

*Stamp: Institute for Biological Research  
„Siniša Stanković“  
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**TEST REPORT FOR ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF  
"Origano Derm" PREPARATION**

**"Baltik Junior"**

**15.01.2019., Belgrade**

**Vučičev prolaz 20a**

**Beograd**

**Subject: Additional particulars to the Letter dated 10. 09.2018**

Company "**Baltik Junior**" has applied to the Institute for Biological Research "Siniša Stanković" in Belgrade (hereinafter referred to as IBRSS), for their expert opinion on potential antibacterial and antifungal effects of the product "**Origano Derm**".

Based on the documents submitted by the applicant and upon review of the relevant literature and laboratory analysis results, we are providing the following

## EXPERT OPINION

The kinds of bacteria used in the research are: *Streptococcus pyogenes* (IBRS S003), *Staphylococcus aureus* (ATCC 25923) and methicillin-resistant *Staphylococcus aureus* (MRSA 11). The bacteria used for testing of antifungal activities *in vitro* are *Candida albicans* (ATCC 750) and *C. krusei* (IBRS lflacl), *Trichophyton mentagrophytes* (IBRS DM), *Microsporum canis* (IBRS MC) and *Trichophyton tonsurans* (IBRS TT). All microorganisms are stored in the Mycological Library at Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", University of Belgrade.

The following antibiotics have been used for resistance control (fusidic acid; gentamicin; bacitracin + neomycin; chloramphenicol) and antimycotics (nystatin; ketoconazole; clotrimazole; miconazole).

It has been established that "**Origano Derm**" has bacteriostatic and bactericidal effect, since it inhibited growth, but also prevented further growth of all tested Gram (-) and Gram (+) bacteria. The test showed that the most sensitive bacteria was *Staphylococcus aureus* with inhibitory concentration of 1.0 mg/ml and bactericidal concentration of 2.50 mg/ml. The most resistant bacteria to the tested product was *Streptococcus pyogenes* with inhibitory/bactericidal concentrations of 3.75/7.50 mg/ml respectively (Table 1.). It has been established that this product has effect even against the most resistant bacteria, including Methicillin resistant bacteria *S. aureus* (MRSA), which is known for being one of the most resistant and most impervious bacteria of all (Soković et al., 2010).

Effects of a few tested antibiotics (fusidic acid, gentamicin, bacitracin + neomycin and chloramphenicol) are shown in Table 1. Fusidic acid has shown effect in Minimum Inhibitory Concentration (MIC) of 0.015-5.00 mg/ml, and Minimum Bactericidal Concentration (MBC) of 0.035-7.50 mg/ml, gentamicin in MIC of 0.005- 1.20 mg/ml, and in MBC of 0.007-5.00 mg/ml, bacitracin + neomycin in MIC of 0.0075-2.50 mg/ml and in MBC of 0.15-10.00 mg/ml, and chloramphenicol in MIC of 0.15-3.75 mg/ml and

in MBC of 0.30-7.50 mg/ml.

Bacterial activity of the cream is better than of fusidic acid and chloramphenicol (except for *S. aureus*), equal to fusidic acid and chloramphenicol (*S. pyogenes*), poorer than of gentamicine, but stronger than of bacitracin + neomycin (Table 1.).

The tested cream has shown better inhibitory activity than fusidic acid (except against *S. aureus*) and equal inhibitory activity against *Streptococcus pyogenes* like chloramphenicol (Table 1.).

In all dilutions the tested sample had inhibitory and fungicidal effects on all treated fungi *Candida albicans* and *Candida krusei*. The product had much stronger effect against *Candida krusei* (0.25/0.30 mg/ml) than against *Candida albicans* (3.75/5.00 mg/ml). In case of *C. albicans*, the cream is equally effective like nystatin, less effective than ketoconazole, and more effective than miconazole and clotrimazole. In case of *C. krusei* the cream showed much stronger antifungal effect than any other tested antimycotic (Table 2.).

