Obtaining and Studying Technology of Dry Substance Silver Nanoparticles Obtained by "Green Synthesis" Method Using Scutellaria Iscandaria L.

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Abstract

Silver nanoparticles were synthesized by the method of "Green Synthesis" using extracts of Scutellaria Iscandaria L. The "Green" pathway for the synthesis of silver nanoparticles has been described using an aqueous extract of the plant Common Skullcap as a reducing and stabilizing agent. A dry powder of silver nanoparticles was obtained from the Synthesized Suspension. The role of Luteolin flavonoid contained in Scutellaria Iscandaria L. extract in the synthesis of silver nanoparticles as a reducing and stabilizing agent is shown, the possible mechanism of nanoparticle formation is given. The synthesized silver nanoparticles have been studied by infrared spectroscopy, mass spectroscopy (ICP-MS), and high-performance liquid chromatography (HPLC). The size and shape of the obtained nanoparticles were characterized by the Atomic Force Microscopy (AFM) method. The antimicrobial activity of dry powder and aqueous suspension with silver nanoparticles was also studied at the Strains Test: St.aureusATCC 25923, B.subtilis ATCC 6633, Candida alb. 885-653.

Keywords: *Silver nanoparticles, extract of Scutellaria Iscandaria L., green synthesis, dry powder, infrared spectroscopy, Atomic Force Microscope (AFM), HPLC, Luteolin, antimicrobial activity, St. aureus ATCC 25923, B.subtilisATCC 6633, Candida alb. 885-653.*

Introduction

Over the last two decades, there has been a significant growth in research on the properties of nanoscale particles and various nanomaterials, which is associated with a wide range of opportunities for their practical application[1]. Applied developments in this direction abroad are being successfully introduced into production by enterprises in the nanoindustry; there is reason to believe [2] that further progress in this direction will make it possible to successfully solve many problems (energy supply, ecology, raising living standards, fighting epidemics and reducing mortality from various diseases, etc.) that arise at the present stage of development of human civilization.

Silver nanoparticles destroy the cell walls of bacteria[4], causing their immediate death from physical destruction rather than from toxin poisoning. Bacteria break down and die, and nanoparticles are able to function further.[5] This way of destroying bacteria leaves them unable to adapt, develop a protective mechanism and pass it on to future generations[3]. The undoubted advantage of nanoparticles over silver ions is that the surface coating of nanoparticles is protective on the one hand and can act as a platform for the transfer of various active substances on the other[6].

Nowadays, with the development of nanotechnologies, interest in silver as an antibacterial and bactericidal agent has increased greatly[7]. Nanotechnology has made it possible to reduce the cost of silver-based drugs and make them more affordable in the treatment of many infectious diseases. Among more than 800 items of consumer nano-goods, the greatest demand in the world is for products containing silver nanoparticles[8].

To date, a new method for obtaining nanoparticles of metals is considered the method of "green synthesis"[9].

The approach based on the method of "Green Synthesis" is non-toxic, environmentally friendly and economically efficient in comparison with other method, as in the method vegetable extracts are used as reducing agents and stabilizers of nanoparticles[10] .

The main advantage of plant-based biological systems[11] for the production of nanoparticles is the biological safety of the process and the possibility of obtaining[12] the necessary volume of production without additional costs[13].

The most frequently used method for obtaining metal nanoparticles in aqueous solutions are those based on the reduction of metal ions in the solution under conditions conducive to the formation of nanoparticles. The method differ mainly in the type of reducer and stabilizer (a substance that helps to preserve nanoparticles in solution in nanosize state). Nanoparticles can be synthesised by chemical reduction - with the use of chemical reducers and stabilisers (pure chemical synthesis) or with the use of current or radiation sources providing reduction by hydrated electron and other reducing particles (photochemical, radiation-chemical and electrochemical synthesis) and biological reduction - with the use of water media containing biological reducers and stabilisers (biological or "green" synthesis). In the latter case, water extracts from various biological objects (green plants, fungi, bacteria, etc.) are used.

To create safe medicines or materials containing silver nanoparticles, it is necessary to obtain aqueous solutions of such nanoparticles, which have high antimicrobial activity and at the same time low enough toxicity for the human body. To solve this problem, over the past 10-15 years, studies have been conducted on the action of the same solution of nanoparticles on bacterial cultures and normal culture cells. Unfortunately, as far as we know, such parallel research is very few, which does not allow us to draw an unambiguous conclusion about the possibility of creating appropriate drugs.

