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Quality Assessment Of Aqueous Herbal/Medicinal Products Sold On The Ghanaian Market.

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Abstract

The microbial quality of sixteen (16) decoctions sold on the Ghanaian market were investigated and the isolated microbes characterized to the species level. The results indicated that, almost all the decoctions were contaminated with aerobic bacteria and/or fungi. The highest microbial counts greater than 1.0×10^9 cfu/ml were found in three of the samples with only one out of the sixteen samples having the lowest aerobic bacterial count of 1.0×10^2 cfu/ml. Fungal contaminations were found in thirteen (81.3%) of the samples with only three found to be free from fungal contamination. The highest fungal contamination of 3.2×10^5 cfu/ml was found in only one sample. The characterization of the isolates revealed six bacterial genera and eight fungal genera with *Bacillus subtilis* (50.0%) and *Cladosporium herbarum* (34.5%) being the predominant bacterial and fungal isolates respectively. The analysis of the samples after three months storage showed that the microbial load of the decoctions were within the acceptable limits whilst the microbial isolates also showed a reduction for *B. subtilis* (23.8%) and *C. herbarum* (14.3%). The study revealed that most of the decoctions sold on the Ghanaian market are contaminated with both bacteria and fungi.

Key words: herbal medicine, aerobic plate count, spoilage microbes, microbial characterization

1.0 Introduction

World Health Organization estimates that, 80% of the populations of most countries of the world rely on herbal or indigenous forms of medicine for some aspect of primary healthcare and that about 74% of the 119 plant-derived pharmaceutical medicines are used in modern medicine in ways that correlate directly with their traditional uses as plant medicine in native cultures (WHO, 2003). In Africa, herbal medicine represents a way of life of the people, where it has proven very effective, less intrusive and less harmful than conventional drugs like antibiotics (Hoareu and Da Silva, 1999). Herbal medicine is the first line of choice for the home treatment of nearly two thirds of children with high fever from malaria in Ghana, Mali and Zambia (Aschwanden, 2001). The reasons for the high patronage of herbal medicine are the high cost of very effective orthodox medicines and the problem of drug resistance which is very common in developing countries (Okeke *et al*., 1999; Hack, 2005).

Herbal medicine is more accessible to most of the populations in developing countries and is the staple of medical treatment in many developing countries (Abbiw, 1990). Traditional herbalists use various herbal preparations to treat various types of ailments, including diarrhoea, cough, convulsions, skin diseases and others (Sofowora, 1982). Visits to Orthodox doctors are reserved for life-threatening or hard-to-treat disorders (Abbiw, 1990). Increased use of plant medicines therefore has the potential of improving public health and lowering health care costs (Calixto, 2000).

Ghana today has realized an increase in the public awareness and usage of herbal medicinal products in the treatment and/or prevention of diseases. The relatively high cost of the conventional pharmaceutical dosage forms and inaccessibility of the orthodox medical services to most people particularly in the rural areas are contributing factors. With this increased usage, health authorities and health professionals are concerned about the safety, efficacy and quality of these medicines. The methods used in harvesting, handling, processing, storage and distribution of herbal medicines subject them to contamination by various microorganisms, some of which may be responsible for spoilage (Abou-Arab *et al*., 1999). Medicinal plant materials normally carry a large number of microbes originating from the soil while various kinds normally adhere to leaves, stem, flowers, seeds, and roots (Adeleye *et al*., 2005). Additionally, contaminants may be introduced during harvesting,

handling, and production of various herbal remedies since no conscious efforts are made to decontaminate the herbs other than by washing them (Adeleye *et al*., 2005). This study is designed to investigate the bacterial and fungal contaminants that contribute to spoilage of aqueous herbal medicinal products from selected herbal products in Ghana. The objective is to culture, isolate and identify specific microbes, to determine the microbial load of each product and to characterize each isolate.

2.0 Materials and Methods

2.1 Study Area

The study was conducted at the Microbiology Laboratory of the Centre for Scientific Research into Plant Medicine, Mampong Akwapim, Ghana.

2.2 Sample collection

Over a six-month period, a total of sixteen (16) decoctions were sampled for their microbial quality. The selection of the samples were not random. The study monitored the samples which were brought for analysis at the Centre during the period of the study. The weights of these herbal medicines were 330ml each and all the finished products were freshly made at the time of the study.

2.3 Method

For each sample, 1ml quantities were dissolved in 9ml of sterile peptone water in test tubes and homogenized using the Vortex mixer for 10 seconds. Ten-fold serial dilutions were made and viability assessed using the pour plate and/or spread plate method in triplicates and plates incubated at 30°C for 3 days in the case of bacteria and at 25°C for 3-5 days for yeasts and moulds. All isolates were maintained on nutrient agar slants and stored at -4 ºC.

