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TEST REPORT ON THE ANTIBACTERIAL AND FUNGICIDAL EFFECTS OF THE PRODUCT

''Herba Sept Strong''

''Baltik Junior''
Vučićev prolaz 20 a
Beograd

08.02.2017. Belgrade

SUBJECT: Response to the Letter of 9th December 2016.

The company "**Baltik Junior"** has addressed the Institute for Biological Test "Siniša Stanković" in Belgrade (hereinafter referred to as the: "IBISS") asking for an expert opinion on potential antibacterial and fungicidal effects of the product "Herba Sept Strong".

Having reviewed the documents submitted by the applicant, along with related literature and performed laboratory analysis, we hereby provide the following

EXPERT OPINION

The “Herba Sept Strong” sample was analyzed for gram-positive (Gram +) and gram negative (Gram -) bacteria. In the course of the Test the following species of bacteria were used: *Streptococcus pyogenes* (IBRS S003), *Streptococcus mutans* (IBRS S001), *Lactobacillus acidophilus* (IBRS L001), *Streptococcus salivarius* (IBRS S006), *Streptococcus sanguis* (IBRS S002), *Pseudomonas aeruginosa* (IBRS P001), *Proteus mirabilis* (clinical isolate), *Staphylococcus aureus* (ATCC 25923) and methicillin-resistant *Staphylococcus aureus* (MRSA) 11. For testing fungicidal activity *in vitro*, *Candida albicans* (IBRS MH4) and *Candida krusei* (IBR 1flac1) were used. All microorganisms were submitted to the Mycoteca of the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Test ‘’Siniša Stanković’’, University of Belgrade. Examined isolates were taken from patients’ mouth.

I*n vitro* microdilution method (Hanela and Raether, 1988; Soković et al., 2010) was used in analyses. The product was assessed in its original concentrated form as well as in 4 dissolved forms:

Ir-Herba Sept Strong concentrates

IIr-Herba Sept Strong dissolved form (1ml of concentrate+ 0.5ml of saline solution)

IIIr-Herba Sept Strong dissolved form (1ml of concentrate+ 1 ml of saline solution)

IVr-Herba Sept Strong dissolved form (1ml of concentrate+ 2 ml of saline solution)

Vr-Herba Sept Strong dissolved form (1ml of concentrate+ 3 ml of saline solution)

For the purpose of the positive control, the following commercial antibiotics were used: Ospamox, Pancef, Sinacilin, Klacid, Cephalexin and Streptomycin and the polyene antifungal Nystatin.

Test results confirmed that the product ‘’Herba Sept Strong’’ had both bacteriostatic and bactericidal effect i.e. inhibited growth and prevented further growth of the examined gram-positive (Gram +) and gram negative (Gram -) bacteria along with Candida species (*Candida albicans and Candida krusei*). The product demonstrated antibacterial and fungicidal effect in all tested dilutions (Table 1, Figure 1 and 2).

When in concentrated form, the product demonstrated the highest antibacterial potential, minimum inhibiting concentration (MIC) of 0.025-0.30mg/ml and bactericidal concentration (MBC) of 0.10.- 0.40 mg/ml. In II dissolved form (1ml of concentrate+ 0.5ml of saline solution) the product demonstrated good antibacterial effect (MIC 0.005-0.60 mg/ml, MBC 0.4-0.8 mg/ml). In III dissolved form (1ml of concentrate+ 1 ml of saline solution) the product demonstrated strong antibacterial effect, too (MIC 0.15-0.40 mg/ml and MBC 0.20-0.80 mg/ml). In IV dissolved form (1ml of concentrate+ 2 ml of saline solution), the product demonstrated inhibiting effect to all bacteria except *Staphylococcus aureus* (MIC 0.40-0.80 mg/ml). Bactericidal effect was demonstrated with 0,80mg/ml on six (6) bacteria while in the case of *Staphylococcus aureus*, *Staphylococcus aureus* MRSA, *Streptococcus salivarius*, *Streptococcus sanguis* and *Pseudomonas aeruginosa* the product in such proportion did not have any bactericidal effect. In V dissolved form (1ml of concentrate+ 3 ml of saline solution) the product had inhibiting effect on all bacteria (MIC 0.40-0.80 mg/ml) except *Staphylococcus aureus* MRSA and *Streptococcus pyogenes* while it had bactericidal effect on six bacteria (MBC 0.80 mg/ml) and no effect on *Staphylococcus aurues, Staphylococcus aureus MRSA, Streptococcus pyogenes, Streptococcus salivarius and Pseudomonas aerugionsa*.