From the literature it is known that the search for new effective means to combat cancer has shown, in particular, that, in addition to antimicrobial activity, silver nanoparticles also have cytotoxicity for various types of malignant cells. Such data have been obtained for aqueous solutions of nanoparticles in studies of their action on the viability and functional activity of various cell cultures [14]. This suggests that solutions of these nanoparticles can be used to create drugs for oncology that are sufficiently effective and at the same time do not cause such serious side effects as those currently used in chemotherapy. But even in this case, it is necessary to obtain solutions of nanoparticles, which show, in the same concentration, high toxicity for malignant cells of this type and low toxicity for normal cells of the organ or tissue concerned.

To date, it has been established that the action of silver nanoparticles on cultured cells of both bacteria and mammals is significantly dependent on the way the nanoparticles are produced. So, for the development of both antimicrobial and antitumor drugs based on silver nanoparticles, the actual task is to create a method for obtaining aqueous solutions of these nanoparticles, ensuring their high efficiency as an active component in drugs and at the same time low toxicity to the human body.

It is known that formation of silver nanoparticles occurs at the expense of various biologically active substances that are in medicinal plant raw materials. In particular, plant flavonoids may include metal ions in the chelating complex and restore them. Flavonoids contain various functional groups that can cause the formation of nanoparticles. On the territory of Uzbekistan there is a sufficient number of medicinal plants rich in flavonoids, in particular Scutellaria IscandariaL. The -OH groups inherent in flavonoids may be responsible both for the reduction of silver ions to nanoparticles and for their role as stabilizers[15].

In this regard, today it is relevant to study the possibility of biosynthesis of metal nanoparticles, including silver, using domestic plant raw materials and justification of the possibility of formation of nanometals by modern physical and chemical method.

Some flavonoids have the ability to chelate metal ions through their carbonyl groups or π-electrons, the presence of such mechanisms may explain the ability of flavonoids to adsorb on the surface of the emerging nanoparticle. This means that they take part in the initiation stages of nanoparticle formation (nucleation) and further aggregation in addition to the bioremediation stage. In addition, isolated flavonoids and flavonoid glycosides have the ability to induce formation of metallic nanoparticles.

Among the great diversity of flavonoids containing plants growing on the territory of Uzbekistan, special attention is drawn to Scutellaria Iscandaria L. from

Labiatae (ScutellariaLamiaceae), which along with various flavonoid compounds is rich in other biological active substances such as glycosides, essential oils, organic acids, macro and micro elements, tannins, etc.[16].

"Green synthesis" method, as opposed to chemical synthesis, are cheaper. They allow nanoparticles to be produced[19] in unlimited quantities without significant energy costs[17], as they are considered cost-effective, and are also non-toxic. Biological method are environmentally safe as they do not use toxic compounds[18]. According to many authors[21], a variety of plant metabolites including terpenoids, flavonoids, polyphenols, sugars, alkaloids, phenolic acids and proteins are transferred to water extracts. These biologically active substances contain the hydroxo and aldehyde groups responsible for the reduction of silver ions, and the carboxylic group responsible for stabilizing the resulting nanoparticles.

A number of silver nanoparticles obtained in this way exhibit antibacterial and fungicidal properties, which makes them promising for use in new medical and cosmetological preparations. Antibacterial properties of silver nanoparticles depend on their size: the smaller the particle, the stronger is the bactericidal effect, as smaller nanoparticles have a larger surface area, which provides better interaction with the bacteria.

Silver nanosuspensions are in demand in various industries. Taking into account that the production of silver nanosuspension is mass-produced, it becomes clear that their production must be rational, efficient and these products must meet high quality and safety requirements. For example, in the production of pharmaceuticals or cosmetics with silver nanoparticles, it is more efficient to produce a dry powder of silver nanoparticles, where the exact quantity of silver nanoparticles can be calculated. Thus, dry silver nano-silver powder is more efficient than a suspension of silver nanoparticles.

In connection with the studied data, we find it interesting to study the formation of silver by the method of "green synthesis" using Scutellaria Iscandaria L. and study their antibacterial properties.

Research objective is to obtain an aqueous suspension and dry substance with silver nanoparticles by the method of "Green Synthesis" using a plant of the genus Common Skullcap, as well as the study of their physical, chemical and antimicrobial properties.

Materials and Method

The above-ground part of Scutellaria Iscandaria L. collected in July in the Pontifical district of Namangan region of the Republic of Uzbekistan was used as plant raw material, while the silver ion source was 0.01 M nitrate.

