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Evaluation of microbial and physico-chemical qualities of some cough syrups marketed in Sana'a city, Yemen

[Evaluación de la calidad físico-química y microbiológica de jarabes para la tos comercializados en la ciudad de Sana'a, Yemen]

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Abstract

Context: Microbial contamination of cough syrups can bring clinical hazards to the users or patients as well as physical and chemical changes in the product.

Aims: To evaluate the microbial and physicochemical characteristics of two hundred samples of four different types of cough syrups marketed in Sana'a city, Yemen.

Methods: All collected samples were subjected to the following examinations: the total microbial count, type of isolated microorganisms, physical parameters, and concentration of active ingredients were identified and assessed by standard techniques described in US Pharmacopeia.

Results: All the cough syrup samples used contained viable microbial load within acceptable limit according Pharmacopeia specifications. *Bacillus subtilis*, *Micrococcus fulvum*, and *Staphylococcus epidermidis* were the most commonly recovered bacteria. However, *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium notatum*, *Mucor* sp., and *Aspergillus flavus* were the most fungi isolated. The physical properties represented in the appearance, density, and pH of the analyzed samples complied with Pharmacopoeia standards. The concentrations of diphenhydramine HCl (92,51 - 108,78%), pseudoephedrine HCl (94,55 - 109,07%), and triprolidine HCl (98,20 - 104,19%) were recorded.

Conclusions: All cough syrups marketed in Sana'a City had good microbiological and physico-chemical qualities.

Keywords: Diphenhydramine; microbiological assay, physical assay, pseudoephedrine; triprolidine.

Resumen

Contexto: La contaminación microbiana de los jarabes para la tos puede traer riesgos clínicos a los usuarios o pacientes, así como los cambios físicos y químicos en el producto.

Objetivos: Evaluar las características microbianas y físico-químicas de doscientas muestras de cuatro tipos diferentes de jarabes para la tos que se comercializan en la ciudad de Sana'a, Yemen.

Métodos: Todas las muestras recogidas se sometieron a los siguientes exámenes: recuento microbiano total, tipo de microorganismos aislado, parámetros físicos, y la concentración de ingredientes activos fue identificada y evaluada por técnicas estándares descritas en la Farmacopea de EE.UU.

Resultados: Todas las muestras de jarabe para la tos contenían carga microbiana viable, dentro de límites aceptables según las especificaciones de la Farmacopea. *Bacillus subtilis*, *Micrococcus fulvum y Staphylococcus epidermidis* fueron las bacterias más comúnmente identificadas. Sin embargo, *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium notatum*, *Mucor* sp., *Aspergillus flavus* fueron los hongos aislados. Las propiedades físicas, representadas en la apariencia, densidad, y el pH de las muestras cumplieron con los estándares de la Farmacopea. Se registraron las concentraciones difenhidramina HCl (92,51 - 108,78%), pseudoefedrina HCl (94,55 - 109,07%), y de triprolidina HCl (98,20 - 104,19%).

Conclusiones: Todos los jarabes para la tos comercializados en la ciudad de Sana'a tuvieron buenas cualidades microbiológicas y físico-químicas.

Palabras Clave: Difenhidramina; ensayo físico; ensayo microbiológico; pseudoefedrina; triprolidina.

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INTRODUCTION

Pharmaceuticals are used for the purpose of prevention, treatment, and diagnosis of diseases. In current years, manufacturers of pharmaceuticals have improved the quality of non-sterile pharmaceuticals such that today such products contain only minimal bioburden (Denyer et al., 2004).

Non-sterile pharmaceutical products are not required to be sterile, but are subject to certain restrictions on the number and types of acceptable microorganisms to make sure their efficaciousness and safety (Jimenez, 2004).

Syrups are non-sterile liquid dosage form that contain active medicaments and constitute the most convenient dosage form for babies, children and the elderly. Syrups are generally prepared for oral administration in children since tablets and capsules cannot be easily or suitably administered to them. The administration of contaminated syrups to these people pose a real danger, even at low levels of contamination, because their immune system are poorly developed (Mendie et al., 1993; Muhammed and Umoh, 2009).

