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Technology Of Obtaining and Studying the Process of Green Synthesis of Medicinal Substance with Gold Nanoparticles

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ABSTRACT

*I*n the work, gold nanoparticles were synthesized using the "Green Synthesis" method with the use of Scutellaria Iscandera L. extracts. The results of the study of gold nanoparticles using the methods of SF spectrophotometry, Inductively coupled plasma mass spectrometry (ICP-MS), and atomic force microscopy (AFM) for visualization of the resulting nanoparticles are presented. The antimicrobial activity of gold nanoparticles was also studied using the microbiological method.

Keyword: *nanotechnology, substance, gold nanoparticles, green synthesis, AFM, SF spectrophotometry*

1. INTRODUCTION

Nanoparticles are objects that have isolated, distinct boundaries with the environment, spheroidal shape, 1-100 nm in size. Currently, nanotechnology has begun to be widely used in medicine and is called nanomedicine. (1)

At the moment, scientists are working on the development of nanoparticles, the main task of which will be the fight against various infections. Nanoparticles can also play a protective role for drugs that are otherwise easily broken down in the body.(2)

Over the past decade, it has been shown that many biological systems, including plants and algae, diatoms, bacteria, yeast, fungi, and human cells, can convert inorganic metal ions into metal nanoparticles through a reduction process carried out by proteins and metabolites contained in these organisms.(3)

An effective and safe method for synthesizing metal nanoparticles is the use of plant extracts. Plants contain certain biologically active compounds such as flavonoids, phenols, citric and ascorbic acids, polyphenols, terpenes, alkaloids, and reductases, which act as reducing agents.(9,10) Based on scientific publications and studies, a green synthesis method was developed aimed at obtaining a dry extract of the herb Scutellaria Iscandari L. (Scutellaria Iscandari) containing gold nanoparticles. (4)

The wide practical application of nanoparticles is due to a number of their unique properties. Currently, various physical and chemical processes are used to synthesize metal nanoparticles, allowing to obtain nanoparticles with specified properties. However, despite their widespread use, these are usually expensive, labor-intensive methods associated with risk and potential danger to the environment and living organisms. Thus, there is an obvious need for alternative cost-effective and at the same time safe and environmentally friendly methods of producing nanoparticles.(5)

"Green" synthesis is a method for obtaining metal nanoparticles of various morphologies from salts of the corresponding metals using plant extracts as reducing and stabilizing agents. The method allows to obtain metal nanoparticles from 10 to 500 nm in size of spherical, triangular, pentagonal and hexagonal shapes. The synthesis of nanoparticles requires three key components - a dissolving medium, reducing and stabilizing substances. (6)

Gold is one of the first metals discovered by man, the history of its study and use goes back at least several thousand years. The first information about colloidal gold can be found in the treatises of Chinese, Arab and Indian scientists, who already in the 5th-4th centuries BC obtained colloidal gold and used it, in particular, for medicinal purposes (Chinese "gold solution" - jin ye, chin-tau, Indian "liquid gold" - Kushta Tila Kalan, Makaradhwaja). (7)

Gold nanoparticles have been proposed for use in, in particular, genomics, biosensorics, immunoassay, clinical chemistry, detection and photothermolysis of microorganisms and cancer cells, targeted delivery of drugs, DNA and antigens, optical bioimaging and monitoring of cells and tissues.

Gold nanoparticles have an increased cross-section of absorption and scattering of light, and the nature of their absorption spectrum depends on their size and shape. Thus, gold nanoparticles can be carriers or transporters of various molecules, such as drug molecules, large biomolecules such as proteins, DNA and RNA, and genes. Like other nano-objects, gold nanoparticles have a number of unique properties. In addition to their tiny size and large surface area, they are also characterized by high physical and chemical activity. Depending on their charge and coating, gold nanoparticles can have different affinities for water and, accordingly, be hydrophilic, hydrophobic, and even amphiphilic, which enables them to penetrate various barriers, including biomembranes. High adsorption capacity enhances their catalytic properties.(8)

The aim of this work is to study the process of green synthesis of a medicinal substance with gold nanoparticles..

