

# Researches on the Antimicrobial Impact of Immunosept Product

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**Abstract** – The hereby paper is a study referring to the antimicrobial action of the Immunosept preparation based on its composition. In this study, its microbial properties were tested on various pathogen and high pathogenic germs, observing the way in which the product behaves in vitro, towards the increase and development of various bacteria.

In comparison with the other actions mentioned by the manufacturer, the study shows the efficiency of the preparation in combatting some pathogenic germs. The products components are represented by: bee honey; Echinacea (*Echinacea purpurea*) – soft extract of aerial parts; lemon (*Citrus limonum*) – fruits fresh; ginger (*Zingiber officinale*) – rhizome fresh; horse radish (*Armoracia rusticana*) – fresh from roots; acerola (*Malpighia punicifolia*) – aqueous dry extract with 50% of vitamin C; Propolis with 6% polyphenols; essential oils of: cloves (*Eugenia caryophyllata*); eucalypt (*Eucalyptus globulus*); tea – tree (*Melaleuca alternifolia*), determined us to test also the antimicrobial action of this preparation taking into consideration that it is a bio product, which does not contain antibiotic additions, preservatives or other compounds which could have side effects on the organism.

**Keywords** – Immunosept, Antimicrobial Action, Pathogenic Germs.

## I. INTRODUCTION

This study has at the base the components composition and properties which proved a strong antimicrobial action.

The bee honey, by its content in inhibin, a factor which assures the antimicrobial and antifungal action, is the support in which the other components embed. (Necula Valentin and Mihaela Babii – 2010).

The propolis, this significant component with miraculous properties which the ancient Greeks used as medicine has anti-inflammatory and antimicrobial action. The propolis contains amino – acids, flavonoids, terpene and derivatives of cinnamic acids having antimicrobial, antifungal action and favouring the issuance of prostaglandins, leukotrienes and histamine. Its antifungal actions is explained by these. (H Wu – 2009).

Also, the ethanol extracts of propolis can represent the basis of some drug preparations due to the multitude of pharmaceutical effects. It has to give a significant attention to the method of extraction, processing and storing of ethanol extracts of propolis whereas they decompose easily at the light and heat action. (LL Vlaia, V Vlaia, IV Olariu, AM Mut-2016 - revistadechimie.ro). The apiarian products, among which the propolis, due to their

composition, represented by phenolic compounds, among which the 3, 4 – dimethoxycinnamic acid, being the major isolated component, have antimicrobial action. The antibacterial activity of propolis is manifested on the Gram positive bacteria at concentrations higher than 0, 2 mg/l. The antibacterial spectre of propolis is extended also on Gram negative bacteria, with this meaning having inhibitor action and on *Salmonella enterica*. (Adell A.A Mohaly, Awada A.Mohamoud.. and colab.-2015). The antimicrobial action of propolis is manifested also on bacteria which are involved in the appearance and development of dental caries, by this the propolis being able to represent an ingredient in various pharmaceutical products, intended to control their bacteria from dental biofilms. (BS Machado and colab. – 2016). The propolis has intense antimicrobial action also on the *Streptococcus mutans* from the dental biofilms (Julia Gabiroboertz Cardoso.. and colab.-2016).

The cloves (*Eugenia caryophyllata*), by its basic component represented by eugenol has antimicrobial, bacterial, virulicidal, antifungal and antiparasitic action. There are used mainly the volatile oils obtained after the distillation by drive with water vapors. The main component from the oil excerpted by distillation is the eugenol, but also other compounds which contain terpenoids, monoterpene, sesquiterpene, aliphatic hydrocarbons with low molecular weight, acids, alcohol, aldehydes, esters, lactone. The terpenes and alcohols, among which the eugenol have a strong antimicrobial action. The terpenes and eugenol have action on some pathogenic germs like: *Acinetobacter calcoaceticus*, *Aeromonas hydrophila*, *Alcaligenes faecalis*, *Bacillus subtilis*, *Citrobacter freundii*, *Clostridium sporogenes*, *Enterococcus faecalis* (etc). (H.J.D. Dorman, S.G. Deans-2015).