Production of silver nanoparticles was carried out by "green synthesis" method.

Extract Obtaining: Water extraction of dried grass was used as a regenerating agent. For this purpose, 1 kg of shredded raw material was placed in the extractor, 10l of purified water was poured, and then left to swell for 24 hours and received the first drain. Then we added 10 liters of purified water and got the first drain. Both drains were combined and filtered. The received suspension was stored in a refrigerator at temperature 20°C[22].

Silver Nitrate Preparation: To produce 0.01 m of silver nitrate, 80 l of distilled water was added to 136 g of silver nitrate powder. Thoroughly stirred until completely dissolved.

Phytosynthesis of Silver Nanoparticles: For the phytosynthesis of silver nanoparticles, a solution of silver nitrate ($1*10^{-3}$ mol/l) in a ratio of 1:4 was added to the resulting extract. Nanoparticles were synthesized at room temperature until light changes. The pH value of the solution was 5.5.

Production of dry substance with silver nanoparticles: To obtain a dry powder of silver nanoparticles, the liquid extract was first converted into a dense form using a rotary evaporator, thus obtaining 5 liters of the dense extract. Dry extract powder with silver nanoparticle from a dense suspension was obtained by means of Lyophilic drying.

Quantitative determination of the content of micro- and macro-elements in the dry substance by the method of ICP-MS.

By results of studying the elemental composition of dry substance of silver nanoparticles by method ICP-MC it is possible to make calculation of silver for carrying out physico-chemical, microbiological analyses.

The method of impurity(Li, Be, Sc, Cr, Mn, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Y, Nb, Mo, Rh, Ag, Pd, Cd, Sn, Sb, Te, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, W, Re, Ir, Pt, Au, Tl, Pb, Bi,

Th и U) elements in soils, Soils and bottom sediments are based on a highly sensitive multi-element analysis method - mass spectra with inductively coupled plasma (ICP-MS).

IR-spectroscopy research: IR spectra of the initial extract of Scutellaria Iscandaria L. and the slurry after phytosynthesis were recorded on an IR spectrophotometer (Cary 630 Ftir Agilent Technologies USA) in the frequency range of 4000-400 cm^{-1} (resolution 4 cm^{-1}, number of scans of the sample 50). A certain amount of solution was applied to the KRS substrate, dried for about 5 minutes and the spectrum was recorded.

Study of identification and quantitative determination of flavonoid content: The identification and quantification of flavonoids in Scutellaria Iscandaria L. was studied by HPLC with photometric detection on Agilent 1100. Luteoline, Apigenin, Rutin, Quercetin (ICC lnc, USA Indofine) standards were used. For the preparation of a standard sample with a concentration of 0.05 mg/cm^3, 5 mg standard is placed in a measuring flask with a capacity of 100 cm^3, bring the volume to the mark with methanol. To prepare the sample for the suspension with silver nanoparticles add 2 ml of hydrochloric acid and heated in a water bath with a reverse refrigerator for 1 hour 30 minutes. The solution is quantitatively transferred into a measuring flask for 100 cm^3 and brought to the mark 70% ethanol. Then the flask is placed for 5 minutes in an ultrasonic bath.

Further, the sample for HPLC analysis is filtered through a membrane filter and used for analysis. Conditions for chromatographic analysis: column: 5 µm, 250×4.6 (e.g. Agilent 5 µm C18(2)); mobile phase: acetonitrile - 0.1% trifluoroacetic acid solution pH 2.6 (40:60); mobile phase rate: 1.0 cm^3/min; Column Temperature: 30^0C; Detection: UV, λ= 254 nm. Volume of Introduced Sample: 10 mm^3.

The content of the indicator components is calculated according to a graduation chart or a formula:

$X = C \times S1 \times V\ S2 \times m$,

C — concentration of the corresponding standard solution, mg/cm^3;

S1 – the area of the peak determined by the component in the analysed sample;

S2 — area of the peak determined by the component in the standard sample;

V — is the total dilution volume of the sample, cm^3;

m — hinge mass, gr.

AFM (Atomic force microscopy) study of silver nanoparticles: Method - atomic force microscopy (AFM) to visualize the resulting nanoparticles. The procedure for preparing samples for atomic force microscopy consists in their immobilization on an even substrate. The substrate material can be varied in a wide range depending on the tasks at hand. Traditionally atomic-smooth substrates made of mica, graphite and other layered materials as well as various glasses, polymeric materials and metal surfaces are used as substrates.