Bacteria most resistant to the examined product were *Staphylococcus aurues and Staphylococcus aureus MRSA*. The bacterium which was most sensitive to the examined product effect was *Streptococcus salivarius*. It was determined that this product in all dissolved forms had effect even on the most resistant gram positive bacteria, including *Pseudomonas aeruginosa*, which is considered one of the most impervious and most resistant bacteria (Soković et al., 2010).

The tested antibiotics demonstrated a strong antibacterial effect on all tested bacteria except for Ospamox, which didn’t have any effect on *S. aureus MRSA, S. pyogenes and S-sanguis*, Pancef, which didn’t have bactericidal effect on *S. aureus, S. aureus MRSA and P.aeruginosa*; Sinacilin, which didn’t have any effect on *S. sanguis* in examined concentration (0.0005- 0.50mg/ml) (Table 1, Figure 1 and 2).

In concentrated form and in II dissolved form the product demonstrated better effect of antibiotics Sinacilin and Ospamox on bacteria *S.aurues and S. sangius*.

In all dissolved forms, the examined sample demonstrated inhibiting effect (0.15- 0.060 mg/ml) and fungicidal effect (0.40-0.80 mg/ml) on treated fungi *Candida albicans and Candida krusei*. The product **‘’Herba Sept Strong’’** demonstrated equal intensity on the both species of Candida.

Nystatin, which was used as a control, demonstrated inhibiting effect of 0.002- 0.0007mg/ml and fungicidal effect 0.003- 0.0015 mg/ml. The examined product demonstrated weaker effect than the commercial medicament.

Having in mind that the microorganisms’ resistence to the current synthetic antibiotics has been on the rise, and considering accompaying toxicity of commercial products on humane cells, it is clear that there is a need for a new polysynthetic or natural antimicrobials with no harmful effects on human health.

With that regard and on the grounds of the reviewed literature and performed *in vitro* analysis, we can provide the following **conclusion**:

The examined product ‘’Herba Sept Strong’’ has demonstrated good antibacterial and fungicidal effects. It may be concluded that the product demonstrates strong antimicrobial effect in all dissolved forms and can be used in several dilutions (1:1, 1:2, 1:3) in which it still retains good antimicrobial potential.

The use of the product ‘’Herba Sept Strong’’ is justified in the prevention of various bacterial and fungicidal infections caused by the above mentioned species. The properties of the product, along with the fact that presence of resistance to natural products is significantly lower, altogether contribute to this conclusion.

Reference:

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., Glamočija 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.

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Dr. Pavle Pavlović

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Principal Test Fellow
Director of IBISS

In the course of the Test the following species of bacteria were used: *Streptococcus pyogenes* (IBRS S003), *Streptococcus mutans* (IBRS S001),*Lactobacillus acidophilus* (IBRS L001), *Streptococcus salivarius* (IBRS S006), *Streptococcus sanguis* (IBRS S002), *Pseudomonas*
aeruginosa (IBRS P001), *Proteus mirabilis* (clinical isolate), *Staphylococcus aureus* (ATCC 25923) i methicillin-resistant *Staphylococcus aureus* (MRSA) 11.
For the purposes of the examination of the fungicidal effects *in vitro, Candida albicans* (IBRS MH4) and *C.krusei* (IBRS 1flac1) were used.
All microorganisms are deposited in the Mycoteca of the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Test Siniša Stanković, University of Belgrade.