The horse radish (*Amoracia rusticana*) is an ingredient which contains isothiocyanates, substances with rubefiant and antiphlogisticum activity. With these major components, the rest of the ingredients have a significant role too by activating the blood flow, but also by the antiphlogisticum and calming activity (K. Chaieb, H. Hajlaoui, T. Zmantar – 2007). Also, the horse radish by the isothiocyanates which it contains has also antitumor action. The horse radish root due to the allyl isothiocyanates has a strong anti-inflammatory and antipyretic action at the level of the human immunity system cells by the PGE2 and leukotriene synthesis. (Corinna Herzend colab. 2016).

## II. MATERIAL AND METHODS

For the hereby study we used the imunosept preparation under liquid form packed in metallic foil and dosed under the form of sachets, each with a dose of 10 millilitres. There were performed antibiogram on which pure cultures of isolated pathogenic germs and stems of ATCC reference were tested, observing the detection of these pathogenic germs.

Petry plates were used, in which Muller – Hinton environment was added, approximately 20 millilitres per plate. After the solidification of the environment 1 ml of inoculum from the bacterial cultures, which we tested, was inoculated on the plate and which was dispersed with a loop on the entire surface of the Muller – Hinton agar and separating further the excess of inoculum.

The inoculated plates were introduced in the thermostat, in the microaerophilic atmosphere, for 30 minutes.

Further the inoculated plates with liquid suspensions from the tested and thermostatic bacterial cultures were pulled out of the thermostat.

Rondeaus of filter paper were made with a diameter of 0, 5 centimetres and which were impregnated with the imunosept preparation.

These rondeaus impregnated with the imunosept preparation were placed with a sterile dental tuck, on the surface of the inoculated agar with suspension of liquid bacterial culture and were introduced to the thermostat at 37°C, for 24 hours.

At 24 hours after thermostat the Pertym plates, with the bacterial inoculum and the rondeaus impregnated with imunosept were examined and the results were interpreted depending on the produced area of lysis, which manifested as a clear area on the matte or pigmented background of the inoculated Muller – Hinton agar.

In the case of the imunosept product 5 lots of the imunosept preparation production were tested each with 5 samples from each lot.

Two plates were inoculated both with liquid suspension of various isolated bacterial cultures of the cadavers of some animals, slinks, but also from food products, parallel with the same germs came from the cultures of ATCC reference. In this way, it could be observed the action of the imunosept preparation on a varied range of pathogenic germs widely spread in the environment and which affects both the animals and human being, being able at the same time to contaminate food. Using the reference cultures of ATCC type is significantly important to serve as reference level for the activity of the tested product in comparison with some known bacterial cultures.

The following pathogenic germs were tested: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Aeromonas salmonicida*, *Pseudomonas aeruginosa*, *Salmonella infantis*, *Salmonella glucester*, *Aeromonas aerogenes*, *Acinetobacter hemoliticus*.

The ginger – (*Zingiber officinale* Rose) is significantly active having a pronounced antifungal and antibacterial action. The ginger extracts are active, among other towards the *Fusarium oxysporum* and *Pseudomonas solanaceum*. (GN Dake - Journal of Spices- 2016).

## III. RESULTS AND DISCUSSION

5 lots of the imunosept product were examined, from each lot being sampled for the experiment five units. The imunosept action on the tested bacterial cultures, both on those isolated from various pathologic materials, and ATCC cultures were uniform, which proves the good isolation of the bacteria originating from food and pathologic materials. At all these, it is added the uniform action of imunosept against the same used bacterial cultures.

Examining the activity of the imunosept product in the plates inoculated with bacterial suspension we obtained the following results: (Fig. no. 1 and 2).



Fig. 1. The lysis area on the Muller – Hinton agar of the imunosept product against the *Pseudomonas aeruginosa*.



Fig. 2. The lysis area on the Muller – Hinton agar of the imunosept product against the *Staphylococcus epidermidis*.

The imunosept product, due to its represented components has bacterial action on some bacteria with high degree of pathogenicity.

For the performed study we used the following types of microorganisms, on which the imunosept preparation had partial intense bacterial effects.