Contamination of oral liquid pharmaceuticals with microorganisms not only makes them hazardous from the infectious standpoint, but may also change the physical, chemical, and organoleptic properties of the drugs, alter the contents of active ingredients, or convert them to toxic products that lead to reduction in their shelf life and efficacy (Shaikh et al., 1988; Moniruzzaman et al., 2012).

So, a drugs may be considered microbiologically spoiled in this situation, depending on its intended use. The presence of even a low level of acutely pathogenic microorganisms, higher levels of opportunist pathogens, or toxic microbial metabolites that persist even after death of the original contaminants may render the product ineffective. Physicochemical deterioration as a result of microbial growth is a acceptable reason to consider the product harmful for patients (Denyer and Baird, 1990).

Numerous reports have also been published describing clinical risks that are attributable to microbiologically contaminated pharmaceuticals (Mugoyela and Mwambete, 2010).

The microbiological quality of a pharmaceutical product may represent contamination from raw materials, industrial equipments, environment, workers and containers (Muhammed and Umoh, 2009).

Within Sana'a City, cough syrups are among the most available syrups in the market which suggest strong likelihood of variation in the level of microbiological purity among these brands. There is therefore, the need to carry out investigations with the view to ascertaining the microbial and physicochemical qualities of these preparations, which are currently available in the market.

MATERIAL AND METHODS

Samples collection

Two hundred samples of cough syrups were collected at random from pharmaceutical stores of five districts located in Sana'a City, Yemen. The samples were produced by four different pharmaceutical companies and coded A, B, C, and D, which are listed in the Table 1.

Table 1. Brand code, sample size, active ingredients, theoretical concentration, and supplier of collected cough syrup samples.

Brand code	Sample size	Active ingredients	Theoretical concentration (mg/5 mL)	Supplier
A	50	DPH HCl	14	Yemen
B	50	DPH HCl	14	Saudi Arabia
C	50	PED HCl	30	Yemen
D	50	TPD HCl + PED HCl	30 + 1.25	Germany

DPH HCl= Diphenhydramine hydrochloride, TPD HCl= Triprolidine hydrochloride, PED HCl = Pseudoephedrine hydrochloride.

Assessment of microbiological qualities

Total microbial count

Ten mL of the sample was diluted to bottle contained 90 mL of trypticase soy broth (Merck, Germany). A quantity of 0.1 mL of the diluted sample was spread on the surface of tryptone soy agar (TSA) and Sabouraud dextrose agar (SDA) plates (Himedia, India). The TSA plates were incubated at 35°C for three days, while the SDA plates were incubated at 25°C for five days with daily observation. Colonies were counted and the mean number of colony forming units (CFU) per mL was calculated and recorded (Clontz, 2009).

Characterization and identification of isolated microbial

Identification of the isolated bacterial and fungal contaminants was preliminarily performed by macroscopic (morphology and growth characteristics) and microscopic (gram staining and lactophenol cotton blue stain technique) examination. Selective, differential culture media, and biochemical test were used to conform the microbial species (Moubasher, 1993; Don et al., 2004; Leboffe and Pierce, 2011).

Physical properties examinations

The appearance was assessed by visual examination to determine the color. The taste was assessed by using the appropriate relevant sense organs. The pH value and density were measured once and determined to use an instrument (Metrohm, Switzerland, model 827pH Lab.) and (Mettler Toledo, Japan, model DA-100M), respectively (USP, 2007).

Chemical examinations

Determination of diphenhydramine hydrochloride (DPH HCl) concentration

Preparation of working standard solution: Fourteen mg of DPH HCl was weighted and dissolved in 50 mL of distilled water (DW) into a volumetric flask (50 mL).

Preparation of sample solution: Five mL from each tested sample was transferred into a volumetric flask (50 mL) containing 45 mL of DW. The working standard and sample solution were sonicated for 10min. Moreover, filtered by membrane filter 0.45 µm before used (USP, 2007).

