EFFECT OF SILVER NANOPARTICLES ON THE PHYSICAL AND CHEMICAL PROPERTIES OF PLANT OILS AND THEIR ANTIMICROBIAL ACTIVITY

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The aim of our research was to investigate the influence of silver nanoparticles on the physical and chemical features of plant oils of dogrose, flax, cedar, amaranth and watermelon and their antimicrobial activity. Plant oils were saturated with silver nanoparticles using electron-beam technology for depositing a molecular stream of metal in a vacuum. To characterize the rancidity of plant oils, the acid, iodine, peroxide, ester and saponification values were determined. A sharp drop in the iodine number and an increase in the peroxide number in oils saturated with silver nanoparticles were observed, as compared to pure oils, indicating a decrease in the number of unsaturated bonds in fatty acids and the formation of peroxides in oils. All pure plant oils and a separate sample of silver nanoparticles suppressed the growth of only \( E. \text{faecalis} \) colonies. Plant oils that were saturated with silver nanoparticles delayed the growth of \( S. \text{aureus}, S. \text{epidermidis}, E. \text{faecalis}, E. \text{coli}, P. \text{aeruginosa}, \) and \( C. \text{albicans} \); the greatest delay in the growth of colonies was caused by flaxseed oil.

Thus, the physical and chemical features of the plant oils under study essentially changed after they are saturated with silver nanoparticles. It can be assumed that the metal acted as a catalyst for peroxide oxidation of lipids in the investigated plant oil samples, the products of which caused toxic effects on cultures of bacteria and fungi in the experiment.

Key words: plant oils of dogrose, flax, cedar, amaranth, watermelon, nanosilver, physical and chemical features, antimicrobial activity.

Nanotechnology has got a tremendous impact on the development of medicine and the pharmaceutical industry. Nano-sized carriers can improve the therapeutic effect, biocompatibility and activity of remedies [1]. Due to the increased resistance of pathogenic microorganisms to antibiotics, many researchers have tried to develop new effective antimicrobial drugs using nanoparticles [2].

Infectious diseases continue to be one of the biggest health problems worldwide, affecting millions of people annually, despite the rapid advances in the development of new medicines [3]. The intensity of the manifestation of antimicrobial properties depends on the technology of particle synthesis, its size, the chemical nature of the coating, the stability of the systems obtained, the type of microorganism, etc. [4].

Among inorganic antibacterial agents, silver is widely used. The development of the resistance of pathogenic microorganisms to this metal has not been found, which is characteristic of antibiotics and chemotherapeutic agents, since metal exhibits various inhibitory effects on pathogenic bacteria, which means they will have to develop a multitude of mutations at the same time to protect themselves. Thus, silver ions are used as antibacterial components in complex pharmaceutical preparations and in coatings of medical devices [5–8]. Nanosilver intensively studied around the world, however, many
important questions regarding these nanoparticles remains open [9, 10].

One of such problematic issues is the search for ways to improve the quality of a number of existing drugs in order to enhance their pharmacological action. It is known that in the process of processing and storage of fats, the deterioration of their quality as a result of oxidative processes, the depth and speed of which depend on the natural properties of fat, temperature, the presence of oxygen and light, is possible. These factors can cause peroxide oxidation of fats [11].

The process of auto-oxidation of fats is significantly accelerated in the presence of moisture, light and catalysts. Such catalysts can be easily oxidized metals. The catalytic action of metals is related to their ability to easily attach or give electrons, which leads to the formation of free radicals from hydroperoxides of fixed acids. Peroxides and hydroperoxides are unstable compounds, and their decomposition occurs with the formation of free radicals. In this case, there are further diverse reactions that result in the accumulation of secondary products: oxides, aldehydes, ketones, low molecular weight acids and others [11]. Consequently, silver nanoparticles in the studied samples of plant oils can cause the formation of peroxides and hydroperoxides, which have a toxic effect on pathogenic microorganisms.

The aim of our study was to investigate the effect of silver nanoparticles on the chemical properties of plant oils and their antimicrobial activity. In this message, we analyze our experimental data on the chemical indicators of the quality of plant oils after saturation with silver nanoparticles; antibacterial and antifungal effects of plant oils saturated with silver nanoparticles to determine the possibility of potentiation of antibacterial action of studied substances and assess the prospects of their use as antiseptics.

