Part 3. Biosafety

UDC 619:615.281.9:[665.52+547.54+546.57-022.532]

ANTIBACTERIAL EFFECT OF VEGETABLE ESSENTIAL OILS BASED ON METAL NANOPARTICLES *IN VITRO*

Kovalenko V. L.[1], **Ponomarenko O. V.**[2], **Korniyenko V. I.**[2], **Harkusha I. V.**[2], **Gordiyenko A. D.**[2]
[1] State Scientific Control Institute of Biotechnology and Strains of Microorganisms, Kyiv, Ukraine, e-mail: kovalenkodoktor@gmail.com
[2] Kharkiv State Zooveterinary Academy, Kharkiv, Ukraine

Summary. The paper presents the results of microbiological studies to determine the bactericidal activity of essential oils in relation to standard test microorganisms.

The bactericidal activity of some natural essential oils in the complex with benzalkonium chloride and silver nanoparticles has been investigated. The general characteristic of active substances of vegetable oils in relation to their influence on the organism of animals and people with the manifestation of anti-inflammatory and stimulating action has been presented.

Microorganisms, in prolonged contact with silver nanoparticles and essential oils, practically do not produce resistance to them, which is a significant advantage of essential oils in comparison with antibiotics and it may be used in human and veterinary medicine.

Keywords: essential oils, disinfection, test microorganisms, silver nanoparticles, benzalkonium chloride, broth cultures, *Escherichia coli, Staphylococcus aureus*, bactericidal activity

Introduction. There are no almost ecologically clean and safe disinfectants in veterinary practice that could be used for sanitation of veterinary supervision objects. Every year, the number of resistant and low-sensitive forms of bacteria increases significantly. In connection with this, there is a need to study new antibacterial agents that do not cause the development of bacterial resistance.

The essential oils may be used as an alternative to chemical disinfectants. Due to their properties they can effectively affect pathogenic microorganisms and show a therapeutic effect. Essential oils consist from the chemical compounds and some chemical elements. The primary elements responsible for the essential oil function are carbon, hydrogen, and oxygen. The components of essential oils are represented by various compounds, which can be arranged in the following order according to their biological properties:

Aldehydes (Melissa, lemon grass, lemon verbena, lemon eucalyptus, etc.) have anti-inflammatory, sedative and antiviral effects.

Alcohols. This is one of the most useful compound groups that have an antiseptic and antiviral effect. These include linalool (present in lavender, linaloe and rose wood), citronelol (present in rose, lemon, eucalyptus, and geraniums), geraniol (present in palmarose), as well as borneol, menthol, nerol, terpineol, farnesol, veteryrol and cetrol.

Phenols. They have a bactericidal activity and can also irritate the skin. The most common phenols of essential oils include eugenol (present in carnation and laurel), thymol (present in the thyme) and carvacrol (present in oregano and thyme).

Terpenes. Terpenic carbohydrates include limonene (an antiviral substance contained in 90% of citrus oils) and pinene (an antiseptic contained in large quantities in pine and sap oils), as well as camphene, cadinin, cariofillin, chedrine, dipentene, phellandrin, sabineen, myrcene etc. Some substances related to terpenes have pronounced anti-inflammatory and bactericidal properties.

Ketones. This is a class of chemical compounds that have a wound healing property and facilitate the secretion of mucus. Among the essential oils with high content of ketones there are also the oils of rosemary, sage, eucalyptus spherical and hyssop.

Sesquiterpenes consist of very long carbon chains. They are contained, in particular, in the essential oils of chamomile, sandy everlasting, tansy, yarrow and marigold. Sesquiterpenes have anti-inflammatory, sedative and antiviral properties, as well as bacteriostatic and immune stimulating effects.

Oxides. The most important oxide is cineol (or eucalyptol), which is presented in eucalyptus oil and has an expectorant property. It was also found in rosemary, tea tree and cajeput oils.

Esters are the most representative group of substances found in essential oils. These include linalin acetate (present in bergamot, muscat, sage and lavender) and geranyl acetate (present in marjoram). Esters have an antifungal effect.

Lactones. This is a group of esters with an additional carbon ring. They belong to the most powerful anti-inflammatory compounds. There is a lot of lactones in arnica essential oils. Some lactones help to separate mucus even more efficiently than ketones.

Phenylpropane esters have a powerful harmonizing effect on the nervous system. They have antiseptic, stimulating, expectorant, anesthetic and diuretic properties. Cinnamon, carnation, anise, basil, tarragon and parsley oils are rich in phenylpropane esters.