Study of antimicrobial activity by microbiological method: The antimicrobial activity of silver nanoparticles was determined[23] by the Diffusion method in Agar and serial dilutions on solid nutrient media[24].

Diffusion in agar on a dense medium (nutrient medium) by comparing the size of the growth depression zones of test microbes.

The following strains were selected: St. aureus ATCC 25923, B.subtilisATCC 6633, Candida alb. 885-653.

Nutrient media: Wednesday No.1, Wednesday No.2.

Nutrient media were prepared in accordance with the manufacturer's instructions.

Autoclaving - 1/atm (121°C) for 15 minutes.

Inoculum Preparation: Neglected daily culture St. Aureus, B. subtilis, Candida alb. grown in a densely nutritious environment. Microbial suspension was prepared by suspending isolated colonies from a 24-hour culture to a standard turbidity of 0.5 McFarland.

Solution Preparation:

Working Solutions:

Tested solution #1: Dry powder with silver nanoparticles obtained by green synthesis method using aqueous extract of common skullcap.

Distilled water was used as a solvent and diluent.

All solutions were applied 100 µl each in wells with one cup of Petri. Then they were kept at room temperature for 1-2 hours.

The cups were incubated in a thermostat at 36 (±1) C^0 for 18-24 hours. After incubation the growth delay zones were measured to the accuracy of 1 mm with the HiAntibiotic Zone Scale CPW 297. The results were taken into account by changing the growth delay zones of microbes around the well (including the diameter of the well itself).

Results

During our Green Synthesis studies, we observed that the color of the observed suspensions was changing. During 1 hour to 4 hours the color of the solution changed from yellow - brown to dark brown light. The change in color indicates an increase in the concentration of silver nanoparticles, as well as an increase in particle size. The liquid extract was then thickened to a thicker form using a rotary evaporator. A 5-liter thick suspension was obtained. Then it was dried by sublimation. From 1 kg of raw material 75 g of dry substance with silver nanoparticles was obtained. Dry fine amorphous powder is dark brown, hygroscopic, slightly crumbling, the smell is weak and peculiar.

The elemental composition of dry substance was studied quantitatively. In particular, the amount of silver in the studied samples was 2500 ppm (2500 mg/kg), which is - 0.25% .

It is known that silver deposition in concentrations of 10-20 µg/ml causes reduction of tumor necrosis factor, interleukin-1, interleukin-6, and at concentration of nanoparticles at the level of 100 µg/ml 50% survival rate of A549 lung cancer cells is achieved. According to EPA (USA) recommendations, the daily permissible dose of silver (RfD) that does not cause cell necrosis is 5 µg per 1 kg of body weight per day.

IR spectroscopy study of Scutellaria Iscandaria L. extracts and extract suspensions with formed silver nanoparticles are presented in Fig.1 and 2.