In the table from below we give the types of tested microorganisms and the imunosept effect on them. (Table no. 1)

Table 1. The imunosept effect on the tested microorganisms

The type of tested microorganism	The effect produced by imunosept on the respective microorganism
<i>Pseudomonas aeruginosa</i>	+++++ 2,5 cmm
<i>Aeromonas aerogenes</i>	-
<i>Aeromonas salmonicida</i>	-
<i>Proteus mirabilis</i>	-
<i>Salmonella glucester</i>	-
<i>Salmonella infantis</i>	-
<i>Staphylococcus epidermidis</i>	+++ 1,8 cmm
<i>Staphylococcus aureus</i>	-
<i>Acinetobacter hemoliticum</i>	-

From the analysis of the data contained in table no.1, it is observed that the most intense bacterial action is manifested on *Pseudomonas aeruginosa* and on the stem of *Staphylococcus epidermidis*. The other tested microorganisms are resistant to the imunosept product action.

Analysing the positive effects, bacterial, we observe a bigger sensitivity to *Pseudomonas aeruginosa* fata de *Staphylococcus epidermidis*.

The lysis produced by imunosept on the stem of *Pseudomonas aeruginosa* is of 2,5 centimetres (fig.1) while the lysis produced on *Staphylococcus epidermidis* is smaller, of only 1,8 centimetres (Fig. 2).

In what regards the *Salmonella* type, in the study which we performed we saw that the Imunosept product, in the form in which it was imagined, does not have action on *Salmonella infantis* and *Salmonella glucester*. The studies performed by H.J.D Dorman and S.G .Deans in 2015 showed that the Eugenol and Propolis, ingredients which are found in the imunosept product have action on *Salmonella enterica*.

The action of the eugenol and terpene of cloves is manifested on the staphylococcus aureus; results which are communicated in the studies by H.J.D. Dorman, S.G. Deans and Adeel A.A. Mohaly – in 2015. In comparison with the data communicated by these authors, in the study which we performed, the Imunosept product, is active also against *Staphylococcus epidermidis*. In what regards the staphylococcus aureus the doses of volatile clove and propolis oil of Imunosept did not action bacterial on it.

Another pathogenic germ which was subject to the test with Imunosept is *Acinetobacter hemoliticum* which we isolated in the pure culture from a sheep slink. The Imunosept product did not have antibacterial effect on this pathogenic germ.

H.J.D. Dorman and S.G. Deans in 2015, tell in the performed studies the bacterial action of eugenol on a stem of *Acinetobacter calcoacetica*, these results can be explained by the increases pathogenicity of the stems of *Acinetobacter hemoliticum* in comparison with the stem of *Acinetobacter calcoacetica*, but also by the doses of clove oil which are found in Imunosept, in comparison with the data communicated by H.J.D. Dorman and S.G. Deans in 2015. This performed study emphasises the antibacterial action of Imunosept, against the studied stems, actions which the manufacturer did not mention on the product prospectus and which the study emphasized as being beneficial. There were mentioned in the product prospectus, actions regarding the antiphlogisticum, antipyretic, sudorific and regulating effect of the pulmonary function.

We can explain these results as being due, mainly, to propolis, clove extract and inhibin, component with antimicrobial and antifungal role, existing in the bee honey.

#### IV. CONCLUSION

The herbal preparations have beneficial effects on body removing the side effects.

The components of the Imunosept product have anti-inflammatory and bacterial action for certain microorganism. In the determinations we did, using the stems of ATCC reference, is very important for the accuracy of the obtained results.

For the *Pseudomonas aeruginosa* type, the imunosept product has strong bacterial action, the lysis area on the Mülle – Hinton agar being of 2,5 centimetres, in comparison with the one produced on the *Staphylococcus epidermidis* type, which is of 1,8 centimetres.

On *Acinetobacter hemoliticum*, the imunosept preparation does not have antimicrobial action. The same component from imunosept, at bigger doses, has inhibited action on *Acinetobacter calcoacetica*, existing with this purpose also literature review.

The imunosept product by its components is active in comparison with *Pseudomonas aeruginosa* and in comparison with *Staphylococcus epidermitis*, being able to be used in diseases produced by this germs.

