

# Evaluation of bacterial and mycotoxin contamination of local medicinal herbs

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## ABSTRACT

One hundred and sixty eight representative samples of unpacked and packed herbal medicine plants were examined for their contamination with salmonella, *Clostridium perfringens*, *Bacillus cereus*, *Staphylococcus aureus* and mycotoxin. The samples were collected over a period extending from January 2003 to April 2004.

The results showed that the total aerobic plate count in herbal samples packed and unpacked product ranged from  $5.2 \times 10^3$  to  $1.9 \times 10^6$  and  $1.2 \times 10^4$  to  $2.6 \times 10^6$ , respectively. The sporeforming bacteria (*B. cereus*, *Cl. perfringens*) were isolated from packed and unpacked. The result showed that 25.0%, 28.6%, 32.1% and 53.6%, respectively. All samples under investigation were free from salmonella, Ochratoxin A, aflatoxin B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. The presence of Aflatoxin B<sub>1</sub> was detected and found to be in the range of 17.7% and 35.7% packed and unpacked herbal medicine products, respectively. Zearalenone was determined and estimated to be 10.7% and 35.1% in packed and unpacked herbal medicine products, respectively.

The statistical analysis showed that no significant differences for all microorganisms between packed and unpacked herbs samples except between yeast, coliform and *Staph. aureus*.

## INTRODUCTION

Recently, the importance of medicinal plants are greatly increased in Egypt. Such plants are known by their use as a safe source for human health remedies, (Owolbai et al., 1995) and as raw materials in the manufacture of pharmaceutical and cosmetic products.

Medecinal plants as raw materials normally contains a great number of molds often from the soil, current practices of harvesting and handling. Production can contribute in contamination and microbial growth, El-Tahan et al. (2001), such processes which may lead to potential carcinogenic effects.

*Aspergillus flavus* and *A. parasitius* have been reported to produce aflatoxins. These metabolites show toxic and carcinogenic properties and their presence as nature contaminants in several food stuffs has been studied (Davis et al., 1966 and Mourean, 1971).

In developing countries, aflatoxicosis are major threats to both animal production and human public health. These factors can play significant difficulties for trade in food and feed (FAO, 1997).

Several herbal medicines are commonly used in different forms in human food. The condition of their production (i.e. cultivation, processes

and storage) may lead to the contamination with some fungi and microorganisms.

The objective of this study is to assess some pathogenic bacteria i.e. *Salmonella* spp., *Clostridium perfringens*, *Bacillus cereus*, *Staphylococcus aureus* and Faecal coliform in the herbal medicine plants. In addition, the evaluation of the occurrence aflatoxins, zearalenone and ochratoxin in the herbal medical plants.

## MATERIALS AND METHODS

One hundred and sixty eight representative samples of packed and unpacked medicinal plants were purchased from local markets in Cairo. The medicinal plant were divided into 14 category including (Renal, slimming, rheumatic, flu, intestinal, colic, laxative, calm, acne, lactagogue, sedative, headache, cough and regime). Six samples of each packed and unpacked medicinal plants from each category were prepared and subjected to microbiology and mycotoxin examination in order to determine its hygienic quality.

### Microbial analysis :

Preparation of tested samples, initial suspension and decimal dilution were carried out according to ISO 6887 (2001). Ten grams of tested sample were weighed and placed in a sterile stomacher bag and 90 ml diluent (buffered peptone water) was added and blended for 1-2 min, then decimal dilution to  $10^{-7}$  in buffer peptone was made to perform enumeration of total aerobic bacterial count, Yeast, Moulds, Enterobacteriaceae, Coliform, Faecal coliform, *E. coli*, Enterococci, *Clostridium perfringens*, *Bacillus cereus* and *Staphylococcus aureus*, as follows:

- \* Enumeration of the total aerobic bacterial count (30°C/72 hrs) was performed using *plate count agar* (ISO 4833-2002).
- \* Yeasts and moulds count were determined using *yeast chloramphenical agar* (30°C/72 hrs) ISO 7954-1988.
- \* Count of Enterobacteriaceae (37°C/24 hrs) was determined using *crystal violet neutral red bile dextrose* (VRBD) ISO 7402-1993.
- \* Total coliforms count was done using *crystal violet neutral red bile lactose* (VRBL) (37°C/48 hrs, ISO 4832-1991).
- \* Presence of faecal coliform was determined using *crystal violet neutral red bile lactose* (VRBL) 44.5°C/24 hrs (NMKL 68 1998). *Escherichia coli* was isolated on *VRB-Mug* (44.5°C/24 hrs) suspected colony detected under UV Lamb at 365 nm (ISO 4832-1991 modified).

