Microbiological status and Pharmaceutical Evaluation of Commercially Available Herbal Medicinal Products in Lagos,Nigeria

ABSTRACT

Some herbal products were sourced from traditional medicine practitioners purposely to evaluate their microbial and pharmaceutical qualities. These products were 15 in number and were of different dosage forms. Phytochemical tests were carried out to determine their phyto-constituents. Also, the microbial qualities were determined and the pharmaceutical evaluation carried out through tablet disintegration of powders, crushing and friability. The powdered formulations were subjected to particle size determination and angle of repose. The density of the suspensions and solutions were taken. Results indicated presence of alkaloids, anthraquinones, tannins and cardenolides. The microbial load of the products varies. Seven (46.67%) of the products were contaminated with *Salmonella*, ten (66.67%) were contaminated with fungi, ten (66.67%) were contaminated by *Staphylococcus aureus* and nine (9) were contaminated with *E.coli*.(60%). The angle of repose for the powder dosage forms were high, this indicate that the powdered products were not free flowing and not highly cohesive. Also, acceptable crushing strength and friability was shown by the tablet formulation but it failed the disintegration time. Out of the fifteen products, two of the products were registered with NAFDAC (13.33%), all the products indicated their manufacturing and expiry dates while ten (66.67%) have their contents stated. All the products have their therapeutic claims indicated on their containers. In conclusion, there should be control of standards of herbal medicine in Nigerian markets as well as constant evaluation and monitoring of the various products.

Keywords: Traditional medicine, Pharmaceutical evaluation, Microbial load, Herbal products, Phytochemical tests.

INTRODUCTION

Traditional medicine has been described by WHO as one of the surest ways to achieve total healthcare delivery to the ever growing world population (1). A survey carried out by WHO indicated that about 85% of the world population particularly the developing world rely on non-conventional medicine majorly of herbal sources (2). Therefore, herbal medicine is increasingly becoming popular both in developing and developed countries of the world.

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There is encouragement by WHO for the rational use of plant based products having noticed that most people have now accepted traditional medicine and have even gone further to develop guidelines for the assessment of herbal products (3,4).

Of recent, there has been general awareness towards the usage of herbal products in Nigeria. This may not be unconnected to the high cost of conventional drugs as well as aggressive marketing embarked upon by herbal medicine producers (5). It has been widely reported that in the rural areas, there is general inaccessibility to conventional drugs as such majority of the rural dwellers depend on traditional medicine for their healthcare needs. Also, there has been reports of prevalence of fake and substandard drugs in the rural communities this inadvertently made traditional medicine a ready alternative (6). WHO in an attempt to coordinate the usage of herbal medicine came up with Good Manufacturing Practice Guidelines designed to provide technical assistant to regulatory bodies who are saddled with the responsibility of determining the safety and efficacy of the traditional medicine products (2,8). It should be noted that herbal medicine products are always subject to adulteration, substitution, contamination, incorrect preparation, lack of standardization, misidentification, improper labelling and bogus claims (5).

Most herbal products of recent, for acceptability, have been formulated into conventional dosage forms. Some are now in tablets, solutions, suspensions and capsules but majority are in powders. Presently in Nigerian supermarkets and pharmacy outlets, these products can be abundantly found therefore, it is necessary to determine their pharmaceutical qualities and ascertain the various claims made by the traditional herbal medicine practitioners. This study is designed to evaluate the pharmaceutical properties and microbial qualities of 15 herbal products found in various herbal sales shops and pharmaceutical outlets in Lagos, Nigeria.

MATERIALS AND METHODS

Materials used include Nutrient agar, MacConkey agar, Nutrient broth, Sabauraud dextrose agar and Salt nutrient agar. Fifteen (15) different herbal products were obtained from traditional medicine practitioners' outlets as well as from pharmacy retail outlets. Presented in Table 1 is the manufacture and expiry dates, type of dosage form and the therapeutic indications.

