

Research On the Study of The Specific Activity of a Medicinal Substance with Gold Nanoparticles

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ABSTRACT

The paper presents the results of a study on the specific activity of the medicinal substance "Dry extract of *Scutellaria Iscandera* L. herb with gold nanoparticles". The ability of gold nanoparticles to act both as a component generating active forms of oxygen and to inhibit the oxidation process by absorbing free radicals can be used to create new drugs that combine antioxidant potential and antibacterial efficacy. The results of the studies showed that the diameter of growth inhibition of *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* was 33-38 mm and 30-34 mm, respectively. The antimicrobial activity of the studied samples showed a high rating against *Escherichia coli* - where the diameter of the inhibition zone was 24-28 mm, *Bacillus subtilis* - 20-25 mm, *Bacillus pumilus* 21-25 mm and *Candida albicans* was 32-37 mm, *Staphylococcus aureus* 20-25 mm.

Keyword: specific activity, gold nanoparticles, dry extract, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, *Staphylococcus epidermidis*, *Staphylococcus aureus*.

1. INTRODUCTION

Gold has been used and highly valued by mankind for over 7 thousand years due to its unusual properties. References to various uses of gold were found in ancient China, Egypt and India. The Egyptians believed that gold not only protected against spells and magic, but also had life-giving properties. In India, it was used for rejuvenation and revitalization in old age.[1]

Gold preparations were used to treat melancholy, fainting, epilepsy, nervous excitement, scrofula and syphilis. However, the use of gold in these diseases was based on purely empirical assumptions about its medicinal properties. A new impetus to the use of gold preparations in medicine was given by the research of the German bacteriologist R. Koch, who in 1890 established the bacteriostatic effect of gold cyanide in vitro in relation to the tuberculosis bacillus.[2]

In the last decade, compared to the previous ones, more intensive use of gold in medicine has been observed. This is due, first of all, to the rapid development of nanotechnology in general and nanomedicine in particular. Nanotechnology is a science that studies the production, processing and use of substances and materials in the size range from 1 to 100 nm.[5]

The rapid development of microorganism resistance to modern antibacterial drugs requires the search for new, alternative methods of therapy. It is known that some organisms, such as plants, algae, and fungi, are capable of converting inorganic metal ions into metal nanoparticles through a reduction process carried out by proteins, sugars and metabolites contained in the tissues and cells of these organisms.[3]

Thus, metal nanoparticles obtained by the method of "green" synthesis from extracts of such plants can become an alternative to many modern antibacterial drugs that currently exist. The antibacterial mechanism of action of nanoparticles depends on the type of microorganisms that are affected, as well as on the type of nanoparticles, their concentration, size, and the method of their production.[4,9,10]

It is known from the literature that intestinal bacteria (*E. coli*) feel relatively good on gold, but die on nanostructured metal. Silver exerts its effect by releasing ions, but this explanation does not work in the case of gold. And Japanese researchers have found that the smallest gold nanoballs, only 20 nanometers in diameter, are most effective in the fight against bacteria. [6]

According to some theories, nanoparticles can help inhibit respiratory chain enzymes, thereby separating oxidation and oxidative phosphorylation processes; interact with nucleotides, disrupting DNA stability; interact with cell wall peptidoglycans, blocking the ability to transfer oxygen; or act as a catalyst, promoting the oxidation of protoplasm with

oxygen dissolved in water. [7]

The mechanism of action depends on both the type of microorganisms that are affected and the type of nanoparticles, their concentration, size, and the method of their production. [8]

The aim of the study. To study the specific activity of the medicinal substance "Scutellaria Iscandera L. dry herb extract with gold nanoparticles"

2. MATERIALS AND METHODS

of the study. The object of the study is the medicinal substance "Scutellaria Iscandera L. dry herb extract with gold nanoparticles".

Studies on the specific activity of the medicinal substance "Scutellaria Iscandera L. dry herb extract with gold nanoparticles" were conducted in the microbiological laboratory of the Scientific Center for Standardization of Medicines LLC.

It was determined by the method of diffusion in agar on a dense nutrient medium by comparing the sizes of the zones of inhibition of the growth of test microbes formed when testing solutions of certain concentrations. Sterile Petri dishes of the same diameter with a smooth flat bottom were used for the analysis. 20 ml of a nutrient medium of a certain composition, infected with an 18-20 hour culture of test strains of *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Bacillus pumilus*, were poured into dishes placed on a horizontal table. The corresponding nutrient media were used for the studies.