- \* Enumeration of Enterococci (37°C/48 hrs) was determined using KF streptococcus agar followed by confirmation of suspected colony using API 20E (NMKL 185-2003).
- \* *Clostridium perfringens* was isolated on T.S.C. agar (37°C/24 hrs) anaerobic followed by confirmation of suspected black colony using API20A (ISO 7937, 1997).
- \* *Bacillus cereus* count was done using MYP agar (30°C/48 hrs), ISO 7932-1993. *Staph. aureus* was isolated on Baird parker agar (37°C/24 hrs) ISO 6888-1998. The suspected black colonies were confirmed by coagulase test.
- \* *Salmonella* spp., 25 g tested sample was performed in 225 ml non selective enrichment buffer peptone water 37°C (16-20 hrs), 1 ml and 0.1 ml of incubated (BPW) were transferred into two tubes containing 10 ml tetra thionate broth (TTB) and rappart-vassliadis broth (RV) 41.5°C/24 hrs, it was isolated on xylose-lysine deoxycholate medium (XLD) and phenol red brillent green agar (37°C/24 hrs) ISO 6579-2003.

General guidelines on quality assurance for the preparation of culture media in the laboratory according to ISO 11133 -2-2002).

#### **Mycotoxin standard :**

Aflatoxin standards sigma (USA) (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>) 0.5 and 2.5 µg/ml were prepared in benzene acetonitrile (812 V/V) according to the procedure described in A.O.A.C. (1998). Zearalenone standard (Sigma) 50 µg/ml was prepared in benzene, according to the procedure in A.O.A.C. (1998). Ochratoxin standard (Sigma) 5 µg/ml was prepared in acetic acid-benzene (1:99) according to the procedure described in A.O.A.C. (1998).

#### **Mycotoxin analysis :**

All chemicals and solvents used were of ACS grade. Thin layer chromatography (TLC) was performed using 20 x 20 cm TLC glass plates precoated with 0.25 mm silica gel 60 (Merck).

Aflatoxin, zearalenone, Ochratoxin were extracted by B.F. method as described in A.O.A.C. (1998). The detection limits were 5 µg/kg, 10 µg/kg, 5 µg/kg for aflatoxin, zearalenone and ochratoxin, respectively. Extracts were dissolved in 200µL chloroform and vortexed, 20 µl aliquot and 10 µL of the standards were brought on TLC plates and developed in a dark room with diethylether : methanol : water (96:3:1). After drying, the spots were examined with U.V. at wave length of 366 nm. Positive samples were subjected to conformation using HPLC according to the method described by A.O.A.C. (1998).

**Statistical analysis** statistical analysis were determined using SAS (1998).

## RESULTS AND DISCUSSION :

The results obtained from analysis of 168 samples from different kinds of local herbal medicines (un-packed and packed) are shown in Tables (1, 2, 3 and 4). The study showed that the total aerobic plate count in samples collected from herbal shops (unpacked and packed product) ranged from  $1.2 \times 10^4$  to  $2.6 \times 10^6$  and  $5.2 \times 10^3$  to  $1.9 \times 10^8$  (Table 1,2). These results were in accordance with Kaul and Teneja (1989) who stated that large variation in total aerobic plate counts were found among herbs samples of different origin.

The results presented in Table (1,2) showed that all unpacked herbs samples were contaminated with yeast in amount  $<10$  cfu/g, whereas 24 product of unpacked samples were contaminated with yeast ranging from  $1.1 \times 10^2$  to  $2.7 \times 10^3$ . These results agree with Guarino et al., 1973 who stated that in general, yeast are seldom detected. However, moderate to high levels of yeast can be found in herbs such as dill and basil, because of moderate climate in which they were grown (Pafumi et al., 1986). The data in Table (1,2) showed that there was no apparent difference in mold count between unpacked and packed herbs medicinal plants.

Spore forming bacteria *Bacillus cereus* and *Clostridium perfringes* were isolated from both packed and unpacked herbal, the result showed that 25.0%, 28.6%, 32.1% and 53.6% respectively.

*Staph. aureus* was presented in 5 packed sample ranging from  $1.1 \times 10^2$  to  $3.1 \times 10^2$  whereas 6 unpacked product samples were contaminated with *Staph. aureus* ranging from  $1.1 \times 10^2$  to  $2.1 \times 10^3$ . These results agree with Zaied et al., 1996, who stated that coagulase positive of staphylococci are not characteristically found the data in Table (1,2) demonstrated that all examined samples were free from salmonella.