2. MATERIALS AND METHODS

of research. The herb Scutellaria Iscandaria L., collected in July in the Pap district of the Namangan region of the Republic of Uzbekistan (VFS 42 Uz-15842845-3731-2019), was used as plant raw materials.

The absorption spectrum of plant extracts and solutions of gold nanoparticles was recorded in the wavelength range of 450-1000 nm at 25 ° C on a Perkin Elmer UV-VIS Lambda 35 spectrophotometer (scan speed 480 nm.min, cuvette thickness 10 mm).

Quantitative determination of gold was carried out by mass spectrometry (ICP-MS). About 0.1 g (exactly weighed) of the extract is placed in microwave oven cuvettes and 2.5 ml of concentrated nitric acid and 1.5 ml of hydrochloric acid are added. Then the cuvette is placed in a microwave oven and heated to 100 °C for 10 minutes, then the temperature is increased to 200 °C and the sample is held at this temperature for 10 minutes. After cooling, the contents of the cuvette are diluted to 100 ml with purified water and mixed. The resulting solution is filtered through a paper filter (blue tape) and then measured in an Agilent 7800 ICP-MS chromatograph.

Based on the results of studying the elemental composition of the substance of gold nanoparticles using the ICP-MS method, the amount of gold is calculated for physicochemical and microbiological analysis.

A detailed picture was visualized using a microscopic method - atomic force microscopy (AFM) to visualize the resulting nanoparticles. The procedure for preparing samples for atomic force microscopy involves immobilizing them on a flat substrate. The substrate material can vary widely depending on the tasks at hand. Traditionally, atomically smooth substrates made of mica, graphite and other layered materials, as well as various glasses, polymeric materials and metal surfaces are used as a substrate.

Studies on the antimicrobial activity of medicinal substances with gold nanoparticles were conducted in the microbiological laboratory of the Scientific Center for Standardization of Medicines, LLC. The antimicrobial activity of medicinal substances with gold nanoparticles was determined by diffusion in agar on a dense nutrient medium by comparing the sizes of growth inhibition zones of test microbes formed when testing solutions of certain concentrations. Sterile Petri dishes of the same diameter with a 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 Candida albicans, Pseudomonas aeruginosa, Staphylococcus aureus, 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 Iscanderi L. substance with silver nanoparticles were taken for the test. The concentrations of solutions containing small, medium and large doses were in a multiple ratio (1:2:4). On the solidified surface of the agar, wells were made in the center 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 ± 1) ° C for 18-24 hours. After incubation in the 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 1 mm. Microbiological activity was estimated by the sizes of the zones.

In the development of the technology, modern technological, physical, physicochemical methods were used.

Experimental part. Phytosynthesis of gold nanoparticles is carried out using plant extracts, in which biologically active substances, in particular, flavonoids, act as an agent that reduces gold ions to nanoparticles. The study material used was an extract of the herb Scutellaria iskander and a substance with gold nanoparticles obtained by the green synthesis method using an extract of the herb Scutellaria iskander. (Fig. 1)