Preparation of the inoculum: pure daily cultures of microorganisms grown on dense nutrient media were used to prepare the inoculum. Several identical, clearly isolated colonies were selected. A small amount of material from the tops of the colonies was transferred with a loop into a test tube with a sterile 0.9% NaCl solution, bringing the density of the inoculum to exactly 0.5 according to the McFarland standard. The inoculum was used within 15 minutes after preparation.

Analysis: Three solutions of the *Scutellaria Iscandera* L. substance with silver nanoparticles were taken for the test. The concentrations of the solutions containing small, medium and large doses were in a multiple ratio (1:2:4). Wells were made in the center of the solidified agar surface with a glass cylinder. The studied substances were added to the wells in the specified concentrations in seven Petri dishes.

Incubation: The dishes were placed in a thermostat at a temperature of $(36 \pm 1)^{\circ}\text{C}$ for 18-24 hours. After incubation in the thermostat, the zones of inhibition of microorganism growth formed by the solutions of the substance with gold nanoparticles were measured with a microbiological ruler with an accuracy of 1 mm. The microbiological activity was assessed by the size of the zones.

3. RESULTS AND DISCUSSION

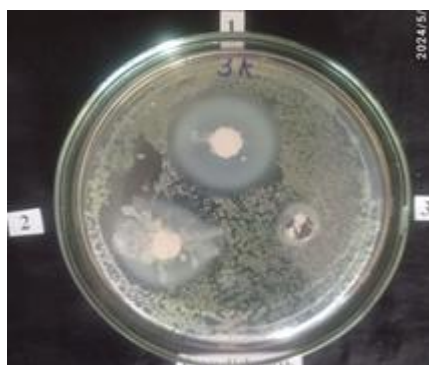
After incubation in a thermostat, the zones of inhibition of microorganism growth formed by solutions of the substance with gold nanoparticles were measured with a microbiological ruler with an accuracy of up to 1 mm. Microbiological activity was assessed by the sizes of the zones. The results of the experiment are given in Table 1.

Table 1 Zones of inhibition of microorganism growth under the influence of a substance with gold nanoparticles

substance with gold nanoparticles Zones of inhibition of microorganism growth, mm				
№	Test strains	1:1	1:2	1:4
1	<i>Pseudomonas aeruginosa</i>	$38,0 \pm 0,5$	$35,6 \pm 0,5$	$33,2 \pm 0,2$
2	<i>Candida albicans</i>	$37,6 \pm 0,5$	$35,2 \pm 0,2$	$32,5 \pm 0,5$
3	<i>Staphylococcus aureus</i>	$25,5 \pm 0,5$	$22,5 \pm 0,3$	$20,5 \pm 0,3$
4	<i>Staphylococcus epidermidis</i>	$34,2 \pm 0,5$	$32,5 \pm 0,2$	$30,0 \pm 0,5$
5	<i>Bacillus subtilis</i>	$25,2 \pm 0,2$	$22,5 \pm 0,5$	$20,2 \pm 0,3$
6	<i>Bacillus pumilus</i>	$25,2 \pm 0,2$	$23,2 \pm 0,2$	$21,2 \pm 0,2$
7	<i>Escherichia coli</i>	$28,5 \pm 0,2$	$26,0 \pm 0,2$	$24,3 \pm 0,2$

The obtained research results showed that the highest growth inhibition diameter of *Candida albicans* at a ratio of 1:1 was 37.6 ± 0.5 mm, *Staphylococcus epidermidis* at a ratio of 1:1 was 34.2 ± 0.5 mm, and when testing *Pseudomonas aeruginosa* it was found that at a ratio of 1:1 the growth inhibition diameter was 38.0 ± 0.5 mm, which refers to the highly sensitive group.

It follows that of all seven microorganisms at a ratio of 1:1 the first is the most rational. A clear picture of the conducted research can be seen in Figure 1.



