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ANTIMICROBIAL PROPERTIES OF SOME ORGANIC ESSENTIAL AND FATTY OILS-BASED DIETARY SUPPLEMENTS

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AIMS

This study has had as aim to demonstrate the efficacy as antimicrobial preservation of some active ingredients from the Hofigal honey-based dietary supplements, multi- and unidose primary packaging, *products without added preservatives in their manufacturing formulation.*

BACKGROUND

We have carried out a study on a number of two finished products as solutions of oral use multidose and unidose, products honey-based in mixture with other organic active ingredients, without preservatives, [6] and [7].

The exemplification will be done on the following finished products:

- HOFIMEL S, container x 400g, dietary supplements as internal solution multidose

[Honey with seabuckthorn (Hippophae rhamnoides) fatty oil and essential oils of Menthe piperita aetheroleum, Lavandulae aetheroleum and Salvia aetheroleum].

- THYME ESSENTIAL OIL IN HONEY, dietary supplements as oral solution as

monodose, honey with Thyme vulgaris essential oil (Thyme vulgaris aetheroleum), Linum usitatissimum oleum and Hippophae rhamnoides oleum.

The study of these products was performed:

- with the objective of demonstrating their own antimicrobial activity, added activity to those of election, in addition at the benefic effect on the human body, [8];

- with the objective of scientifically demonstrating to maintain the microbiological quality for a multidose oral product, particularly for multidose containers during normal conditions of use, [9];

- with the objective of additional scientifically proofs as support for the Hofigal product stability in general and particularly to aqueous preparations, to prevent proliferation or to limit microbial contamination which, during normal conditions of storage and use could occur in a product and present a hazard to the patient from infection and spoilage of the preparation. Antimicrobial preservative properties of the formulation must not be used as a substitute for good manufacturing practice, these products are the results of sustained and prolonged research and development activity, a continue care for the product's development and improvement;

- with the objective of demonstrating that the antimicrobial efficacy may be enhanced by one compound or more of the formulation in which it is incorporated or by the container and closure used;

- with the object of sustaining the Company's mission: natural products, without

preservatives, in the fight for the human's health and safety, for a clean environment, in

respect for the nature.

Experimental [1], [2], [3], [4], [5]

Test for efficacy of antimicrobial preservation consists of challenging the preparation, with a prescribed inoculums of suitable micro-organisms which present a hazard to the user from infection or spoilage of the preparation, storing the inoculated preparation at a prescribed temperature withdrawing samples from the containers at specified intervals of time and counting the organisms in the inoculated samples in the specified medium culture.

We carried out all tests under the following standard conditions in compliance with European Pharmacopoeia 7th edition requirements:

- agar medium B for bacteria
- agar medium C without the addition of antibiotics for fungus
- recently grown stock culture of each of the specified test micro-organisms asfollowing (a

concentration of about 10^8 micro-organisms per milliliter):

- *Pseudomonas aeruginosa ATCC 9027;*
- *Staphylococcus aureus ATCC 6538;*
- *Candida albicans ATCC 10231;*
- *Aspergillus brasiliensis ATCC 16404;*
- *Escherichia coli ATCC 35218*

(Single-strain challenges are used and the designated micro-organisms are supplemented, where appropriate, by other strains or species that may represent likely contaminants to the preparation).

It is recommended that *Escherichia coli* will be used for all oral preparations.

- a working inoculum from each test organisms to give an inoculum suspension of

10^5 to10^6micro-organisms per milliliter or per gram of the preparation;

- incubate the bacterial cultures at 30-35 ^{0} C for 18-24 h, the culture of *Candida albicans* at 20-25 ^{0} C for 48 h, and the culture of *Aspergillus brasiliensis* at 20-25 ^{0} C for one week or until good sporulation is obtained;
- determine the number of colony-forming units per milliliter in each suspension by plate count or membrane filtrate;
- the suspensions shall be used immediately after preparation;
- the volume of the suspension of the inoculum does not exceed 1 per cent of the volume of the product and mix thoroughly to ensure homogeneous distribution;
- ensure that any residual antimicrobial activity of the product is eliminated by dilution, by filtration or by the use of a specific inactivate;
- maintain the inoculated product at 20-25 ^{0} C, protected from light;
- the appointed times are:

-for Oral preparations:

T_0= time 0 of contact between sample (preparation, culture medium, micro-organism and witness (culture medium and micro-organism) for bacteria and fungus

T_1= after 14 days of contact, for bacteria and fungus

T_2= after 28 days of contact, for bacteria and fungus.

