Adeleye*et al*. (2005) have all reported that the pathogens frequently isolated in herbal products were *S. aureus*, *E. coli.* Except for*Scedosporium*which was not isolated in some reports mentioned above. This work varies by reporting a higher count of *Bacillus* spp. Contamination by *Bacillus* spp could have arisen during growth of the herbs as the bacterium is commonly found in soils. *Escherichia coli*, a major faecal coliform may have been introduced from the water used during processing of the herbs.

Microbial contamination of herbal mixture as shown in this study, may also be as a result of the plant materials, utensils used during preparation, poor hygiene of the manufacturer or even the packaging vessel after processing as also suggested by many authors earlier repoted. Microorganisms are present everywhere and can easily contaminate any substrate. Considering the packaging materials, it is worthy of note that this contributes greatly to microbial contamination as the final stage of the processing is packaging. Most of the packaging cans used are not sterilized and are usually picked up where they are found littered along the road or in public places and barely washed before being used to package finished herbal products. It was observed that fungal growth was more than bacterial growth and this is attributable to the low pH value of all the herbal formulation samples which is favorable for fungal growth. The high microbial load and presence of specific pathogens in the tested herbal formulation heave serious clinical as well as pharmaceutical implications. Clinically, consumers of any of these products are at risk of contracting infections by the different pathogens which may be of great consequence if not identified and treated appropriately. Presence *of Eschericha coli* in the sample indicates poor hygiene practices and lack of adequate handling of the products. According to the European pharmacopoeia 2007, no *Salmonella* spp or *Eschericha coli* strain should be present in samples (Oluyege and Adelabu, 2010). The intake of a high concentration of accumulated toxins produced by organisms such as *Bacillus cereus*, *Staphylococcus aureus* and *Aspergillus niger* may lead to undesirable reactions in consumers. Olusegun (2007) reported that the presence of *Bacillus* spp. in herbal preparations is an indication that the water used in the preparation of the products is not from a good source. The risk is greater if the consumer is a young child with undeveloped immunity, an elderly with diminished immunity or the immunocompromised patients. Incidentally, these groups of consumers are the most in need of herbal medicines for the treatment of many diseases to which they are susceptible

CONCLUSION

Staphyococcus aureus, Escherichia coli, Enterobacter spp*, Bacillus* spp*Scedosporidum*spp*Penicilliumspp, Aspergillus niger*, and *Phialophoraparasiticum*sppwere isolated and identified as micro- organisms associated with contamination of herbal formulations sold in markets within Makurdi metropolis, with *Staphylococcus aureus* having the highest bacterial colony count and *Scedosporium*spp having the highest fungal count.

REFERENCES

Acharya, Deepak and ShrivastaAnshu (2008): Indigenous Herbal Medicine: Tribal Formulation and Traditional Herbal Practice, Aaviskhkar Publishers Distributor, Jaipur India Pp 440.

Addo, V.N. (2007). Herbal Medicines: Socio-Demographic Characteristics and Pattern of Use by Patients in a Tertiary Obstetrics and Gynaecology Unit. *Journal of Science and Technology* 27(3): 149-155.

Adeleye IA, Okogi G and Ojo EO. (2005). Microbial contamination of herbal preparation in Lagos, Nigeria. *Journal of Health, Population and Nutrition,* 23(3),296-297.

Akande, T., Agbulu C.O., and Oche, (2013). Microbial Contamination of Herbal Mixtures (Local Concoctions) Used in the Treatment of Typhoid Fever, Malaria Fever, and Dysentery in Makurdi Metropolis.

Alakali, J.S., Ismaila, A.R., Alaka, I.C., Faasema, J. and Yaji, T.A. (2016). Quality Evaluation of Herbal Tea Blends from GingerandPavettacrassipes. *European Journal of Medicinal Plants* 12(4): 1-8

Bisset,N.G.(1994). Herbal Drugs and Phyto pharmaceuticals. CRC Press, Boca Raton. 82p.

Kafaru, E. (1994). Immense help formative workshop. In:131in the treatment of diabetes in Southwestern Nigerian. *Journal of Medicine and Medical Sciences* 4 (11): 423-432.

Khan, R., Islam, B., Akram, M., Shakil, S., Ahmad, A., Ali, M.S., Sadiqui, M., Khan, A.U. (2008). Antimicrobial Activity of Five Herbal Extracts against Multi Drug Resistant (MDR) strains of Bacteria and Fungus of Clinical Origin. Molecules13:97-103

Kneifel W, Czech E and Kopp B. (2002). Microbial contamination of medicinal plants-A review. Planta Medica, 5-15, 68.

Kong, J.,Goh,N..Chia, L. and Chia, T. (2003).Recent Advances in Traditional Plant Drugs and Orchids.Acta Pharmacology 24(1):7-21

Nyarko, K.N., Aseidu-Gyekye, I.J. andSittie, A.A. (2005). A Manuel of Harmonized Procedures for Assessing the Safety, Efficacy and Quality of Plant Medicines in Ghana, Ministry of Health: The Ghana National Drugs Programme. 202pp.

Ochei (2007). Medical Laboratory Sciences, Theory and Practical pp 568-9. Kafaru, Elizabeth, immerse “Help from Nature’s Workshop”. Guide Lines on to Use Herbs to Achieve a Healthy Living, as an individual responsibility.

Ogbonnia, S.O., Mbaka, G.O., Emordi J., Nkemhule, F., Joshua, P., Usman, A., Odusanya, P.and Ota, D. (2013). Antimicrobial evaluation and acute and sub-acute toxicity studies on a commercial polyherbal formulation, “Ade & Ade Antidiabetic®” used

Okeniyi,J.A., Ogunlesi,T.A., Oyelami,O.A. and Adeyemi,L.A. (2007). Effectiveness of dried Caricapapayaseeds against human intestinal parasitosis: a pilot study. *Journal of Medicine and Food*10:194-196.

Oluyege J.O. and Adelabu, D.M. (2010). Microbial Contamination of some Hawked Herbal Products in adoekiti, Nigeria. Continental Journal of Microbiology.4: 8 -14

Olusegun V. Oyetayo (2007):Microbial Load and Antimicrobial Property Of Two Nigerian Herbal Remedies. African Journal of Complementary and Alternative Medicines 5(1):74-78

Oreagba, I.A., Oshikoya, K.A. and Amachree, M. (2011). Herbal medicine use among urban residents in Lagos, Nigeria. *BMC Complementary and Alternative Medicine* 11:117-125.

Sofowora, A. (1996). Research on Medicinal Plants and Traditional Medicine in Africa. *Journal of Alternative and Complementary Medicine* 2(3):365-372.

WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues (2007).

Yidana, J.A. and Bayorbor, T. (2002). The Multi-Purpose Value of Medicinal Plants and the Potential for Cultivation. *Journal of the Ghana Science Association* 4(2): 146-158.