

# TESTING THE EFFECTIVENESS OF INHERENT ANTIMICROBIAL PRESERVATIVES OF A COSMETIC PRODUCT WITH ORGANIC ACTIVE INGREDIENTS

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**ABSTRACT.** In this study we formulated, developed and tested body care milk that contains two active ingredients of plant origin from Organic Agriculture, obtained by cold pressing, namely: vegetable greasy oil from sea buckthorn [*Hippophaerhamnoides*.L.] and greasy oil from flaxseeds [*Linumusatissimum*.L.], with coenzyme Q10.

The purpose of this paper is to demonstrate the antimicrobial efficacy of a cosmetic product with its own antimicrobial properties, product what contained these two organic active ingredients.

Milk Body Care formula contains no preservatives in the preparation and therefore in this study we aim to demonstrate the antimicrobial efficacy of cosmetic product components in accordance with the requirements of the European Pharmacopoeia monograph ed. 7-a / 2010.

**KEYWORDS:** organic active ingredients, cold pressing, antimicrobial efficacy

## INTRODUCTION

Body care products help the skin to regain properties after washing / etching using reconstruction protective film or is to help skin become firmer, tonic and a tendency to do away with cellulite.

Milk, lotions and body are mostly emulsions with plenty of water and some grease substances which are designed to moisturize and nourish the skin.

As in most cosmetic products on the market can find a number of controversial ingredients such as preservatives critics unidentified perfumes, oils and waxes of questionable quality, and recommended numerous little texture such as PEG, we proposed the formulation, implementation and testing of milk for body care that contain two active ingredients of plant origin from OA obtained by cold pressing, namely: red sea buckthorn oil vegetable fat [*Hippophaerhamnoides*.L.] [2] [6] [7] [8] [9] [12] [14] [15] [16] [17] and fatty oil from flaxseed [*Linumusatissimum*.L.] [1] [5] [10] [11] [13] with coenzyme Q10.

The purpose of this paper is to demonstrate the

antimicrobial efficacy [4] own a cosmetic product with two organic active ingredients.

Milk for body care formula contains no preservatives in the preparation and therefore in this study we aim to demonstrate the antimicrobial efficacy of the components of this cosmetic product in accordance with the requirements of the European Pharmacopoeia monograph ed. 7 - a / , 2010.

## MATERIALS AND METHODS

### 1. Culture media [3] [4] [18]

1.1. To study the properties of antimicrobial preservatives are necessary following culture media:

- To bacteria: agar culture medium B (Casein Soya Bean Digest Agar)

- For fungus: C agar culture medium (Sabouraud Glucose Agar without Antibiotics)

1.2. Test of promoting the growth of bacteria and fungus on culture media and their sterility test is performed according to the procedure of the monograph "microbial contamination" and check the microbiological quality culture media.

**2. Microorganisms test [3] [4] [18]****2.1. Bacteria :**

- *Staphylococcus aureus* ATCC 6538 ( Gram + )
- *Pseudomonas aeruginosa* ATCC 9027 ( Gram - )

**2.2 . Fungus :**

- *Candida albicans* ATCC 10231 ( yeast )
- *Aspergillus brasiliensis* ATCC 16404 ( filamentous fungus ) .

2.3. Passages of the test strains of microorganisms to perform according to the monograph "Testing the effectiveness of antimicrobial preservatives " , as follows:

- 3 passages were performed on the culture medium B inclined agar tubes ( Casein

Soya Bean Digest Agar ) inclined at an interval of 24 hours, fresh cultures obtained from *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 9027, the cultures were incubated at 30 -35 °C, for 18-24 hours.

- 3 passages were performed on agar tube culture medium C level ( Sabouraud Glucose

Antibiotics with Agar ) every 48 hours and 7 days, cultures of *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* 16404. Tubercle ATCC medium was incubated at 20 -25 °C for 48 hours for *Candida albicans* and 7 days for *Aspergillus brasiliensis*.

**Tabel 1.** Passage test strains of microorganisms

Micro organisms test	Category preparati on	Number of passages		
		1	2	3
<i>Aspergillus brsiliensis</i> ATCC 16404	topical	9.06.13	16.06.13	23.06.13
<i>Candida albicans</i> ATCC 10231	topical	19.06.13	21.06.13	23.06.13
<i>Staphylococcus aureus</i> ATCC 6538	topical	21.06.13	22.06.13	23.06.13
<i>Pseudomonas aeruginosa</i> ATCC 9027	topical	21.06.13	22.06.13	23.06.13

**3. Preparation of inoculum [3] [4] [18]** of bacterial and fungal strains is performed after the third passage, according to pharmacopoeial monograph "Testing the effectiveness of antimicrobial preservatives."

