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QUALITY ASSESSMENT OF SOME TOPICAL POLYHERBAL PREPARATIONS ON THE GHANAIAN MARKET

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ABSTRACT

The aim of this project was to assess the quality of some topical polyherbal ointments available on the Ghanaian market. The indices of quality measured were grouped as follows: organoleptic properties; (appearance, colour, odor and texture), physicochemical properties; (pH, loss of drying and specific gravity), microbial type and load; (*Staphylococccus aureus*, aerobic bacteria, yeast/molds and *Pseudomonas aeruginosa*) and heavy metals and elements; (Sulphur). A total of eleven (11) polyherbal topical ointments on the Ghanaian market were sampled and evaluated for various parameters. A further study was done to find out the possibility of adulteration or contamination in some of these polyherbal ointments with chemically defined active substances. These chemically defined active substances may be used to boost the therapeutic activity of these ointments. These adulterants were benzoic acid, salicylic acid and Sulphur. Results obtained in the study showed the presence of Sulphur in four samples 4/11 (36.36%). The classification of these as herbal products therefore is questionable. Following chromatographic separation and identification, none of the samples tested were found to contain salicylic acid or benzoic acid. As many as eight of the samples 8/11 (72.72%) had a pH out of the range of the natural skin surface pH; on average below 5, which is beneficial for its resident flora. Two out of the eleven ointments 2/11 (18.18%) had a fungal above the acceptable limit of 10^3 cfu/g and as many as seven 7/11 (63.63%) tested positive for *Staphylococcus aureus*. The results on loss on drying and specific gravity were satisfactory. Given that these products were duly registered; post marketing testing must be rigorously pursued in order to guarantee safety.

KEYWORDS: adulteration, quality control, herbal therapy, sulphur, fungal.

INTRODUCTION

Herbal therapy and herbal medicines predominates in traditional medicine as well as in alternative medicine, practiced in both the developed and the developing world. In many developing countries including Ghana, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal products in order to meet healthcare needs (IARC Monograph, 2002). The popularity of herbal medicines notably increased in the past years due to rapid increase in allopathic drugs price and reports on their safety. People are often using herbal products especially in rural areas because of its availability, accessibility and affordability. There is also a general public perception that herbal products are safer and harmless and without any adverse side effects because of their natural plant based origin/material. Skin and skin related diseases are ranked top among the various indications where traditional herbal medicines are used (Rajasree et al., 2012). Some topical polyherbal preparations used in the treatment of these diseases have been found to be adulterated contaminated with allopathic drugs such as dexamethasone, a powerful corticosteroid (Ofori Kwakye et al., 2014). The quality assessment of herbal formulations is of paramount importance in order to justify their acceptability in modern system of medicine. One of the major problems faced by the herbal industry is the unavailability of rigid quality control profiles for herbal materials and their formulations (Pulak et al., 2013). The reason for the scarcity of data on quality, safety and efficacy is attributed to the inadequate and accepted research methodologies for evaluating natural products (Liang et al., 2004). Quality issues of herbal medicines can be classified into two categories: external and internal. External issues include contamination (e.g. toxic metals, pesticides residues and microbes), adulteration and misidentification. Complexity and non-uniformity of the ingredients in herbal medicines are the internal issues affecting the quality of herbal medicines (Zhang et al., 2012).

Contamination is a repeated problem due to lack of quality control. Raw plant materials may be contaminated with pesticide residues (physicochemical contaminant) and microorganisms including fungi (biological contaminant) (WHO, 2007). Clinically important morbidity may occur from contamination with heavy metals such as arsenic, lead, cadmium, and mercury (WHO, 2007). An analysis on ten (10) brands of herbal medicines sold in Ghana intended to be administered orally showed the presence of trace metals in them and some of the products were found to contain an unusually high content of the trace metals (Anim et al., 2012).

These contaminants could be avoided and controlled through quality assurance measures such as good manufacturing practices for herbal products and post marketing testing of products on the markets.

Another quality and safety issues with herbal preparations is with their microbiological profiles. A study conducted in Ghana analysed 31 herbal preparations for their microbiological quality by testing for their total aerobic count and the presence of pathogenic bacteria such as Salmonella spp. A total of 8 samples showed the presence of all the microorganisms analysed for including the pathogenic ones. Assessment of the safety and efficacy of these preparations is an important issue for the health professions (Ampofo et al., 2012).