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Table 1. List of dosage used for the study

Product code	Dosage form	Date of manufacture	Expiry date	Country of manufacture	NAFDAC registration	Contents	Therapeutic claim
A	Tablet	April 2022	March 2025	Ghana	No	Not stated	Stroke, Ulcer, Waist pain, Back bone, Rheumatism, Menstrual pains, Muscular pain
B	Capsule	Dec.2021	Nov.2024	Nigeria	Yes	Citrus auantifolia, Lawsonia inermis, Nauclea latifolia, Morinda lucida,Theobroma cacao,Ficus capensis	Anti-malaria
C	Capsule	June 2023	May 2026	Nigeria	No	Sorgum bicolour, Hibiscus sabdariffa, Gongronema latifolium	Energy booster, Blood normalizer, Immune booster.
D	Capsule	Jan.2021	Dec.2024	Nigeria	No	Strophanthus sarmentosus, Pyrenanca ntha	Cough, Chronic fever
E	Solution	Jan.2023	Dec.2024	Nigeria	No	Citrus aurantifolia,Occimum,Morinda lucida,Ananas comosus, Azaridacta	Malaria

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F	Solution	Mar.2023	Feb.26	Nigeria	Yes	Alstonia bonei, Citrus medica, Varacida, Phyllantus reticulates, Lawsonia inermis, Anthoderta nobilis, Zingiber officinale	Anti-malaria
G	Solution	Jan 2023	Dec.26	Nigeria	No	Cassia alata, Allium sativa,Xylopia aethopica, Sacchatrium officinum	Pile, dysentery, stomach problems, ulcer, waist pain, menstrual pain
H	Solution	Feb.2022	Jan.2025	Nigeria	No	Daniella Oleveri, Alstonia congensis, Opuntia ssp, Makamia tormentosa, Eugenia aromatica	Skin rashes, ringworm, Eczema, acne

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I	Solution	May 2023	Apr.2026	Nigeria	No	Xylopia aromatic,Allium sativa, Tetrapleura tetraptera	Small pox, Skin rashes, Measles and Fever.
J	Powder	Jan.2021	Dec.2024	Nigeria	No	Cymbogon citratus, Camilla sinensis,Hibiscus sabdariffa, Gongronema latifolium	Typhoid fever
K	Powder	Nov.2022	Oct.2025	Nigeria	No	Vernonia amygdalina, Mormodia charantia,Phyllanthus reticulate,	Diabetes
L	Powder	Feb.2022	Jan.2025	Nigeria	No	Not stated	Antimalaria
M	Powder	Jun.2021	May 2024	Nigeria	No	Not stated	Skin infections
N	Powder	Jan.2021	Dec.2023	Nigeria	No	Not stated	Typhoid fever
O	Powder	Feb.2022	Jan.2025	Nigeria	No	Not stated	Aphrodisiac

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Determination of tablet/capsule properties

Ten (10) tablets/capsules were weighed separately with an electronic balance (OHAUS CS Series. U.S.A) and the mean was calculated. The crushing strength of the tablets was also evaluated at room temperature and determined. The friability tests of the tablets was determined using 230 Lcd Digital Frability apparatus (Double Drum), India which operated at 25 rpm for 5mins. To determine the disintegration time, the tablets/capsules were put in

distilled water at 37^{O}C using an Erweka ZT 720 disintegration Tester. All experiments were in triplicate and results given are the means (16).

Determination of powder properties

The particle size distribution and shape of the medicinal products in powder forms were determined using Optical microscopy. Approximately 300 particle per sample was used. The angle of repose was measured by pouring 5g of each powder into a cylindrical glass fixed to a flat based diameter of 28mm so as to determine the flow properties of the powders. The angle of repose was calculated using the equation"

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Determination of solution properties

The density is measured as the mass per unit volume of the preparation. The density of the solutions and suspensions were actually determined taking the weight of 1ml of each of the samples.

Phytochemical tests

To test for alkaloids**,** Wagner and Dragendoff's reagent were used in which 0.5g of the sample was added 5ml of 1% aqueous HCl on a steam bath. It was filtered and few drops of Drangendof's reagent was added to 1ml of the filtrate. Also, Wagner's reagent was also added to another 1ml of the filtrate (12). Formation of precipitate indicated the presence of alkaloids. To test for saponins, blood heamolysis method was used. For anthraquinones, 0.5g of the sample was vigorously shaken with 5ml of chloroform for about 5mins(12). The extract was filtered and the filtrate was thereafter shaken with an equal volume of 10% ammonia solution (12). The presence of pink,red or violet colour in the lower layer (ammoniacal layer) showed that anthraquinones are present. Ferric chloride test giving deep green colouration indicated the presence of tannins. To test for cardenolides, the Keller – Kiliani test was used in which 0.5g of the sample was dissolved in 2ml of glacial acetic acid containing one drop of ferric chloride solution which was then fluxed with 1ml of concentrated sulphuric acid (12). The presence of brown ring at the interphase indicated the presence of cardenolides.

