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Evaluation of microbial quality of herbal tea pharmaceutical products found in the Egyptian market

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ABSTRACT

The presence of microbial contaminants in non-sterile pharmaceutical herbal preparations not only cause spoilage of products but also proved to be a potential health hazard to the consumer. Accordingly, non-sterile preparations have to pass microbial limit tests. The aim of present study was to evaluate the microbial quality of oral herbal non-sterile pharmaceutical preparations available in the Egyptian market.

A total of 32 non-sterile herbal tea pharmaceutical preparations were subjected to microbial limit testing which included microbial enumeration tests using the spread plate technique and tests for specified microbial contaminants using standard conventional techniques. The data was interpreted according to the United states Pharmacopoeia (USP) guidelines. All the tested products contain bacteria within the limits stated in USP for botanicals treated with boiling water, while 5 products (15.6%) have total fungal counts that exceed the USP limits. Two out of the 32 tested products (6.25%) were contaminated with *E. coil* while 4 products (12.8%) found to have *S. aureus*. Our results indicates that the microbiological quality of the examined products was, in general, adequate. with exception of few products.

Key words: Non-sterile pharmaceuticals, herbal tea, microbial limit tests, microbial quality.

INTRODUCTION

Herbal drugs are complex active substances which are either used by patients as original herbal drugs or which serve as starting materials for the manufacture of finished medicinal products ***(European Pharmacopoeia, 2006).*** About, 80% of the world's population, living in developing countries use herbal medicines as their primary health care source ***(WHO, 1998a, Mukherjee, 2002).***

National regulation and registration of herbal medicines vary from country to country. In many countries herbal products are launched into the market without proper evaluation ***(Bauer and Tittel, 1996).*** The regulatory process is a mechanism for evaluating the safety, efficacy and quality of herbal medicinal product. Each country should adopt a regulatory system to manage the appropriate use of herbal medicines. Adopting a regulatory mechanism is important to ensure the quality of herbal medicines, including the microbiological quality ***(WHO, 1998b; WHO, 2004).***

Herbal medicines are considered as non sterile pharmaceuticals, that may contain limited microflora. Microbial contamination of non sterile pharmaceuticals refers to the presence of specified undesired pathogenic microbes, or higher than the acceptable levels of aerobic bacteria and fungi **(Muhammed and Umoh, 2009**). For medicinal herbs, the United States Pharmacopoeia (USP) discussed microbial requirements of the botanical ingredients and their products under the chapter; "microbiological attributes of non sterile nutritional and dietary supplements" (**USP, 2006**). For the

first category, powdered botanicals, fluid extracts and pharmaceuticals supplements with botanicals, USP requires not more than 10^5 CFU/g aerobic bacteria, not more than 10^3 CFU/g fungi, not more than 10^3CFU/g Gram negative *Enterobacteriaceae* and the absence of *Salmonella spp.* and *E. coli* in 10 grams. For the second category (botanicals to be treated with boiling water before use), USP requires not more than 10^5 CFU/g aerobic bacteria, not more than 10^3 CFU/g fungi and the absence of *E. coli* in 10 grams (**USP, 2006**).

The current study aims to evaluate the microbial quality of oral herbal teas found in the Egyptian market according to the United States Pharmacopoeia regulations.

Materials and methods

1. Sample collection

A total of thirty two herbal teas products representing products of the most popular Egyptian companies were collected from local pharmacies and subjected to qualitative and quantitative assessment of microbial quality. A total of 96 batches were evaluated, three batches for each of the tested herbal tea products (**Table 1**).

2. Microbial Enumeration Tests

A portion of each sample (10g) was dispersed in trypton soya broth medium to make 100 ml under aseptic conditions, polysorbate 80 (1 g/L) was added to assist the suspension of poorly wettable substances**.** Ten fold serial dilutions were made to the previously prepared sample then total bacterial counts and total fungal count **(USP, 2007)**.