Sample assayed by HPLC: Perkin Elmer HPLC system (Avenue, USA) consisting of LC-20AD pump, an autosampler (Model SIL-20A), and photodiode array UV-Visible detector was used. The column used was a Macherey-Nagel® EC250 mm × 4.6 mm, Nucleosil, 100-10CN. The mobile phase with a flow rate of 1.5 mL/min was composed of acetonitrile 200 mL to 800 mL of ammonium dihydrogen phosphate (8.57 g/L) (Himedia), and adjusted the pH at 3.0 with phosphoric acid. The injection volume was 20 µm and the detection wavelength was fixed at 225 nm for DPH HCl assay (USP, 2007).

Determination of triprolidine hydrochloride (TPD HCl) pseudoephedrine hydrochloride (PED HCl) concentrations

Preparation of working standard solution: TPD HCl 20 mg and PED HCl 240 mg were weighed and dissolved into a volumetric flask (200 mL) containing 200 mL of HCl 0.01 M (Scharlau, Spain).

Preparation of sample solution: Ten mL of sample was transferred into a volumetric flask (50 mL) containing 40 mL of HCl 0.01 M. The working standard and sample solution were sonicated for 10 min. Moreover, filtered before used (USP, 2007).

Sample assayed by HPLC: Waters HPLC system (Singapore) consisting of Binary pump Waters (Model 1525, Singapore), an autosampler (Model 2707, Netherlands) and UV/Visible detector Waters (Model 2489, Singapore) was used. The Merck® SI 250 ×4.6 mm L3, 5 µm Silica was used. The mobile phase with a flow rate of 1.5 mL/min was composed of mixture of 850 mL of ethanol and 150 mL of ammonium acetate solution (4 g/L). The injection volume was 20 µm and the detection wavelength was fixed at 254 nm for TPD HCl and PED HCl (USP, 2007).

The analysis of results was made as follow:

Percentage (%) = (Absorbance of sample/absorbance of standard) × (concentration of standard/concentration of test) × 100 (USP, 2007).

Statistical analysis

The obtained data were performed for statistical purpose using the IBM SPSS Statistics software (version 20.0, 2011). Differences in the microbial count, isolated microbial, physical and chemical parameters were compared using the ANOVA test. Values of $P < 0.01$ were considered as statistically significant.

RESULTS

Microbiological assays

Table 2 shows the total microbial count of microorganisms present in the analyzed cough syrup samples.

The bacteria and fungi number isolated from tested cough syrup samples are showed in Fig. 1. There is a significant difference in the isolated number of bacteria and fungi results when compared between cough syrup samples with an individual confidence level of 99%.

Physical properties

The results of physical parameters include the color, description, taste, pH, and density (g/mL) that obtained from analyzed cough syrup samples are listed in Table 3.

Chemical concentrations

Table 4 shows the DPH HCl, TPD HCl, and PED HCl concentration in the analyzed cough syrups samples compared with an acceptable limit.

Table 2. Total microbial count in the tested cough syrup samples.

Brand code	Total bacteria (CFU/mL)	Total fungi (CFU/mL)	Acceptable limit (CFU/mL) (USP, 2007)		*P* value
			Bacteria	Fungi	
A	$<10^1$ (90%)				
	$<10^2$ (10%)	$\leq 10^1$ (100%)			
B	$<10^1$ (88%)				
	$<10^2$ (12%)	$\leq 10^1$ (100%)	$<10^2$	10^1	$>0.01^*$
C	$<10^1$ (86%)				
	$<10^2$ (14%)	$\leq 10^1$ (100%)			
D	$<10^1$ (90%)				
	$<10^2$ (10%)	$\leq 10^1$ (100%)			

*The P value ($P > 0.01$) which obtained by variance analysis between groups A, B, C and D in the total microbial count is not statistically significant difference when compared by ANOVA test.