Currently it is doubtful if herbal preparation producers in Ghana adhere to complete quality control and good manufacturing procedures including microscopic, physical, chemical and biological analyses. There is therefore the need for measures and techniques to control the quality and therefore the safety of herbal topical preparations especially those on the Ghanaian market.

The current study was aimed at assessing the quality status of some commonly used and generally accepted topical polyherbal ointments based on indices of quality such as organoleptic properties, microbial load and the presence of some allopathic topical preparations such as benzoic acid, salicylic acid and sulphur. This is because the use of topical herbal remedies for the treatment of various skin ailments has increased drastically over the years and therefore there is the need to take issues concerning quality, safety and efficacy serious.

MATERIALS AND METHODS

MATERIALS

Pure benzoic acid, pure salicylic acid, sodium nitroprusside, ammonium hydroxide, Iron (III) Chloride, Chloroform, butan-1-ol, activated charcoal, methanol were obtained and used.

Sample selection

A total of eleven (11) herbal ointments that were manufactured and marketed in Ghana for topical administration were randomly purchased from traditional medicine distributors and retail Pharmacy outlets in Kumasi located in the Ashanti region of Ghana. These samples

were all in the semi-solid state, un-expired and popular for their effectiveness in the treatment of various skin diseases. Products sampled were those that have been registered with Ghana's Food and Drugs Authority (the country's main regulatory body for drugs and food).

Table 1: Description of topical herbal ointments sampled in Ghana.

Samples	Labeled contents	Therapeutic indication	FDA Number
THO1	*Carica papaya, Cassia alata, cashew, Alchornea cordifolia, Aloe vera*	Facial spots, eczema, boils, foot rot, razor bumps	FDB/HD-07-10138
THO2	*Danlellia ogea, Khaya ivorensis*, mineral oil, paraffin wax	Boils, ringworm, foot rot, eczema	FDB/HD-08-9151
THO3	*Cassia alata* *Gossypium arboretum* *Daucus carota* *Carica papaya* Petroleum jelly	Candidiasis (white), skin rashes, boils, eczema, pimples, ringworm, footrot	FDB/HD 07/4051
THO4	*Cassia alata* *Aloe forex*	Skin rashes	FDB/HD 02-4062
THO5	*Butyprosperum parkii,* *Zingiber officinale* *Piper guineense* *Cassia alata* *Eugenia aromatica* *Aframomum melegueta*	Boils, ringworm, Footrot, Shingles, bodily pains, rheumatism	FDB/HD 03-7032
THO6	*Elaeis guinnensis, Raphia hokeri, Securidaca longipedunculata, Zingiber officinale*, Rashes, boils, foot rot, body pains		FDB/HD-03-12160
	Mangifera indica, *Pachypodandhum standtii,* *Piper guineensis*		
THO7	*Butyprosperum parkii* *Allium sativum* *Zingiber officinale* *Citrus medica,* *Piper guineense* Sodium benzoate 0.2%	Skin rashes, Boils, body pains, waist pains, rheumatism	FDB/HD 09-10195
THO8	*Bombax buonopozense, Musa sapientum, Theobromo cacao*	Eczema, ringworm, skin rashes, footrot, shingles, pimples, boils	FDB/HD 02-12096
THO9	*Aloe barbadensis, Azadirahta indica* *Tridax procumbens* *Gossypium arboretum*	Eczema, foot rot, dandruff, acne, piles, candidiasis	FDB/HD 11-11102

	Petroleum jelly		
THO10	*Daucus carota, Syudreda, lime, Cassia alata*, petroleum jelly, Fragrance	Boils, pimples, foot rot, eczema, chicken pox	0603
THO11	*Alchornia cordifolia, Cassia alata, Guto, Terminalia Superba, Aloe vera*	Ringworm, foot rot, eczema, shingles	FDB/HD-04-7075

