Microbial Contamination of Herbal Mixtures (Local Concoctions) Used in the Treatment of Typhoid Fever, Malaria Fever, and Dysentery in Makurdi Metropolis.

Akande, T., Agbulu C.O., and Oche, M.
Department of Biological Sciences, College of Science, University of Agriculture, Makurdi, Nigeria
Email: atitilayo@ymail.com

ABSTRACT

Microbiological analysis of Herbal mixtures (local concoctions) used in the treatment of typhoid fever, malaria fever and dysentery was carried out to investigate and evaluate the microbial qualities. The study was carried out on five sales outlets namely; North Bank, Wurukum, High Level, Modern Market and Wadata. The viable plate count ranges between 1.0-9.4 X 10^{-5}cfu/ml for bacteria and 1.0 - 8.0 x 10^{-5}cfu/ml for fungi. The highest viable count of bacteria were from typhoid fever samples collected from modern market which was about 9.4 x 10^{-5}cfu/ml and it shows a higher level of contamination with *Escherichia coli* and *Klebsiella species.* Malaria fever samples collected from Modern Market also had the highest viable count of fungi which was about 8.0x10^{-5} and it also shows a high level of contamination with *Alternaria species* and *Rhizoid species*. The quality of most of the herbal medicine was unaccepted as *Escherichia coli, Candida species*, *Salmonella species, Bacillus species, Alternaria* were isolated. Most of the herbal medicine can cause harm to consumers and can be source of enteropathogenic organisms that cause anthrax, typhoid fever, hepatitis which can lead to death. There is need to institute not only sanitary measures, but also infrastructural development since most of these herbal medicine were contaminated due to failed storage, packing, handling of raw materials.

Keywords: Microbial, Herbal Mixtures, Typhoid, Malaria, Dysentery

INTRODUCTION

Mankind has used plants in an attempt to cure diseases and relieve physical suffering in earliest time. Primitive people in all ages had some knowledge of medicinal plants and herbs derived as a result of trials and error. These primitive attempts at medicine were

based on speculation and superstition (Sharma, 2006). 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).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) After dark ages, there came the period of the herbalist and encyclopedists. It was a time the herbalist believes the harmony represented in the plants will transfer into optimal success in treating ailments (Gaster *et al.,* 2000).Oxford advanced dictionary of biology defined herbs as plants with medicinal or culinary uses. Herbal medicine also refers to phytomedicine or botanical medicine or herbalism or herbology and phytotherapy is the use of plants in a wide variety of forms for their therapeutic value for treatment of diseases and other ailments caused by micro-organisms or other toxins (Acharya *et al.*, 2008). Herbal medicine have been used and practiced long before pharmaceutical discoveries. Herbal plants have been of immeasurable value to mankind in the provision of drugs after extraction of active ingredients in them in orthodox medicine for curing ailments traditionally. 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). According to Dr. I .A Adeleye, concoction used to treat typhoid, malaria and dysentery are contaminated with microbes. He reported that the level of microbial contamination clearly exceeded the tolerable limits and the presence of a large number of pathogenic organisms. The organisms he said were *Staphylococcus aureus, Eschericha coli, Rhyzopus stolonifer, 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). Since local concoctions or herbal medicine are widely consumed for treating disease and illness, the study will help ascertain the microbial quality and thus aid in determining their safety for concoctions. The result of this study will also help in providing information on these products which will promote healthier handling of the products by herbalist, the sellers and the consumers.

Therefore, the aim of this study was to ascertain the extent of contamination of local concoctions (Agbo) used in treatment of malaria fever, typhoid fever and dysentery. Also, to isolate and identify the common microorganisms associated with local concoctions sold to people in Makurdi metropolis.