The preservative properties of the preparation are adequate if, in the conditions of the test, there is a significant fall or no increase, as appropriate, in the number of micro-organisms in the inoculated preparation after times and at the temperatures prescribed.

The criteria of acceptance, in terms of decrease in the number of micro-organisms with time, vary for different types of preparations according to the degree of protection intended: Oral preparation, Topical preparation or Ophthalmic preparation.

The criteria for evaluation of antimicrobial activity are given in terms of the log reduction in the number of viable micro-organisms against the value obtained for the inoculum.

The test of the efficacy of the antimicrobial activity is not intended to be used for routine control purposes, batch by batch of finished products.

RESULTS AND DISCUSSIONS

Antimicrobial properties of the Hofigal products studied by the test for efficacy of antimicrobial preservation were demonstrated and could be emphasize by the curves‘ trend from Graphs number 1, 2, 3, 4, 5, 6,7,8,9 and 10.

Results obtained at the test of the products antimicrobial activity:

- THYME ESSENTIAL OIL IN HONEY, dietary supplements as oral solution

as monodoses x 2 ml, on:

- micro-organism *Pseudomonas aeruginosa ATCC 9027* are presented in Graph 1;
- micro-organism *Escherichia coli ATCC 35218* are presented in Graph 2;
- micro-organism *Candida albicans ATCC 10231* are presented in Graph 3;
- micro-organism *Staphylococcus aureus ATCC 6538* are presented in Graph 4;
- micro-organism *Aspergillus brasiliensis ATCC 16404* are presented in Graph 5.

The product mentioned above presents antimicrobial activity on *Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 35218 and Pseudomonas aeruginosa ATCC 9027,Aspergillus brasiliensis ATCC 16404* and *Candida albicans ATCC 10231 because we could observe a reduction of the bacteria number* by *a factor of at least 10^2/ml product within 14 days as log reduction log=5*.

After this time the micro-organism NI (= No Increase).

The product could achieved the A criteria and justified the efficacy of antimicrobial preservation, in compliance with criteria of acceptance requested by European Pharmacopoeia, current edition, for an Oral preparation.

- HOFIMEL S, container x 400g, dietary supplements as internal solution

multidose, *on:*

- micro-organism *Pseudomonas aeruginosa ATCC 9027* are presented in Graph 6;
- micro-organism *Escherichia coli ATCC 35218* are presented in Graph 7;
- micro-organism *Candida albicans ATCC 10231* are presented in Graph 8;
- micro-organism *Staphylococcus aureus ATCC 6538* are presented in Graph 9;
- micro-organism *Aspergillus brasiliensis ATCC 16404* are presented in Graph 10.

The product mentioned above presents antimicrobial activity on *Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 35218* and *Pseudomonas aeruginosa ATCC 9027, because we could observe a reduction of the bacteria number* by *a factor of at least 10^2/ml product within 14 days as log reduction log=5.*
The product has presented antifungical activity on Aspergillus brasiliensis ATCC 16404 and Candida albicans ATCC 10231 with a reduction of the fungi number by a factor of at least 10^1/ml within 14 days as log reduction log=5.

After this time the micro-organism NI (= No Increase).
The product could achieved the A criteria and justified the efficacy of antimicrobial preservation, in compliance with criteria of acceptance requested by European Pharmacopoeia, current edition, for an Oral preparation.

CONCLUSIONS

1. Test for antimicrobial activity was demonstrated by the Test for efficacy of antimicrobial preservation in compliance with cEuropean Pharmacopoeia for studied Hofigal'Oral and Topical preparations unidose and multidose and Cosmetics WITHOUT Preservatives on Gram positive bacteria, Gram negative bacteria, yeast and filamentous fungus, in according it the criteria of acceptance requested by cEuropean Pharmacopoeia for these categories of finished products.

2. Results obtained the demonstrated antimicrobial activity of these natural vegetal preparations from Hofigal'portfolio are enhanced by the active constituents, by the formulations in which it is incorporated and by the kind of the primary packaging/the container and closure used.