For bacterial and fungal counts, trypton soya agar (TSA) media or Sabouraud dextrose agar (SDA) were used, respectively. About 20 ml of each media was poured into 9 cm Petri dishes at 45° C and allowed to solidify. At least three Petri dishes were used for each medium and each level of dilution. A 100 µL of the prepared samples were spread plated on prepared plates. Each set of plates were incubated aerobically at 37° C for 24h for bacteria and for 5 day for fungi. Developed colonies were enumerated and CFU/g was calculated and recorded for the total aerobic count and total fungal count**.**

3. Tests for Specified Microorganisms.

A) Detection of *Escherichia coli*

The product was prepared as described previously and incubated at 37ºC for 2 hr. One ml of sample was dispensed in 100 ml of MacConkey broth then incubated at 37 ºC for 48 hr, growth was sub-cultured on surface of Eosin methylene blue (EMB) agar. The formation of black colonies with greenish metallic sheen indicated presence of *E. coli* **(USP, 2006)**.

B) Detection of *Salmonella spp.*

The product was prepared as described previously and incubated at 37ºC for 2 hr. One ml of sample was transferred to 10 ml of tetrathionate broth then incubated at 37ºC for 24 hr. By using inoculating loop, growth was on sub-cultured on Xylose-lysine-deoxycholate (XLD) agar then incubated at 37ºC for 24 hr. The presence of well developed red colonies indicates presence of *Salmonella spp.* **(USP, 2006)**.

C) Detection of *Staphylococcus aureus*

The product was prepared as described previously and incubated at 37ºC for 2 hr. About 0.1 ml of sample was subcultured on a plate of Manitol-Salt Agar (MSA) and incubated at 37ºC for 48 hr. The presence of *Staphylococcus aureus* is indicated by development of yellow colonies surrounded by yellow zone **(USP, 2007).**

Table (1): List of pharmaceutical herbal tea products included in this study

Company	Product name	code	Active constituents
Q1	**Isis Green tea**	I1	Green tea
	Isis Cinnamon	I2	Cinnamon
	Isis Hibiscus	I3	Hibiscus
	Isis Chamomile	I4	Chamomile
	Isis Caraway	I5	Caraway
	Isis Ment	I6	Ment
	Isis Anise	I7	Anise
	Isis Tilio	I8	Tilio
	Isis Ginger & cinnamon	I9	Ginger, cinnamon
Q2	**Royal Caraway**	R1	Caraway
	Royal Hibiscus	R2	Hibiscus
	Royal Anise	R3	Anise
	Royal Guava leaf	R4	Guava leaf
	Royal Cinnamon	R5	Cinnamon
	Royal Lemon & Ginger	R6	Lemon, Ginger
	Royal Green tea	R7	Green tea
	Royal Piperment	R8	Piperment
	Roya pect	R9	beba scum, thymus, cinnamon, menthe, psydium
	Royal	R10	Chicory- nettle, liqurice, marioram, senna, clerey
Q3	**Sekem Intestinal**	S1	Senna, fennel, anise, liqurice
	Sekem Renal	S2	Ammivisnaga, cymbopgon, liqurice, peppermint, ambrosia, achillea, chicory
	Sekem Cough	S3	Chamomile, liqurice, fennel, dill, anise, caraway, marjoram
	Sekem Laxative	S4	Caraway, foenugreak, dill, anise, nettel, chamomile, fennel
	Sekem Calm	S5	Tilia, guava, verbscum, menth, fennel, liqurice, majoram
	Sekem Lactaguge	S6	Curcuma, rosemary, senna, liqurice, achillea, chicory, fennel
	Sekem Flu	S7	Chamomile, thyme, caraway, pepperorh, anise
	Sekem Hepatic	S8	Curcuma, rosemary, senna, liqurice, achillea, chicory, fennel
	Sekem Baby calm	S9	Chamomile, thyme, caraway, pepperorh, anise
Q4	**Root Tilio**	T1	Tilio root
	Root Hibiscus	T2	Hibiscus
	Root Cinnamon	T3	Cinnamon
Q5	**Pharco Baby drink**	P1	Baby drink

