



## THE MICROBIAL LEVEL CONTAMINATION IN DRIED PLANT MATERIAL EVALUATED BY THE STANDARD PLATE COUNT

Poiata ANTONIA<sup>1</sup>, Tuchilus CRISTINA<sup>1</sup>, Gille ELVIRA<sup>3</sup>, Clara  
APROTOSOAI<sup>2</sup>, Stanescu URSULA<sup>2</sup>, Hancianu MONICA<sup>2</sup>

1 Microbiology Dept., Faculty of Medicine, University of Medicine and Pharmacy, Iasi, Romania

2 Pharmacognosy Dept., Faculty of Pharmacy, "Gr. T. Popa" University of Medicine and Pharmacy,  
Iassy, Romania

3 National Institute of R&D for Biological Sciences "Stejarul"-Biological Research Center, Piatra  
Neamt, Romania

### SYNOPSIS

#### Key words:

Microbial  
contamination,  
plant material.

For the control of microbial content, the plant materials (*Origanum vulgare*, *Ocinum sanctum*, *Majorana hortensis*, *Foeniculum vulgare* and *Hyssopus officinalis*) are tested to determine the number of microorganism per gram sample (total aerobic count) by standard plate count method. The interpretation of the results is in concordance with the monograph recommendations on the microbiological quality of herbal preparation, excepted one sample of *Foeniculum vulgare* (Category 4) (European Pharmacopoeia, 2006).

### INTRODUCTION

Many plants are used in traditional medicine as herbal preparations for human benefit. It is estimated that 70 to 75 % of the population living in the developing countries are dependent on traditional medicine for their health needs (Taneja & Qazi, 2007)

According to European Pharmacopoeia (EDQM, 2006) definition the herbal drugs are whole fragmented or cut, plants, parts of plants, algae, fungi, lichen in an unprocessed state, usually in dried form but sometimes fresh.

Depending upon the cultivation collection, harvesting, drying, fragmentation and storage conditions, the medicinal herbs can be highly susceptible to microbial contamination. The presence in sufficient numbers, the microorganisms may be harmless to consumers and can cause medicinal plants quality problems.

In this paper we determine the microbial quality of some medicinal herbs samples by the standard plate-count method.

## MATERIAL AND METHODS

### PLANT MATERIAL

Aerial parts of plants (*Origanum vulgare*, *Ocinum sanctum* (three samples), (*Majorana hortensis* (three samples), *Foeniculum vulgare* and *Hyssopus officinalis*) were collected in the blooming stage on July period 2008 on the Bacau areas, Eastern Romania.

The plants were identified in the Herbarium Section, faculty of Pharmacy, University of Medicine and Pharmacy, Iassy, Romania, in which a voucher specimen in the each species was deposited.

After collection, the plant material (9 samples) was air-dried at room temperature. The dried plant material is shown in Table 1.

**Table 1. Samples of dried plant material investigated.**

Sample	Plant material
1	<i>Majorana hortensis</i> second collection, var 3
2	<i>Majorana hortensis</i> second collection, var 1
3	<i>Hyssopus officinalis</i> 1 <sup>st</sup> year, Secuieni
4	<i>Ocinum sanctum</i> second collection, var 2
5	<i>Ocinum sanctum</i> second collection, var 3
6	<i>Majorana hortensis</i> second collection, var 3
7	<i>Origanum vulgare</i> 1 <sup>st</sup> year, Secuieni
8	<i>Ocinum sanctum</i> second collection, var 1
9	<i>Foeniculum</i> CCB- Stejarul

### EVALUATION OF THE MICROBIAL QUALITY OF PLANT MATERIAL BY THE STANDARD PLATE-COUNT METHOD

Standard plate-count method was performed by a pour plate procedure standard method (Group of authors, 1992). The test is used for quantitative evaluation of mesophilic bacteria and fungi that grow aerobically. A sample of 10 g of the each plant material is suspended in 100 ml sterile distilled water and mix vigorously. Because the plant samples are recognized to have a significant microbial contamination, serial dilutions are prepared so that the number of colony-forming units (CFUs) in Petri dishes will be less than 300. Duplicate 1.0 ml aliquots of a each dilution sample are pipette into two separate sterile Petri dishes 9 cm in diameter and 20 ml of a liquefied nutritive agar medium suitable for the cultivation bacteria, respectively Sabouraud agar for the cultivation of fungi, at not more than 45°C. After

solidification of the soft agar, the Petri plates are incubated at 35°C for bacteria and 25°C for fungi, for five days. The number of microorganisms in each sample is evaluated by multiplying the average number of colonies per plate by the dilution used. The results are expressed as number of CFUs per gram material plant.

## RESULTS AND DISCUSSION

For the control of microbial content, the plant materials are tested to determine the number of microorganism per gram sample (total aerobic count). The standard plate count method is commonly used for detecting and determining the number of microorganisms in plant material.

The pour plate procedure is easy to perform and is relatively accurate. The plant material samples are tested for the presence of *Escherichia coli* belonging to the coliform bacteria.