SAMPLE COLLECTION

Samples were obtained from North Bank market, Wurukum market, High-level Market, Modern market and Wadata market respectively. Also, Identification of the plants were done by the forestry department of the University of Agriculture, Makurdi. Material samples for treatment of typhoid fever include: *Cochlo spermum, Carica papaya, Citrus microcarpa, Moringa oleifera, Annickia chlorantha, Citrus aurantifolia*.Material samples for the treatment of dysentery include: *Khaya senegelensis, Tetrapleura tetraptera, Alliumvineale, Piper Guieense, Parinari specie, Eugenia camyophyllus, Ancistrophyllum secundiflorum.*

Materials samples for the treatment of malaria fever include: *Annickia chlorantha*, bark of *Mangifera indica, Latifolia, Citrofortunella mitis, Nauclea, Hippocratea indica*.

MEDIA PREPARATION/ CULTURAL PREPARATION

Macconkey Agar (MA), Nutrient Agar (NA), Potatoes Dextrose Agar (PDA), and Cysteine Lactose Electrolyte-Deficient (CLED) were prepared according to the manufacturer's instruction.

RESULTS

The results obtained from the microbiological analysis of concoctions used in the treatment of Malaria fever, Typhoid fever, and Dysentery are shown below:

Table 1 shows the viable bacteria count and organisms isolated in concoction for malaria fever. Bacillus species was found in Wadata, Wurukum concoction for malaria fever, *Moraxella* in high level concoctions, *Enterococcus* species in north bank and *Klebsiella* in Modern Market. Modern Market had the highest viable count of $7.1X10^{-5}$cfu/ml and Wurukum with the least viable bacteria count of $1.2X10^{-5}$cfu/ml.

Table 2 shows the viable bacteria count and organisms found in concoction for typhoid fever. It was observed that bacillus species was found in north bank, Wurukum, Wadata samples. *Klebsiella, Escherichia coli* in Modern Market, *Salmonella* Species in High Level. Modern Market has the highest viable

bacteria count of $9.4X10^{-5}$cfu/ml and North bank had the least viable count of $1.2X10^{-5}$cfu/ml.

Table 3 shows the viable bacteria count and organisms present in the concoction for dysentery. *Escherichia coli* were found in North Bank and Wadata, *Pseudomonas* species in Wurukum, *Salmonella* species, *Moraxella species* in High Level. Wadata had the highest viable bacteria count of $7.6X10^{-5}$cfu/ml and High Level had the least of $3.1X10^{-5}$cfu/ml.

Table 4 shows the fungi colony count of malaria fever and organisms isolated from sample. *Alternaria* species was found in malaria fever concoction for North bank, High level, and Modern Market. *Penicillin* species from Wurukum concoction, *candida* species from Wadata concoction. Modern market had the highest viable fungi count of $8.0X10^{-5}$cfu/ml and North Bank, Wurukum the least count of $1.0X10^{-5}$cfu/ml.

Table 5 show the fungi count and organisms present in concoction for typhoid fever. *Alternarian* was observed to contaminate most of the concoction samples. Wadata had the highest fungi count of $8.0X10^{-5}$cfu/ml while Wurukum had the least fungi count of $3.3X10^{-5}$cfu/ml.

Table 6 shows that north bank market and modern market had the highest fungi count of $4.4X10^{-5}$cfu/ml in dysentery concoction.

Table 7 shows that *Bacillus* had the highest percentage occurrence of 33% and *Staphylococcus* species, *Streptococcus pneumoniae* had the least percentage occurrence of 6.67%.

Table 8 shows that *Altenaria* had the highest occurrence of 53% and *Fusarium, penicillium* and *Mucor* with least percentage of 6.67%.

Table 9 shows the total and average colony count of bacteria and fungi. Concoction for typhoid had the highest total colony count of $25.8X10^{-5}$cfu/ml while Malaria concoction had the total of $16.3X10^{-5}$cfu/ml for bacteria. Concoction for typhoid had the highest total colony count of $23.1X10^{-5}$cfu/ml while dysentery had the least total count of $15.5X10^{-5}$cfu/ml.

Table 10 shows the biochemical characteristics of isolates.

Figure 1 shows the histogram of frequency of occurrence of bacteria and fungi isolates in all the samples analyzed.

Table 1: Bacterial colony count of concoction for malaria fever and organisms isolated from all sampling sites.