Anti-inflammatory action is caused by the influence of aromatic components on vascular-tissue reactions: reduction of vascular wall permeability, optimization of vascular reactions, astringent and anti-edema activity, optimization of oxygen metabolism, stimulation of proliferation and regeneration. The most effective in acute inflammation essential oils are: tea tree, carnation, sage, oregano, muscat, basil, thyme, juniper, bergamot, myrtle, pine, schizandra, citronella, eucalyptus. In case of subacute inflammation: anise, verbena, geranium, chamomile, lavender, rose, hyssop, myrrh, marjoram, rosewood, tea tree, myrtle, muscat, oregano, spruce, pine. For chronic inflammations: orange, lemon, grapefruit, ylang, incense, neroli, petitgrain, sandalwood.

Immunostimulating effect. Aromatic carbohydrates stimulate the activity of cellular and humoral immunity, optimize the production of T- and B-lymphocytes, phagocytic activity of macrophages, destroy unbound biogenic amines, enhance the production of histaminase by intestines and kidneys — an enzyme producing an oxidative catalysis of histamine, serotonin, norepinephrine, adrenaline, tyramine, etc., resulting in a non-toxic product.

Thus, it is possible to interpret the effect of most essential oils as immunostimulating and anti-inflammatory. The most pronounced such an effect is expressed in bergamot, valerian, geranium, grapefruit, ylang, hyssop, cajeput, cedar, lavender, incense, myrtle, juniper, neroli, rose, petitgrain, rose wood, chamomile, pine, tea tree, sage, eucalyptus (Nikolaevskiy, 2000; Bergonzelli et al., 2003; Sartoratto et al., 2004; Borodina, 2004).

The aim of this study. The purpose of the research was to study the antibacterial effect of plant essential oils on the basis of nanoparticles of metals in vitro with the use of test microorganisms.

Materials and methods. There have been conducted studies of biological properties and the composition selection of a bactericidal preparation based on nanoparticles of silver, benzalkonium chloride and essential oils *in vitro* with the use of test microorganisms.

Broth cultures of *S. aureus* (strain P-209) were used in experiments. To prepare the broth culture, 25 cm^3 of the nutrient medium was poured into the flask and then 0.25 cm^3 of the overnight broth culture of microorganisms was added. A day later, broth culture was filtered through a sterile gauze-wool or paper filter. In test tubes with various dilutions of disinfectant (5 cm^3 in each), 0.5 cm^3 of overnight broth culture of tested microorganisms was added. After 10 min samples were taken from the flasks by a platinum loop and transferred to a Petri dishes with meat-peptone agar.

The indicated types of work were conducted in compliance with the conditions of sterility. After 30 min, keeping the same interval, samples were taken again and the next sowing on agar was carried out. After that, Petri dishes were placed in a thermostat with a temperature of 37 °C. The culture was viewed in 24 and 48 hours.

A similar method was also used in conducting a study to determine the bactericidal effect concerning *E. coli* (strain 1257).

For control, there was carried out the sowing of broths cultures *S. aureus* and *E. coli* according to generally accepted methods (Kovalenko, 2014; Obrazhei et al., 2008). In this case, microbiological indicative test cultures *E. coli* (strain 1257) and *S. aureus* (strain P-209) were used.

Results. In the study of bactericidal activity in the laboratory, 1.0% solution of essential oils with silver nanoparticles and benzalkonium chloride in the concentration of the basic solution 1:50 was prepared with a progressive reduction of the active substance in a solution of 1:4 by serial dilutions.

At the same time, a suspension of overnight culture *E. coli* (strain 1257) and *S. aureus* (strain P-209) in liquid media was prepared, containing microorganisms in concentration $2{\times}10^9$ CFU/cm^3, which was exposed to the investigated solution of a certain dilution at exposures of 10 and 30 min without protein and in the presence of protein (inactivated cattle blood serum). The results of the study of bactericidal activity of the drugs are presented in Tables 1 and 2.

The received data demonstrate determination of the protein index showed that in the presence of the protein substance, tested solutions 1.4 times reduced their bactericidal activity concerning *E. coli*, and 1.9 times — concerning *S. aureus*. Obtained results give grounds to assume that these preparations can be used for the sanitation of various objects.