Coliform bacteria are gram-negative bacilli that are found in intestinal tract of humans and animals and can ferment lactose in 24-48 h at 35°C. For this purpose we have used the selective agar medium Mc Conkey. The probable presence of *E. coli* is indicated by the growth of red-pink non-mucoid colonies that is confirmed by biochemical tests, such as indole production (Speck, 1992, Helrich, 1990).

The results of microbial content evaluation by the plate-count method are presented in Tables 2. The interpretation of the results is in concordance with the monograph recommendations on the microbiological quality of herbal preparation (Category 4) (EDQM, 2006).

The herbal medicinal products are divided in two categories and should comply with the following criteria:

- A. Herbal medicinal preparations to which boiling water is added before use:
  - Total viable aerobic count: not more than  $10^7$  bacteria and  $10^3$  fungi per gram
  - Not more than  $10^2$  *E. coli* per gram
- B. Herbal medicinal preparations to which boiling water is not added before use:
  - Total viable aerobic count: not more than  $10^5$  bacteria and  $10^4$  fungi per gram
  - Absence of *E. coli* and *Salmonella* per gram

According to the microbiological requirements specified in the monograph, all fresh and dried plant material samples comply with the criteria A and B of the category 4 (herbal medicinal products). The number of *E. coli*, according to the criteria B (absence of *E. coli* per gram) exceeded the accepted limits for one sample of dried plant material.

The results for the dried material plant samples were in good agreement with the microbial number calculated per gram proposed by the monograph (category 4, A and B criteria) excepted one sample of *Foeniculum*.

Table 2. Microbial quality of dried plant material expressed as CFU/g product

Sample	Total aerobic count	Criteria		Fungi	Criteria		<i>E. coli</i>	Criteria	
		A	B		A	B		A	B
<i>Majorana hortensis</i> 2 nd collection, var 1	34x10 <sup>2</sup>	a	a	14x10 <sup>2</sup>	a	a	0	a	a
<i>Hyssopus officinalis</i> 1 <sup>st</sup> year, Secuieni	95x10 <sup>2</sup>	a	a	4x10 <sup>2</sup>	a	a	0	a	a
<i>Ocinum sanctum</i> , 2 nd, var 2	24x10 <sup>2</sup>	a	a	9x10 <sup>2</sup>	a	a	0	a	a
<i>Ocinum sanctum</i> 2 nd collection, var 3	59x10 <sup>2</sup>	a	a	3x10 <sup>2</sup>	a	a	0	a	a
<i>Majorana hortensis</i> 2nd collection, var 3	41x10 <sup>2</sup>	a	a	8x10 <sup>2</sup>	a	a	0	a	a
<i>Origanum vulgare</i> 1 <sup>st</sup> year I, Secuieni	99x10 <sup>2</sup>	a	a	10x10 <sup>2</sup>	a	a	0	a	a
<i>Ocinum sanctum</i> , 2nd collection var 1	5x10 <sup>2</sup>	a	a	1x10 <sup>2</sup>	a	a	0	a	a
<i>Foeniculum</i> CCB- Stejarul	47x10 <sup>3</sup>	a	a	59x10 <sup>2</sup>	a	a	21x10 <sup>1</sup>	a	ua**

Legend: \* a=acceptable; \*\* ua- unacceptable

## CONCLUSION

The microbial quality of the plant material analyses, performed by the pour plate count procedure showed that all samples are in accordance with criteria A and B of category 4 (total viable aerobic count) of monograph.

One dried sample cannot be accepted because *E. coli* contamination exceeds the limits for products described in category 4.

Many medicinal plant, are used to obtain herbal drug preparations. The raw materials of vegetable origin are submitted to treatments such as solvent extraction, distillation expression, fractionation, purification, concentration, fragmentation. During of these treatments may be obtained a reduction of microbial contamination and in this way the herbal drugs comply with the requirements on the microbiological quality.

## ACKNOWLEDGEMENTS

The work is sustained from the PNCDI-2 Program 4: 61-039, financed by National Authority for Scientific Research - Romanian Government.

**REFERENCES:**

- Group of authors, 1992: Standard methods for the examination of water and wastewater, 18<sup>th</sup> edition. - American Public Health Association (APHA), American Water Works Association (AWWA), Water Environmental Federation (WEF), Washington, DC, 385 pp.
- EDQM (European Directorate for the Quality of Medicines), 2006: European Pharmacopoeia, 3<sup>rd</sup> edition, Strasbourg: Council of Europe, 3308 pp.
- HELRICH, K. 1990: Official Methods of Analysis of the Association of Official Analytical Chemists, Fifteenth Edition, Arlington, Virginia (USA), pp: 430-431.
- SPECK, M.L. 1992: Compendium of Methods for Microbiological Examination of Foods. - American Public Health Association Intersociety/Agency Committee on Microbiological Methods for Foods, Washington DC: 979 pp.
- TANEJA, S.C. & QAZI, G.N. 2007: Bioactive molecules in medicinal plants: a perspective on their therapeutic action. - In: Chorghade, M. S., Wiley, J. (Eds), New York Drug discovery and development, pp: 1-50.

Original Research article

Received: 13 July 2010.