Materials and Methods

For research we have selected plant oils of dogrose, flax, cedar, amaranth and watermelon made by the method of the first cold expression in the Scientific and Production Company “Elitphito”, Ivano-Frankivsk [12]. The specified oils are saturated with silver nanoparticles of a certain size with a given concentration of metal and particle size using electron-beam technology for deposition of a molecular stream of metal in a vacuum at a laboratory installation UE-142 [13].

The following chemical parameters were determined to characterize the exhaustion of plant oils: acid value, iodine value, peroxide value, saponification value and ester value using classical methods [14].

Screening of antimicrobial activity of plant extracts was carried out using a micromethod of diffusion in agar. It is characterized by high sensitivity and discriminatory ability, which allows to reliably differentiate active extracts from inactive ones [15]. In petrie dishes, located on a strictly horizontal and even surface, was poured 30 ml of agar. After solidification of growth-supporting microenvironment wells with diameter 4.0 mm were made. The agar was uniformly seeded with a suspension of test culture (concentration 1×10^7 CFU/ml). In the experimental wells, 20 ml of test samples of vegetable oils were introduced. After culturing for 24 h, the diameters of the growth inhibition zones of microbial cultures were determined. Digital images of bacterial inoculation on petrie dish, were processed using the computer program Image Tool 2.0 (UTHSCSA Image Tool 2.0, The University of Texas Health Science Center in San Antonio, © 1995-1996) [15].

Results and Discussion

Dogrose (Rosa canina L.) is widely known as a valuable source of polyphenols and vitamin C. Fruits have anti-inflammatory, antioxidant and anti-obesity. The biological activity of fruits is manifested through a wide range of biologically active compounds, including galactolipids, vitamin C, phenolic compounds, lycopene, lutein, zeaxanthin, and others. Oil of rose fruits has antimicrobial effect, stimulates regeneration of the skin and mucous membranes [12]. Different drugs from dogrose fruits were demonstrated antioxidant and anti-inflammatory effects. The lipophilic components take part in these mechanisms [16, 17]. The anti-inflammatory activity of three basic biologically active compounds of the dogrose: galactolipid, linoleic acid and α-linolenic acid was established [18]. Carotenoids of dogrose fruits inhibit harmful photochemical reactions, particularly oxidation, and thus exhibit antioxidant activity that is directly related to their structure.

The widespread use of amaranth oil for health of people due to the unique composition, the presence of amino acids, microelements, minerals, vitamins, proteins, fixed acids, choline, bile acids, steroid [19]. Amaranth oil
contains 6–8% squalene, most important component. It is known that squalene is the source of oxygen needed for metabolism, and it helps to normalize processes of tissue respiration, has antimicrobial, anticarcinogenic and fungicidal properties [20]. In particular, an increase in the antimicrobial action of pessaries with fluconazole was observed after introducing an amaranth oil preparation [21].

It is recommended to use cedar oil as a source of essential polyunsaturated fixed acids, phospholipids, fat-soluble vitamins for healthy people, with increased physical and mental stress, to increase natural immunological protection, especially during epidemics, acute respiratory viral diseases [12]. It also has a regenerating and bactericidal property, which is important in the treatment of skin diseases, ulcers and erosive processes [22].

Oil flax contains polyunsaturated acids (linoleic, linolenic), saturated fixed acids and vitamins F, A, E, B, K, so it is effectively used for the treatment of allergic skin diseases, lower blood cholesterol levels [12].

Oil of watermelon has a wide range of therapeutic and preventive action, due to the high content of zinc and selenium, tocopherol, fat, fixed acids, vitamins A, C, E, PP. It dissolves and washes out mucus, as well as removes inflammatory processes in the urinary system; promotes rapid healing of any skin blemishes, wounds and burns [12].

Using electron-beam technology for depositing a molecular stream of metal in a vacuum on a laboratory installation UE-142 was obtained colloid with nanoparticles of silver metal with a given concentration and particle size. The average particle size range and distribution of the particles were determined using the method of laser correlation spectroscopy (LCS) [13]. The analysis results are shown in Table 1.