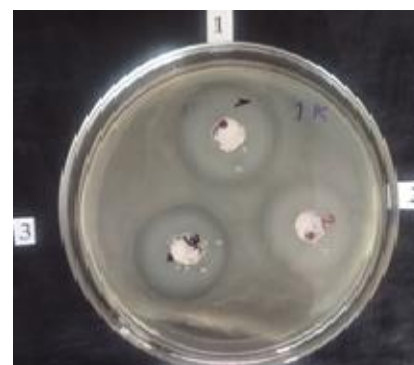
Bacillus subtilis

I₁-1:4, I₂-1:2, I₃-1:1



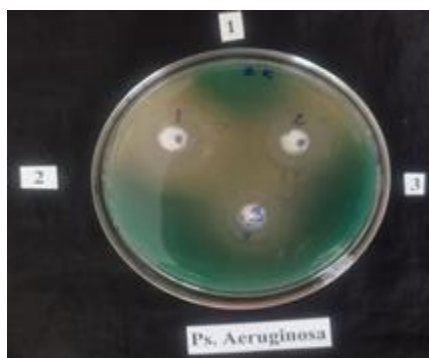
Escherichia coli

I₁-1:4, I₂-1:2, I₃-1:1



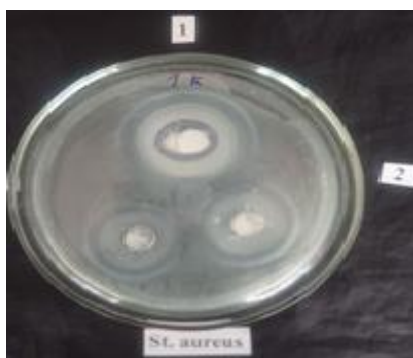
Candida albicans

I₁-1:4, I₂-1:2, I₃-1:1



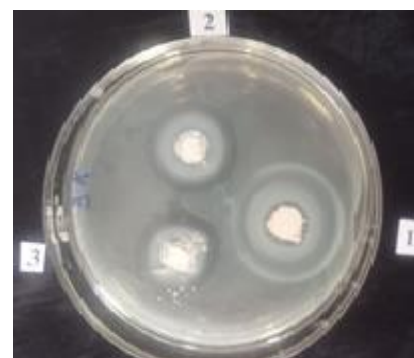
Pseudomonas aeruginosa

I₁-1:4, I₂-1:2, I₃-1:1



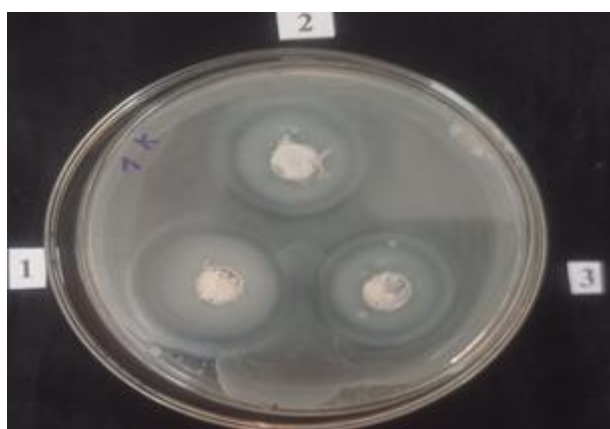
Staphylococcus aureus

I₁-1:4, I₂-1:2, I₃-1:1



Staphylococcus epidermidis

I₁-1:4, I₂-1:2, I₃-1:1



Bacillus pumilus

I₁-1:4, I₂-1:2, I₃-1:1

Figure 1. Results of the zone of inhibition of microorganism growth under the influence of a substance with gold nanoparticles

4. CONCLUSION

It is known that the cell wall of gram-positive bacteria is thicker than that of gram-negative bacteria (from 20 to 80 nm), most of its mass (40–90%) is peptide glycan, while in the cell wall of gram-negative bacteria it is much less (5–10%). Increasing the resistance of microorganisms to known antimicrobial drugs is a pressing problem that requires a non-standard approach to its elimination. The creation of a pharmaceutical drug substance based on nanoparticles may be one of the options for such a solution. It is known from the literature that significant surface binding of gold nanoparticles with subsequent intracellular absorption disrupted the morphology of cells and caused noticeable damage to the cell membrane. The interrupted kinetics of bacterial growth, loss of cellular respiration, increased production of intracellular ROS and leakage of cytoplasmic contents clearly indicated a strong interaction of gold nanoparticles with the outer surface of the cell and intracellular components, which ultimately led to cell death and destruction of biofilms. The obtained pharmacopeial medicinal substance contains both the extract of Iskander's Skullcap (*Scutellaria Iscanderi* L.) and, accordingly, as biologically active substances - the sum of flavonoids, and gold nanoparticles. Based on the data presented in "Table-1" and "Figure-1", antibacterial, antimicrobial and high antifungal activity were proven in several in vitro studies

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