Enterobacteriaceae, coliform, *F. coliform*, *E. coli* and *Enterococci* were presented in unpacked herbs and estimated to be ( $1.1 \times 10^4$  to  $1.5 \times 10^3$  cfu/g), ( $1.1 \times 10^2$  to  $2.6 \times 10^4$  cfu/g), ( $1.1 \times 10^2$  to  $4.3 \times 10^3$  cfu/g), ( $<10$ ) and ( $1.1 \times 10^2$  to  $3.0 \times 10^3$  cfu/g), respectively.

**Table (1): Bacterial counts in unpacked herbs samples collected from two local markets.**

Product	T.P.C.	Yeast	Molds	Entero-bacteria	Coliform	F. coliform	E. coli	Entero-cocci	Cl. perf.	B. cereus	Staph. aureus	Salmo-nella
Renal	C	7.7x10 <sup>4</sup>	1.8x10 <sup>3</sup>	1.3x10 <sup>3</sup>	3.5x10 <sup>3</sup>	5x10 <sup>2</sup>	4.3x10 <sup>3</sup>	<10	3x10 <sup>3</sup>	2.5x10 <sup>3</sup>	<10	<10
	D	8.2x10 <sup>4</sup>	1.2x10 <sup>2</sup>	4 x 10 <sup>2</sup>	2.3x10 <sup>3</sup>	3.5x10 <sup>2</sup>	3.1x10 <sup>2</sup>	<10	3.2x10 <sup>3</sup>	1.6x10 <sup>2</sup>	<10	<10
Slimming	C	1.6x10 <sup>4</sup>	2.7x10 <sup>2</sup>	2.2x10 <sup>2</sup>	1.4x10 <sup>3</sup>	1.4x10 <sup>2</sup>	1.1x10 <sup>2</sup>	<10	1.5x10 <sup>3</sup>	1.5x10 <sup>2</sup>	<10	<10
	D	6x10 <sup>4</sup>	2.5x10 <sup>2</sup>	2.5x10 <sup>2</sup>	1.9x10 <sup>2</sup>	<10	<10	<10	2.6x10 <sup>2</sup>	1.1x10 <sup>2</sup>	<10	<10
Rheumatic	C	3.5x10 <sup>4</sup>	4x10 <sup>2</sup>	1.5x10 <sup>3</sup>	4.5x10 <sup>2</sup>	<10	<10	<10	<10	1.5x10 <sup>3</sup>	<10	<10
	D	2.6x10 <sup>4</sup>	2.1x10 <sup>2</sup>	<10	3.1x10 <sup>2</sup>	1.3x10 <sup>2</sup>	<10	<10	2.1x10 <sup>2</sup>	<10	<10	<10
Flu	C	1.9x10 <sup>4</sup>	1.9x10 <sup>2</sup>	4.1x10 <sup>2</sup>	<10	<10	<10	<10	1.1x10 <sup>2</sup>	3.1x10 <sup>2</sup>	<10	1.6x10 <sup>2</sup>
	D	3.8x10 <sup>4</sup>	1.7x10 <sup>2</sup>	2.5x10 <sup>2</sup>	1.6x10 <sup>2</sup>	<10	<10	<10	<10	<10	<10	1.1x10 <sup>2</sup>
Intestinal	C	7.1x10 <sup>4</sup>	2.2x10 <sup>2</sup>	1.4x10 <sup>2</sup>	<10	<10	<10	<10	1.2x10 <sup>3</sup>	<10	3.2x10 <sup>3</sup>	<10
	D	6.2x10 <sup>4</sup>	3.5x10 <sup>2</sup>	2.2x10 <sup>2</sup>	1.9x10 <sup>2</sup>	1.2x10 <sup>2</sup>	<10	<10	1.1x10 <sup>2</sup>	<10	<10	1.2x10 <sup>2</sup>
Colic	C	2.2x10 <sup>4</sup>	<10	2.1x10 <sup>2</sup>	<10	1.1x10 <sup>2</sup>	<10	<10	1.6x10 <sup>2</sup>	<10	ND	ND