Tables

Table 1-*Efficacy of antimicrobial preservation-Criteria of acceptance for Oral preparations*

	Log reduction	
	14 d	**28 d**
Bacteria		3
NI*		
Fungus		1
NI		

NI* = no increase

Graphs:

Graph 1 .-Test for efficacy of antimicrobial preservation for THYME ESSENTIAL OIL IN HONEY, oral solution monodoses x 2 ml, inoculated with *Pseudomonas aeruginosa ATCC 9027*

Graph 2.-Test for efficacy of antimicrobial preservation for THYME ESSENTIAL OIL IN HONEY, oral solution monodoses x 2 ml, inoculated with *Escherichia coli ATCC 35218*

Graph 3.-Test for efficacy of antimicrobial preservation for THYME ESSENTIAL OIL IN HONEY, oral solution monodoses x 2 ml, inoculated with *Candida albicans ATCC 10231*

Graph 4.-Test for efficacy of antimicrobial preservation for THYME ESSENTIAL OIL IN HONEY, oral solution monodoses x 2 ml, inoculated with *Staphylococcus aureus ATCC 6538*

Graph 5-Test for efficacy of antimicrobial preservation for THYME ESSENTIAL OIL
IN HONEY, oral solution monodoses x 2 ml, inoculated with *Aspergillus brasiliensis*
ATCC 16404

Graph 6 -Test for efficacy of antimicrobial preservation for HOFIMEL S, container x 400 ml, oral solution multidose, inoculated with *Peudomonas aeruginosa ATCC 9027*

Graph 7-Test for efficacy of antimicrobial preservation for HOFIMEL S, container x
400 ml, oral solution multidose, inoculated with *Escherichia coli ATCC 35218*

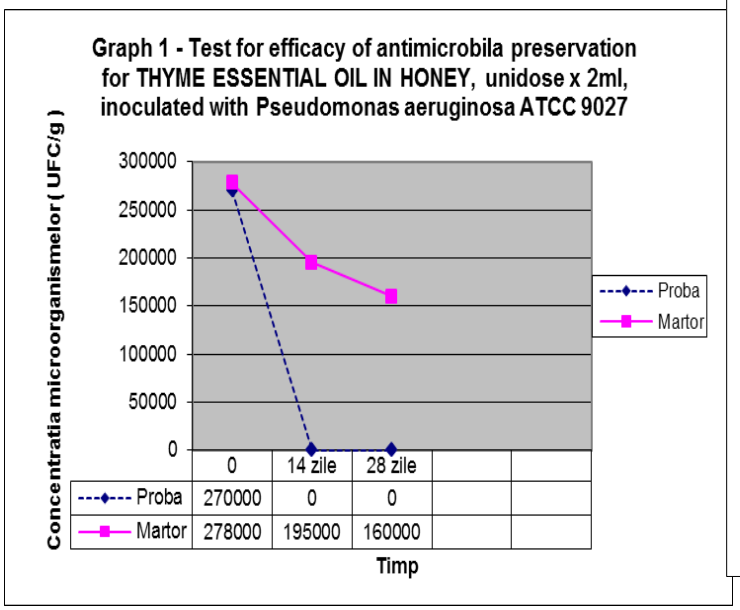
Graph 8 -Test for efficacy of antimicrobial preservation for HOFIMEL S, container x 400 ml oral solution multidose, inoculated with *Candida albicans ATCC 10231*

Graph 9-Test for efficacy of antimicrobial preservation for HOFIMEL S, container x 400 ml, oral solution multidose, inoculated with *Staphylococcus aureus ATCC 6538*

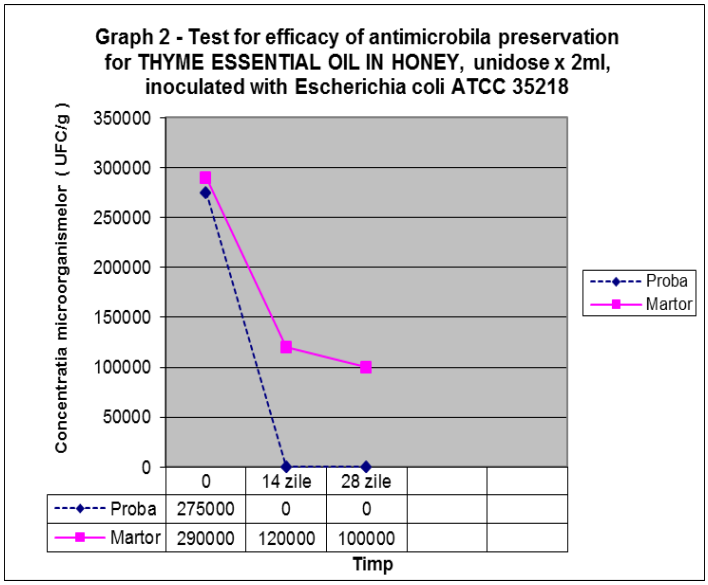
Graph 10-Test for efficacy of antimicrobial preservation for HOFIMEL S, container x 400 ml, oral solution