To obtain bacterial strains and fungal inoculum in the crop area was washed with isotonic NaCl, pH 7.0.

Bacterial and fungal culture from each scale using nephelometric suspensions were obtained with a concentration of approximately  $1 \times 10^8$  / mL micro test.

**4. Technique [3][4][18]** is performed according to the monographs "Testing the effectiveness of antimicrobial preservatives" and "Determination of microbial contamination."

*The sample:* In four 250 ml bottles were distributed 20 ml of the analyte and 0.1 ml each bacterial or fungal suspension (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus brasiliensis*) to obtain a concentration of the microorganism approximately  $1 \times 10^5$  / ml.

*Blank:* In four 250 ml bottles were distributed 20 ml isotonic NaCl pH 7.0 and 0.1 ml each bacterial or fungal suspension (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus brasiliensis*) for to obtain a concentration of approximately  $1 \times 10^5$  microorganisms/ ml.

For these vials was 1 ml taken at time 0 ( $T_0$ ) to 24 hours ( $T_1$ ), 2 days ( $T_2$ ), 7 days ( $T_3$ ), 14 days ( $T_4$ ), to 21 days ( $T_5$ ), and 28 days ( $T_6$ ) and determined the number of colony forming units of microbial contamination technique.

At the intervals specified initial goal was to reduce the number of viable microorganisms and logarithmic reduction was calculated from time 0 ( $T_0$ ).

**5. Test preparation: Milk for body care**

**5.1. Programming test data of the preparation** is shown in Table 2 below [3][4][18]:

**Tabel 2.** Programming test data

Category preparati on	Testing				
	0 days ( $T_0$ )	2 days ( $T_1$ )	7 days ( $T_2$ )	14 days ( $T_3$ )	28 days ( $T_4$ )
Milk for body care / 3 consecutive batches	02.07.13	04.07.13	11.07.13	18.07.13	01.08.13

**Abbreviations**

CCM = Microbial Contamination Control

ATCC = American Type Culture Collection - International Reference Culture Collection [3][4][18]

UFC = Colony Forming Units [3][4][18]

**RESULTS AND DISCUSSION [3][4][18]**

Antimicrobial activity evaluation criterion of "Milk body care " was the calculation of the sub

logarithmic (log) number of viable organisms in the sample relative to the control values obtained at time zero.

Antimicrobial efficacy test of the product was evaluated at time 0 ( $T_0$ ) to 24 hours ( $T_1$ ), 48 hours ( $T_2$ ), 7 days ( $T_3$ ), 14 zile ( $T_4$ ), 21 days ( $T_5$ ) and 28 days ( $T_6$ ), inoculation test it with the following microorganisms: *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404.

The results obtained from testing the antimicrobial efficacy against *Staphylococcus aureus* ATCC 6538 are presented in Table 3 and 4 and Figure 1.

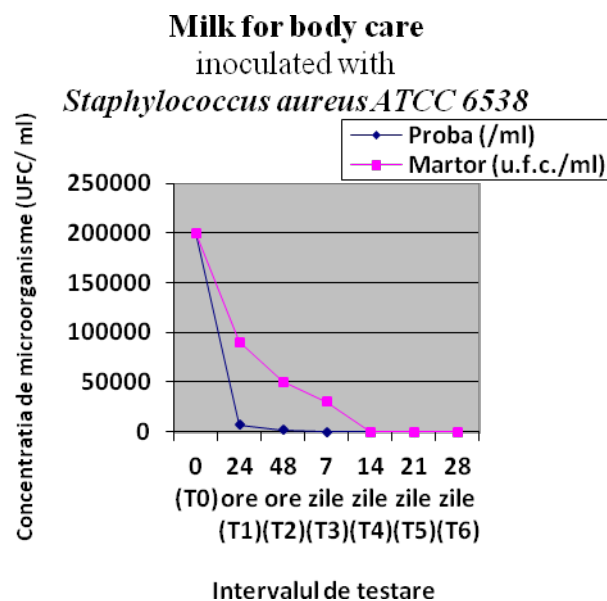
**Table 3.** The concentration of microorganisms for "Milk for body care" with inoculum *Staphylococcus aureus* ATCC 6538