	D	3.5x10 <sup>4</sup>	1.4x10 <sup>2</sup>	<10	<10	<10	<10	<10	2.2x10 <sup>2</sup>	2.5x10 <sup>2</sup>	<10	<10
Laxative	C	8.1x10 <sup>4</sup>	2.9x10 <sup>2</sup>	1.9x10 <sup>2</sup>	1.1x10 <sup>2</sup>	<10	<10	<10	3.1x10 <sup>2</sup>	1.3x10 <sup>2</sup>	5.1x10 <sup>2</sup>	ND
	D	5.2x10 <sup>4</sup>	1.1x10 <sup>2</sup>	1.1x10 <sup>2</sup>	1.9x10 <sup>2</sup>	<10	<10	<10	2.2x10 <sup>2</sup>	3.2x10 <sup>2</sup>	<10	<10
Calm	C	1.6x10 <sup>4</sup>	1.5x10 <sup>2</sup>	3.1x10 <sup>2</sup>	3.5x10 <sup>2</sup>	1.3x10 <sup>2</sup>	<10	<10	<10	3.1x10 <sup>2</sup>	<10	<10
	D	2.1x10 <sup>4</sup>	<10	<10	2.2x10 <sup>2</sup>	1.9x10 <sup>2</sup>	<10	<10	<10	2.6x10 <sup>3</sup>	<10	<10
Acne	C	3.5x10 <sup>4</sup>	<10	1.2x10 <sup>2</sup>	<10	<10	<10	<10	1.2x10 <sup>2</sup>	<10	1.2x10 <sup>2</sup>	ND
	D	5.7x10 <sup>4</sup>	1.2x10 <sup>2</sup>	2.2x10 <sup>2</sup>	<10	<10	<10	<10	3.6x10 <sup>2</sup>	<10	ND	ND
Lactagogue	C	2.5x10 <sup>4</sup>	1.4x10 <sup>2</sup>	1.1x10 <sup>2</sup>	1.6x10 <sup>2</sup>	1.3x10 <sup>2</sup>	<10	ND	<10	<10	<10	ND
	D	4.2x10 <sup>4</sup>	<100	<10	2.5x10 <sup>2</sup>	2.2x10 <sup>2</sup>	<10	ND	<10	<10	<10	<10
Sedative	C	3.5x10 <sup>4</sup>	2x10 <sup>2</sup>	<10	<10	<10	<10	4.5x10 <sup>2</sup>	1.1x10 <sup>2</sup>	1.6x10 <sup>2</sup>	1.6x10 <sup>2</sup>	ND
	D	4.1x10 <sup>4</sup>	<10	1.2x10 <sup>2</sup>	1.1x10 <sup>2</sup>	<10	<10	3.2x10 <sup>2</sup>	1.7x10 <sup>2</sup>	1.7x10 <sup>2</sup>	>10	>10
Headache	C	3.6x10 <sup>4</sup>	2.7x10 <sup>2</sup>	<10	3.6x10 <sup>2</sup>	3.1x10 <sup>2</sup>	<10	<10	<10	<10	1.2x10 <sup>2</sup>	ND
	D	2.9x10 <sup>4</sup>	1.9x10 <sup>2</sup>	<10	2.2x10 <sup>2</sup>	2.1x10 <sup>2</sup>	<10	<10	<10	<10	2.1x10 <sup>2</sup>	>10
Cough	C	3.9x10 <sup>4</sup>	2.1x10 <sup>2</sup>	<10	4.5x10 <sup>2</sup>	2.6x10 <sup>2</sup>	<10	ND	<10	<10	<10	ND
	D	2.7x10 <sup>4</sup>	1.1x10 <sup>2</sup>	1.3x10 <sup>2</sup>	1.7x10 <sup>2</sup>	1.1x10 <sup>2</sup>	<10	ND	1.1x10 <sup>2</sup>	<10	<10	ND
Regime	C	8.4x10 <sup>4</sup>	2x10 <sup>2</sup>	<10	<10	<10	<10	<10	1.2x10 <sup>2</sup>	<10	1.3x10 <sup>3</sup>	>10
	D	7.2x10 <sup>4</sup>	2.6x10 <sup>2</sup>	<10	<10	<10	ND	1.6x10 <sup>2</sup>	<10	<10	<10	>10