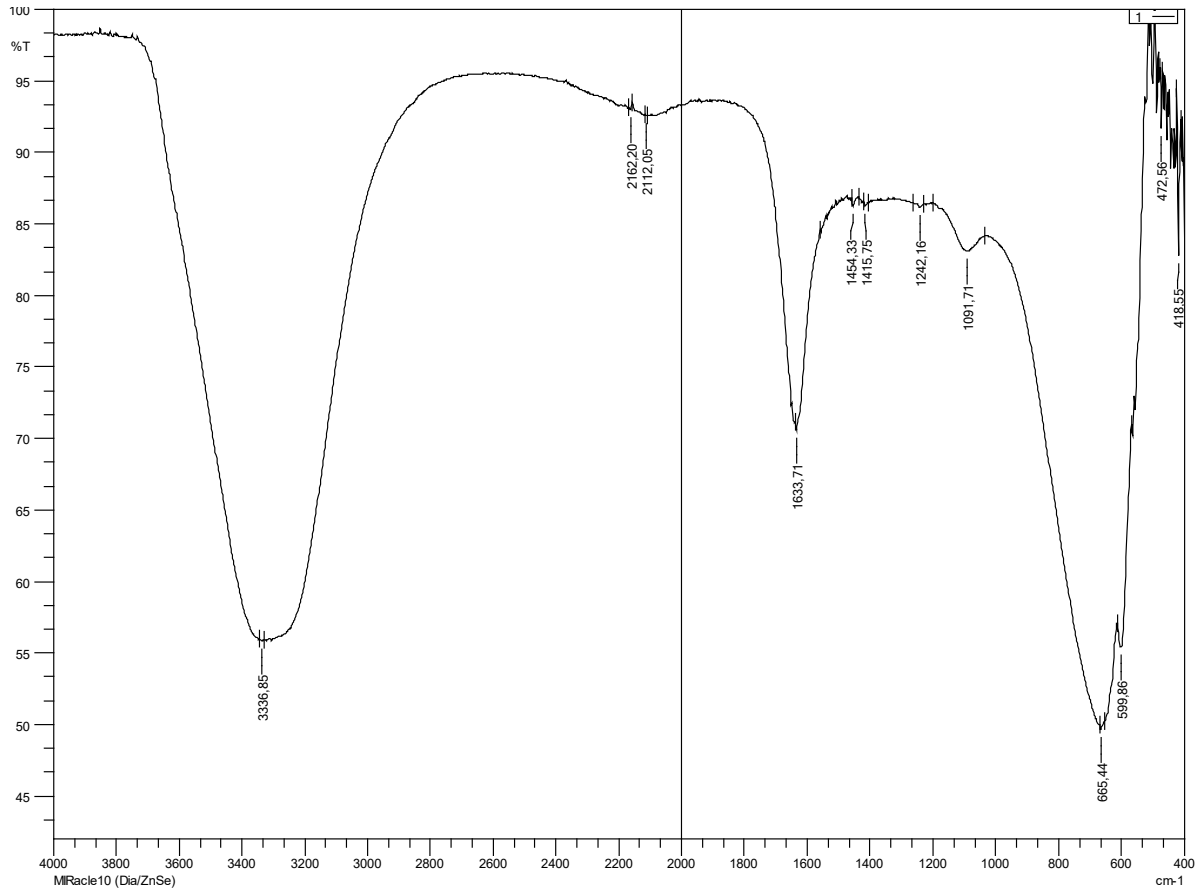

Fig. 1. IR absorption spectrum of Scutellaria Iscandaria L. extract.