Range test	concentration microorganisms (CFU/ml)	
	Sample	Blank
0 ( $T_0$ )	$2 \times 10^5$	$2 \times 10^5$
24 hours ( $T_1$ )	$7 \times 10^3$	$9 \times 10^4$
48 hours ( $T_2$ )	$1,7 \times 10^3$	$5 \times 10^4$
7 days ( $T_3$ )	0	$3 \times 10^4$
14 days ( $T_4$ )	0	$1,0 \times 10^2$
21 days ( $T_5$ )	0	$1,0 \times 10^2$
28 days ( $T_6$ )	0	0

**Table 4.** Logarithmic reduction achieved for "Milk for body care" with inoculum *Staphylococcus aureus* ATCC 6538

Product	Logarithmic reduction					
	24 h ( $T_1$ )	48 h ( $T_2$ )	7 d ( $T_3$ )	14 d ( $T_4$ )	21 d ( $T_5$ )	28 d ( $T_6$ )
Milk for body care inoculated with <i>Staphylococcus aureus</i> ATCC 6538	2	2	3	0	0	0

**Figure 1.** Graphic representation of the efficacy of the product on the microorganism *Staphylococcus aureus* ATCC6538



As is apparent from these data, the product has reduced the number of bacteria (*Staphylococcus aureus*), 24 hours ( $T_1$ ) and 48 hours ( $T_2$ ), the logarithmic reduction = log 2; 7 days ( $T_3$ ) after the inoculation, logarithmic reduction was log = 3, to 14 days ( $T_4$ ), to 21 days ( $T_5$ ) and 28 days ( $T_6$ ) after the inoculation, no *Staphylococcus aureus* is revealed (see Figure 1). The results obtained from testing the antimicrobial efficacy against *Pseudomonas aeruginosa* ATCC 9027 are presented in Table 5 and 6 and Figure 2.

**Table 5.** The concentration of microorganisms for "Milk for body care" with inoculum *Pseudomonas aeruginosa* ATCC 9027:

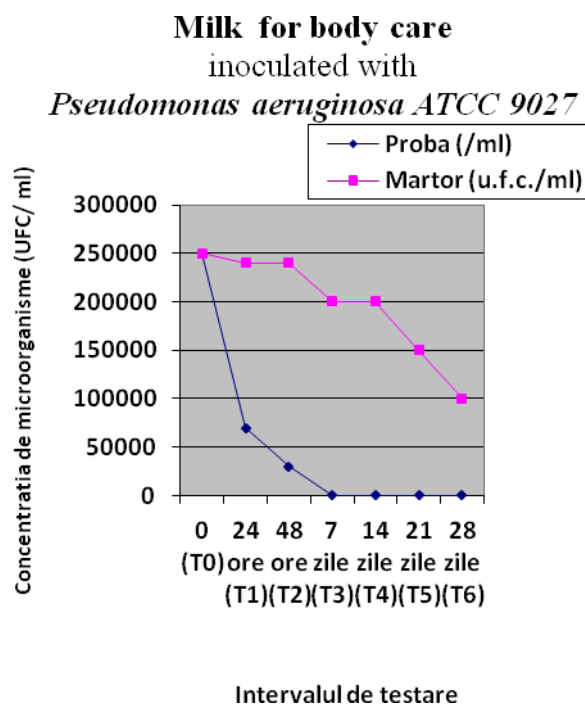
Range test	Concentration microorganisms (CFU/ml)	
	Sample	Blank
0 ( $T_0$ )	$2,5 \times 10^5$	$2,5 \times 10^5$
24 h ( $T_1$ )	$7 \times 10^4$	$2,4 \times 10^5$
48 h ( $T_2$ )	$3 \times 10^4$	$2,4 \times 10^5$
7 d ( $T_3$ )	0	$2 \times 10^5$
14 d ( $T_4$ )	0	$2 \times 10^5$
21 d ( $T_5$ )	0	$1,5 \times 10^5$
28 d ( $T_6$ )	0	$1 \times 10^5$

