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QUALITY ASSESSMENT OF SIX LIQUID HERBAL PREPARATIONS MARKETED IN ANAMBRA STATE, NIGERIA

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ABSTRACT

Acute toxicity and microbiological evaluations were carried out to assess the quality of six different herbal medicinal products in Nigerian market. Also, HPLC-DAD analysis of the herbal preparations was carried out to identify the phyto-compounds. Although all the products here found to be non-toxic, only three of the six herbal preparations passed the acceptance criteria for microbiological quality. The HPLC screening of the herbal preparations revealed the presence of similar phyto-constituents occurring in most of the products. The results of this study indicate that some herbal medicinal products sold in open markets across Nigeria do not meet specifications as they contain microbial loads above recommended levels for herbal products for oral administration.

KEYWORDS: ***Quality assessment, herbal preparations, acute toxicity, microbiological evaluation, HPLC analysis***

INTRODUCTION

Herbal medicines, also known as botanical medicines or phytomedicines, refer to the medicinal products of plant roots, leaves, barks, seeds, berries or flowers that can be used to promote health and treat diseases [1]. The relative affordability and availability of herbal medicine has led to the rapidly growing adoption of herbal medicines as primary health therapy and care, especially in developing countries where finance is a major constraint.

Despite its existence and continued use over many centuries, and its popularity and extensive use during the last decade, traditional medicine has not been officially recognized in most countries. Consequently education, training and research in this area have not been accorded due attention and support [2-3].

The biggest problem with herbal medicine in Nigeria is a lack of standardization and safety regulations [4] The quantity and quality of the safety and efficacy data on traditional medicine are far from sufficient to meet the criteria needed to support its use world-wide. The reasons for the lack of research data are due to not only to health care policies, but also to a lack of adequate or accepted research methodology for evaluating traditional medicine [2-3].

Standardization of a herbal medicine that may contain hundreds of chemical constituents with little or no evidence indicating which might be responsible for the presumed or proven therapeutic effect is still not practical [4,5]. These challenges retard the acceptability of herbal medicine and the growth of its

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industry. Though, herbal medicines are frequently considered to be safer than conventional medicines because of their better tolerance. However, there are also reports of side effects and adverse reactions that have been related to herbal medicines [6-7] The public's belief of herbal and natural products being safer than synthetic medicines can only be strengthened by imposing regulatory standards on these products, which should be manufactured using good practices [8]. While some of the reported side effects and adverse drug reactions may be due to the intrinsic bioactive secondary metabolites present in the herbal materials, many are due to the poor qualities of the products which may be attributed to such factors as contamination (with chemicals, pesticides, microorganisms and heavy metals), adulteration with pure drug compounds and poor quality control measures. An acceptable herbal product must be safe, stable and presented in a suitable dosage form and package [7,9].

This research, thus, investigates the microbiological quality and acute toxicity of six commercial herbal preparations procured from open markets in Awka Metropolis in Anambra State, Nigeria.

MATERIALS AND METHOD

Acute Toxicity Assay

The acute toxicity study of the herbal preparations was carried out according to the method employed by [10], with modification. For each sample, total of 21 mice were used which were divided into 7 groups of 3 animals per group. Each mouse was weighed and the respective weight recorded. The herbal samples were evaporated so as to establish the dry weight, after which the required weight for each mouse was measured out. The assay was carried out in two phases. In the first phase, for each sample, test animals were grouped into 3 groups of 3 mice per group. Groups 1, 2 and 3 were respectively administered 10, 100 and 1000 mg/kg body weight of the herbal sample p.o., to possibly establish the range of doses producing any toxic effect. The animals were then constantly monitored for mortality the next 2 h, then intermittently for the next 6 h, and finally over a period of 24 h. Because there was no deaths recorded in the first phase, further specific doses (2000, 3000, 4000 and 5000 mg/kg) of the samples were administered to Groups 4, 5, 6 and 7 respectively in the second phase.

Microbiological Analysis

Microbiological analysis of the herbal preparations was carried out according to the European Pharmacopeial specification for the microbiological quality of pharmaceutical preparations for oral administration containing raw materials of natural (animal, vegetable or mineral) origin for which antimicrobial pre-treatment is not feasible and for which the competent authority accepts microbial contamination of the raw material exceeding 10^3 viable micro-organisms per gram or per millilitre [11].