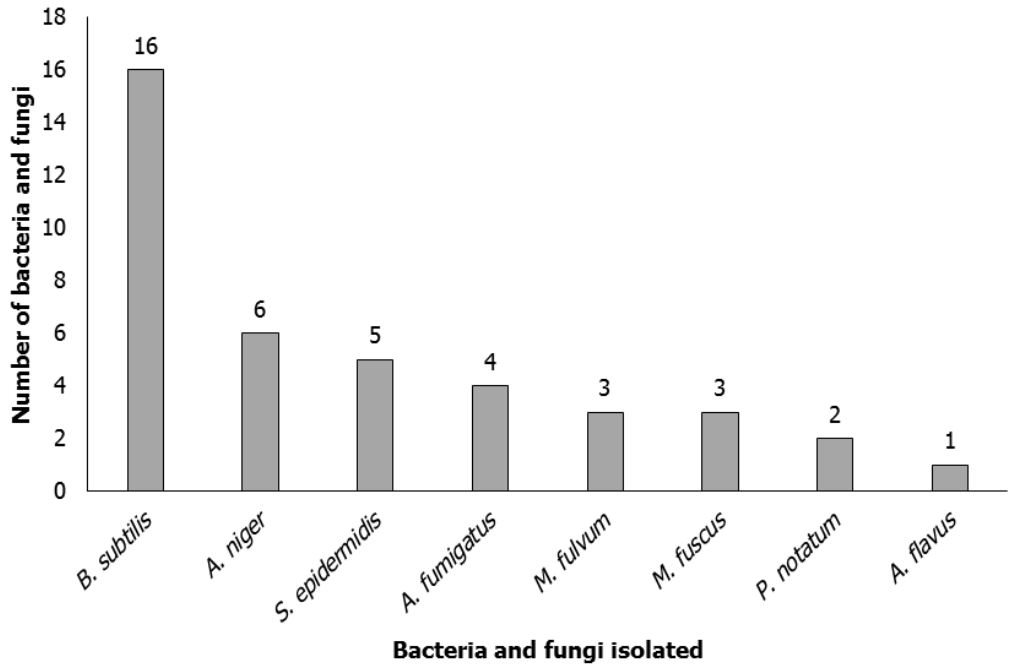

Figure 1. The number of bacteria and fungi isolated from analyzed cough syrup samples.

Table 3. Physical parameters of the different brands of cough syrups.

Parameters	Cough brand code				*P* value
	A	B	C	D	
Color	Green	Brown	Orange	Red	
Description	Clear solution	Clear solution	Clear solution	Clear solution	
Taste	Bitter	Bitter	Bitter	Bitter	
pH	5.03 – 5.10	5.12 – 5.20	5.12 – 5.18	6.01 – 6.10	<0.01**
Density (g/mL)	1.231 – 1.234	1.264 – 1.270	1.198 – 1.203	1.225 – 1.234	<0.01**

**The P value (P <0.01) which obtained by variance analysis between groups A, B, C and D in the pH and density results is statistically significant difference when compared by ANOVA

Table 4. The DPH HCl, TPD HCl, and PED HCl concentration results of analyzed syrup samples.

Brand code	Active ingredients	Theoretical content (mg/5 mL)	Results (%)	*P* value
A	DPH HCl	14.0	94.81 – 108.78	
B	DPH HCl	14.0	92.51 – 105.80	
C	PED HCl	30.0	94.55 – 106.78	<0.01**
D	PED HCl	30.0	98.21 – 109.07	
	TPD HCl	1.25	98.20 – 104.19	

**The P value (P <0.01) which obtained by variance analysis between groups A, B, C and D in the active ingredients is statistically significant difference when compared by ANOVA test.

DISCUSSION

In this research, the result showed that the samples tested had satisfactory total microbial count levels compared to the USP (2007) specification and this in agreement with Brooks et al. (2012).

It is gratifying to note that very low levels of microbial contamination recorded in this investigation, this could be due to the adoption of Good Manufacturing Practice, effective preservative agents, and adequate quality control program (Ogbulie et al., 2009).