RESULTS

The microbial quality of 32 herbal teas was assessed, collected samples were subjected to total bacterial count, total fungal count and detection of specific microorganisms **(Table 2).** The data shown in **Table 2** was evaluated according to USP guidelines for botanicals that are treated with boiling water before use and the results are shown in (**Table 3.** All the tested products contain bacteria within the limits stated in USP, while 5 products (15.6%) have total fungal counts that exceed the USP limits. Two out of the 32 tested products (6.25%) contain *E. coil*, in addition 4 products (12.8%) found to have *S. aureus*, while *Salmonella* was not detected in any of the products.

Table (2): Microbial quality of some herbal teas from different companies

	Total bacterial count /1g	**Total fungal count /1g**	**Specific organism /10 g**
I1	$1.09\pm0.06 \times 10^2$	1.6 ± 0.3	-
I2	$1.47\pm0.3 \times 10^2$	1.53 ± 0.5	-
I3	$1.34\pm0.2 \times 10^2$	**$2.3\pm0.3 \times 10^3$**	***E. coli***
I4	$2.87\pm0.2 \times 10^2$	$0.2 \pm 0 \times 10^2$	-
I5	$3.07\pm0.9 \times 10^2$	1.77 ± 0.7	-
I6	$4.4\pm1.27 \times 10^2$	$0.2 \pm 0 \times 10^2$	-
I7	$1.26\pm0.1 \times 10^3$	2.3 ± 0.3	-
I8	$4.6 \pm2.0 \times 10^2$	1.1 ± 0.17	-
I9	$1.2\pm0.2 \times 10^2$	$0.1 \pm 0 \times 10^2$	-
R1	$1.17\pm0.06 \times 10^2$	2.67 ± 0.6	-
R2	$2.69\pm0.29 \times 10^2$	2.2 ± 0.03	-
R3	$2.97 \pm0.35 \times 10^2$	3.0 ± 0.5	
R4	$2.93 \pm0. 6 \times 10^2$	3.53 ± 0.68	-
R5	$1.3\pm0.17 \times 10^3$	2.43 ± 0.15	-
R6	$2.0\pm0.5 \times 10^2$	$0.15 \pm 0 \times 10^2$	-
R7	$1.6\pm0.35 \times 10^2$	$0.2 \pm 0.01 \times 10^2$	-
R8	$0.4\pm0.75 \times 10^2$	$0.23 \pm 0 \times 10^2$	-
R9	$3.1\pm0.29 \times 10^2$	$2.43 \pm 0.15 \times 10^2$	-
R10	$2.43\pm0.15 \times 10^2$	$0.13 \pm 0 \times 10^2$	-
S1	$1.25\pm0.08 \times 10^2$	**$1.53\pm0.4(10^3)$**	-
S2	$2.87\pm0.23 \times 10^3$	$0.22 \pm 0 \times 10^2$	-
S3	$1.22\pm0.1 \times 10^3$	$0.17 \pm 0 \times 10^2$	-
S4	$1.22\pm0.1 \times 10^2$	0.76 ± 0.0	***E. coli***
S5	$2.87\pm0.23 \times 10^2$	2.3 ± 1.0	-

S6	1.6±0.42 x 10^2	1.8±0.3	-
S7	1.11±0.1 x 10^3	**1.4±0.5 (10^3)**	-
S8	1.21±0.08 x 10^3	**2.2±0.2(10^3)**	***S. aureus***
S9	0.87 ±0.4 x 10^2	0.18 ± 0 x 10^2	-
T1	1. 9±0.34 x 10^3	1.43±0.0	***S. aureus***
T2	1.2±0.1 x 10^3	4.3 ±0.0	***S. aureus***
T3	1.17±0.15 xx10^3	**1.95±0.9(10^3)**	-
P1	1.33±0.3 x 10^2	1.46±0.06	***S. aureus***