T.P.C. = Total plate count.

N.D = Not detected

A = Mean of three samples collected from first market.

B = Mean of three samples collected from 2<sup>nd</sup> market.

**Table (2): Bacterial counts in packed herbs samples collected from two local markets.**

Product		T.P.C.	Yeast	Molds	Entero- bacteria	Coliform	<i>F. coliform</i>	<i>E. coli</i>	Entero- cocci	<i>Cl. perf.</i>	<i>B. cereus</i>	<i>Staph.</i> <i> aureus</i>	<i>Salmo-</i> <i>nella</i>
Renal	A	2X10 <sup>6</sup>	<10	2.2x10 <sup>3</sup>	2.3x10 <sup>4</sup>	2.7x10 <sup>3</sup>	1.1x10 <sup>3</sup>	<10	<10	<10	1.1x10 <sup>3</sup>	1.1x10 <sup>2</sup>	N.D
	B	7.7x10 <sup>4</sup>	<10	<10	1.2x10 <sup>4</sup>	1.1x10 <sup>2</sup>	<10	<10	<10	<10	<10	2.1x10 <sup>3</sup>	N.D
Stimring	A	3.1x10 <sup>4</sup>	<10	1.9x10 <sup>3</sup>	2.1x10 <sup>3</sup>	1.1x10 <sup>4</sup>	1.2x10 <sup>2</sup>	<10	1.2x10 <sup>2</sup>	<10	<10	<10	N.D
	B	3.6x10 <sup>6</sup>	<10	3.4x10 <sup>2</sup>	1.1x10 <sup>2</sup>	<10	<10	<10	<10	<10	<10	<10	N.D
Rheumatic	A	6.2x10 <sup>4</sup>	<10	3.2x10 <sup>4</sup>	7.5x10 <sup>3</sup>	2.5x10 <sup>2</sup>	<10	<10	1.3x10 <sup>2</sup>	<10	1.6x10 <sup>2</sup>	<10	N.D
	B	2.8x10 <sup>6</sup>	<10	2.3x10 <sup>6</sup>	3.2x10 <sup>3</sup>	1.5x10 <sup>2</sup>	<10	<10	<10	<10	<10	<10	N.D
Flu	A	5.2x10 <sup>4</sup>	<10	8.2x10 <sup>2</sup>	4.1x10 <sup>4</sup>	3.5x10 <sup>2</sup>	1.7x10 <sup>2</sup>	1.1x10 <sup>2</sup>	<10	1.3x10 <sup>2</sup>	<10	<10	N.D
	B	3.5x10 <sup>6</sup>	<10	2.6x10 <sup>3</sup>	2.3x10 <sup>3</sup>	2.6x10 <sup>2</sup>	2.2x10 <sup>2</sup>	2x10 <sup>2</sup>	<10	2.5x10 <sup>2</sup>	3.1x10 <sup>2</sup>	<10	N.D
Intestinal	A	2.5x10 <sup>4</sup>	<10	3.9x10 <sup>2</sup>	1.5x10 <sup>6</sup>	1.7x10 <sup>3</sup>	1.2x10 <sup>2</sup>	<10	1.5x10 <sup>2</sup>	<10	<10	<10	N.D
	B	6.2x10 <sup>6</sup>	<10	<10	<10	<10	<10	<10	2.5x10 <sup>2</sup>	1.6x10 <sup>2</sup>	<10	1.6x10 <sup>3</sup>	
Colic	A	3.5x10 <sup>6</sup>	<10	1.4x10 <sup>4</sup>	8.2x10 <sup>3</sup>	1.3x10 <sup>2</sup>	1.3x10 <sup>2</sup>	1.1x10 <sup>2</sup>	3.2x10 <sup>2</sup>	<10	<10	<10	N.D
	B	2.6x10 <sup>6</sup>	<10	<10	3.6x10 <sup>3</sup>	2.3x10 <sup>2</sup>	3.2x10 <sup>2</sup>	1.2x10 <sup>2</sup>	<10	<10	<10	<10	N.D
Laxative	A	7x10 <sup>6</sup>	<10	4.1x10 <sup>3</sup>	1.5x10 <sup>4</sup>	2.5x10 <sup>2</sup>	1.7x10 <sup>2</sup>	1.1x10 <sup>2</sup>	1.6x10 <sup>2</sup>	<10	<10	<10	N.D