METHODS

TLC assay of topical herbal preparations

For benzoic acid and salicylic acid

0.2g of the sample was weighed into a clean 25 ml beaker previously rinsed with a small amount of chloroform. 5ml of the chloroform was added, warmed on a water bath for 30 seconds with frequent shaking to dissolve the ointment completely. The resulting solution was then cooled and filtered using a filter paper previously wet with chloroform. Separate standard solutions were prepared in the same solvent containing 30mg benzoic acid in 5ml and 30mg salicylic acid in 5ml. 5µl of each solution (both standard and test) was applied using a capillary tube at separate points 2.5 cm from the bottom edge of a 20×20 cm thin-layer chromatographic plate coated with 0.25 mm layer of chromatographic silica gel mixture and the spots allowed to dry. The chromatogram was then developed in a solvent system consisting of equal volumes of butan-1-ol, acetone and ammonium hydroxide until the solvent front has moved about three-fourth of the length of the pre-coated plate.

Sodium fusion test for sulphur

A sodium metal (about a quarter size of a pea) was put in a fusion tube. A small weight of the ointment was added onto the sodium metal in the tube. The fusion tube was then heated in naked flame until the sample melted, pointing the mouth from people. The tube was then immersed in an about 5ml of distilled water and the tube grounded with a pestle in a porcelain mortar. The solution was then filtered and the filtrate which is water-clear collected into a clean test tube. Few drops of dilute solution of sodium nitroprusside were added to the filtrate. An intense pink colouration indicated the presence of sulphur.

Organoleptic test

Appearance

All the ointments were tested for appearance with no lumps by visual inspection.

Color and odor

The color was determined by visual inspection. An amount of the ointment was taken onto a petri dish. Air was blown over it towards the nostrils to determine the odor (Quality Control of Herbal Materials).

Texture

A small amount of the ointment was rubbed around the back of the neck (Quality Control of Herbal Materials).

pH

The pH of a solution was determined by digital pH meter. The pH meter was operated according the manufacturer's instructions. First the apparatus was calibrated using buffer of 4, 7 and 9 pH. 1.0g of ointment was dispersed in 100 ml of distilled water and the measurement of pH of each ointment was done in triplicate and average values calculated (Rajasree et al., 2012).

Specific gravity

A clean dry pycnometer which has been previously calibrated by determining its weight and the weight of recently boiled water contained in it at 25^{o}C was used. The pycnometer was filled with the molten ointment whose temperature was adjusted at a temperature of 20^{o}C. The temperature was re-adjusted to 25^{o}C, any excess of the ointment was cleaned and the pycnometer with the molten ointment weighed. The tare weight of the pycnometer was subtracted from the filled weight of the pycnometer and the specific gravity calculated.

The specific gravity of the substance is the quotient obtained by dividing the weight of the substance contained in the pycnometer by the weight of water contained, both determined at 25^{o}C (The USP, 1990).

Loss on drying

About 2.0g of ointment was accurately weighed into a previously dried petri dish. The sample was then dried in an oven at a temperature of 100-105^{0}C. Two consecutive readings were then taken. The difference between the initial reading and the reading after drying is the loss of weight in per cent w/w (Quality Control of Herbal Materials).

Evaluation of microbial load and presence of specific microorganisms

Microbial enumeration tests

Procedure

A weight (0.12g) of ointment was dissolved with 2.0ml of sterile tween 80 (a non-inhibitory emulsifying agent) and warmed to not more than 45^{o}C to obtain a viscous suspension. 1.0 ml of the suspension was accurately measured using a pipette in nutrient broth to make 10ml. it was mixed carefully while maintaining the temperature for the shortest time necessary for the formation of an emulsion. A further 1: 10 dilution was done to obtain a less viscous suspension.

Preparatory testing

About 1.0ml of a 10^{-3} dilution of a 24-hour broth culture of *Staphylococcus aureus* and *Pseudomonas aeruginosa* each was inoculated into a volume of the less viscous suspension. The tube was then incubated for 48 hours for growth. This test is done to demonstrate that the test specimens to which they are applied do not, of themselves, inhibit the multiplication, under the test conditions, of microorganisms that may be present.

Total aerobic count

Plate Method

1.0ml of the final dilution was pipetted onto each of the two sterile petri dishes. 20.0ml of nutrient agar that has been previously melted and cooled to approximately 45^{o}C was added promptly to each dish. The petri dishes were covered, rotated to mix the sample with the agar and the contents left to solidify at room temperature. The dishes were then inverted and incubated for 48 hours. After incubation, the plates were examined for growth, the number of colonies counted and the average expressed for the two plates in terms of the number of microorganisms per gram of specimen.