multidose, inoculated with *Aspergillus brasiliensis ATCC 16404*

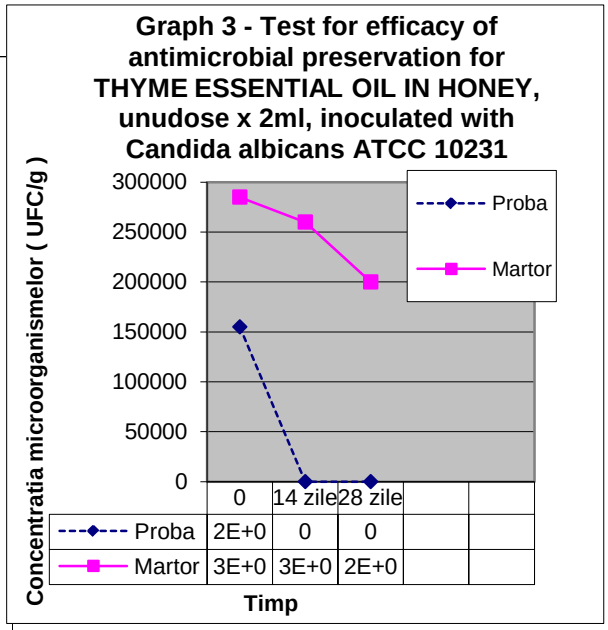
Graph 1-Test for efficacy of antimicrobial preservation for THYME ESSENTIAL OIL IN HONEY, monodoses x 2 ml, inoculated with *Pseudomonas aeruginosa ATCC 9027*:



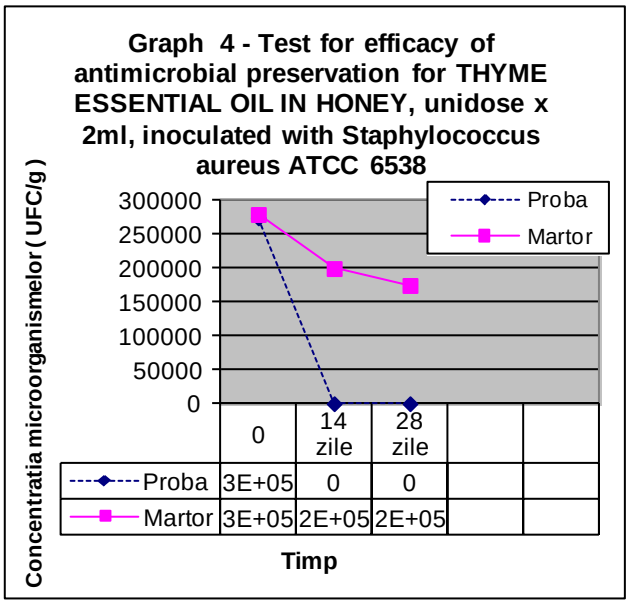
Graph 2-Test for efficacy of antimicrobial preservation for THYME ESSENTIAL OIL IN HONEY, monodoses x 2 ml, inoculated with *Escherichia coli ATCC 35218:*