Total Viable (Heterotrophic) Plate Count (For Bacterial and Fungal Counting)

The total viable aerobic plate count was carried out using the pour plate method as described by the European Pharmacopeia (EP, 2005). Ten (10)-fold serial dilutions of each sample solution were repeatedly prepared in 4 test tubes (labelled 1-4), each containing the stock, 10^{-1}, 10^{-2} and 10^{-3} dilutions respectively. A volume of 0.1ml of each dilution of each sample was aseptically pipetted from each test tube into a sterile 9 cm Petri plate respectively and 20ml of sterilized molten agar medium (that has been kept below 45^{o}C) was added to the plate. This was then carefully swirled to properly mix the medium with inoculums. This was done in duplicates for each sample, and the mean value of each count was recorded. For bacterial counting, Tryptone Soy Agar (Oxoid, UK), also called Soybean Casein Digest Agar, was used while for fungal enumeration, Sabouraud Dextrose Agar (Oxoid, UK) was used. After the solidification of the agar, plates were incubated in an inverted position at 35-37^{o}C (for bacterial counting) and 25-27^{o}C (for fungal counting) for at least 5 days. Plates were observed daily for presence of countable colonies.

Total Enterobacteria (*Coliforms*) and *E. coli* Count

For the isolation and counting of the total enterobacteria/coliforms, Chromocult-Coliform Agar (Merck, Germany) was used. The Chromocult-Coliform Agar is a selective and differential chromogenic culture medium that enables the detection, differentiation and enumeration of *Escherichia coli* and Coliforms. It gives reddish or pinkish coloration to Coliforms, except for *E. coli* which may appear as dark blue or violet colonies. For this evaluation, the same procedure described above in the total viable aerobic (heterotrophic) plate count was employed.

Total *Staphylococcus aureus* count

For isolation and counting of *S. aureus,* Mannitol salt Agar (Oxoid, UK) was used, and the same procedure described above in the total viable aerobic (heterotrophic) plate count was employed.

Total *Salmonella* Count

For counting of *Salmonella,* Salmonella-Shigella Agar (Oxoid, UK) was used, and the same procedure described above in the total viable aerobic (heterotrophic) plate count was employed.

HPLC-DAD Analysis of the Herbal Preparations

Chromatographic separation were carried out using Eurospher-100 C18 (5µm) filled separation column (125 x 2 mm, ID) with integrated pre-column (Knauer; Berlin Germany) and a dionex photodiode array detector UVD 340S. A volume of 1 ml of each formulation was completely freeze-dried and then reconstituted with HPLC grade methanol prior to analysis. The chromatogram monitored at 254 and 340nm was analysed for the retention time, UV Visible spectra as well as the peak distribution of the constituents from the extract. The solvent gradient used started with MeOH, nano pure water adjusted to PH 2 with phosphoric acid to 100% MeOH in 60mins. Autosampler injected 20µL samples which consisted of about 2 mg of the fraction in 1 ml of the solvent system.

RESULTS AND DISCUSSION

The results of the acute toxicity profile investigation on the herbal medicinal products showed that the product are relatively non-toxic as none caused death of the test animal (Table 1). Tables 2 and 3 gave an overview of the European Pharmacopeia specifications for the acceptance of herbal medicinal products, with only three of the products (LB, BE, and BC); passing the microbiological analyses carried out. The three other products (KM, GC, and YB) failed these acceptance criteria, recording microbial levels which are above the specified limit. This microbial contamination is of serious concern and might be due to the manufacturer not observing Good Manufacturing Practices (GMP) in the course of manufacture, leading to an increase in the microbial burden present in the product. This increased bio-burden could lead to adverse health and/or toxic effects on consumers [12].

As it is well known, the efficacy of traditional herbal medicines has characteristics of a complex mixture of chemical compounds present in the herbs. The specific identity of the constituents of the herbal preparations was not confirmed using only HPLC-DAD analysis, but similar compounds were identified in these mixtures, which could be responsible for their similar ethnomedicinal properties. It can be observed from Figure 3, that YB, BC and GC were found to contain the same components, looking at the UV-spectrum of their constituents in comparism to the other drugs. The chromatogram for BE showed the presence of a single compound, which is unusual and quite interesting for a herbal formulation. This singular peak of BE was also observed in GC at the same retention time of 18.72 min and same λmax of 229.5, 274.2 and 229.6, 274.2 respectively. KM and LB also shared similar constituents. Of all the tested herbal preparations, KM contained the most phytoconstituents as evident by the crowded peaks.
Chromatographic finger print analysis serves as a promising quality control tool for traditional medicines. This study suggests a complex relationship between chromatographic finger prints and efficacy of the herbal medicines which is an important aspect of quality control of herbal drugs.

