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Microbiological and Physicochemical Analyses of Pharmaceutical Raw Materials Used in Some Pharmaceutical Products

Okolosi-Patani O.E[1]* and Omonigho S.E[2]

[1]Pharmaceutical Microbiology Department, Faculty of Pharmacy, Delta State University, Abraka, Nigeria
[2]Department of Microbiology, Faculty of Life Sciences, University of Benin, P.M.B. 1154, Benin City, Nigeria

*Corresponding author; Email: elohous@yahoo.com, Tel: +2348033119273

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ABSTRACT: This study was carried out to investigate the microbiological and physicochemical characteristics of pharmaceutical raw materials used in some non-sterile preparations. A total of 16samples of pharmaceutical raw materials and 3 formulated non sterile preparations were subjected to microbial limit test, isolation and characterization of microbial contaminants and physicochemical analysis. From the result there were no bacterial contamination in all samples analyzed but 7 out of the 16 raw materials had fungal count ranging from 0.30±1.50 x10² cfu/ml (glycerol) to 1.61±0.83 x 10^2cfu/ml (raspberry juice) The formulated paracetamol elixir recorded the highest fungal count (3.33 ± 1.43 x 10^2cfu/ml) while pediatric ferrous sulphate mixture had the least fungal count (0.33 ± 0.04 x 10^2cfu/ml). The fungal isolates and their frequency of occurrence in the raw materials were *Aspergillus niger* (25%), *Rhizopus stolonifer* (6.25%) and *Candida albicans* (12.50%). Similarly, the fungal isolates from the formulated oral preparations were *Aspergillus niger* (100%), *Rhizopus stolonifer* (33.33%) and *Candida albicans* (33.3%). The physicochemical characteristics of the various raw materials and formulated preparations revealed the colour, odour, pH, density, total solids and moisture content of the various samples. The study has revealed that the microbiological and physicochemical characteristics of pharmaceutical raw materials determine their suitability for pharmaceutical preparations. Most of the analyzed samples were well within the stipulated limits of the United States Pharmacopeia. It is therefore advised that pharmaceutical raw materials be evaluated at all stages of the production process.

Keywords: Pharmaceutical raw materials, microorganisms, physicochemical properties, non-sterile

Introduction

Pharmaceutical products are chemical compounds developed by a pharmaceutical company administered to humans or animals for prophylactic, curative, palliative or diagnostic purposes. Microbiologically, pharmaceutical products can be grouped into non sterile and sterile products. Non-sterile products are pharmaceutical preparations that are not sterilized but must meet the microbiological criteria as stated in the pharmacpoeia. Drugs of this nature may likely contain different types of microbial species. One major reason for contamination of pharmaceuticals is the source of the raw materials used (James, 2013). Variation in manufacturing conditions, handling of raw materials and poor preservative system may also determine the microbial bioburden or contamination of medicaments (Moniruzzaman *et al*., 2012; James, 2013). The presence of microbes in a medicinal preparation or product may not only cause them to be hazardous from the infectious standpoint, but also cause modification in the physical, chemical, and organoleptic properties of such drugs, alter the contents of active constituents, or convert them to toxic products (Kabir and Hossain, 2013). This study

was done to investigate the microbiological and physicochemical characteristics of pharmaceutical raw materials used in some non-sterile preparations.