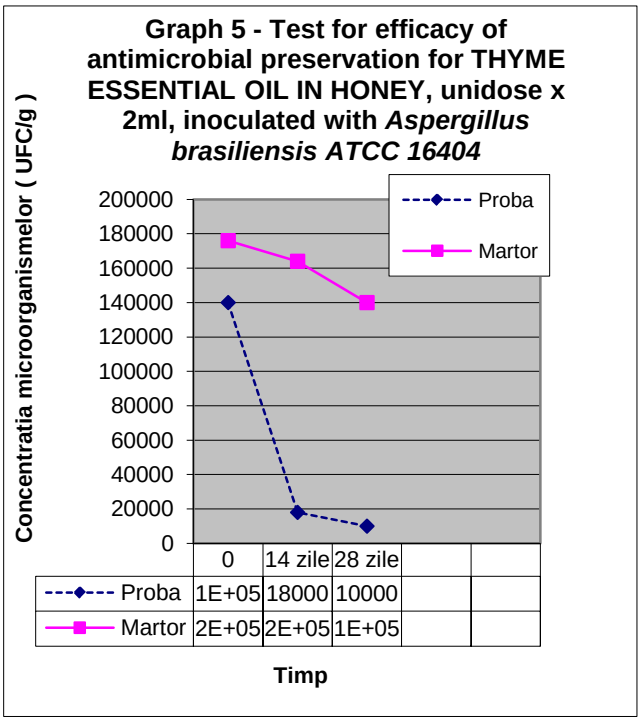
Graph 3-Test for efficacy of antimicrobial preservation for THYME ESSENTIAL OIL IN HONEY, monodoses x 2 ml, inoculated with *Candida albicans ATCC 10231:*



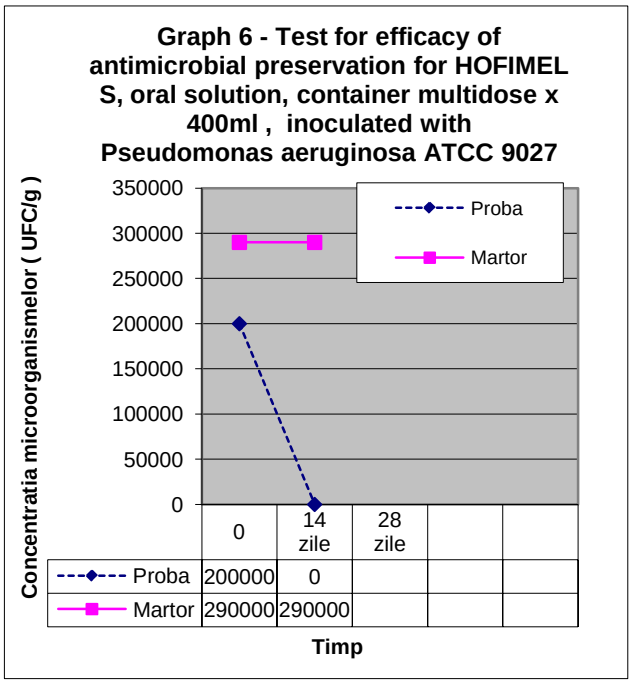
Graph 4-Test for efficacy of antimicrobial preservation for THYME ESSENTIAL OIL IN HONEY monodoses x 2 ml, inoculated with *Staphylococcus aureus ATCC 6538*:



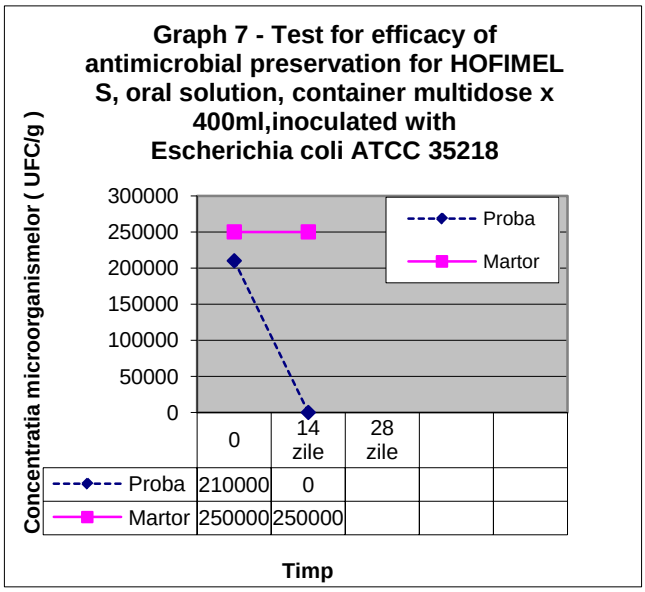
Graph 5-Test for efficacy of antimicrobial preservation for THYME ESSENTIAL OIL IN HONEY, monodoses x 2 ml, inoculated with *Aspergillus brasiliensis ATCC 16404:*