	B	7.3x10 <sup>6</sup>	<10	4.2x10 <sup>3</sup>	2.1x10 <sup>4</sup>	1.7x10 <sup>3</sup>	2.2x10 <sup>2</sup>	1.6x10 <sup>2</sup>	1.3x10 <sup>2</sup>	3.1x10 <sup>2</sup>	<10	<10	N.D
Calm	A	2.6x10 <sup>4</sup>	<10	9x10 <sup>3</sup>	1.6x10 <sup>4</sup>	5.5x10 <sup>2</sup>	1.6x10 <sup>2</sup>	<10	<10	<10	6.1x10 <sup>2</sup>	<10	N.D
	B	2.1x10 <sup>4</sup>	<10	<10	1.2x10 <sup>3</sup>	3.6x10 <sup>2</sup>	1.1x10 <sup>2</sup>	<10	2.1x10 <sup>2</sup>	3.5x10 <sup>2</sup>	<10	<10	N.D
Acne	A	3.5x10 <sup>6</sup>	<10	1.5x10 <sup>3</sup>	4.3x10 <sup>4</sup>	2.5x10 <sup>3</sup>	1.9x10 <sup>2</sup>	<10	<10	5.1x10 <sup>2</sup>	<10	<10	N.D
	B	4.1x10 <sup>4</sup>	<10	2.3x10 <sup>3</sup>	3.8x10 <sup>3</sup>	1.7x10 <sup>2</sup>	1.3x10 <sup>2</sup>	<10	<10	<10	<10	<10	N.D
Lactagogue	A	1.1x10 <sup>3</sup>	<10	8.2x10 <sup>2</sup>	2.5x10 <sup>4</sup>	8.1x10 <sup>3</sup>	3.4x10 <sup>2</sup>	<10	<10	<10	2.3x10 <sup>2</sup>	2.7x10 <sup>2</sup>	N.D
	B	2.1x10 <sup>4</sup>	<10	6.1x10 <sup>2</sup>	1.7x10 <sup>3</sup>	1.6x10 <sup>2</sup>	1.1x10 <sup>2</sup>	<10	<10	<10	<10	<10	N.D
Sedative	A	3.5x10 <sup>3</sup>	<10	<10	4.5x10 <sup>2</sup>	<10	<10	<10	2.2x10 <sup>2</sup>	5x10 <sup>2</sup>	<10	<10	N.D
	B	4.5x10 <sup>4</sup>	<10	3.6x10 <sup>3</sup>	3.2x10 <sup>2</sup>	1.6x10 <sup>3</sup>	<10	<10	<10	<10	<10	<10	N.D
Headache	A	2.6x10 <sup>4</sup>	<10	<10	1.5x10 <sup>3</sup>	4.5x10 <sup>3</sup>	2.3x10 <sup>2</sup>	1.1x10 <sup>2</sup>	<10	<10	3.6x10 <sup>2</sup>	<10	N.D
	B	3.2x10 <sup>6</sup>	<10	<10	2.5x10 <sup>3</sup>	8.2x10 <sup>3</sup>	5.1x10 <sup>2</sup>	2.1x10 <sup>2</sup>	<10	<10	<10	<10	N.D
Cough	A	1.6x10 <sup>6</sup>	<10	<10	3.2x10 <sup>3</sup>	2.5x10 <sup>4</sup>	1.9x10 <sup>2</sup>	1.1x10 <sup>2</sup>	<10	<10	<10	3.1x10 <sup>2</sup>	N.D
	B	2.1x10 <sup>6</sup>	<10	3.9x10 <sup>3</sup>	3.8x10 <sup>2</sup>	1.9x10 <sup>3</sup>	1.6x10 <sup>2</sup>	1.3x10 <sup>2</sup>	<10	<10	<10	<10	N.D
Regime	A	1.2x10 <sup>6</sup>	<10	3.9x10 <sup>4</sup>	4.5x10 <sup>3</sup>	2.9x10 <sup>4</sup>	2.3x10 <sup>2</sup>	2.6x10 <sup>2</sup>	<10	2.5x10 <sup>2</sup>	<10	<10	N.D
	B	1.6x10 <sup>6</sup>	<10	<10	5.6x10 <sup>3</sup>	4.5x10 <sup>3</sup>	3.1x10 <sup>2</sup>	1.6x10 <sup>2</sup>	<10	<10	3.2x10 <sup>2</sup>	<10	N.D