The Test included the following dissolved forms of the product Herba sept strong:

Ir-Herba Sept Strong concentrates

IIr-Herba Sept Strong dissolved form (1ml of concentrate+ 0.5ml of saline solution)

IIIr-Herba Sept Strong dissolved form (1ml of concentrate+ 1 ml of saline solution)

IVr-Herba Sept Strong dissolved form (1ml of concentrate+ 2 ml of saline solution)

Vr-Herba Sept Strong dissolved form (1ml of concentrate+ 3 ml of saline soluti

Table 1. Antimicrobial effect of the examined product ‘’Herba Sept Strong’’(mg/ml).

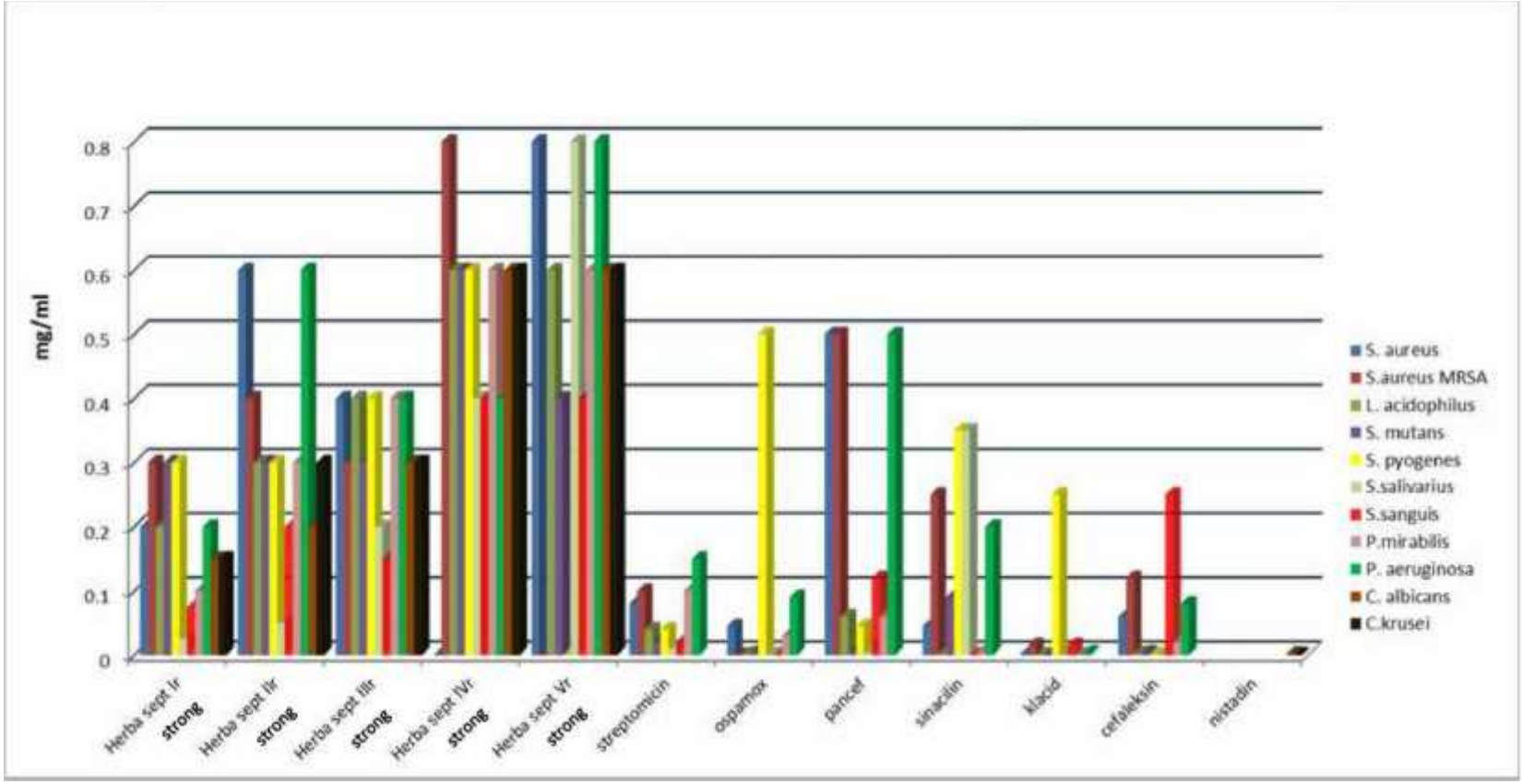
		S. aureus	*S. aureus* MRSA	*L. acidophilus*	*S. mutans*	*S. pyogenes*	*S. salivarius*	*S. sanguis*	*P. mirabilis*	*P. aeruginosa*	*C. albicans*	*C. krusei*
Herba sept strong Ir	MIK	0.20	0.30	0.20	0.30	0.30	0.025	0.075	0.10	0.20	0.15	0.15
	MBK/ MFK	0.40	0.40	0.40	0.40	0.40	0.10	0.10	0.20	0.40	0.40	0.40
Herba sept strong IIr	MIK	0.60	0.40	0.30	0.30	0.30	0.05	0.20	0.30	0.60	0.20	0.30
	MBK/ MFK	0.80	0.80	0.40	0.40	0.40	0.40	0.40	0.40	0.80	0.40	0.40
Herba sept strong IIIr	MIK	0.40	0.30	0.40	0.30	0.40	0.20	0.15	0.40	0.40	0.30	0.30
	MBK/ MFK	0.80	0.40	0.80	0.40	0.80	0.40	0.20	0.80	0.80	0.40	0.40
Herba sept strong IVr	MIK	-	0.80	0.60	0.60	0.60	0.40	0.40	0.60	0.40	0.60	0.60
	MBK/ MFK	-	-	0.80	0.80	0.80	-	-	0.80	-	0.80	0.80
Herba sept strong Vr	MIK	0.80	-	0.60	0.40	-	0.80	0.40	0.60	0.80	0.60	0.60
	MBK/ MFK	-	-	0.80	0.80	-		0.80	0.80	-	0.80	0.80
streptomicin	MIK	0.08	0.10	0.04	0.02	0.04	0.01	0.02	0.10	0.15	-	-
	MBK	0.16	-	0.08	0.04	0.08	0.02	0.04	0.20	0.20		
ospamox	MIK	0.04 5	-	0.002	0.006	0.50	0.001	-	0.03	0.09	nt	nt