2.4 Microbial load count

Bacteria were enumerated on Plate Count Agar, Violet Red Bile Agar, deMann-Rogosa-Sharpe Agar and Glucose Yeast extract Calcium Carbonate Agar. Yeasts and moulds were enumerated on Malt Extract Agar containing specific antibiotics. The viable aerobic bacterial count were assessed using well established methods.

2.5 Microbial identification

The pure isolates were examined by their colonial and cell morphology, Gram reaction and other biochemical tests. Identification of species were carried out by assaying pure cultures in Analytical Profile Index (API) galleries (Biomerieux, France).

3.0 Results and Discussion

3.1 Results

The results presented in Table 1.0 shows the mean microbial load counts of the sixteen decoctions that were analyzed. All the sixteen freshly prepared decoctions were contaminated to various extents by aerobic bacteria with only seven (43.8%) out of the sixteen counts falling within the acceptable range. Microbial counts greater than 3.0 x 10^9 cfu/ml were found in ASA, MOD and CPT whilst the lowest aerobic bacteria count of 1.0 x 10^2 cfu/ml was found in DDA. Fungal contaminant were found in thirteen (81.3%) out of the sixteen decoctions with MMN, ETA and LXV not contaminated by any fungus. The highest fungal contamination of 3.2 x 10^5cfu/ml was found in CPT. In all only four out of the sixteen products achieved the EP 2007 specifications for fungal counts. The results of the aerobic plate counts (APC) of the decoctions after three months (Table 1.0) showed reductions in the microbial load for all sixteen samples analyzed with none of the counts exceeding the acceptable limits. In the case of the counts on malt extract agar, it was found that only two failed to achieve the specifications after three months (European Pharmacopoeia, 2007). The results of this study have shown that the microbial load count of the decoctions after three months storage was of better microbial quality compared with the freshly prepared products. The microbial content of the decoctions investigated (Table 2.0) confirmed the presence of six bacterial and twelve fungal species in the freshly prepared samples. Of the isolated bacteria, *Bacillus subtilis* accounted for 50.0% (11/22) of the total bacteria isolated from the products. *Bacillus coagulans, Bacillus licheniformis* and *Enterobacter aerogenes* accounted for 18.2% (2/22), 13.6% (3/22) and 9.1% (2/22) respectively. Both *Klebsiella oxytoca* and *Serratia odorifera* accounted for 9.1% of the bacteria population isolated.

The fungal population of the freshly prepared decoctions revealed that 34.5% (10/29) of the isolates were *Cladosporium herbarum*. *Aspergillus ustus, Aspergillus oryzae, Aspergillus sulphureus, Saccharomyces*

kluyverii, Rhodotorulla minuta, Candida membranifasciens and *Sporobolomyces salmonicolor* together accounted for 24.1% (7/29) of the total mycopopulation. *Trichosporon mucoides* and *Penicillium digitatum* both accounted for 27.6% (8/29) of the total mycopopulation, while *Aspergillus niger* and *Mycelia sterilia* accounted for 6.9% each.

The results of the investigation on the decoctions after three months of production revealed the isolation of seven microbial genera consisting of one bacteria genus, two yeasts and four moulds. The most isolated species were *Bacillus subtilis* (5/21), followed by *Zygosaccharomyces spp* (4/21), and *Cladosporium herbarum* (3/21), accounting for 23.8%, 19.1% and 14.3% of the total microbial population respectively.

Table 1.0: Mean Microbial load of finished herbal products (Decoctions).

Key: - = No growth**,** PCA= Plate Count Agar, MEA= Malt Extract Agar**,** TNTC= Too Numerous To Count