Microbial content determination

To determine the microbial content for the solid samples which comprises of tablets and powders, 1g of sample is dissolved in 9 mL sterile distilled water and for the liquid samples, 1 mL is added to 9 mL of distilled water after which serial dilutions were made. Pour plate

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method was used in determining the microbial assay. The plates were incubated at 37^{o}C for 24 hours. The number of colony forming units was taken using the colony counter and the microbial content was determined with the mean of triplicate experiments. For fungal growth detection, Agar disc diffusion method was used in which 56 g of Nutrient agar was dissolved in 2000ml deionized water in a conical flask. This was autoclave for 15 minutes at 121^{o}C to increase the solubility of the agar (14,15).

Table 2: Physicochemical properties of the Herbal Medicine Products

Product code	Dosage form	Weight uniformity (mg)	Crushing strength (N)	Friability (%)	Disintegration time (min)	Weight (g/ml)	Mean particle size	Angle of repose	Bulk density(g/cm^3)
A	Tablet	539±0.3	126.4±0.7	0.7±0.1	114.0±2.3	-	-	-	-
B	Capsule	485±0.2	-	-	35.0± 3.5	-	-	-	-
C	Capsule	500±0.2	-	-	40.1±4.0	-	-	-	-
D	Capsule	360±0.3	-	-	55.3±2.5	-	-	-	-
E	Suspension	-	-	-	-	1.345±0.01	-	-	-
F	Suspension	-	-	-	-	1.043±0.04	-	-	-
G	Suspension	-	-	-	-	1.099±0.02			
H	Suspension	-	-	-	-	1.017±0.05			
I	Suspension	-	-	-	-	1.002±0.03			
J	Powder	-	-	-	-	-	21.56±2.42	54.4±0.5	0.23±0.03
K	Powder	-	-	-	-	-	13.22±0.98	60.0±2.2	0.43±0.04
L	Powder	-	-	-	-	-	15.22±1.12	66.9±1.8	0.37±0.03
M	Powder	-	-	-	-	-	18.65±0.65	52.4±1.1	0.44±0.05
N	Powder	-	-	-	-	-	24.70±1.84	59.5±0.9	0.32±0.02
O	Powder	-	-	-	-	-	65.33±0.56	55.4±	0.48±0.03

Values are in means±SD of three triplicates

Results and discussion

The herbal products selected for this study are one (1) tablet 6.67%, five (5) solution 33.33%, three (3) capsules 20% and six (6) powders 40%. It was observed that all the products were within their shelf life at the time of this study. Out of the fifteen products, only one is manufactured in Ghana (Table 1). Two (13.33%) of the fifteen products have been registered with NAFDAC despite the strict regulation that only products duly registered can be advertised and sold within the country( ). It was noted that five (33.33%) of the herbal products don't have their contents stated. This negates the regulation of Nigeria Agency for

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Food Drug Administration and Control, NAFDAC, The European Agency for the Evaluation of Medicinal Products, EMEA and WHO.

The results obtained for weight uniformity might falls within the acceptable limits by African and European pharmacopoeia which states that for tablets ≥ 250 mg may not deviate from the average weight by more than 5% w/w and capsules of ≥ 300 mg may not deviate by more than 7.7% w/w (1,2). It was observed that the results obtained for weight uniformity tests for capsules and tablets were within acceptable limits. The tablet product A will be able to resist chipping and breakage under conditions of handling and storage as indicated in the results obtained for its crushing strength and friability. The acceptable disintegration time for tablets is within 15 minutes (8,9). The observed disintegration time of 114 minutes for the tablet is unacceptably high which implies that there will be delay in the release of active ingredients. For capsules, the acceptable disintegration time is within 30 minutes. Products B-D did not fall within the acceptable limit. The suspensions (Products E –I) all contained about 1g per mL of the suspensions with the standard deviation of the variation in weight in the range of ≤ 5%. The mean particle sizes of the powder products (J – O) fall within the acceptable limit. The values obtained for the angle of repose for the powders indicated that the particles are highly cohesive and definitely will not flow freely which may make the dose dispensed not to be uniform. The bulk density values obtained for the powder products fall within the acceptable pharmacopoeia limits.