T.P.C. = Total plat count.

N.D = Not detected

A = Mean of three samples collected from first market.

B = Mean of three samples collected from 2<sup>nd</sup> market.

While in packed herbs were ( $1.1 \times 10^2$  to  $4.5 \times 10^3$  cfu/g), ( $1.1 \times 10^2$  to  $2.9 \times 10^4$  cfu/g), ( $1.1 \times 10^2$  to  $2.3 \times 10^3$  cfu/g), ( $1.1 \times 10^2$  to  $2.6 \times 10^3$  cfu/g) and ( $1.1 \times 10^2$  to  $3.2 \times 10^2$  cfu/g), respectively.

The result indicate that hygienic condition of packed herbs is better than unpacked herbs. The results agree with Pafumi 1986 and Satchell et al. (1989) whom they stated that coliform are often detected in herbs with *Enterobacteria* spp. and *Escherichia coli* in samples from various retail markets but usually in low number.

The manner and environment in which they were grown, harvested and handing, as well as the chemical nature of medicinal plants, directly impacts its microbiological quality. In general, roots, berries and herbs carry a greater microbiological load than the bark and seed items. While a considerable number of vegetative cells were killed during the drying process, many bacteria and molds may survive (Kiss and Farkas 1988 and ICMSF 1980).

Raw agricultural commodities, medicinal plants and herbs commonly harbor large numbers of bacteria and fungi including potential spoilage organisms and occasionally organisms of sanitary and public health significances (Guarino 1973).

#### **Mycotoxin analysis :**

All samples under investigation were free from ochratoxin A, aflatoxin B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. The detection limits were 5 µg/kg for aflatoxin and ochratoxin.

Table (3) showed that the aflatoxin B<sub>1</sub> and zearalenone at the same prevalence of 35.7% of the unpacked herbs. The aflatoxin mean concentration ranged from 8 to 25 µg/kg, while zearalenone mean concentration ranged from 10 to 60 µg/kg for unpacked herbs.

The results in Table (4) showed that the prevalence of aflatoxin B<sub>1</sub> and zearalnone were 17.9% and 10,7% of the packed herbs, respectively. Aflatoxin mean concentration ranged from 6 to 12 µg/kg, while zearalonone mean concentration ranged from 10 to 20 µg/kg in packed herbs.

The obtained results are in agree with those reported by Ellis et al. (1991), who mentioned that not every *A. flavus* or strains produce aflatoxin, as the genotype of each strain determines whether it is aflatoxigenic or not.

Detection of natural occurrence of mycotoxins revealed that presence of potential toxigenic *A. flavus* on the herbs samples density always mean that these products contain aflatoxins. However, various environmental factors also play a part in aflatoxin production, El-Tahan et al. (2001).

From the present results, it can be considered that raw agricultural commodities, medical plants and herbs commonly contain large numbers of bacteria and fungi including potential spoilage organisms hence, it is recommended that herbs should be produce from selected good quality plants with hygenic processing conditions and handling to protect consumers health.

The statistical analysis of the data where the test were employed and the results quoted in Table (5). The results showed that no significant differences for all microorganisms were found between packed and unpacked herbs samples, except between yeast, coliform and *Staph. aureus*.

**Table (3): Mycotoxin in different unpacked herbs sample collected from two local markets.**

Product	Aflatoxin B <sub>1</sub> µg/kg			Zearalenone µg/kg			Ochra-toxin µg/kg
	Detect-ion	Concen-tration	Mean conc. range	Detect-ion	Clonc.	Mean conc. range	
Renal A	ND			ND		<10 (0-<10)	ND
B		10	10 (10)	ND		<10	ND
Slimming A	ND		<5(0-<5)	ND		>10(0-<10)	ND
B	ND			ND			ND
Rheumatic A	ND		<5(0-<5)	ND		<10(0-<10)	ND
B	ND			ND			ND
Flu A		15	11.5(8-10)		60	40(20-60)	ND
B		8			20		ND
Intestinal A	ND		<5(0-<5)	ND			ND
B	ND			ND			ND
Colic A	ND		<5(0-<5)		15	20(15-25)	ND
B	ND				25		ND
Laxative A		9	10(9-11)	ND		40(0-<10)	ND
B		11		ND			ND
Calm A	ND			ND		<10(0-<10)	ND
B	ND			ND			ND
Acne A		8	8(8)	ND		<10(0-<10)	ND
B	ND			ND			ND
Lactagogue A	ND		<5(0-<5)		40	25(10-40)	ND
B	ND				10		ND
Sedative A	ND		<5(0-<5)		20	17.5(15-20)	ND
B	ND				15		ND
Headache A		25	20(15-25)	ND		<10(0-<10)	ND
B		15		ND			ND
Cough A		9	10.5(9-12)	ND		<10(0-40)	ND
B		12		ND			ND
Regime A	ND		<5(0-<5)		25	22.5(20-25)	ND
B	ND				20		ND

A = Mean of 3 samples collected from first market.

B = Mean of 3 samples collected from 2<sup>nd</sup> market.