Total Combined Molds and Yeasts Count

The same method used in the plate method is used, except for using the same amount of sabouraud Agar Medium, instead of Nutrient Agar Medium, and except for the inverted petri dishes incubated for 5 to 7 days at 25^{o}C.

Test for *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Using a sterile inoculating loop, the growth found on the surface of the nutrient agar was removed and streaked on the surface of mannitol-Salt Agar Medium and of cetrimide Agar Medium for *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively, each plated

on sterile petri dishes. The dishes were covered and inverted and incubated. After incubation, the plates were examined and the colonies compared with the characteristics listed in the table. The presence of any colonies with any of the characteristics signifies the presence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Organoleptic evaluation

Table 2: Results for the organoleptic properties of the ointments

Sample	Color	Odor	Texture	Appearance
THO1	Light Green	Characteristic	Gritty	No lumps
THO2	Light Yellow	Characteristic	Gritty	No lumps
THO3	Light Yellow	Characteristic	Smooth	No lumps
THO4	Light Orange	Characteristic	Smooth	No lumps
THO5	Green	Characteristic	Smooth	No lumps
THO6	Brown	Characteristic	Gritty	No lumps
THO7	Yellow	Characteristic	Smooth	No lumps
THO8	Brown	Characteristic	Smooth	No lumps
THO9	Yellow	Characteristic	Gritty	No lumps
THO10	Blue	Characteristic	Smooth	No lumps
THO11	Deep Green	Characteristic	Gritty	No lumps

Microbiological profile

Presence of *S. aureus and P. aeruginosa*

Table 3: Results for the presence of *S. aureus and P. aeruginosa.*

Samples	Total aerobic count	Total combined molds and yeasts	*S. aureus*	*P.aeruginosa*
THO1	4.0×10^{2}	2.0×10^{2}	+	_
THO2	2.3×10^{1}	1.1×10^{2}	+	_
THO3	$1.\ 6\times10^{5}$	4.8×10^{3}	+	_
THO4	6.0×10^{3}	1.6×10^{3}	+	_
THO5	1.2×10^{5}	4.0×10^{3}	+	_
THO6	3.2×10^{4}	1.2×10^{3}	_	_
THO7	<10	1.6×10^{4}	_	_
THO8	2.0×10^{5}	8.0×10^{2}	_	_
THO9	1.2×10^{4}	8.0×10^{3}	+	_
THO10	5.2×10^{3}	1.2×10^{4}	_	_
THO11	1.84×10^{4}	2.0×10^{3}	+	_

Detection of adulterants and contaminants

Table 4: Results for the benzoic acid and salicylic acid.

Samples	Sulphur	Benzoic acid	Salicylic acid
THO1	+	-	-
THO2	+	-	-
THO3	+	-	-
THO4	-	-	-
THO5	-	-	-
THO6	-	-	-
THO7	-	-	-
THO8	-	-	-
THO9	+	-	-
THO10	-	-	-
THO11	-	-	-

Table 5: Results for the pH analysis on the ointments.

Samples	Av. pH	Av. Specific Gravity	Av. % loss of drying
THO1	4.95	0.8351	6.50
THO2	7.03	0.8793	5.08
THO3	3.78	0.8437	1.64
THO4	6.88	0.8363	1.50
THO5	10.10	0.8717	3.00
THO6	6.65	0.9224	1.40
THO7	7.12	0.8340	1.11
THO8	7.33	0.8804	4.02
THO9	7.09	0.9295	1.30
THO10	4.60	0.8470	1.63
THO11	7.20	0.8582	0.95