Furthermore, it can be inferred that probably an excessive quantity of preservative is used in the preparation of non-sterile pharmaceutical syrups that prevented the growth of microorganisms (Shaikh et al., 1988).

The incorporation of trisodium citrate, together with a sugar content of the syrups, probably are responsible for the lower contamination rates seen in cough syrups. These compounds provide high osmotic pressure and could inhibit many microorganisms (Tukur et al., 2012).

The absence of indicator microorganisms such as *Salmonella* spp., *Escherichia coli*, and *Candida albicans* is an absolute requirement (USP, 2007). In this study, microorganisms were absent, and the samples complied with USP (2007) standards. These findings are similar to Gad et al. (2011) and Mustapha et al. (2013).

In the current study, the absence of *E. coli* and *Salmonella* spp. in all the samples studied indicated that the water used by the pharmaceutical industries may not contaminated by coliform bacteria. The organisms of these types are water–borne and frequently contaminate liquid pharmaceutical product (Denyer et al., 2004).

B. subtilis, *M. fulvum*, and *S. epidermidis* were the most important isolated bacteria, while the *A. niger*, *A. fumigatus*, *P. notatum*, *M. fuscus*, *and A. flavus* were the most important isolated fungi from samples. A similar observation was reported by Oyeleke et al. (2005).

Bacillus subtilis reported to be the most frequent in syrups, and also found to be the number one contaminant of non-sterile pharmaceutical in Spain (Rosa et al., 1993).

Although the organisms that are detected in the syrup samples are not pathogenic, they are "objectionable" since they can bring about the destruction of active ingredients. Thus, they interfere with the function of the therapeutic product (Shaikh et al., 1988).

Gram–positive cocci can also be survived in the environment and thus contaminate the medicaments. *S. epidermidis* reported being the most frequent in oral and topical medicaments. Whereas, the *Micrococcus* sp. was isolated from liquid and solid drugs (Rosa et al., 1993).

However, *S. aureus* and *S. epidermidis* found as contaminants reflect easy contamination of processing unit. The organisms being normal floral of the body easily contaminate products during handling and processing by personnel. Though, favorable growth environment for microorganisms is prevented by high sugar concentration (Coker, 2005).

Isolated *Aspergillus*, *Penicillium* and *Mucor* species are producers of possible allergic and toxin compounds. *Aspergillus* sp. causes aspergillosis while *Aspergillus flavus* produces aflatoxin that is carcinogenic (Prescott et al., 2008).

From the finding made in this study, it could be inferred that the isolated microorganisms were either of processing unit, during handling, and human flora types, principally, Gram-positive bacteria, or air-borne fungi.

The physical examination results showed that the cough syrup samples appearance were varied from clear green, brown, orange, and red with a bitter taste and this agreement with Edebi et al. (2011).

However, the density results in the cough syrup samples were ranged from 1.198 – 1.270 g/mL.

The pH values in the cough syrup contained TPD HCl, and PED HCl should be between 4.6–6.6 (USP, 2007). In the same manner, the pH values in the cough syrup contained DPH HCl should be between 4.0–6.0.

From these results, it can be observed that all the pH value results obtained from cough syrup samples are within the acceptable range for pH value results according to USP (2007) specifications. The acidity and alkalinity of the syrups determine the effectiveness in administration.

The USP (2007) stipulates that an oral cough solution should contain not less than 90.0% and not more than 110.0% of the labeled amount of DPH HCl, TPD HCl, and PED HCl and it also states that for maximum stability. From the results of Table 4, product A, B, C, D showed values within the USP (2007) specifications for a product to be passed. These results agree with Edebi et al. (2011).

CONCLUSION

This study showed the compliance of cough syrups with the good manufacturing practice; especially with the aspects related to the control of microbial levels of all raw materials and water used, which ensure production of cough syrups with low microbial counts. The low contamination level could be maintained during the shelf life by the inclusion of suitable preservants where necessary.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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