Product Name	**Mean Microbial load (cfu/ml)**			
	Freshly Prepared		**After 3 Months storage**	
	PCA	**MEA**	**PCA**	**MEA**
ASA	TNTC	$9.0x10^4 \pm 2.00$	$7.2\ x10^4 \pm 4.93$	$1.8\ x10^3 \pm 2.52$
NGR	$1.0x10^4 \pm 0.58$	$1.0x10^5 \pm 0.58$	$5.4\ x10^2 \pm 4.16$	-
JDA	$3.0\ x10^6 \pm 1.53$	$3.0x10^3 \pm 0.58$	$6.2\ x10^3 \pm 5.57$	-
NMA	$3.0\ x10^6 \pm 1.73$	$3.3x10^4 \pm 2.65$	$1.1\ x10^2 \pm 2.00$	$1.4x10^2 \pm 2.52$
ETA	$2.0x10^4 \pm 2.65$	-	-	-
MOD	TNTC	$3.1x10^5 \pm 2.65$	$6.2x10^4 \pm 3.51$	$7.0x10^2 \pm 2.00$
TNA	$1.2x10^5 \pm 1.00$	$1.2x10^3 \pm 3.00$	-	$8.0x10^2 \pm 1.73$
CPT	TNTC	$3.2x10^5 \pm 2.52$	-	$2.1x10^3 \pm 3.51$
DSP	$3.2x10^3 \pm 3.00$	$7.0x10^4 \pm 2.00$	-	$4.3\ x10^2 \pm 3.00$
CGH	$3.2x10^8 \pm 5.51$	$1.0x10^4 \pm 1.00$	$5.7\ x10^4 \pm 3.00$	-
SOD	$2.7x10^4 \pm 2.52$	$3.0x10^3 \pm 1.00$	-	$1.4x10^2 \pm 3.61$
AGT	$1.8x10^5 \pm 5.13$	$2.0x10^3 \pm 1.00$	$8.3x10^3 \pm 5.29$	$5.0x10^2 \pm 1.00$
LXV	$4.3\ x10^2 \pm 3.61$	-	-	-
MZA	$6.0x10^3 \pm 1.53$	$6.0x10^2 \pm 2.00$	$1.9\ x10^2 \pm 2.52$	-
DDA	$1.0x10^2 \pm 2.65$	$4.0x10^3 \pm 1.00$	-	$8.0x10^2 \pm 2.00$
MMN	$6.0x10^5 \pm 0.58$	-	-	-

Table 2.0. Microbial content of decoctions.

Name	Microbial isolate		
	Freshly prepared decoction		After 3 months storage
	Bacteria	**Fungi**	
ASA	*Bacillus licheniformis* *Bacillus subtilis* *Enterobacter aerogenes*	*Cladosporium herbarum* *Penicillium digitatum* *Trichosporon mucoides*	*Bacillus licheniformis* *Rhizopus stolonifer* *Penicillium digitatum*
NGR	*Bacillus subtilis* *Serratia odorifera*	*Aspergillus oryzae* *Cladosporium herbarum*	*Bacillus subtilis*
JDA	*Bacillus subtilis*	*Cladosporium herbarum* *Penicillium digitatum* *Mycelia sterilia*	*Bacillus subtilis*
NMA	*Bacillus coagulans*	*Cladosporium herbarum* *Trichosporon mucoides*	*Bacillus coagulans* *Rhizopus stolonifer*
ETA	*Bacillus subtilis*	-	*Saccharomyces kluyveri*
MOD	*Bacillus subtilis*	*Cladosporium herbarum*	*Bacillus subtilis* *Cladosporium herbarum* *Zygosaccharomyces spp*
TNA	*Bacillus licheniformis* *Bacillus subtilis*	*Mycelia sterilia* *Cladosporium herbarum* *Saccharomyces kluyverii*	*Saccharomyces kluyveri*
CPT	*Bacillus coagulans* *Klebsiella oxytoca*	*Aspergillus sulphireus* *Cladosporium herbarum* *Trichosporon mucoides* *Rhodotorula minuta*	*Cladosporium herbarum*
DSP	*Bacillus subtilis*	*Penicillium digitatum,* *Aspergillus niger* *Candida membranifasciens*	*Aspergillus flavus* *Zygosaccharomyces spp*
CGH	*Bacillus coagulans*	*Aspergillus ustus* *Aspergillus niger* *Penicilliun digitatum*	*Bacillus subtilis*
SOD	*Bacillus subtilis*	*Cladosporium herbarum* *Sporobolomyces salmonicolor*	*Zygosaccharomyces spp*
AGT	*Bacillus subtilis*	-	*Bacillus subtilis* *Cladosporium herbarum*
LXV	*Bacillus coagulans*	*Cladosporum herbarum*	-
MZA	*Bacillus licheniformis*	*Cladosporium herbarum*	*Bacillus licheniformis*
DDA	*Bacillus subtilis* *Enterobacter aerogenes*	*Trichosporon mucoides*	*Zygosaccharomyces spp*
MMN	*Bacillus subtilis*	-	-