Materials and methods

Collection of samples: A total of 16 samples comprising 3 APIs and 13 excipients were obtained from the Pharmaceutics and Pharmaceutical Technology Departmental Laboratory were carefully and aseptically transported to the laboratory in their packages for analysis.
Media and reagents: The media employed included Nutrient Agar (BIOTECH, TM 341, India), Mueller Hinton Agar (BIOTECH, TM 339, India), Nutrient Broth (BIOTECH, TM 350, India), Sabouraud Dextrose Broth (BIOTECH, TM 361, India), Sabouraud Dextrose Agar (BIOTECH, TM 387, India).All reagents and media were of analytical grades and were prepared following standard microbiological procedures.
*Physicochemical analysis of samples***:** Each sample was analyzed in turn for colour, odour, density, pH, moisture content and total solids. Each set of data were recorded accordingly.
*Treatment of samples and enumeration of associated microorganisms***:** Samples were aseptically weighed or measured out around the zones of a Bunsen flame and the total viable bacterial and fungal counts of the samples were determined using serial dilution and spread plate method (El-Housseiny *et al*., 2013; Islam *et al*., 2015). In the process, one g or ml of each sample was serially diluted in 9 ml of sterile distilled water to achieve 1:100 serial dilution and 0.1 ml aliquot of each sample from the dilution 10^{-2} was spread on to sterilized Petri dishes containing different growth media such as nutrient agar (N.A) for enumerating the total viable bacteria count and Sabouraud dextrose agar (SDA) for the estimation of fungal load. The plates were incubated at 37°C for 24hours and 25°C for 48 to 72 hours, respectively (El-Housseiny *et al*., 2013). All experiments were done in triplicates and emerging colonies on both the nutrient agar plates and Sabouraud dextrose agar plates were enumerated and recorded as cfu/ml or cfu/g representing total viable bacterial and fungal counts.
Isolation of Microbial Contaminants: Fungal identification was done by macroscopic (cultural and morphological characteristics), microscopic (lactophenol cotton blue technique) and biochemical (germ tube test) characteristics. These characteristics were compared to features outlined in photographic atlas for the microbiology laboratory (Leboffe and Pierce, 2011). The substantive pure isolates were lastly stored at 4°C until further use.
*Preparation of the pharmaceutical formulations***:** The formulations were made using the modified Pharmaceutical Codex methods (Walter and Royal Pharmaceutical Society of Great Britain, 2009) for preparing non sterile oral liquid preparations with a mortar and pestle.
*Preparation for paracetamol elixir***:** The quantity required of each ingredient for a 100 ml solution to be prepared was calculated. Each ingredient was accurately weighed and/or measured. Paracetamol powder weighing 2.40 g was dissolved in 10 ml of alcohol then 0.10 g of benzoic acid dissolved in 10ml of propylene glycol was also added. Concentrated raspberry juice measuring 2.50 ml diluted with the 27.50 ml invert syrup and 0.20 ml of amaranth solution were also added to the ensuing preparation. Lastly 49.80 ml of glycerol was added to make up to the required volume and was thoroughly triturated.
Preparation of ferrous sulphate mixture: The quantity required of each ingredient for 100 ml solution to be prepared was calculated. The Ascorbic acid powder of 0.20 g was weighed and dissolved in 40 ml of sterile distilled water and this was used to dissolve the 1.20 g of ferrous sulphate. Furthermore, 10 ml of the orange syrup was added to the ensuing solution. It was finally made up to the volume by the adding 50 ml of the sterile distilled water and was thoroughly triturated
Preparation of kaolin mixture: The required quantity of each ingredient for a 100 ml solution to be prepared was calculated. Each ingredient was accurately weighed and/or measured. Light kaolin weighing 20.0 g, light magnesium carbonate (5.00 g), benzoic acid (0.10 g) and sodium bicarbonate (5.0 g) were triturated using a mortar and pestle. This was made up to 97.50 ml by adding measured sterilized distilled water gradually while mixing the powders until a smooth paste was formed then peppermint emulsion of 2.50 ml was further added before dispensing into sterilized screw capped bottle prior to analysis.
*Statistical analysis:*Data obtained were subjected to descriptive (mean and standard error of mean) (Ogbeibu, 2005) and inferential (Chi-square test and ANOVA) using SPSS version 20, Chicago, USA.