Sample sites	Organisms Isolated	Cfu/ml or cfuml^{-1}
North Bank Market	*Enterococcus species*	4.0X10^{-5}
Wurukum Market	*Bacillus species*, *Escherichia coli*, *Pseudomona species*	1.2X10^{-5}
High Level Market	*Moraxella species*	2.0X10^{-5}
Wadata Market	*Bacillus species*	2.0X10^{-5}
Modern Market	*Klebsiella species, Escherichia coli*	7.1X10^{-5}
Total counts		**16.3X10^{-5}**

Table 2: Bacterial colony count of concoction for typhoid fever and organisms isolated from all sampling sites.

Sample sites	Organisms Isolated	Cfu/ml or cfuml^{-1}
North Bank Market	*Bacillus species*	1.2X10^{-5}
Wurukum Market	*Streptococcus pneumoniae*, *Pseudomonas species*	4.5X10^{-5}
High Level Market	*Salmonella species*	3.1X10^{-5}
Wadata Market	*Eschericha coli*	7.6X10^{-5}
Modern Market	*Bacillus species Klebsiella species Escherichia coli*	9.4X10^{-5}
Total counts		**25.8X10^{-5}**

Table 3: Bacterial colony count of concoction for dysentery and organisms isolated from all sampling sites.

Sample Sites	Organisms Isolated	Cfu/ml or cfuml^{-1}
North Bank Market	*Esccherichia coli*, *Staphylococcus species*	$3.8X10^{-5}$
Wurukum Market	*Bacillus species*, *Escherichi, coli, Pseudomona species*	$7.4X10^{-5}$
High Level Market	*Moraxella* Species	$2.0X10^{-5}$
Wadata Market	*Bacillus species*	$2.0X10^{-5}$
Modern Market	*Klebsiella species, Escherichia coli*	$7.1X10^{-5}$
Total counts		**$22.3X10^{-5}$**

Table 4: Fungi colony count of concoction for malaria fever and organisms isolated from all sampling sites.

Sample Sites	Organisms Isolated	Cfu/ml or cfuml^{-1}
North Bank Market	*Alternaria species*	$1.0X10^{-5}$
Wurukum Market	*Penicillium species*	$1.0X10^{-5}$
High Level Market	*Alternaria species*	$2.0X10^{-5}$
Wadata Market	*Candida species*	$7.0X10^{-5}$
Modern Market	*Rhizopus species*, *Alternaria*	$8.0X10^{-5}$
Total counts		**$19.0X10^{-5}$**

Table 5: Fungi colony count of concoction for typhoid fever and organisms isolated from all sampling sites.

Sample Sites	Organisms Isolated	Cfu/ml or cfuml^{-1}
North Bank Market	*Alternaria species*	$4.0X10^{-5}$
Wurukum Market	*Alternaria species*	$3.3X10^{-5}$
High Level Market	*Alternaria species, Candida species*	$4.0X10^{-5}$
Wadata Market	*Fusarium species*	$8.0X10^{-5}$
Modern Market	*Rhizopus species, Alternaria species*	$3.8X10^{-5}$
Total counts		**$23.1X10^{-5}$**

Table 6: Fungi colony count of concoction for dysentery and organisms isolated from all sampling sites.

Sample Sites	Organisms Isolated	Cfu/ml or cfuml^{-1}
North Bank Market	*Pennicillium species, Alternaria species, Rhizopus species, Candida species*	4.4X10^{-5}
Wurukum Market	*Candida species*	3.0X10^{-5}
High Level Market	*Alternaria species*	2.0X10^{-5}
Wadata Market	*Alternaria species*	1.7X10^{-5}
Modern Market	*Candida species, Alternaria species*	4.4X10^{-5}
Total counts		**15.5X10^{-5}**

Table 7: Percentage occurrences of bacterial isolates in local concoctions used in the treatment of Malaria fever, Typhoid fever and Dysentery