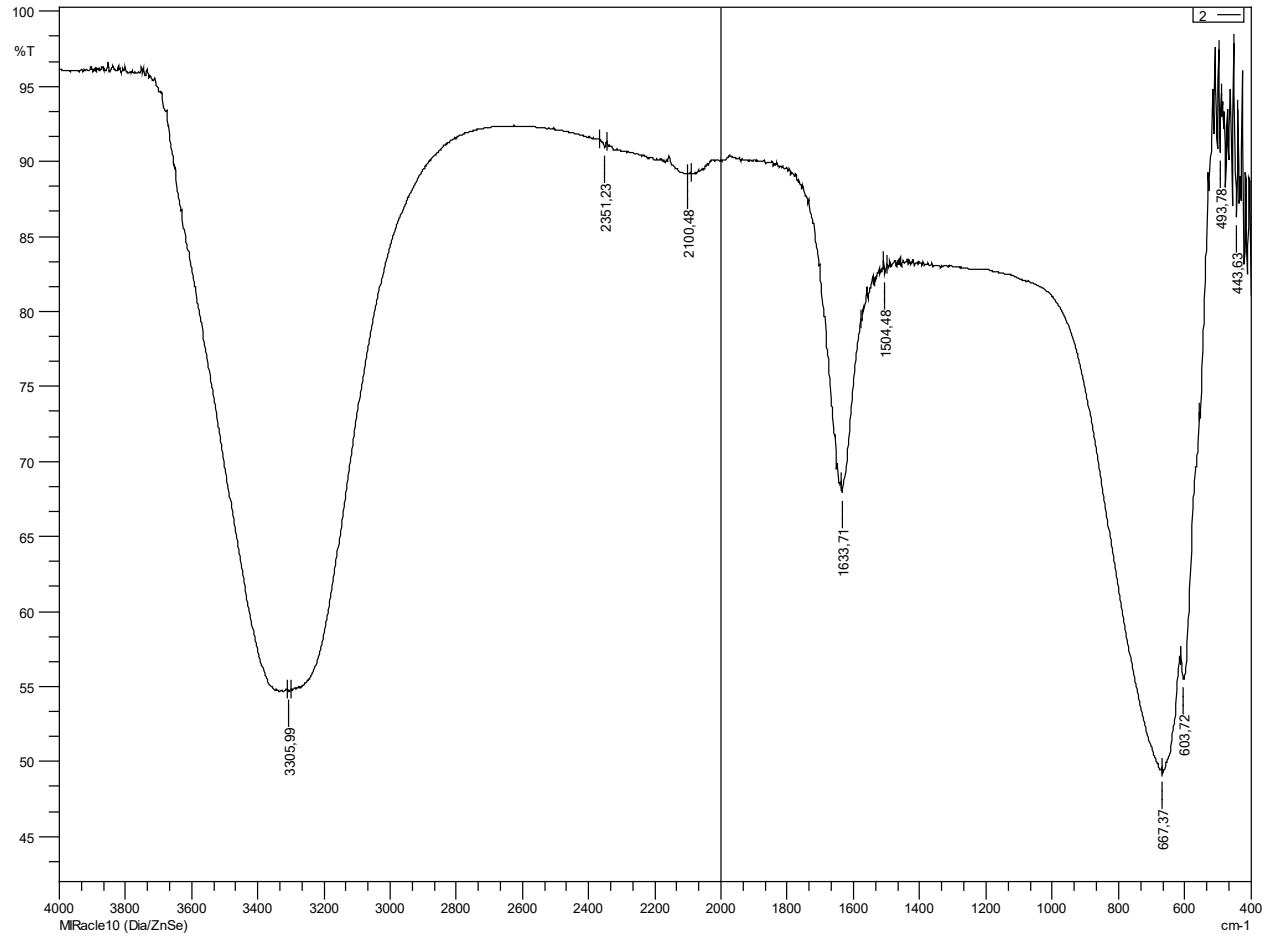

Fig 2. IR spectrum of silver nanoparticles absorption using Scutellaria Iscandaria L. extract.

In the presented infrared spectra, the following characteristic peaks in the absorption area can be distinguished: 3336-3305 cm^{-1}, 2112-2100 cm^{-1}, 1633 cm^{-1}, 1454-1504 cm^{-1}, 1091 cm^{-1}, 400-667 cm^{-1}. It is known that these peaks are connected with valence oscillations of hydroxyl groups in alcohols or phenolic compounds, functional groups C-O of phenol, C-H of aromatic ring.

Removed IR spectra (Fig.1, 2) showed the fluctuations of the temple ring at 1633 cm^{-1}. In the spectrum of the extracts under study there is a wide absorption band in the region of 3336 cm-1, which refers to the valence oscillations of OH-groups in alcohols and phenolic compounds.

After the interaction of $AgNO_3$ solution with Scutellaria Iscandaria L. extract, instead of the band at 3336 cm^{-1}, there is a band at 3305 cm^{-1}, which suggests that hydroxyl groups of phenolic compounds that are part of the extract take part in the restoration of silver ions and formation of nanoparticles.

Absorption bands at 1415 and 1454 cm^{-1} refer to the valence vibrations of the benzene ring at low intensity. Absorption bands in the area of 1242 cm^{-1} belong to the valence oscillations of C-O phenol, while absorption bands in the area of 665 cm^{-1} characterize the deformation oscillations of C-H of the aromatic ring. Taking into account that Scutellaria Iscandaria L., contains flavonoid compounds, which participate in formation of silver nanoparticles, we have carried out a comparative chromatographic study of water extraction of Scutellaria Iscandaria L. and suspensions with silver nanoparticles.