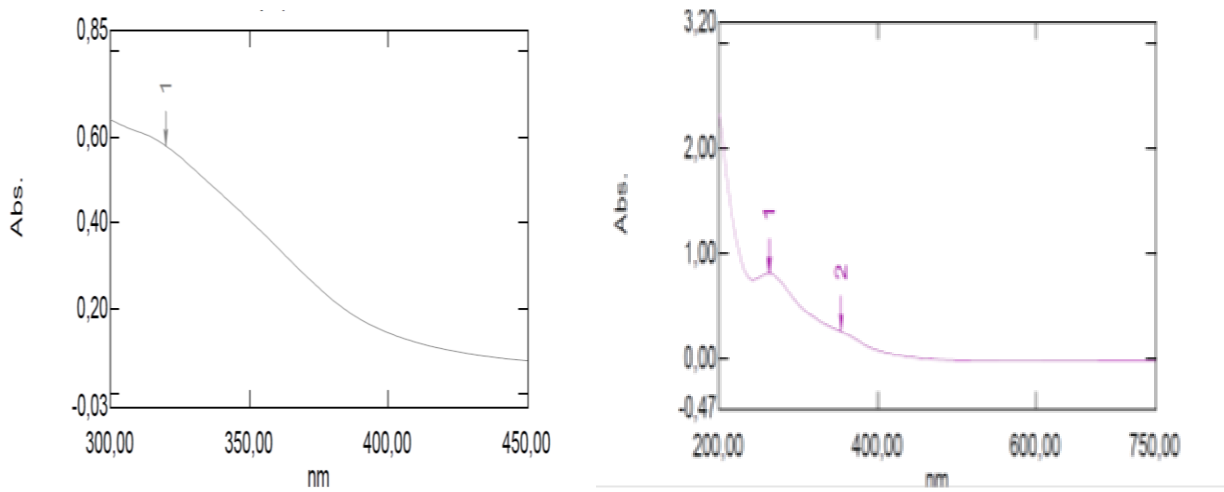
Figure-1. Suspension with gold nanoparticles

It is known from the literature that gold nanoparticles have different colors - from weak shades of pink to intense red, and sometimes - blue and green. The color of the solutions is determined by the size and shape of the nanoparticles.

3. DISCUSSION OF THE RESULTS OBTAINED

UV-VIS spectra of the plant extract showed an absorption peak at 320 nm, indicating the presence of phenolic compounds (Fig. 2.a).

Moreover, the UV-VIS spectra of the finished substance showed a strong absorption peak at a wavelength of about 270 nm, confirming the successful formation of gold nanoparticles using an aqueous extract of Iskander Skullcap. The results are shown in Figure 2.b.



а) б)

Figure 2. UV-VIS spectra of the aqueous extract of Iskander Skullcap (a), UV-VIS spectra of the substance with gold nanoparticles

The next stage of our research was to establish the quantitative content of gold in the obtained substance.

Quantitative determination of gold was carried out by mass spectrometry (ICP-MS) in the Central Laboratory of the State Committee for Geology and Mineral Resources of the Republic of Uzbekistan.

The elemental composition of the substance was quantitatively studied. (Table 1)

Table 1.Quantitative content of the elemental composition of the substance

№	Metal standards	Results
1	Li (Lithium)	Not detected
2	Na (Sodium)	1,11 mg/kg
3	K (Potassium)	6,3 g/kg
4	Mg (Magnesium)	7,34 g/kg
5	Ca (Calcium)	25,0 mg/kg
6	Se (Selenium)	0,176 mg/kg
7	Zn (Zinc)	45,6 mg/kg
8	Cu (Copper)	0,14 mg/kg
9	Cd (Cadmium)	Not detected
10	Ni (Nickel)	Not detected
11	As (Arsenic)	Not detected
12	Pb (Lead)	Not detected
13	Cr (Chromium)	Not detected
14	Fe (Iron)	1,253 g/kg
15	Co (Cobalt)	0,094 mg/kg
16	Sn (Tin)	Not detected
17	Au (Gold)	0,131 mg/g
18	Ag (Silver)	0,054 mg/kg

Based on the results of studying the elemental composition of the dry substance with gold nanoparticles by the ICP-MS method, it is possible to calculate gold for conducting physicochemical and microbiological analyses.

From the data in the table it is clear that the quantitative content of gold is 0.131 mg/g.

For further study of our substance with gold nanoparticles, it was advisable to examine the shape and size of the formed nanoparticles.