Microbial Isolates	Number of samples Isolates	Total number of samples examined occurrence	Percentage
Enterococcus species	1	15	6.67%
Bascillus species	*5*	15	33.33%
Escherichia coli	*3*	15	20.00%
Moraxella species	*2*	15	13.33%
Klebsiella Species	2	15	13.33%
Salmonella species	3	15	20.00%
Staphylococcus Species	1	15	6.67%
Streptococcus pneumoniae	1	15	6.67%
Pseudomonas species	3	15	20.00%

Table 8: Percentage occurrence of fungi isolates in local concoctions used in the treatment of malaria fever, typhoid fever and dysentery

Microbial Isolates	Number of samples Isolates	Total number of samples examined occurrence	Percentage
Alternaria species	8	15	53.33%
Candida species	*4*	15	26.67%
Fusanium species	*1*	15	6.67%
Rhizoid species	*2*	15	13.33%
Penicillium species	1	15	6.67%
Mucor	1	15	6.67%

Table 9: Showing total and average numbers of bacteria and fungi from fifteen samples collected.

	Total number of colony count (cfu/ml) Bacteria	Average count	Total number of colony count (cfu/ml) Fungi	Average count
Malaria fever	16.3	$3.26x10^{-5}$	19.0	$3.8X10^{-5}$
Typhoid fever	25.8	$5.16x10^{-5}$	23.1	$4.62X10^{-5}$
Dysentery	22.3	$4.46x10^{-5}$	15.5	$3.1X10^{-5}$

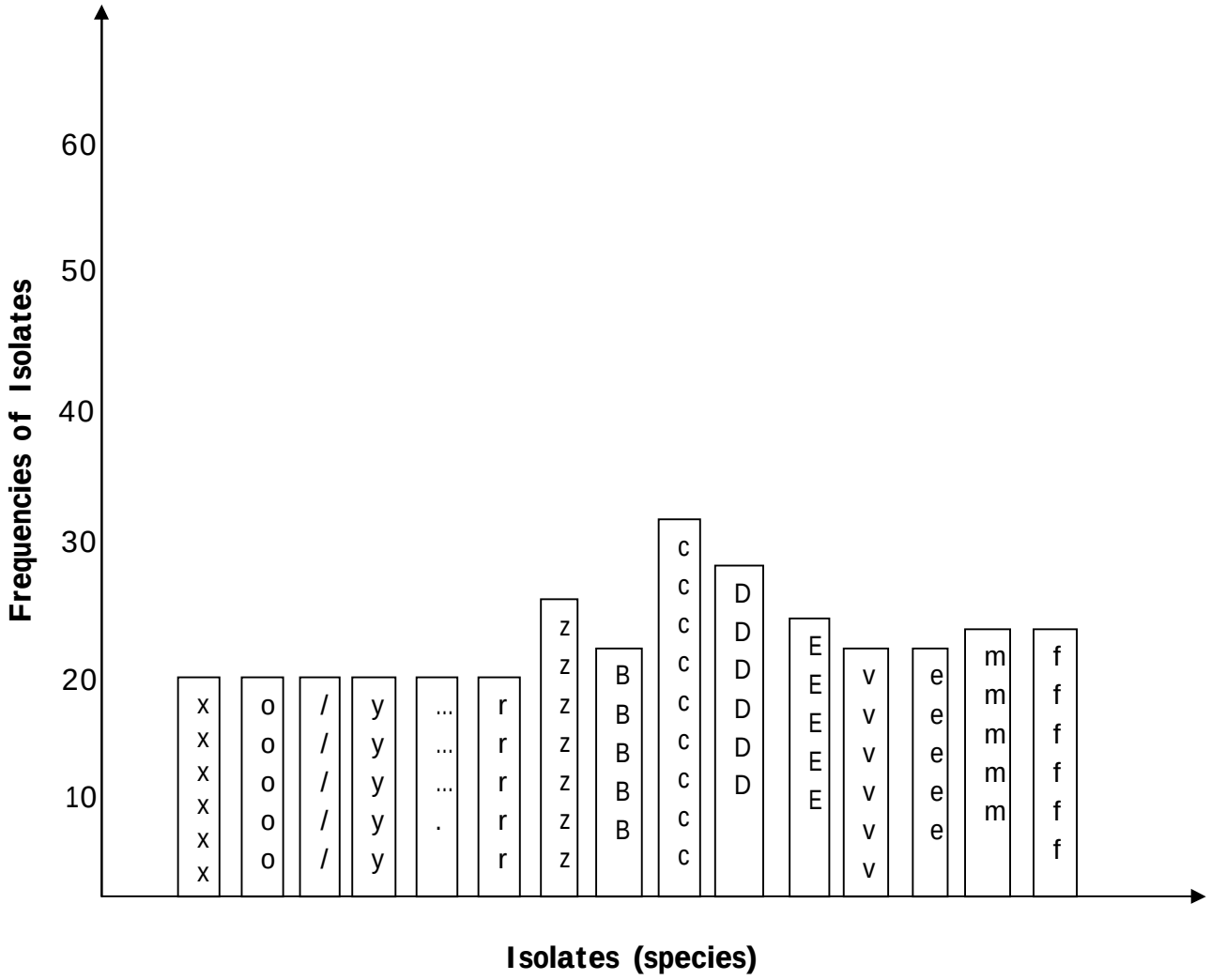
Table 10: Biochemical Characteristic of bacterial isolates