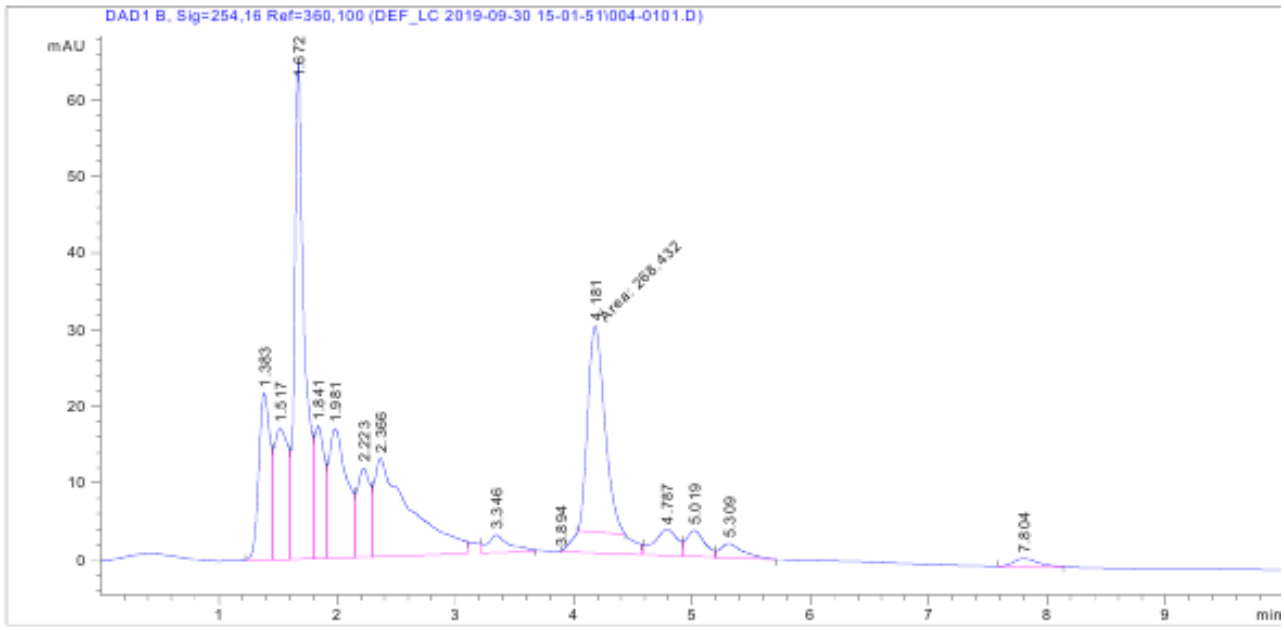

Fig. 3. Luteolin chromatogram.

To begin with, chromatograms of Lutheonine standards were taken (Fig. 3).

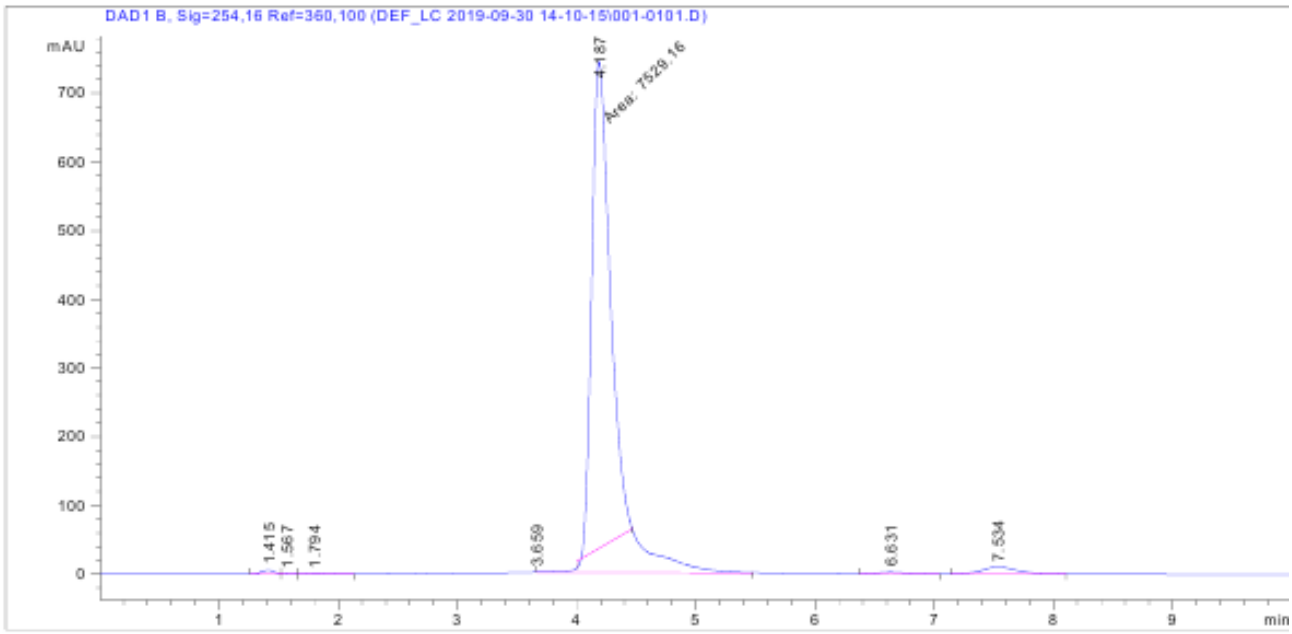

Fig. 4. Water extraction chromatogram of Scutellaria Iscandaria L. (Determination of flavonoids: Luteolina).

The chromatogram shows that the peak holding time for the Luteolin standard is 4.1 minutes.

Further, the water chromatogram of Scutellaria Iscandaria L. was taken. As can be seen from the picture below, the chromatogram also shows peaks in the area of 254 nm, whose retention time (min) characterizes the presence of only Luteolin - 4.1 minutes.