Due to the small size nanoparticles can penetrate directly through the skin, respiratory system, digestive system, cell membranes and pores distributed throughout the body [23].

To characterize the rancidity of plant oils, we use the method of determining free fixed acids by acid value and bound fixed acids by the ester value [11, 14].

The acid value is an important indicator of the quality of oil, which characterizes its suitability for eating. The lower it is, the higher nutritional value of the oil. Increased acid value indicates poor quality of raw materials, oil deterioration during prolonged storage. Fresh fats have almost neutral pH levels [11, 24].

We have found that all the oils with silver nanoparticles increase the acid value (Fig. 1). After saturation of silver nanoparticles almost 2 times increase acid value in oils of dogrose, flax and watermelon, 2,5 times — in amaranth oil. In cedar oil acid number increases by 35%, due to a high content of vitamin E, which exhibits antioxidant properties.

Iodine value is the most important chemical indicator of fats and plant oils, which characterizes the degree of unsaturation of organic substances. Iodine value is expressed in

Fig. 1. Acid value of pure oils and oils with silver nanoparticles

Hereinafter: * — $P < 0.05$ compared with control (the corresponding oil without Ag nanoparticles)
the number of grams of iodine which can join for unsaturated bonds into the substance weighing 100 g. The higher iodine value shows the greater degree of unsaturation compounds in the substance [11, 24].

After saturation of plant oils with silver nanoparticles iodine value of all oils decreased by 11–26% (Fig. 2). Reducing the iodine value indicates a decrease the amount of unsaturated bonds in fatty acids, which was caused by the influence of silver nanoparticles.

The characteristic of oxidative rancidity of fat is carried out by the definition of peroxide number, which is expressed as a percentage of iodine spent on the destruction of peroxides [11].

The primary oxidation products are peroxides that activate the oxidation of other molecules. Due to this, the oxidation reaction is chain-shaped. First, unsaturated fatty acids are oxidized, but saturated acids can also be oxidized to form hydroperoxides. Deep oxidation of fats may result in the formation of cyclic peroxides and epoxy compounds.

The content of peroxide compounds in fat is estimated by the value of the peroxide value. This is a very sensitive indicator, and its value is concluded about the beginning and depth of oxidation of fat. There is no peroxide in fresh fat. In the initial stages of oxidation for some time, the chemical and organoleptic parameters of fat are almost unchanged. This period, which has different durations, is called induction. After the induction period, the fat begins to deteriorate. It can be detected by increasing the peroxide value and changing the organoleptic properties of fat [11].

Peroxide value of dogrose oil is not established, since the carotenoids of the oil masked the color, which had to change at the point of equivalence in the titration. In establishing the peroxide value of the studied substances, we found that in linseed oil is the highest index of peroxide value (5.24), which, after saturation of oil by silver nanoparticles, increases 2.2 times (Fig. 3). In cedar oil and watermelon oil this indicator was increased by 26% and 65% respectively. In amaranth oil after saturation with silver nanoparticles, the peroxide value decreased by 63%, due to the high content of squalene, which probably slows down lipid peroxide oxidation processes. In pure dogrose oil, the determination of the peroxide value was impossible.

<table>
<thead>
<tr>
<th>№</th>
<th>The name of the sample</th>
<th>Particle size, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oil of dogrose + Ag *</td>
<td>458</td>
</tr>
<tr>
<td>2</td>
<td>Oil of flax + Ag</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>Oil of cedar + Ag</td>
<td>59</td>
</tr>
<tr>
<td>4</td>
<td>Oil of amaranth + Ag</td>
<td>58</td>
</tr>
<tr>
<td>5</td>
<td>Oil of watermelon + Ag</td>
<td>68</td>
</tr>
</tbody>
</table>

Table 1. The average particle size of silver nanoparticles in colloids with plant oils

Note: * submicron particles.

Fig. 2. Iodine value of pure oils and oils with silver nanoparticles
Saponification value describes the total acid (free and bound in glycerides), comprising the fat. Saponification is alkaline hydrolysis of fats, resulting in the formation of glycerol and fatty acid salts — soaps [11]. After saturation of the plant oils with silver nanoparticles, saponification value increased in all samples. Least increased saponification value of flaxseed oil (4%), the largest — in dogrose oil — 39% (Fig. 4).