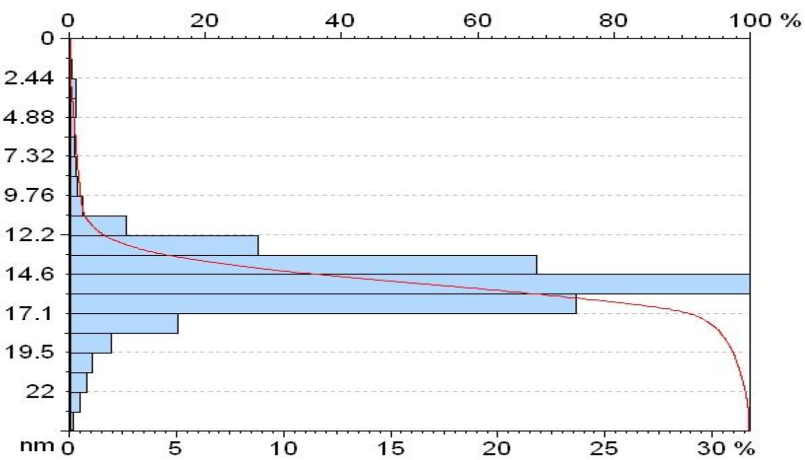


Fig.3- Microscopic study of gold nanoparticles obtained using Scutellaria Iscanderi L. extract using atomic force microscopy

Thus, we took microscopic images of the substance with gold nanoparticles using atomic force microscopy. The images obtained are presented in Fig. 3.

In particular, the sizes of gold nanoparticles obtained using extracts of medicinal plants Scutellaria Iscanderi L. were 12.2 nm (28% of the total number of particles) with a spread of the main fraction (72%) from 2.44 nm to 22 nm.

Figure 4 shows a 3D image of surface ultrastructures with molecular resolution in real time and physiological conditions.

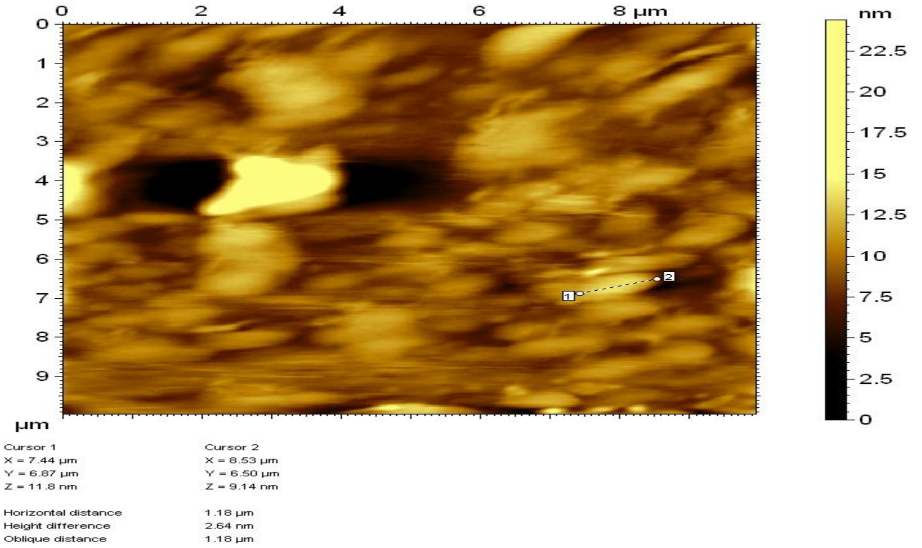


Fig.4. AFM image of metallic gold nanoparticles. Study of shape, length and width of gold nanoparticles

After studying the substance with gold nanoparticles, it was advisable for us to study the antimicrobial activity of the substance.

Studies on the antimicrobial effect of the substance under study were conducted in the microbiological laboratory of the Scientific Center for Standardization of Medicines LLC.

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 size of the zones. The results of the experiment are given in Table 2.