Bacterial	Gram Reaction	MOT	CAT	COAG
Bacillus Species	ve rods in chains and clusters, with Clear colonies	+ve	+ve	ND
Enterococcus Species	-ve cocci in pairs and short chains	-ve	-ve	ND
Eschericha coli	-ve rods in clusters	-ve	-ve	ND
Klebsiella Species	-ve rods	-ve	-ve	ND
Moraxella Species	large and cocci	-ve	-ve	ND
Pseudomonas Species	-ve rods, slightly curved	-ve	-ve	ND
Salmonella Species	-ve rods, slender and scattered	-ve	-ve	ND
Staphylococcus Species	+ve cocci in clusters	-ve	-ve	-ve
Streptococcus Pneumonae	+ve capsulated *diplococci*	-ve	-ve	-ve

KEY

-ve = Negative
+ve = Positive
COAG = Coagulase Test
CAT = Catalase Test
MOT = Motility Test
ND = Not Done

Figure 1: A histogram showing the frequencies of occurrence of bacteria and fungi isolates in all the Samples.



KEYS

xxx xxx	*Enterococcus species*
rrr	*Escherichia coli*
EEE	*Penicillium species*
ooo	*Staphylococcus species*
zzz	*Salmonella species*
ww	*Mucor*
///	*Streptococcus species*
bbb	*Pseudomonas species*
eee	*Rhizoid*
vvv	*Moraxella species*

cccc	*Bacillus Species*
mm	*Candida Species*
….	*Klebsiella Species*
DD	*Fusarium Species*
fff	*Alternaria Species*

DISCUSSION

In this study, it was discovered that *Alternaria species* was the highest among the fungi isolated from the Herbal mixtures with percentage occurrence of 53.33% and *Bacillus* was the highest bacteria isolated from the Herbal mixtures with percentage occurrence of 33.33%. *Enterococus species, Escherichia coli*, *Pseudomonas, Moraxella, Klebsiella, Salmonella, Streptococcus, Streptococcus pneumonae* are found in the local concoctions. Fungi like *Penicillium*, *Candida, Rhizoids, Fusarium, Mucor,* were also fund in the local concoctions. Similar findings have been reported by other writers who examined plant materials and discovered that it normally carry a large number of microbes originating from the soil (Adeleye, 2005)*Escherichia coli, Salmonella* specie and *Pseudomonas* species were found to be the second dominant bacteria in the local concoctions. These species account for more than 50% of all isolates in nosocomial infections. Their widespread involvement in hospital acquired infections can be attributed to their constant in the hospital environment and their survival capabilities. *Escherichia coli* causes a severe diarrhea illness brought on by exotoxines and the rate of infection is higher in crowded tropical regions were sanitary conditions are poor, water supplies are contaminated and where adults carry pathogenic strains. Also low grade fever, nausea and vomiting are symptoms associated with the infections (Kathleen, 2008) *Salmonella specie* had been reported the assaultive agent of many diseases, including typhoid fever. In parts of the world typhoid fever is still serious health problem responsible for 25,000 death each year and probably millions of cases. Removal of the gallbladder may be necessary in individuals with chronic gallbladder