#### REFERENCES

- [1] Adell A.A Mohaly, Awada A. Mohamoud, Mohamed H.H. Roby, Iryna Smatanska, Mohamed Fawzy Ramadan, 2015. Phenolic Extract from Propolis and Bee Pollen: Composition, Antioxidant and Antibacterial Activities.- Journal of biochemistry, volume 39-issue 5 October 2015, 538-547.
- [2] Barzoi Dobre, Sorin Apostu, 2002 - Food Microbiology - Risoprint Publishing House Cluj-Napoca.
- [3] BS Machado, TN Pulcino, AL Silva, D Tadeu, 2002 - Propolis as an alternative in prevention and control of dental cavity immunity.- Brazilian Journal of Microbiology. São Paulo.pg 33 (4): 365-369. 36.
- [4] Corinna Herz, Hoai Thi Thu Tran, Melinda-Rita Márton, Ronald Maul, Susanne Baldermann, Monika Schreiner, and Evelyn Lamy, 2016. Evaluation of an Aqueous Extract from Horseradish Root (*Armoracia rusticana Radix*) against Lipopolysaccharide-Induced Cellular Inflammation Reaction.- Evidence-Based Complementary and Alternative Medicine Volume 2017 (2017), Article ID 1950692, 10 pages.
- [5] Dan Eduard Mihăilescu, Dragos Gudovan and Anca MA., 2014. Control release profiles of *Eugenia Caryophyllata* *Artemisia*, *Artemisia annua* and *carum carvicolat* oils from organic functionalized MCM-41 suport.- Rev. Roum. Chim., 59 (2), 111-116.
- [6] H Wu, GA Zhang, S Zeng, K Lin, 2009 - Pest management science, Wiley Online Library- Comparison of flavour compounds in wasabi and horseradish.
- [7] H.J.D. Dorman, S.G. Deans, 2015. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of applied microbiology - Online ISSN: 1365-2672.
- [8] Hwa-Won Lee, Sang-Guei Lee, Hoi-Seon Lee, 2016. Active component isolated from *Eugenia caryophyllata* leaves and its structural analogues show insecticidal properties against *Pochazia shantungensis* - Applied Biochemical Chemistry - August 2016, Volume 59, Issue 4, pp 609–614.
- [9] Julia Gabiroboertz Cardoso, Natalia Lopes Pontes Ioriob, Luís Fernando Rodriguesb, Maria Luiza Barra Courib, Adriana Farahc, Lucianne Cople Maiaa, Andréa Gonçalves Antonio, 2016. Archives of oral Biology- vol 65 May-2016, 75-81.
- [10] Khayyal MT, el-Ghazaly MA, el-Khatrib AS, 1993. Department of Pharmacology, Faculty of Pharmacy, Cairo University, Egypt – Mecanism involved in the antiinflammatory effect of propolis. Drugs Under Experimental and Clinical Research.
- [11] K. Chaieb, H. Hajlaoui, T. Zmantar, 2007. The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzgium aromaticum* L. Myrtaceae): a short review - Wiley Online Library.
- [12] LL Vlaia, V Vlaia, IV Olariu, AM Mut, 2016. Preparation and Characterization of Inclusions Complexes between Propolis

- Ethanollic Extracts and 2- hydroxypropyl-  $\beta$ - cyclodextrin.  
 Revistadechimie.ro
- [13] Necula Valentin, Mihaela Babii, 2010. Food, Food and Their Impact on Consumer Health - Transylvania University Publishing House of Brasov.
- [14] Nueleanu Veturia Ileana, 2008. Veterinary pharmacology - Risoprint Publishing House Cluj-Napoca.
- [15] Puchianu G., 2012. Microbiological criteria for food safety and hygiene of processing - Food of animal origin. Transylvania University Publishing House in Brasov. ISBN 978-606-19-0097-8, E-mail editura@unitbv.ro.
- [16] Y. Vural,Y. Ugan, A. Yigit, A. Doğru, M. Deryal, E. Uz,H. Vural, S.E. Tunc, 2016. AB0893 Comparison of Anti-Inflammatory Effects of The Gel Included Diclofenac Sodium and The Mixture of Zingiber Officinale, Hypericum Perforatum, Eugenia Caryophyllata and Laurus Nobilis Oil in Collagen-Induced Arthritis Model in Rats — BMJ Journals vol. 75- ISSUE SUPPL 2.