Results and discussion

The total bacterial counts were 0.00 x 10^2 cfu/ml or cfu/g for all 16 samples (Table 1). The bioburden of the raw materials in this study was well within the acceptable limit of not more than 2.00 x 10^2 cfu/g or ml yeast and

mold (USP, 2003). This corroborates the findings in a previous study (Anjum *et al.,* 2014). However, contaminants were of fungal origin as opposed to the bacterial contaminants found in a previous study (Anjum *et al.,* 2014). The presence of fungi in pharmaceutical preparations will give rise to public health risk as they are known to produce toxins that are harmful to humans.

Table 1: Total microbial count of the pharmaceutical raw materials

Pharmaceutical raw materials	**Microbial counts (Mean ± S.E.M)× 10^2**	
	Bacterial (cfu/ml or cfu/g)	**Fungal (cfu/ml or cfu/g)**
Alcohol	0.00 ± 0.00	0.00 ± 0.00
Ascorbic acid	0.00 ± 0.00	0.00 ± 0.00
Raspberry juice	0.00 ± 0.00	1.61 ± 0.83
Light magnesium carbonate	0.00 ± 0.00	0.00 ± 0.00
Sodium bicarbonate	0.00 ± 0.00	0.00 ± 0.00
Propylene glycol	0.00 ± 0.00	0.00 ± 0.00
Benzoic acid	0.00 ± 0.00	0.00 ± 0.00
Peppermint emulsion	0.00 ± 0.00	0.66 ± 0.86
Amaranth solution	0.00 ± 0.00	1.30 ± 0.11
Orange syrup	0.00 ± 0.00	0.67 ± 0.03
Invert syrup	0.00 ± 0.00	0.00 ± 0.00
Glycerol	0.00 ± 0.00	0.30 ± 1.50
Sterile water	0.00 ± 0.00	0.00 ± 0.00
Paracetamol	0.00 ± 0.00	0.67± 1.04
Ferrous sulphate	0.00 ± 0.00	0.00 ± 0.00
Light Kaolin	0.00 ± 0.00	0.33 ± 0.71

Key: S.E.M (Standard error of mean)

The non-sterile preparations also had fungal contaminants within the acceptable limits (Table 2) which is in contrast to the findings of Urmi *et al.* (2014) in his study on antacid, where all samples recorded heavy contamination of both fungal and bacterial origin and microbial count ranged from 10^4 to 10^5 exceeding the USP limits. Reasons for differing contaminant types and counts may be due to different components, diverse products been sampled; use of different dosage forms (Mugoyela and Mwambete, 2010) use of different sampling and cultivation methods. In comparing the fungal counts of the raw materials and formulated preparations there was no significant difference in fungal counts of raw materials and formulated preparations, $p > 0.05$ however the difference in fungal counts between paracetamol elixir, kaolin mixture and pediatric ferrous sulphate mixture was statistically significant ($p < 0.05$).

Table 2: Total microbial count of the non-sterile preparations

Non sterile preparations	**Microbial counts(Mean± S.E.M x10^2)**	
	Bacterial (cfu/ml)	**Fungal (cfu/ml)**
Kaolin mixture	0.00 ± 0.00	0.70 ± 1.05
Paracetamol elixir	0.00 ± 0.00	3.33 ± 1.43
Pediatric Ferrous sulphate mixture	0.00 ± 0.00	0.33 ± 0.04

Key: S.E.M (Standard error of mean)

USP Specification

Total combined mold/yeast count**:** <10^2 cfu/ml

A total of three fungal species were isolated from the pharmaceutical raw materials (Table 3) and were identified as *Aspergillus niger*, *Rhizopus stolonifer* and *Candida albicans*. *Aspergillus niger* was the most predominant contaminant isolated while *Rhizopus stolonifer* was least. Contamination of pharmaceutical preparations with *Aspergillus niger* pose a very serious risk to consumer's health as *Aspergillus* species have the capability to produce a variety of fungal metabolites, also called mycotoxins, these toxins are mostly responsible for several nephrogenic, carcinogenic, hepatogenic, and immunosuppressive effects (Rai and Mehrotra, 2005; Dragan *et al.,* 2010).