The effect of the tested cream on dermatomycetes *Trichophyton mentagrophytes*, *Microsporum canis* and *Trichophyton tonsurans* was inhibitory in concentrations of 25 and 50 mg/ml and fungicidal in concentrations of 50 mg/ml. Fungal pathogen most susceptible to the tested product was *T. mentagrophytes* with inhibition growth zone of 16.67 mm (concentration 25 mg/ml), and in concentration of 50 mg/ml growth of this pathogen dermatomycete was stopped, while the control showed growth of 21.33 mm. The tested product showed only inhibitory activity against dermatomycetes *M. canis* and *T. tonsurans* in concentrations of 25 and 50 mg/ml. In *M. canis* the size of inhibition zones was 6.67 mm in lower tested concentrations and 6.33 mm (concentration of 50 mg/ml), while the growth of control was 9.67 mm. The effect of the tested product on dermatomycete *T. tonsurans* was inhibitory with inhibition zone of 16.33 mm in concentrations of 25 mg/ml and 15.33 mm in concentration of 50 mg/ml, while the growth of control was 19.00 mm.

In lower tested concentrations, the tested antimycotics (nystatin, ketoconazole, miconazole, clotrimazole) showed better inhibitory activity than the cream (25 mg/ml), while higher concentration of the cream (50 mg/ml) completely stopped further growth of fungi. Nystatin had fungicidal effect on *M. canis* and *T. tonsurans* also in concentration of 25 mg/ml (Table 3.).

Taking into account that in recent years, the frequency of microorganism resistance to the existing synthetic antibiotics is increasing, as well as toxicity of commercial preparations to human cells, there is a need for new semi-synthetic or natural antimicrobial agents that do not have any harmful effect on human health.

In that respect and based on the relevant literature and *in vitro* analysis, the following may be concluded:

**The tested product "Origano Derm" has shown good antibacterial and antifungal activity. The use of this product in prevention of various bacterial and fungal infections caused by the above mentioned kinds is justified. The very properties of this product and the fact that in general, resistance to natural preparations is much lower, speak in favour of this conclusion.**

Dr Marina Soković

*Signature*

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Dr Pavle Pavlović

*Signature and seal*

Science advisor

IBRSS Director

## References:

Hanel H. and Raether W. (1988): A more sophisticated method of determining the fungicidal effect of water-insoluble preparations with a cell harvester, using miconazole as an example. *Mycoses* 31, 148-154.

Soković M., Glamočlija J., Marin D.P., Brkić D., van Griensven L.J.L.D (2010): Antibacterial Effects of the Essential Oils of Commonly Consumed Medicinal Herbs Using an *In Vitro* Model, *Molecules*, 15, 7532-7546

**Table 1.** Antibacterial effect of the tested preparation (mg/ml).

		Origano Derm	Fusidic acid	Gentamicine	Bacytracine + neomycin	Chloramphenicol
<i>Staphylococcus aureus</i>	MIC	1.00	1.25	0.005	0.075	0.15
	MBC	2.50	5.00	0.007	0.15	0.30
methicillin-mesistant <i>S. aureus</i>	MIC	2.50	0.015	0.30	0.30	0.30
	MBC	5.00	0.035	0.60	0.60	0.60
<i>Streptococcus pyogenes</i>	MIC	3.75	5.00	1.20	2.50	3.75
	MBC	7.50	7.50	5.00	10.0	7.50

**Table 2.** Antifungal effect of the tested preparation (mg/ml), microdilution method.

		Origano Derm	Nistatin	Ketoconazole	Clotrimazole	Miconazole
<i>Candida albicans</i>	MIC	3.75	3.75	1.50	7.50	5.00
	MFC	5.00	7.50	2.00	15.0	7.50
<i>Candida krusei</i>	MIC	0.25	2.50	0.50	5.00	2.50
	MFC	0.30	5.00	1.00	7.50	5.00

**Table 3.** Antifungal effect of the tested preparation (mm), disc diffusion method.

		Origano Derm 25 mg/ml	Origano Derm 50 mg/ml	Nystatin	Ketoconazole	Clotrimazole	Miconazole	Control
<i>Trichophyton mentagrophytes</i>	mm	16.67	-	6.67	14.33	-	2.67	21.33
<i>Microsporium canis</i>		6.67	6.33	-	4.00	-	5.33	9.67
<i>Trichophyton tonsurans</i>		16.33	15.33	-	9.00	-	5.33	19.00

- no growth of the tested fungal pathogen was observed