**Table 6.** Logarithmic reduction achieved for "Milk for body care" with inoculum *Pseudomonas aeruginosa* ATCC 9027:

Product	Logarithmic reduction					
	24 h ( $T_1$ )	48 h ( $T_2$ )	7 d ( $T_3$ )	14 d ( $T_4$ )	21 d ( $T_5$ )	28 d ( $T_6$ )
Milk body care inoculated	2	2	3	0	0	0

with <i>Pseudomonas aeruginosa</i> ATCC 9027	1	1	0	0	0	0
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**Figure 2.** Graphic representation of the efficacy of the product on the organism *Pseudomonas aeruginosa* ATCC9027:



*Pseudomonas aeruginosa* ATCC 9027 compared to the product reduced the number of bacteria by a factor of  $10^4$ /ml and 48 h and 24 h after inoculation (T<sub>1</sub> and T<sub>2</sub>), the log reduction unit logarithmic = 1, 7 days after the inoculation (T<sub>3</sub>), 14 days (T<sub>4</sub>) and 28 days (T<sub>5</sub>) after the inoculation was not put into evidence the presence of bacteria.

The results obtained from testing the antimicrobial efficacy against *Aspergillus brasiliensis* ATCC 16404 are shown in Table 7 and 8, and Figure 3.

**Table 7.** The concentration of microorganisms for "Milk for body care" with inoculum *Aspergillus brasiliensis* ATCC 16404:

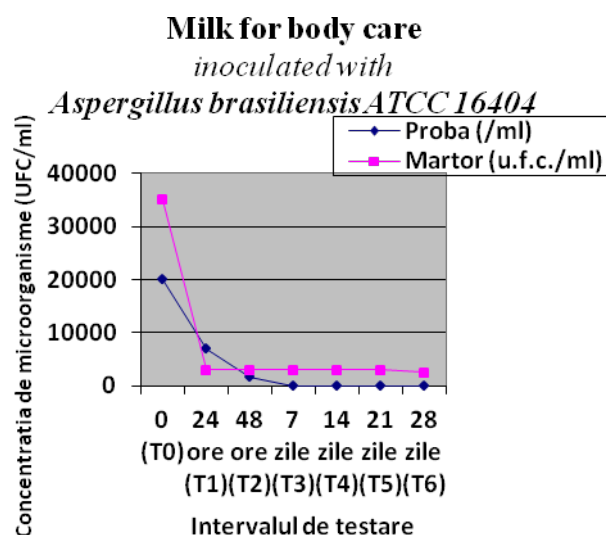
Range test	concentration microorganisms (CFU/ml)	
	Sample	Blank
0 (T <sub>0</sub> )	$2 \times 10^4$	$3,5 \times 10^4$
24h (T <sub>1</sub> )	$7 \times 10^3$	$3 \times 10^3$
48 h (T <sub>2</sub> )	$1,7 \times 10^3$	$3 \times 10^3$
7 d (T <sub>3</sub> )	0	$3 \times 10^3$
14 d (T <sub>4</sub> )	0	$3 \times 10^3$

21 d (T <sub>5</sub> )	0	$3 \times 10^3$
28 d (T <sub>6</sub> )	0	$2,5 \times 10^3$

**Table 8 .** Logarithmic reduction achieved for "Milk for body care" with inoculum *Aspergillus brasiliensis* ATCC 16404:

Product	Logarithmic reduction					
	24 h (T <sub>1</sub> )	48 h (T <sub>2</sub> )	7 d (T <sub>3</sub> )	14 d (T <sub>4</sub> )	21 d (T <sub>5</sub> )	28 d (T <sub>6</sub> )
Body care milk inoculated with <i>Aspergillus brasiliensis</i> ATCC 16404	2	2	-	-	-	-

**Figure 3.** Graphic representation of the efficacy of the product on the organism *Aspergillus brasiliensis* ATCC 16404:



On *Aspergillus brasiliensis* ATCC 16404, the active ingredients in the product reduced the initial number of fungus by a factor of  $10^3$ /ml within 7 days after inoculation (T<sub>3</sub>) at this time logarithmic reduction was log = 2, 14 days (T<sub>4</sub>) and 28 days (T<sub>5</sub>) after the inoculation was not emphasized growth of the fungus. The results obtained from testing the antimicrobial efficacy against *Candida albicans* ATCC 10231 are shown in Table 9 and 10 and Figure 4:

**Table 9.** The concentration of microorganisms for "Milk for body care" with inoculum *Candida albicans* ATCC 10231:

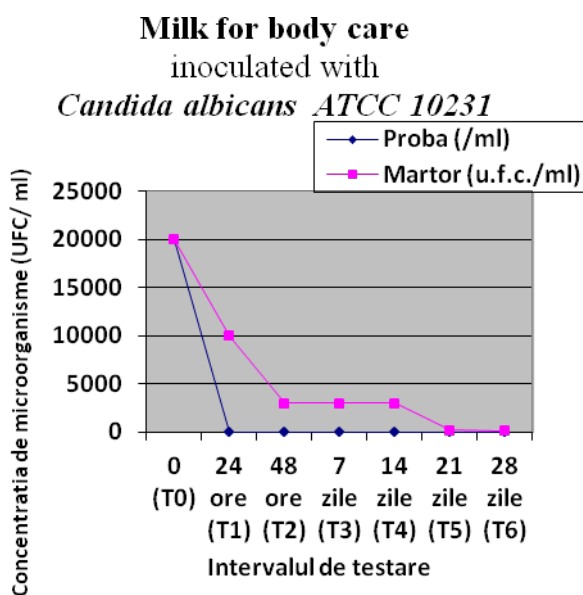
Range test	Concentration microorganisms (CFU/ml)	
	Sample	Blank

0 (T <sub>0</sub> )	2 x 10 <sup>4</sup>	2 x 10 <sup>4</sup>
24 h (T <sub>1</sub> )	0,3 x 10 <sup>1</sup>	1 x 10 <sup>4</sup>
48 h (T <sub>2</sub> )	0	3 x 10 <sup>3</sup>
7 d (T <sub>3</sub> )	0	3 x 10 <sup>3</sup>
14 d (T <sub>4</sub> )	0	3 x 10 <sup>3</sup>
21 d (T <sub>5</sub> )	0	2 x 10 <sup>2</sup>
28 d (T <sub>6</sub> )	0	1 x 10 <sup>2</sup>

**Table 10 .** Logarithmic reduction achieved for "Milk for body care" with inoculum *Candida albicans* ATCC 10231:

Product	Logarithmic reduction					
	24 h (T <sub>1</sub> )	48 h (T <sub>2</sub> )	7 d (T <sub>3</sub> )	14 d (T <sub>4</sub> )	21 d (T <sub>5</sub> )	28 d (T <sub>6</sub> )
Body care milk inoculated with <i>Candida albicans</i> ATCC 10231	2	0	0	-	-	-

**Figure 4.** Graphic representation of the efficacy of the product on the organism *Candida albicans* ATCC 10231:



The active ingredients of the product 'milk body care' reduced the initial number of fungus (yeasts) (*Candida albicans* ATCC 10231) by a factor of 10<sup>1</sup>/ml within 24 hours after inoculation (T<sub>1</sub>), after this time never revealed growth of *Candida albicans*.

## CONCLUSIONS

- The components of the product 'Milk for body care' contains no preservatives in formula preparation had antimicrobial activity against *Staphylococcus aureus* ATCC 6538, reducing the number of bacteria by a factor of 10<sup>3</sup> germs / ml.

The product is effective and meets the criterion recommended acceptance of European Pharmacopoeia, 7th Edition / 2010 [4].

- The components of the product "Milk for body care" had antimicrobial activity against the *Pseudomonas aeruginosa* ATCC 9027, reducing the number of germs 2 logs at 48 hours and 3 log after 7 days after inoculation.

The product is effective against *Pseudomonas aeruginosa* and corresponding recommended acceptance criterion A European Pharmacopoeia 7th Edition / 2010 [4].

- On *Candida albicans* ATCC 10231 the product reduced the number of bacteria by a factor of 10<sup>1</sup>/ml within 24 hours after inoculation with antimicrobial activity against this yeast.

A product is within the acceptance criteria recommended by the European Pharmacopoeia 7th Edition / 2010 [4].

- The product "Milk for body care" reduced the initial number of fungus (*Aspergillus brasiliensis* ATCC 16404) by a factor of 10<sup>3</sup> bacteria / ml at 7 days after inoculation. Components of this product have antimicrobial activity against *Aspergillus brasiliensis* and corresponding recommended acceptance criterion A European Pharmacopoeia 7th Edition / 2010 [4].

This study has provided the scientific basis and demonstrated intrinsic antimicrobial properties (antibacterial and antifungal) milk body care product with active materials and organic vegetable gives results support its use as a topical product for quality and safety for such a product.

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