Although there was no library hit of the UV spectrum for all the compounds detected in the herbal products, the products can be investigated further with advanced spectroscopy to identify the compounds. The information presented in this study will evolve as preceding factor in standardization of these polyherbal formulations, and also for other formulations in Nigerian system of medicine.

CONCLUSION

The results of this study indicate that some herbal medicinal products sold in open markets across Nigeria do not meet specifications as they contain microbial loads above recommended levels for herbal products for oral administration. This reflects the lack of control of quality in the production prior to their distribution. These data point to the need for deployment of effective systems of supervision of the manufacture and marketing of these products, as well as insightful programmes of quality control on the part of National regulatory agencies.

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CONFLICT OF INTEREST

No conflict of interest associated with this work.

Table 1: Result of Acute Toxicity Assay

Samples	Doses	Number of death
KM, BC, BE, GC, LB and YB,	10 mg/kg	0
	100 mg/kg	0
	1000 mg/kg	0
	2000 mg/kg	0
	3000 mg/kg	0
	4000 mg/kg	0
	5000 mg/kg	0

Table 2: Result of Microbiological Analyses

Analysis	European Pharmacopoeial Specification* (Organisms/ml)	Herbal Preparations	Organisms/ml	Remark
Total Viable Aerobic Count (Bacteria)	Not more than 10^4 Bacteria per millilitre (ml)	KM	$2x10^4$	Fail
		GC	$1.1X10^5$	Fail
		YB	$4x10^1$	Pass
		LB	$2x10^1$	Pass
		BE	$1.4X10^2$	Pass
		BC	$5x10^1$	Pass
Total Viable Aerobic Count (Fungi)	Not more than 10^2 fungi per millilitre (ml)	KM	$1.7x10^2$	Fail
		GC	$2x10^1$	Pass
		YB	$7.7x10^2$	Fail
		LB	$2x10^1$	Pass
		BE	$1x10^1$	Pass
		BC	$1X10^1$	Pass
Total Enterobacteria	Not more than 10^2 enterobacteria and certain other gram-negative bacteria per millilitre (ml)	KM	0	Pass
		GC	0	Pass
		YB	0	Pass
		LB	0	Pass
		BE	0	Pass
		BC	0	Pass
Total *E. coli*	Absence of *Escherichia coli* (1 ml)	KM	0	Pass
		GC	0	Pass
		YB	0	Pass
		LB	0	Pass
		BE	0	Pass
		BC	0	Pass
Total *S. aureus*	Absence of *Staphylococcus aureus* (1 ml)	KM	0	Pass
		GC	0	Pass
		YB	0	Pass
		LB	0	Pass
		BE	0	Pass
		BC	0	Pass
Total Salmonella	Absence of Salmonella (10 ml)	KM	0	Pass
		GC	0	Pass
		YB	0	Pass
		LB	0	Pass
		BE	0	Pass
		BC	0	Pass

*(EP, 2005)

Table 3: Summary of microbiological quality the herbal medicinal products

Herbal Preparations	Total Viable Aerobic Count (Bacteria)	Total Viable Aerobic Count (Fungi)	Total Enterobacteria	Total *E. coli*	Total *S. aureus*	Total Salmonella	Final Remark
KM	Fail	Fail	Pass	Pass	Pass	Pass	Fail
GC	Fail	Pass	Pass	Pass	Pass	Pass	Fail
YB	Pass	Fail	Pass	Pass	Pass	Pass	Fail
LB	Pass	Pass	Pass	Pass	Pass	Pass	Pass
BE	Pass	Pass	Pass	Pass	Pass	Pass	Pass
BC	Pass	Pass	Pass	Pass	Pass	Pass	Pass