	MBK	0.06	-	0,004	0,008	-	0,002	-	0.06	0.12	nt	nt
pancef	MIK	0.50	0.50	0.06	0.006	0.045	0.004	0.12	0.06	0.50	nt	nt
	MBK	-	-	0.12	0.008	0.06	0.008	0.50	0.12	-	nt	nt
synacylin	MIK	0.045	0.25	0.002	0.09	0.35	0.35	-	0.006	0.20	nt	nt
	MBK	0.06	0.50	0.004	0.12	0.50	0.50	-	0.008	0.25	nt	nt
klacid	MIK	0.002	0.015	0.0005	0.0005	0.25	0.0005	0.015	0.0005	0.004	nt	nt
	MBK	0.03	0.03	0.001	0.001	0.50	0.001	0.03	0.001	0.008	nt	nt
cephalexin	MIK	0.06	0.12	0.003	0.004	0.001	0.0005	0.25	0.02	0.08	nt	nt
	MBK	0.12	0.50	0.004	0.008	0.002	0.001	0.50	0.03	0.12	nt	nt
nystatin	MIK	nt	nt	nt	nt	nt	nt	nt	nt	nt	0.002	0.0007
	MBK	nt	nt	nt	nt	nt	nt	nt	nt	nt	0.003	0.0015

- no effect on tested microorganisms

nt- not tested

Minimum inhibiting effect of the product Herba Sept Strong and antibiotics/antimycotics



Minimum bactericidal/ fungicidal effect of the product Herba Sept Strong and antibiotics/antimycotics