Ester value is an indicator characterizing the content of complex esters (esters) in the oil. The ester value is the number of milligrams of potassium hydroxide, which is necessary for saponification of all esters contained in 1 gram of fat. The ester value is the difference between the saponification value and the acid value [11]. In all samples of plant oils saturated with silver nanoparticles, this figure increased (Fig. 5).

Thus, it has been found that silver nanoparticles substantially change the chemical indices of the studied oils. The most significant increase was observed in the acid value of amaranth oil saturated with silver nanoparticles (2.5 times), and the acid value in oils of dogrose, flaxseed and watermelon (Fig. 1) increased by almost 2 times. Silver nanoparticles caused a decrease in iodine values in most of the oils by 11–26%, but increased by 25% in the oil of watermelon (Fig. 2). The peroxide value of the oils saturated with nanoparticles most significantly increased in linseed oil (2.2 times) while in the amaranth oil it decreased by 63% (Fig. 3). The saponification value increased in all samples of plant oils with silver nanoparticles (Fig. 4).

Investigation of antimicrobial activity of pure and saturated with silver nanoparticles...
plant oils was performed on clinical isolates of antibiotic-sensitive and antibiotic-resistant microorganisms. Bacterial cultures were identified by biochemical microtests “STAPHYtest 16”, “ENTEROtest 24”, “NEFERMENTtest 24” (Lachema, Czech Republic), as well as taking into account the complex of morphological and cultural properties, in accordance with the recommendations of the 9th edition of the “Bergey’s Manual of determinative bacteriology”. Yeast-fungal cultures were identified on the basis of 40 biochemical tests using the VITEK 2 system (VITEK 2 YST ID card, biomerieux, France) [15].

The results showed (Table 2, Fig. 6) that all samples of pure plant oils and oils saturated with silver nanoparticles inhibited growth only *E. faecalis* colonies. Plant oils, which were saturated with silver nanoparticles showed a much broader spectrum of antibacterial activity, inhibiting the growth of most bacterial cultures, including *S. aureus*, *S. epidermidis*, *E. faecalis*, *E. coli*, *P. aeruginosa* and *C. albicans*. The oils of flax, cedar and watermelon saturated with silver nanoparticles showed antibacterial activity against *S. aureus*, *S. epidermidis*, *E. faecalis*, *E. coli*, *P. aeruginosa*. Fungicidal activity against *C. albicans* was established only for dogrose oil with silver nanoparticles. The largest growth inhibition of *S. epidermidis*, *E. coli* and *P. aeruginosa* colony growth was caused by linseed oil, saturated with silver nanoparticles. Diameters of *E. faecalis* growth inhibition zones for all samples of fatty oils, saturated silver nanoparticles were approximately 30% higher compared to the results of the corresponding pure oils.

It is believed that the mechanism of fungicidal action of silver nanoparticles is similar to the interaction of ionic silver with the cell wall of fungi, in particular *C. albicans*. It consists in irreversible binding to the thiol groups of the cysteine residue in the phosphomannose isomerisation, which blocks the synthesis cell wall followed the loss of irreplaceable nutrients and death [8]. The antifungal effect of silver nanoparticles is associated not only with violations of cell wall permeability due to the interaction of nanosilver with thiol groups of enzymes [25] but also with the induction of apoptosis by an increased number of hydroxyl radicals, whose production is enhanced during exposition of *C. albicans* fungi with silver nanoparticles [26]. The leading role of surface contacts in the implementation of silver nanoparticles antibacterial activity is confirmed by the fact that the separation of the bacterial cell by membrane not permeable for such particles reduced significantly specified action [27].

Antibacterial action of nanosilver depends not only on the size of the particles, but also on the type of microorganism under investigation [28–30], which is confirmed by our research results. Previously, it was found that gram-positive bacteria were more susceptible to nanosilver. It is also noted that *P. aeruginosa* has a high sensitivity to nanosilver due to its properties in enhanced contact with silver nanoparticles [27].