Table 3: Synopsis of microbial isolates and their frequency of occurrence in different pharmaceutical raw materials used in some non-sterile preparations

Organisms	Pharmaceutical raw materials																Occurrence of fungal isolates (%)
	Ac	Aa	Rj	Mc	Sb	Pg	Ba	Pe	As	Os	Is	Gy	Sw	Pc	Fs	Kn	
Aspergillus niger	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	+	25.00
Rhizopus stolonifer	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	6.25
Candida albicans	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	12.50

Key: + (isolated), - (not isolated), Alcohol (Ac), Ascorbic acid (Aa), Raspberry juice (Rj), Light magnesium carbonate (Mc), Sodium bicarbonate (Sb), Propylene glycol (Pg), Benzoic acid (Ba), Peppermint emulsion (Pe), Amaranth solution (As), Orange syrup (Os), Invert syrup (Is), Glycerol (Gy), Sterile water (Sw), Paracetamol (Pc), Ferrous sulphate (Fs) and light Kaolin (Kn)

All 3 non sterile preparations were contaminated with *Aspergillus niger* while *Candida albicans* and *Rhizopus stolonifer* occurred in one preparation each (Table 4). *Candida albicans*, *Bacillus* spp. and *Aspergillus* spp. were also found in some nonsterile pharmaceuticals investigated (Mugoyela and Mwambete, 2010). On the contrary Al-Charrakh (2012) reported the isolation of *E. coli* (5.70 %), *S. aureus* (20.80 %) and *Pseudomonas aeruginosa* (1.90 %), from a range of pharmaceutical products. The isolation of fungal contaminant in pharmaceutical preparations especially *Candida albicans* cannot be overlooked as 33.00 % of the recovered isolates was of the indicator pathogens. *Candida albicans* is a normal floral of the human body which can easily cause pharmaceutical products to be contaminated by personnel's during handling and processing. *Aspergillus species* are opportunist by nature and they have the capacity to causes a wide range of human diseases, especially in immunnocopromised patients (Hossein *et al.*, 2015)

Table 4: Synopsis of microbial isolates and their frequency of occurrence in some non-sterile preparations

Organisms	Pharmaceutical preparations			Frequency of occurrence of fungal isolates (%)
	KnM	PcE	PfsM	
Aspergillus niger	+	+	+	100.00
Candida albicans	-	+	-	33.33
Rhizopus stolonifer.	+	-	-	33.33

Key: + (isolated) - (not isolated); Kaolin mixture (KnM); Paracetamol elixir (PcE); Pediatric ferrous sulphate mixture (PfsM).

The pharmaceutical raw materials were assessed for their physicochemical properties (Table 5). Extreme pH values seen in them could be responsible for no bacterial contamination. It has been reported that microbial attack is unlikely to occur at extreme pH and spoilage is rare at values above 8 (Kamil and Lupuliasa, 2011). No moisture content was recorded in ascorbic and benzoic acids. These raw materials had no microbial counts whereas fungal counts were recorded in some raw materials with varied moisture content. The total solids of the raw materials were also found to influence microbial growth. Alcohol and sterile distilled water had no total solids and also no microbial count recorded whereas raw materials with total solid contents had fungal contamination. Studies on the effect of milk total solid on the activity of starter culture revealed improved growth and activity of starter cultures with higher amount of total solid compared to others (Mahdian and Mazaheri, 2007). This is because microorganisms require nutrients (solid content) to carry out their metabolic activities.