**Table (4): Mycotoxin in packed herbs sample collected from two local markets.**

Product		Aflatoxin B <sub>1</sub> µg/kg			Zearalenone µg/kg			Ochratoxin µg/kg
		Detection	Concentration	Mean conc. range	Detection	Clonc	Mean conc. range	
Renal	C	ND		<5(0-5)	ND		<10 (0-<10)	ND
	D	ND			ND			
Slimming	C	ND		<5(0-5)	ND		>10(0-<10)	ND
	D	ND			ND			
Rheumatic	C	ND		<5(0-5)	ND		<10(0-<10)	ND
	D	ND			ND			
Flu	C	ND		<5(0-5)	ND		10(0-10)	ND
	D	ND			ND			
Intestinal	C	ND		<5(0-5)	ND		<10(0-<10)	ND
	D	ND			ND			
Colic	C		8	8(8)	ND		10(0-10)	ND
	D	ND		<5(0-15)	ND			
Laxative	C	ND		<5(0-15)			10(10)	ND
	D	ND			ND	10		
Calm	C		10	10(10)	ND		<10(0-<10)	ND
	D	ND		<5(0-5)	ND			ND
Acne	C		12	9(6-12)		15	15(15)	
	D		6		ND		<10(0-<10)	ND
Lactagogue	C	ND		<5(0-5)	ND		<10(0-<10)	ND
	D	ND			ND			
Sedative	C	ND		<5(0-5)	ND		<10(0-<10)	ND
	D	ND			ND			
Headache	C		8	8(8)	ND		<10(0-<10)	ND
	D	ND		<5(0-5)	ND			
Cough	C	ND		<5(0-5)	ND		<10(0-<10)	ND
	D	ND			ND			
Regime	C	ND		<5(0-5)	ND	20	20(20)	ND
	D	ND					<10(0-<10)	

A = Mean of 3 samples collected from first market.

B = Mean of 3 samples collected from 2<sup>nd</sup> market.

**Table (5) : The mean values and standard error of packed and unpacked of medical herbs and T test between them.**

Trait	Packet	Unpacket	T-value
Total plate count	322200±99826.28	254828.57±7162.99	0.548 <sup>NS</sup>
Yeast	100±0	332.14±105.99	-2.19*
Molds	1567.50±8161.30	292.86±69.96	1.878 <sup>NS</sup>
Enter bacteriaceae	14326.43±5486.78	923.21±255.06	2.44 <sup>NS</sup>
Coliform	153.93±18.19	2664.29±911.43	2.75*
F. foliform	286.07±83.50	119.64±13.72	1.968 <sup>NS</sup>
E. coli	100±0	204.64±88.90	1.064 <sup>NS</sup>
Enterococci	128.21±1050	342.14±116.42	-1.83 <sup>NS</sup>
Cl. perfringens	159.29±22.59	149.64±14.38	0.36 <sup>NS</sup>
Bacillus cereus	183.57±40.62	425±147.94	-1.573 <sup>NS</sup>
Staph. aureus	112.80±5.28	164.64±15.05	3.25*

NS: non significant

\* : significant

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## مدى تواجد السالمونيلا ، كلوستريديم بيرفرينجيس ، الباسيلس سيرياس والاستافيلوكوكاس أوريس وميكوتوكسين في النباتات الطبية

محمود حلمى الطحان

المعمل المركزى للأغذية والأعلاف / مركز البحوث الزراعية

تم أخذ ١٦٨ عينة ممثلة من النباتات الطبية للمعابة والغير معابة وتم اختيار التحاليل الآتية:  
للسالمونيلا ، الكلوستريديام بيرفرينجيس والباسيلس سيرياس والاستافيلوكوكاس أوريس والميكوكوكسين  
وجمعت العينات فى الفترة من يناير ٢٠٠٣ الى أبريل ٢٠٠٤ .

أوضحت النتائج أن المعدل الكلى للبكتريا الهوائية فى عينات النباتات الطبية غير للمعابة والمعابة  
١,٢ × ١٠ الى ٢,٦ × ١٠ ، ٥,٢ × ١٠ الى ١,٩ × ١٠ على التوالى .

كما وجدت للبكتريا المتجرثمة (باسيلس سيرياس ، كلوستريديام بيرفرينجيس المعزولة من  
عينات النباتات الطبية للمعابة والغير معابة بنسبة ٢٥,٥ % ، ٢٨,٦ % ، ٣٢,١ % ، ٥٣,٦ % على التوالى  
كل العينات تحت الاختبار كانت خالية من السالمونيلا ولوكروتوكسين (A) وفلاتوكسين (B<sub>2</sub> &  
G<sub>1</sub> and G<sub>2</sub>) . بينما وجدت أفلاتوكسين B<sub>1</sub> فى ١٧,٧ % ، ٣٥,٧ % للمنتجات النباتية للطبية المعابة  
والغير معابة على التوالى . كما أظهرت النتائج تواجد لزيرونيون فى ١٠,٧ % ، ٣٥,١ % فى النباتات  
الطبية للمعابة والغير معابة على التوالى .

أظهرت نتائج التحليل الإحصائى عدم وجود فروق معنوية بين معظم الميكروبات تحت الدراسة  
فى العينات للمعابة والغير معابة وماعد فى الخمائر - بكتريا القولون - البكتريا المنقودية .