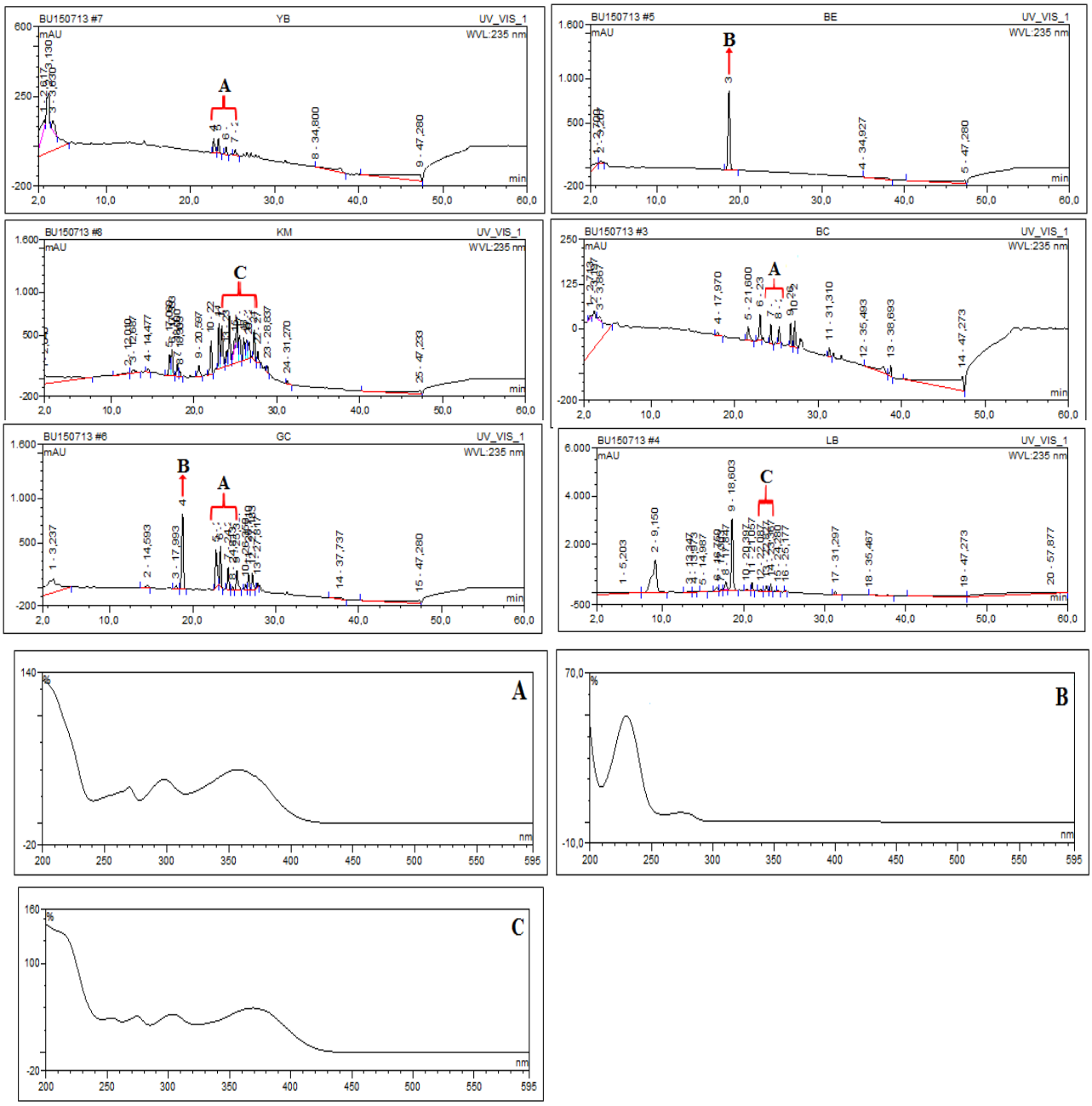

Figure 3: HPLC Chromatograms and UV spectra of similar compounds detected in the herbal preparations: the chromatograms of YB, BC and GC showed the presence of similar components grouped as 'A'; a single major compound 'B' was detected in BE, and this compound was observed in GC; also similar constituents 'C' were also observed in KM and LB.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) and all experiments have been examined and approved by the appropriate ethics committee. Ethical committee approval number: EC/NAU/PHARM/028/16.

CONFLICTS OF INTEREST

The authors have no conflict of interest to declare

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