Table 2 Zones of inhibition of microorganism growth under the influence of a substance with gold nanoparticles

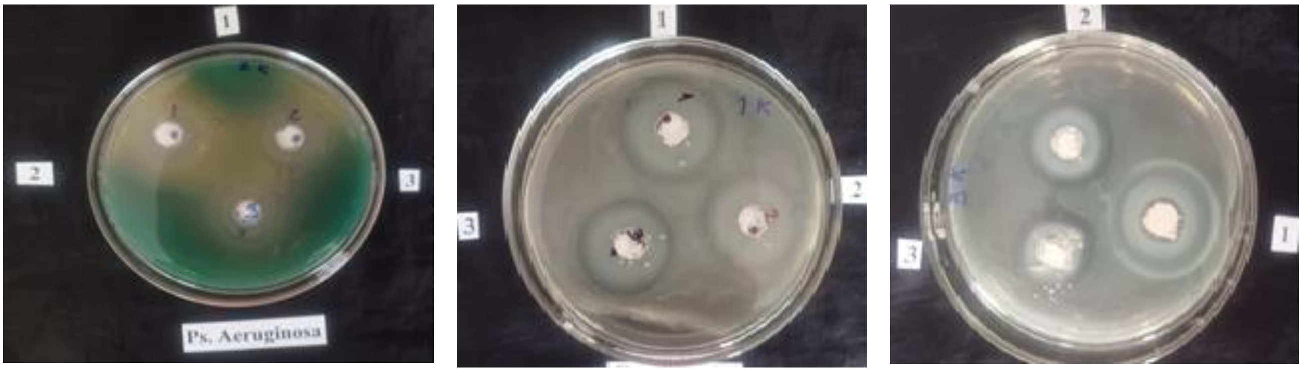
	Zones of inhibition of microorganism, mm			
№	**Test strains**	**1:1**	**1:2**	**1:4**
1	Pseudomonas aeuruginosa	38,0 ± 0,5	35,6 ± 0,5	33,2 ± 0,2
2	Candida albicans	37,6 ± 0,5	35,2 ± 0,2	32,5 ± 0,5
3	Staphylococcus aureus	25,5 ± 0,5	22,5 ± 0,3	20,5 ± 0,3

The results of the conducted studies showed that the highest diameter of inhibition of growth zones of Pseudomonas aeuruginosa at a concentration of 1:4 is 33.2 ± 0.2 mm and belongs to the resistant group. At a concentration of 1:2 it is 35.6 ± 0.5 mm, which is included in the sensitive group, and at a concentration of 1:1 the diameter of inhibition of growth is 38.0 ± 0.5 mm, which belongs to the highly sensitive group.

Also, when checking Candida albicans, it was found that at a concentration of 1:4 the diameter of inhibition of growth is 32.5 ± 0.5 mm, at a concentration of 1:2 it is 35.2 ± 0.2 mm, and at 1:1 the diameter of inhibition of growth was 37.6 ± 0.5 mm, which also belongs to the highly sensitive group. The growth inhibition zone of Staphylococcus aureus microflora for the substance at a concentration of 1:4 was 20.5 ± 0.3 mm, at a concentration of 1:2 it was 22.5 ± 0.3 mm, and at 1:1 the growth inhibition diameter was 25.5 ± 0.5 mm.

It follows from this that, of all three microorganisms, at a substance concentration of 1:1 is the most rational.

The results of the studies can be seen in Figure 5.



Pseudomonas aeruginosa **I_1-1:4, I_2-1:2, I_3-1:1**

Candidaalbicans **I_1-1:4, I_2-1:2, I_3-1:1**

Staphylococcus epidermidis **I_1-1:4, I_2-1:2, I_3-1:1**

Figure 5. Results of the zone of inhibition of microorganism growth under the influence of a substance with gold nanoparticles

4. CONCLUSION

As a result of our research, we obtained gold nanoparticles ranging in size from 2.44 nm to 22 nm.

As a result of the obtained research results, it was established that the highest diameter of inhibition of Candida albicans growth zones at a ratio of 1:1 was 37.6±0.5 mm, Staphylococcus aureus at a ratio of 1:1, the diameter of growth inhibition was 25.5±0.5 mm, also, when checking Pseudomonas aeuruginosa, it was found that at a ratio of 1:1, the diameter of growth inhibition was 38.0 ± 0.5 mm, which refers to the highly sensitive group.

Based on the above studies, the obtained medicinal substance with gold nanoparticles can be used to develop the technology of antimicrobial dosage forms

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