Table 5: The physicochemical characteristics of the pharmaceutical raw materials (excipients and active pharmaceutical ingredients)

Pharmaceutical raw materials	Colour	Odour	pH	Density (g/ml)	Total solid (%)	Moisture content (%)
Alcohol	Colourless	Choking	7.27±0.01	0.79±0.01	0.00±0.00	100.00±0.00
Ascorbic acid	Pale yellow	Odourless	3.98±0.02	1.51±0.01	100±0.00	0.00±0.00
Raspberry juice	Pink	Pleasant	5.25±0.01	1.03±0.01	0.80±0.01	99.20±0.02
Light magnesium carbonate	White	Odourless	9.41±0.03	0.15±0.01	99.11±0.02	0.89±0.01
Sodium bicarbonate	White	Odourless	9.85±0.03	0.98±0.31	99.29±0.01	0.71±0.02
Propylene glycol	Colourless	Odourless	7.99±0.07	1.04±0.05	1.20±0.03	98.80±0.03
Benzoic acid	White	Odourless	3.82±0.04	0.65±0.01	100.±0.00	0.00±0.00
Peppermint emulsion	White	Minty	5.89±0.02	0.83±0.02	0.61±0.02	99.39±0.03
Amaranth solution	Purple	Odourless	8.40±0.02	1.05±0.01	1.40±0.01	98.60±0.00
Orange syrup	Orange	Pleasant	5.40±0.01	1.04±0.00	12.40±0.02	87.60±0.02
Invert syrup	Colourless	Odourless	7.21±0.05	0.93±0.01	0.43±0.03	99.57±0.02
Glycerol	Colourless	Odourless	6.08±0.04	1.30±0.01	0.31±0.05	99.69±0.01
Sterile distilled water	Colourless	Odourless	6.15±0.01	0.89±0.01	0.00±0.00	100.00±0.00
Paracetamol	White	Odourless	5.44±0.01	0.52±0.02	99.17±0.02	0.83±0.07
Ferrous sulphate	Pale green	Odourless	3.92±0.01	0.75±0.01	99.91±0.01	0.09±0.05
Light Kaolin	White	Odourless	7.86±0.02	0.53±0.02	99.96±0.03	0.04±0.07

The physicochemical characteristics of some non-sterile preparations are shown in Table 6. The colour of the non-sterile preparations varied from one another and they generally had pleasant odour. The pH values ranged from 4.21 ± 0.02 (Paracetamol Elixir) to 6.43 ± 0.05 (Kaolin mixture) while the densities ranged from 0.96 ± 0.02 (Kaolin mixture) to 1.13 ± 0.01 (Pediatric ferrous sulphate mixture). The percentage moisture content ranged from 75.60 ± 0.01 (kaolin mixture) to 96.79 ± 0.02 (pediatric ferrous sulphate mixture) while total solid content ranged from 3.21 ± 0.04 (pediatric ferrous sulphate mixture) to 27.40 ±0.01 (kaolin mixture)

Table 6: Physicochemical characteristics of some non-sterile preparations

Physicochemical characteristics	Pharmaceutical Preparations		
	Kaolin mixture	**Paracetamol elixir**	**Pediatric ferrous sulphate mixture**
Colour	Cream	Light Pink	Light brown
Odour	Pleasant	Pleasant	Pleasant
Total solid (%)	27.4±0.01	4.40±0.03	3.21±0.04
pH	6.43±0.05	4.21±0.02	4.63±0.03
Density (g/ml)	0.96±0.02	1.18±0.04	1.13±0.01
Moisture content (%)	75.6±0.01	95.6±0.03	96.79±0.02

Conclusion

The present study reveals that the microbiological and physicochemical characteristics of pharmaceutical raw materials determine their suitability for pharmaceutical preparations. The quality of raw pharmaceuticals cannot be compromised as these will be eventually used in formulating pharmaceutical products. Most of the analyzed samples were well within the stipulated limits of the United States Pharmacopeia except for a few which were contaminated with *Candida albicans*. It is therefore proper that all pharmaceutical raw materials be evaluated before manufacturing so as to ensure safety and efficacy of pharmaceutical products.

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