information. *Klebsiella species* are said to lead chronic lung infection and can cause urinary tract infections. *Escherichia coli* are also reported to cause urinary tract infection and it's currently one of the indicator bacteria to monitor fecal contamination in water, food and dairy products. *Escherichia coli* according to its rationale; if *Escherichia coli* are present in a water sample, fecal pathogens such as salmonella, viruses and even pathogenic protozoa may also be present which can also lead to kidney damage, hepatic anemia. The disease mentioned all can be acquired as a result of taking any concoction of the three which shows t not healthy for consumption, even though it is highly medicinal.

Moraxella are weakly pathogenic but can still cause ear infections and conjunctivitis in humans. *Streptococcus pneumonae* causes upper respiratory tract infection that spread to meningitis (Kathleen, 2008).Staphylococcus most common infection is a mild, superficial inflammation of the hair follicles (follicutitis, boils, cardiac abnormalities). Herbs medicine or concoction harbor all the bacteria mentioned and it had been observed that instead of healing, it causes more damage and destruction to the system of humans. Most of the bacteria inhabitant is soil and water, and that makes it so available in the concoctions. *Candida specie*, one of the fungi isolated from the concoction causes infection of the mouth, skin, alimentary canal, vegina, lungs and even disseminate to internal organs. Volvovaginal conditions (vc) known more commonly as yeast infection has widespread occurrence in adult women, and it's caused by the fungi *Candida species. Candida vaginititis* also possess a risk for neonate, can be infected during child birth for baby which the mother such concoctions during pregnancy and can be transmitted to male partners during sexual intercourse. Candida also infects the oesophagus and anus causing painful bleeding ulceration, nausea, and vomiting, affecting skin and nails called omychomycosis. Candida in the blood is such a serious assault that it causes more human mortalities than any other fungal pathogen (Kathleen, 2008)*Fusarium* a common soil habitant and plant pathogen also found in the concoction occasionally infect the eyes, toe nail, and burned skin, also infect nail bed and cause Tinea urgrum. *Alternaria* causes chromblastomycosis, a disease highly characterized by visible verrucous lesions. Mucormycosis is a disease condition or disease that is contacted by critically ill patients (Kwashiorkor child, Leukemia patients)

it is cause d by mucor. The position of fungi in the biological world has been detected for years causing different damages to human and also reducing human life span. Bacteria commonly reproduce by binary fission, which shows that one cell in a man's system divide in two increasing the population by geometric progression (Michael *et al.*, 2005) and also the infections it can cause. *Bacillus specie* causes anthrax, pseudomonas burn infections. Herbal medicine can be indeed harmful if not well prepared.

CONCLUSION

From the study, some of the organisms discovered were pathogenic. *Bacillus* species is said to cause anthrax in man, *Staphylococcus* species causes boils and food poisoning. Local concoctions carry a lot of microbes which as a result can cause harm when consumed. All other organisms present in the concoctions were due to plant materials used and water. This is because the soil inhabits almost the organism and also water because of the poor hygiene. Herbalist should be enlightened by the government concerning the contamination possible ways and should be taught to produce bacteria and fungi free herbal medicine.

RECOMMENDATIONS

Following the results obtained, listed below are recommendation that should be adopted.

i. A primary visual evaluation which seldom needs more than a simple magnifying lens can be used to ensure that the plant is of the required species.
ii. Good method of harvesting, cleaning, drying, handling and storage of herb stems, root and leaves should be adopted.
iii. Hygiene should be ensured when preparing local concoctions.