The differences in the degree of sensitivity to nanosilver of gram-negative and gram-positive microflora are explained by specific
Table 2. Antimicrobial and antifungal activities of the studied plant oils

<table>
<thead>
<tr>
<th>Oil samples</th>
<th>Test-cultures, (growth inhibition zones, mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus MSSA</td>
</tr>
<tr>
<td>Amaranth oil</td>
<td>0</td>
</tr>
<tr>
<td>Flax oil</td>
<td>0</td>
</tr>
<tr>
<td>Watermelon oil</td>
<td>0</td>
</tr>
<tr>
<td>Dogrose oil</td>
<td>0</td>
</tr>
<tr>
<td>Cedar oil</td>
<td>0</td>
</tr>
<tr>
<td>Amaranth oil+Ag</td>
<td>0</td>
</tr>
<tr>
<td>Flax oil+Ag</td>
<td>4.69 ± 0.56</td>
</tr>
<tr>
<td>Dogrose oil+Ag</td>
<td>0</td>
</tr>
<tr>
<td>Cedar oil+Ag</td>
<td>5.02 ± 0.51</td>
</tr>
<tr>
<td>Watermelon oil+Ag</td>
<td>0</td>
</tr>
<tr>
<td>Ag</td>
<td>0</td>
</tr>
</tbody>
</table>

* — P < 0.05 compared with control (Ag nanoparticles alone); † — compared with appropriate sample of oil without Ag nanoparticles.

Note: MSSA — Methicillin-susceptible S. aureus; MRSA — Methicillin-resistant S. aureus; Ag — silver nanoparticles.

Fig. 6. Growth inhibition zones of microbial cultures under the influence of investigated substances. The numbers indicate the test substance from 1 to 11, which was added to each of 8 Petri dishes with different microorganisms: 1 — amaranth oil, 2 — watermelon oil, 3 — cedar oil, 4 — flax oil, 5 — dogrose oil, 6 — amaranth oil+Ag, 7 — flax oil+Ag, 8 — dogrose oil+Ag, 9 — watermelon oil+Ag, 10 — cedar oil+Ag, 11 — Ag nanoparticles.

In our microbiological studies, we used 8 microorganisms cultivated on 8 petri dishes. 11 samples of pure fatty oils, fatty oils with nanoparticles and silver nanoparticles for comparison were added to each dish. In fig. 6 shows 8 petri dishes, the last, ninth, dish shows the order of application of the test substances from 1 to 11 in each dish. Accordingly, each room (wells) in the last dish is identical in each of the 8 experimental dishes. In each of 8 dishes, 11 samples. In the 9th dish is simply the numbering of the samples. The numbers are decoded with text.
features of cell wall structure [31]. Gram-negative bacteria have a more subtle cell wall that includes the bimolecular layer of peptidoglycan and does not contain teichoic acid. Due to the presence of phospholipid bilayer, polysaccharides and lipopolysaccharide-protein complex in the outer membrane, and enzymes (ribonuclease, phosphatase, penicillinase, etc.) in periplasm Gram-negative bacteria are vulnerable targets for silver. At the same time, gram-positive bacteria, in particular S. aureus, have a simply organized but more powerful cell wall, consisting of multiple layers of peptidoglycan incorporated with unique teichoic acid polymers, which serves as the main framework of a microbial cell. Enzymes containing thiol groups are located on a cytoplasmic membrane, beneath a powerful layer of peptidoglycan (murein). Therefore, inactivation of sulfhydryl groups by silver ions or clusters is weaker and “stretched” over the time in comparison with their effect on gram-negative bacteria [32].

Stabilized by polymers of silver nanoparticles loaded with biologically active substances of plant origin have recently been intensively studied. The high antimicrobial activity of PEG@Ag-nanoparticles loaded with substances of plant origin have recently been intensively studied. The high antimicrobial activity of PEG@Ag-nanoparticles loaded with the water extract of leaves of Thuja occidentalis L. [33] and Tribulus terrestris L. [34] was demonstrated. Similar nanoparticles with Eucalyptus globulus Labill extract. [35], in addition to the harmful effect on planktonic forms of bacteria, inhibit their ability to biofilm formation. Our studies have shown that the combination of silver nanoparticles with plant oils results in antimicrobial effect on microorganisms that were less susceptible to these components, applied separately.