3.2 Discussion

The result of the microbial load count of the decoctions presented in Table 1.0 showed that 50% (8 out of 16) of the decoctions had bacterial load count that exceeded the acceptable limits for aerobic plate count (>$1.0x10^5$cfu/ml), whilst 75% (12 out of 16) had fungal load in high numbers (>$1.0x10^3$cfu/ml). However, the microbial load count of enterobacteria and other Gram negative bacteria were within the acceptable limits (≤$1.0x10^3$ cfu/g). The limits of microbial contamination for finished products are: total aerobic bacteria 10^5cfu/ml, yeasts and moulds 10^3 cfu/ml, Enterobacteria and other Gram negative organisms 10^3cfu/ml and *E. coli, Salmonella* spp and *S. aureus* should be absent per milliliter (European Pharmacopoeia, 2007).
The microbial quality based on APC, coliforms and mould and yeast count were comparable to the findings of Okunlola *et al*., (2007) and Oyetayo (2008) in which herbal medicinal products from Nigeria were found contaminated with various aerobic bacteria and fungi. The very high aerobic plate count (APC) and mould and yeast count in the decoctions is a direct reflection of the quality of the raw materials used coupled with the

storage conditions and processing. Lee and Jo (2006) reported that spores of *Aspergillus* and *Rhizopus* contaminate the air in drying and packaging areas. The result of this study on the freshly prepared decoctions (Table 1.0) shows that only ETA, LXV and MZA out of the sixteen products were safe for human therapeutic use because of their acceptable microbial levels (EP, 2007). Therefore, there is the need to rid the products of such microbes since consumption of such spores could cause human illness such as food poisoning. Eight (50%) out of the sixteen decoctions had no microbial contaminants after the three month storage period (Table 1.0). This could be due to the actions of the preservative used (Azaz *et al*., 2004). The other eight had microbial contaminants that were within the acceptable APC limit established by the European Pharmacopoeia for finished herbal medicinal products, that is $\leq 1.0 \times 10^5$ cfu/ml. Seven out of the sixteen decoctions analyzed had no fungal contaminant whilst seven others had fungal load within the acceptable limits. Only two, ASA and CPT, out of the sixteen samples failed to achieve the acceptable limit, which is $\leq 1.0 \times 10^3$ cfu/ml. The result implied that, after the three months storage as many as 14 (87.5%) out of the 16 products were safe for human therapeutic treatment. It is therefore better for finished herbal products to be stored for some time so that the preservative can exert its effect on the product

The characterization of bacteria in the decoctions (Table 2.0) indicates the predominance of bacteria belonging to the genus *Bacillus* and members of the family Enterobacteriaceae (*Klebsiella, Enterobacter and Serratia)*. The fungal profile of the decoctions assessed in this study revealed the presence of large numbers of *Cladosporium herbarum* (34.5%) and members of the genus *Aspergillus* (17.1%). *Aspergillus* species are capable of producing toxic secondary metabolites with aflatoxins being the most toxic of the mycotoxins produced by *A. flavus* and *A. paraciticus* (Canafoglia *et. al*., 2007; Riba *et. al*., 2008). Rizzo *et al*., (2004) reported of toxigenic fungi such as *A. flavus, A. parasiticus* and members of the genus *Fusarium* from herbal medicinal plants from Argentina, whereas Halt (1998) isolated a wide spectrum of fungi, including *Aspergillus, Cladosporium, Rhizopus,* and *Penicillium* species from Croatian herbal teas and medicinal plants which is not different from the present study. Aflatoxins are responsible for various toxicological effects such as hepatic, gastrointestinal and carcinogenic diseases and have the capacity to cross the placental barrier and cause genetic defects at foetal stages (Maxwell *et al*., 1998).

After three months storage, microbes in the decoctions were again characterized. The results confirmed a reduction in the level of microbial contaminants. This reduction may be attributed to the effect of the methylparaben used as preservative for the decoctions. However, *Bacillus* species, *Cladosporium* species and the yeast *Zygosaccharomyces* species were the most isolated microbes. The presence of *Bacillus* and *Cladosporium* species could be explained by the fact that these organisms produce spores which are resistant to harsh processing, elevated heat and dry conditions (Dutkiewicz *et. al*., 2001). Therefore, these spores can survive for a very long time in the product in a dormant state and re-sprout when conditions become favourable (Dutkiewicz *et al*., 2001). The higher yeast populations could be the result of non-strict GMPs and hygienic conditions during processing and packaging of the products (WHO, 2004).

4.0 Conclusion and Recommendation

The study demonstrated the presence of microbial contaminants in the freshly produced decoctions at levels most times exceeding the acceptable limits of microbial load count. The presence of the *Aspergillus* species isolated has the potential for toxin production in the products. Therefore, strict GMPs and hygienic practices should be followed in order to minimize added contamination. Raw materials of good microbial quality should be used in the production of these medicines. The results of the studies on the decoctions after three months revealed a reduction in the levels of microbial contamination in the products. This occurrence may be explained by the antimicrobial action of the methyl-paraben used as preservative in the decoctions. The antimicrobial property of the preservative inhibits the proliferation of microorganisms in the product and extends the product shelf-life. It is therefore necessary to keep the products for at least three months before consumption so as to allow the preservative to inhibit/destroy the microbes present in the products.

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