Table (3): List of herbal tea products that not comply with the requirements of USP (2006) for medicinal herbs to which boiling water is added before use

Product code	Reasons
I3	Fungal count > 10^3 & Presence of *E. coli*
S1	Fungal count > 10^3
S4	Presence of *E. coli*
S7	Fungal count > 10^3
S8	Fungal count > 10^3
P1	Fungal count > 10^3

DISCUSSION

The occurrence of microbial contamination has been well documented, several reports have been published describing clinical hazards of microbiologically contaminated pharmaceuticals (**USP, 2003**)**.** The major health concern is when such microbial contaminants exceed acceptable limits **(Carstersen and Rhodes, 2000).** In recent years, manufacturers of pharmaceuticals have improved the quality of non-sterile pharmaceuticals to such products contain only minimal bioburden **(Aulton, 2002)**

In the current study, A total of 32 oral herbal pharmaceuticals were screened for microbial contamination. Results revealed that 18.75% of the products were contaminated and that the bioburden levels were generally adequate. All the tested products contain bacteria within the bioburden limits, this come in accordance with the results of **Enayatifard *et al.* (2010)**. While a previous study in Libya reported the presence of aerobic bacteria in some of the tested oral non-sterile products, 10% in syrups and 15% in tablets (**Tamalli *et al.*, 2013),** which indicates higher quality of pharmaceuticals tested in current study. A small proportion of the products (12.5%) was found to have a fungal count that exceeds the bioburden level, the presence of some molds may reflects the storage quality of the herbal pharmaceuticals.

The percentage of products which contain pathogenic microorganisms in this study is 18.75% (6.25% contain *E. coli* and 12.8% contain *Staphylococcus aureus*) which is smaller than the 33.75% reported by other authors for oral non-sterile pharmaceuticals (**El-Houssieny *et al.*, 2013).** Also *Salmonella spp* was not

detected in any of the products in current study**. Enayatifard *et al.* (2010)** detected *Salmonella* in all of the tested herbal products. This may be due to better adherence to 'Good Manufacturing Practices' by pharmaceutical manufacturers.

The presence of *Staphylococcus aureus* (in the products of three companies) may suggest contamination from the equipment and/or raw material, or poor hygiene of the hands during production (**Shaikh *et al.*, 1988**).

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تقييم الجودة الميكروبية للمستحضرات العشبية التي تستخدم عن طريق الفم و المتوفرة في السوق المصري

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تم اجراء دراسه ميكروبيولوجيه علي عدد من المستحضرات العشبية التي تستخدم عن طريق الفم و المتوفرة في السوق المصري. و أشتملت هذه العينات علي ٣٢ مستحضر (٣ تشغيلات من كل مستحضر) .تم دراسه الحد الحيوي للبكتيريا الهوائيه والحد الحيوي للفطريات بالأضافه الي أختبارات الكشف عن تواجد الاشيشرشيا كولاي و المكورات العنقوديه الذهبيه و جنس السالمونيلا و تم تحليل نتائج الدراسة ومقارنتها بارشادات دستور الادوية الامريكي. وقد اظهرت الدراسة ان عدد البكتيريا الهوائية اقل من الحد المذكور بدستور الادوية الامريكي بينما زاد عدد الفطريات في ١٥,٦% من المستحضرات عن الحد المسموح به. وتواجد ميكروب الاشيرشيا كولاي في ٦,٢٥% من المستحضرات كما تواجدات المكورات العنقودية الذهبية في نسبة ١٢,٨ % من المستحضرات قيد الدراسة. و بصفة عامة فان معظم المستحضرات العشبية موضع الدراسة كانت ذات جودة مقبولة باستثناء عدد قليل من المستحضرات.