Thus, our results indicate that the chemical parameters of the studied plant oils change significantly after their saturation with silver nanoparticles, which is reflected in their antimicrobial and antifungal properties. It can be assumed that the metal was a catalyst for lipid peroxidation in the studied samples of plant oils, followed to the formation of peroxides and hydroperoxides, which caused a toxic effect on bacteria and fungi cultures of performed experiments.

The results of our studies offer new opportunities for expanding the range of active substances and excipients to create new antimicrobial drugs based on plant oils of dogrose, flax, cedar, amaranth and watermelon saturated with silver nanoparticles.

REFERENCES

Experimental articles


ВПЛИВ НАНОЧАСТИК СРІБЛА НА ФІЗИКО-ХІМИЧНІ ВЛАСТИВОСТІ РОСЛИННИХ ОЛІЙ ТА ЇХНЮ АНТИМІКРОБНУ АКТИВНІСТЬ

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Метою роботи було дослідити вплив наночастинок срібла на фізико-хімічні властивості рослинних олій шипшини, льону, амаранту і кавуна та їх антимікробну активність. Рослинні олії насичували наночастинками срібла за допомогою електронно-променевої технології осадження молекулярного потоку металу в вакуумі. Для характеристики згіркнення рослинних олій визначали кислотне, йодне, пероксидне, ефірне число та число омилення. Спостерігали різке зниження йодного і збільшення пероксидного числа в оліях, насичених наночастинками срібла, порівняно з чистими оліями, що свідчить про зменшення кількості ненасичених зв'язків у жирних кислотах та про утворення пероксидів. Усі чисті рослинні олії та окремий образець наночастинок срібла пригнічували рост колоній E. faecalis. Рослинні олії, насичені наночастинками срібла, затримували рост S. aureus, S. epidermidis, E. faecalis, E. coli, P. aeruginosa та C. albicans; найбільшу задержку росту колоній вызывало льняное масло.

Таким образом, фізико-хімічні показателі досліджуваних рослинних олій суттєво змінюються після насичення їх наночастинками срібла. Можно припустити, що метал виступав як катализатор пероксидного окиснення ліпідів у досліджуваних зразках рослинних олій, продукти якого зумовили токсичний вплив на культури бактерій та грибів.

ВЛИЯНИЕ НАНОЧАСТИЦ СЕРЕБРА НА ФИЗИКО-ХИМИЧЕСКИЕ СВОЙСТВА РАСТИТЕЛЬНЫХ МАСЕЛ И ИХ АНТИМИКРОБНУЮ АКТИВНОСТЬ

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Целью работы было исследовать влияние наночастиц серебра на физико-химические свойства растительных масел шиповника, льна, кедра, амаранта и арбуза и их антимикробную активность. Растительные масла насыщали наночастицами серебра с помощью электронно-лучевой технологии осаждения молекулярного потока металла в вакууме. Для характеристики прогоркания растительных масел определяли кислотное, йодное, пероксидное, эфирное число и число омыления. Находили резкое снижение йодного и увеличение пероксидного числа в маслах, насыщенных наночастицами серебра, по сравнению с чистыми маслами, что свидетельствует об уменьшении количества ненасыщенных связей в жирных кислотах и об образовании пероксидов.

Все чистые растительные масла и отдельный образец наночастиц серебра подавляли рост только колоний E. faecalis. Растительные масла, насыщенные наночастицами серебра, угнетали рост S. aureus, S. epidermidis, E. faecalis, E. coli, P. aeruginosa и C. albicans; наибольшую задержку роста колоний вызывало льняное масло.

Таким образом, физико-химические показатели исследуемых растительных масел существенно изменяются после насыщения их наночастицами серебра. Можно предположить, что металл выступает как катализатор пероксидного окисления липидов в исследуемых образцах растительных масел, продукты которого обусловили токсическое воздействие на культуры бактерий и грибов.

Ключевые слова: растительные масла шиповника, льна, кедра, амаранта, арбуза, наносеребро, физико-химические свойства, антимикробная активность.