REFERENCES

A. B. Heber D. (2008). "Multi-targeted Therapy of Cancer by Ellagitannins". Cancer Lett 289 (2): 262-268. doi: 10.1016/ J. Canlet. 2008.03.043. PMID 18468784.

Acharya, Deepak and Shrivasta Anshu (2008): Indigenous Herbal Medicine: Tribal Formulation and Traditional Herbal Practice, Aaviskhkar Publishers Distributor, Jaipur India ISBN 978-81-7910-252-7. Pp 440.

Adeleye (2005), Microbial Contamination of Herbal Preparation in Lagos "Nigeria"

Journal Health Population and Nutrition. 23(3) 296-7.

Challen, Jack (January, 1999). "The Problem with Herbs". Natural Health. Retrieved 2010-12-23.

Cunningham, A. B. (1994), Integrating Local Plant Resources and Habitant Management. Biodiv. & Conserve. 3=104-115.

Eric Yarnell, N.D., R. H., and Katthy Abascal, J. D. (2002). "Dilemmas of Traditional Botanical Research". Herbalgram. 55:46-54.

Gaster, B & Holroyd, J (2000). "St. John's Worth for Depression: a Systematic Review". Achieves International Medicine 160 (2): 152-6. doi:10.1001/Archinte. 160.2.152. PMID 106647752.

Gill, L. S. (1988), Ethno Medical uses of Plants in Nigeria Ibadan University Press Pp 276.

Goldman, Peter (2001)."Herbal Medicine Todays and the Roots pf Modern Phamacology" PDF. Annals of Internal Medicine135 (8 pt 1): 594-600. PMID 11601931.

Govender S. DU Plessis-D., Dowing T. G. and Vande Venter M. (2006), Traditional Herbal Medicines: Microbial Contamination. Consumer Safety and "The Need for Standards". South Africa J. Sc. 102: 253-5.

Hearst R, *et al*. (2009). "An Examination of Bacterial and Antifungal Properties of Constituents of Shiitake (Lentinus Edodes) and Oyster (Pleurotus Ostreatus) Mushrooms". Complement their Clin. Pract. 15 (1): 5-7. doi:10.1016/j.ctcp.2008.10.002. PMID 19161947.

Huffman, MA (May, 2003). "Animal Self Medication and Ethno-Medicine: Exploiration and Exploitation of the Medicinal Properties of Plants" (In Hindi). Proc Nutr Soc 62 (2): 371-81. doi: 10. 1079/ pns 2003257. PMID 14506884.

Iwu, M. M. (1993) Handbook of African Medical Plants. Boca Raton Ckc Press Pp 435.

J. Ochei (2007). Medical Laboratory Sciences, Theory and Practical pp 568-9. Kafaru, Elizabeth, Immerse "Help from Nature's Workshop". Guide Lines on how to

Use Herbs to Achieve a Healthy Living, as an individual responsibility.

Stethberger *et al.,* (1996), Economics of Medical and Condiment Plants. Germuse- Muchen 32(2). 117-8.

WHO (1998) Quantity Control Methods for Medical Plants. Determination of Microorganism Behavior. Technical Report Series Number 18 pp 64-73.

Srinivasan K. (2005). "Spices as Influencers of Body Metabolism: an Overview of Three Decades Research". Food Research International 38 (1): 77-86. doi:10.1016/j.foodres.2004.09.001.

Tapsell *et al.,* (August, 2006) "Health Benefits of Herbs and Spices: The Past, the Present, the Future". Med. J. Aust. 185(4 Suppl.): S4-24. PMID 17022438.

Reference to this paper should be made as follows: **Akande T. et al., (2013)** Microbial Contamination of Herbal Mixtures (Local Concoctions) Used in the Treatment of Typhoid Fever, Malaria Fever, and Dysentery in Makurdi Metropolis, *J. of Medical and Applied Biosciences, Vol.5, No.1, Pp. 31-45 .*

Biographical Note: Akande T

Biographical Note: Agbulu C.O

Biographical Note: Oche, M.