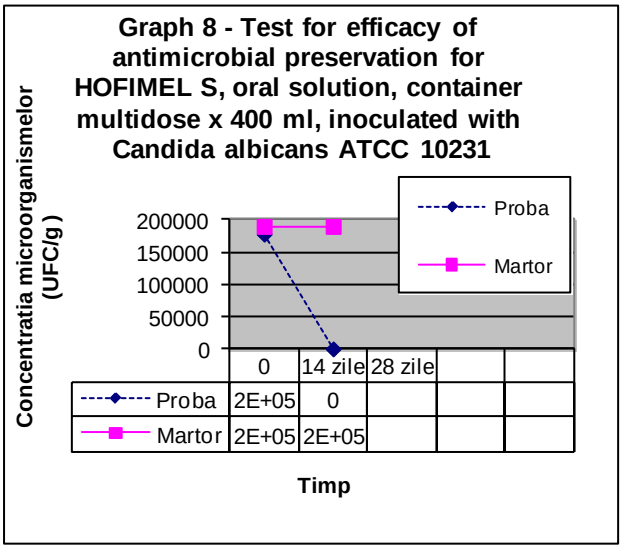
Graph 6-Test for efficacy of antimicrobial preservation for HOFIMEL S, oral solution multidose, container x 400 ml, inoculated with *Peudomonas aeruginosa ATCC 9027:*



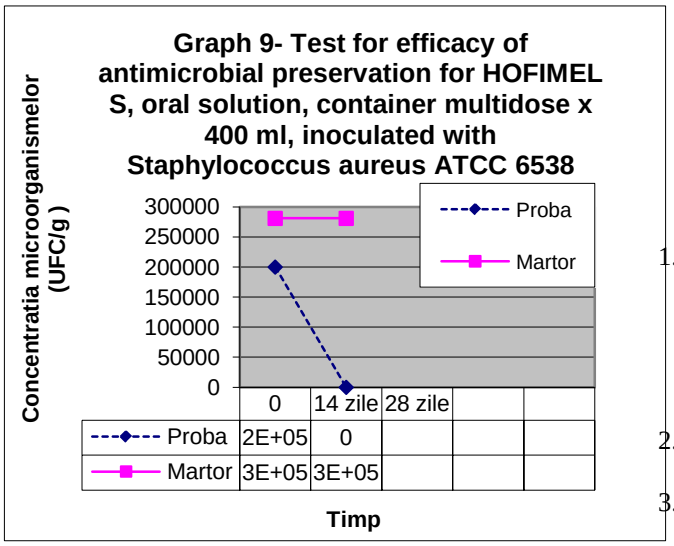
Graph 7-Test for efficacy of antimicrobial preservation for HOFIMEL S, oral solution multidose, container x 400 ml, inoculated with *Escherichia coli ATCC 35218:*



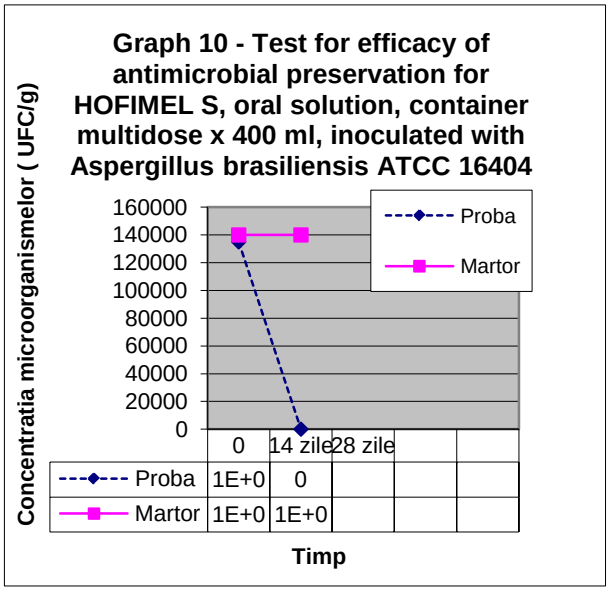
Graph 8 -Test for efficacy of antimicrobial preservation for HOFIMEL S, oral solution multidose, container x 400 ml, inoculated with *Candida albicans ATCC 10231:*



Graph 9-Test for efficacy of antimicrobial preservation for HOFIMEL S, oral solution multidose, container x 400 ml, inoculated with *Staphylococcus aureus ATCC 6538:*



Graph 10-Test for efficacy of antimicrobial preservation for HOFIMEL S,oral solution multidose, container x 400 ml, inoculated with *Aspergillus brasiliensis ATCC 16404:*



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