The phytochemical results as shown in Table 3 indicated that the products contain at least one of the phytochemicals tested. In orthodox medicine, there is always an active component but in herbal medicine, products are made from two or more plants or active components. The products efficacy is not usually based on just one active component. There is synergy between the components of different plants combined. In most cases, the component responsible for therapeutic effect is usually unknown or partially explained. It has also been argued that herbal medicine produced may be different in standards as the production is determined by the practitioner who in most cases do not keep production records or formulation methods data(7). Some of the factors also mitigating against the contamination of herbal medicine products includes, fumigants, toxic metals, pesticides and endotoxins (11,13). All these can make herbal medicine products unsafe for use unlike orthodox drugs.

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Table 3: Phytochemical analysis of the Herbal Medicine Products

Product code	Alkaloids	Saponins	Tannins	Anthraquinones	Cardenolides
A	+	-	+	+	-
B	+	+	-	+	+
C	-	-	+	+	+
D	+	+	-	-	-
E	-	+	-	-	+
F	-	-	+	+	-
G	+	+	-	-	-
H	-	-	+	+	-
I	-	+	+	-	-
J	+	-	+	-	-
K	+	+	+	-	+
L	+	+	-	-	+
M	+	-	+	-	-
N	+	-	+	-	-
O	+	+	-	+	+

- = Absent, + = Present

The microbial contaminants of the herbal medicinal products are shown in Table 4. Due to the unhygienic methods of preparation of some of these products, there is always presence of contaminants from soil and atmosphere. The microbial contamination limits as stated by WHO is yeasts and moulds 10^3 cfu/g, *E.coli* and *Salmonella* should be absent, while Enterobacteria and other gram-negative organisms 10^3 cfu/g (1,2). *Pseudomonas aeruginosa* is primarily a soil bacterium and it was not found in any of the products. Out of the fifteen (15) products studied, seven (46.67%) were contaminated by *Salmonella* while nine (60%) were contaminated by *E.coli*. *E.coli* is known to be an intestinal bacterium and is ever present in faeces. Fungal and *Staphyloccocus aureus* contamination were found in ten products (66.67%). Lack of formal training for herbal medicine practitioners most times influence the microbial quality of their products. Unhygienic environment, quality of raw materials and non-sterilization of production materials can inactivate the therapeutic activity of the products

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(18,19). It has been reported that some infectious outbreaks have been associated with the use of heavily contaminated raw materials of plant origin (10,16,17). There is need for herbal medicine practitioners to ensure that their raw materials and products for formulations are of good microbial qualities.

Table 4: Microbial contaminants of Herbal Medicine Products

Products code	Fungi	*E.coli*	*Pseudomonas aeruginosa*	*Salmonella*	*Staphyloccocus aureus*
A	2.1 x103	4.2×10^3	-	-	1.1×10^4
B	1.4 x 103	2.1×10^3	-	-	3.3×10^2
C	7.2 x 103	7.4×10^3	-	-	2.4×10^3
D	-	-	-	-	-
E	-	6.4×10^3	-	1.3×10^2	2.8×10^4
F	4.6 x 103	1.5×10^3	-	2.5×10^3	1.8×10^3
G	3.5 x 102	-	-	3.6×10^2	-
H	1.2 x 103	-	-	1.6×10^2	3.1×10^3
I	-	2.0×10^3	-	-	5.2×10^3
J	-	3.1×10^3	-	-	2.0×10^4
K	1.0×10^3	5.0×10^3	-	-	1.5×10^3
L	-	-	-	-	-
M	3.3×10^3	2.6×10^3	-	2.1×10^3	4.5×10^3
N	2.0×10^3	-	-	3.0×10^3	-
O	4.3×10^3	-	-	1.5×10^3	-

- = No growth, Cfu/mL = Colony forming unit per mL or g.

Conclusion: Herbal medicine products are improved version of previous traditional methods of production. They are of natural origin and the complexity is in the mixtures without any proven active ingredients. To get a good herbal product, quite a number of steps need to be taken so as to guarantee a quality product as obtained in orthodox medicine products. There is need for quality, standardization and constant monitoring of herbal medicine products which are sold in Nigeria so that there will be conformity with laid down rules and regulations. The government should, as a matter of urgency, train regularly the traditional medicine practitioners as almost 85% of the population directly or indirectly patronize them. There

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should be regular monitoring of herbal products in the markets to stem outbreak of communicable diseases.

Disclaimer

The materials used for this research are commonly and predominantly use materials in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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