Table 1 — Antibacterial action of preparations based on essential oils concerning *E. coli* (strain 1257)

Solutions of preparations (in the ratio)	Bactericidal dilutions, Exposure, min		Average phenolic coefficient	Average protein index
	10	30		
Phenol 1:50	1:98	1:192.8	—	—
Fir+Eucalyptus+Thyme	1:192.8	1:376.5	1.96	
Fir+Eucalyptus+Thyme+Benzalkonium chloride	1:1,033.1	1:3,698.0	14.84	
Fir+Eucalyptus+Thyme+Benzalkonium chloride+Silver nanoparticles	1:3,968.6	1:5,566.0	34.68	
Fir+Eucalyptus+Thyme+Benzalkonium chloride+ Silver nanoparticles+protein	1:2,834.7	1:3,968.6	24.06	1.4

Table 2 — Antibacterial action of preparations based on essential oils concerning *S. aureus* (strain P-209)

Solutions of preparations (in the ratio)	Bactericidal dilutions, Exposure, min		Average phenolic coefficient	Average protein index
	10	30		
Phenol 1:50	1:98	1:192.8	—	—
Fir+Eucalyptus+Thyme	1:98	1:137.2	0.9	
Fir+Eucalyptus+Thyme+Benzalkonium chloride	1:737.9	1:2,024.8	9.0	
Fir+Eucalyptus+Thyme+Benzalkonium chloride+Silver nanoparticles	1:2,834.7	1:3,968.6	24.06	
Fir+Eucalyptus+Thyme+Benzalkonium chloride+ Silver nanoparticles+protein	1:1,464.3	1:2,024.8	12.7	1.9

The mechanism of action of low doses of silver nanoparticles and essential oils on microorganisms consists in reducing the permeability of cytoplasmic membranes, reducing the intensity of metabolism and the activity of aerobic respiration of microorganisms, destruction of cytoplasmic membranes caused by bactericidal doses of essential oils.

Conclusions. The tested drug has high bactericidal activity concerning *S. aureus*, which 12.7 times exceeds the activity of phenol, and 24 times — in relation to *E. coli*.

The bactericide activity 1.9 times decreases growth of Gram-positive microflora and 1.4 times — Gram-negative flora in the surface protein contamination conditions.

The tested solutions of essential oils have a wide spectrum of antimicrobial action and can be used for disinfection in cases of animal diseases, when resistance of pathogens is assimilated to *E. coli*, and in cases when resistance of pathogens can be assimilated to *S. aureus* resistance.

Microorganisms, upon prolonged contact with silver nanoparticles and essential oils, practically do not produce resistance to them. That is their essential advantage over antibiotics, and is useful for their practical application in human and veterinary medicine.

References

Bergonzelli, G. E., Donnicola, D., Porta, N. and Corthésy-Theulaz, I. E. (2003) ‘Essential oils as components of a diet-based approach to management of *Helicobacter* infection’, *Antimicrobial Agent and Chemotherapy*, 47(10), pp. 3240–3246. doi: 10.1128/AAC.47.10.3240-3246.2003.

Borodina, A. V. (2004) ‘Comparative analysis of the antimicrobial activity of essential oils’ [Sravnitel’nyy analiz antimikrobnoy aktivnosti efirnykh masel], *Archives of Clinical and Experimental Medicine [Arkhiv klinicheskoy i eksperimental’noy meditsiny]*, 13(1–2), pp. 65–67. [in Russian].

Kovalenko, V. L. (ed.) (2014) *Methods for control of disinfectants: a handbook [Metody kontroliu dezinfikuiuchykh zasobiv: dovidnyk]*. Kyiv: VSP ‘IPO KNUBA’.

Nikolaevskiy, V. V. (2000) *Aromatherapy: a handbook [Aromaterapiya: spravochnik]*. Moscow: Meditsina. ISBN: 5225045413. [in Russian].

Obrazhei, A. F., Kvachov, V. G., Ayshpur, O. E., Sapeiko, V. P. and Primatchenko, Y. N. (2008) ‘Essential oils as an alternative to antibiotics in respiratory infections treatment and prophylaxis in pigs’, *Veterinary Biotechnology [Veterynarna biotekhnolohiia]*, 12, pp. 147–150.

Sartoratto, A., Machado, A. L. M., Delarmelina, C., Figueira, G. M., Duarte, M. C. T. and Rehder, V. L. G. (2004) ‘Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil’, *Brazilian Journal of Microbiology*, 35(3), pp. 275–280. doi: 10.1590/S1517-83822004000300001.