DISCUSSION

The safety and quality of medicinal herbal products have become a major concern for health authorities, pharmaceutical industries, regulatory bodies and the general public (WHO, 2002). This is partially due to the upsurge in the usage of herbal preparations for the treatment of various human diseases. Most of these topical preparations attributed the effectiveness of their products to the pure herbal ingredients contained in it (Ofori Kwakye et al., 2014). The physicochemical properties such as appearance, colour, texture, pH, loss on drying and specific gravity affects quality of herbal product, its acceptability by its consumers and also the activity of the product. From the results of the experiment, there were no lumps found in the ointments and there were of good homogeneity. This may be due to good preparation and effective mixing of the plant materials with ointment bases. All the ointments had characteristic colours ranging from light blue to deep green with their characteristic aromatic sweet smelling odour. This makes the product readily acceptable and increase patient compliance. With the test on texture, it was found out that some of the ointments were

smooth when applied to the skin whiles others were gritty. A gritty ointment when applied to an affected skin can aggravate the condition by causing abrasions of the skin. An ointment for topical application is expected to be smooth without any grits. The presence of grits may be a sign of physical instability (The International Pharmacopeia, 2015). The loss on drying estimates the amount of moisture or/and volatile matter present in the polyherbal topical ointments. If the amount of moisture present in the products exceeds the acceptable limit, the excess water can facilitate the growth of both harmless and pathogenic microorganisms if present (Quality Control of Herbal Materials). It can also lead to both physical and chemical changes causing spoilage of the ointments. It can cause breakdown of both active substances into less potent or more toxic substances and excipients used in the formulation of the ointments. The % loss on drying is specific for each sample. The specific gravity of any substance is the ratio of the density of the substance to the density of water both determined at 25^{o}C. It gives an idea of how denser the ointment is in relation to water. The specific gravity of the ointments may be a factor of the ointment base used in the formulation process. Different ointment bases have different densities in relation to the density of water. Both the % loss on drying and the specific gravity can be used to assess the consistency of the production process. This is because each production batch of ointments is expected to have the same and % loss on drying and specific gravity of products. This can even help in detecting any contamination or adulteration of a production batch. The pH is a measure of the hydrogen ion concentration of the product. The pH of the ointment must fall in the range of the pH of the natural skin which is 4.0-7.0 with an average of 4.7 (Lambers et al., 2006). The pH of the natural skin protects the skin against both pathogenic and opportunistic infections and also promotes the growth of the microflora of the skin. A pH out of the range of the pH of the skin destroys the protective functions of the skin and the microflora of the skin. Certain chemicals and factors can tamper or destroy the pH of the natural skin and makes it susceptible to so many infections of the skin. From the results, only four (4) out of the eleven ointments had their pH in this range. The other seven (7) had their pH range either above or below the pH of the skin. This makes the skin susceptible to infections from both pathogenic organisms and opportunistic organisms. The study showed that 63.6% of the local traditional medicines sold on the market are not safe for use due to their poor microbial quality. Most of the products examined had bacteria and fungi in them, and 18.2% had combined moulds and yeast counts in excess of the stipulated and acceptable limits of 10^3 per gram (WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues). The products might have been contaminated from the raw materials, packaging

used, during production, display and handling. Microbial contamination of herbal products can have many adverse effects on the user. Some of these microbial contaminants are potential pathogens of man, and may thus predispose the patients using these products to the risk of acquiring infections. These contaminated medicinal preparations may also facilitate transfer of strains of microorganisms which harbor antibiotic resistance genes. They may also lead to spoilage through physical (colour, texture) or chemical change (hydrolysis, oxidation) and thus render the active constituents less potent and even toxic thereby presenting potential health hazard to consumers. From the experimental results, four (4) out of the eleven ointments sampled was found to contain Sulphur; even though their labels did not have Sulphur as one of its ingredients. The presence of the Sulphur in these ointments may be as a result of intentional addition because of its antiseptic effect and its usage in the treatment of many skin conditions such as acne, eczema or psoriasis and seborrheic dermatitis (Sulphur revised, 2006). The Sulphur contained in these ointments may be in high amount and long-term use of these ointments on the skin may results in adverse health effects from the side effects of Sulphur (swelling and irritation). None of the ointments tested positive for benzoic acid or salicylic acid. One of the ointment that contained sodium benzoate (2%) as preservative did not test positive for benzoate.

CONCLUSION

It is evident from the study that the quality of some of the topical polyherbal ointments used in the treatment of skin ailments available on the Ghanaian market are not up to standard. This is because these topical herbal ointments are contaminated with microorganisms and also adulterated with other chemicals which may be in excess to increase their therapeutic efficacy. There is therefore the need for the regulatory bodies for medicines to increase their post marketing surveillance in order to curb the abusive use of herbal medicines.

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