According to the results of the analysis, the content of Luteolin in the samples of Scutellaria Iscandaria L. extract is - 0.0062 mg/g (fig.), and in the suspension with formation of silver nanoparticles - 0.0039 mg/g (fig.).

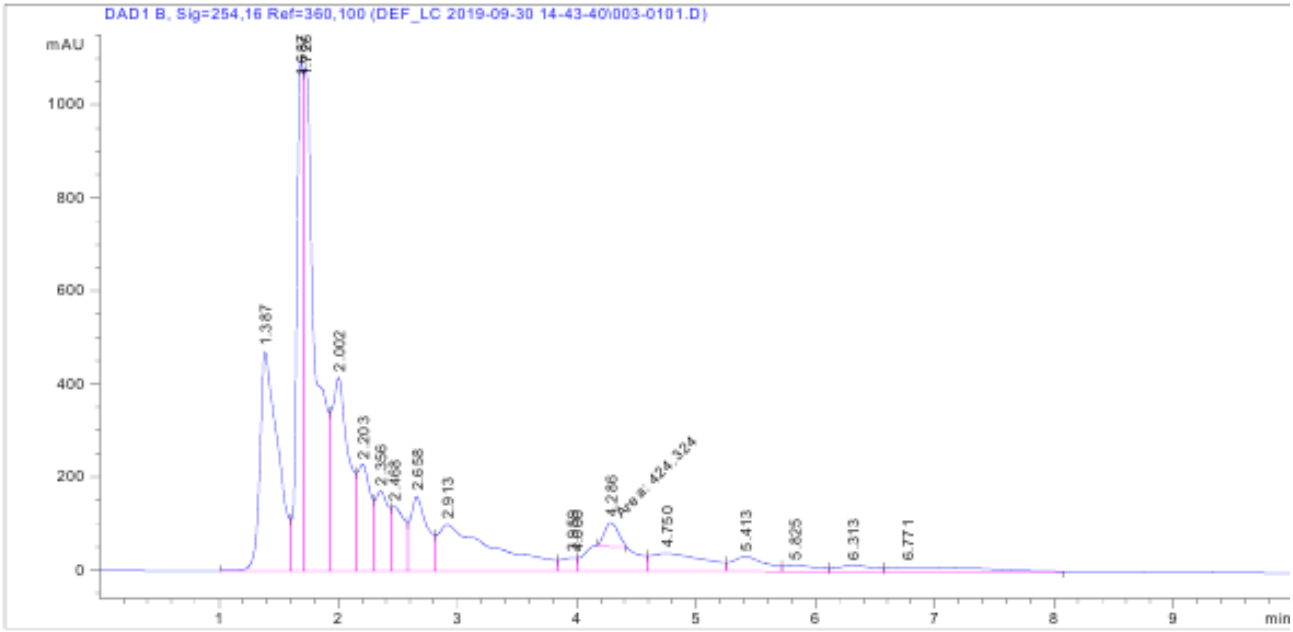

Fig. 5: HPLC - water extraction chromatogram of Scutellaria Iscandaria L. with silver nanoparticles.

Table 1: The generalized results of chromatographic analysis are presented.

Standard Names	Contents of Scutellaria Iscandaria L. water recovery standards.	Contents of Scutellaria Iscandaria L. water extraction standards with silver nanoparticles.
Concentration of mg/g		
Luteolin	0,0062	0,0039

As can be seen from the data presented, the flavonoids contained in Scutellaria Iscandaria L are actively involved in the formation of silver nanoparticles[25].

As is known from literature data[26], the rich phytochemical composition of the extracts used suggests its complex action, for example, as a reducing, stabilizing agent. The mechanism of nanoparticles formation consists mainly of three stages: ion recovery, clustering, and further growth of nanoparticles. Features of each stage depend on the nature of the reducing agent, its concentration, pH, $AgNO_3$: concentration of the reducing agent. The -ON groups present in flavonoids such as luteolin are responsible for the reduction and play the role of stabilizers, therefore, the resulting suspension with nanoparticles can be stored as colloids.

As is known from the literature, the rich phytochemical composition of the extracts used suggests its complex action, for example, as a restorative and stabilizing agent. There is no corresponding literature explaining the mechanism of flavonoid recovery and stabilization of silver nanoparticles. The mechanism of formation of nanoparticles consists mainly of three stages: restoration of ions, clusterization and further growth of nanoparticles. Features of each stage depend on the nature of the reducing agent, its concentration, pH and concentration of the reducing agent. According to some researchers, the -OH groups present in flavonoids, such as Luteolina, may be responsible for the recovery and play the role of stabilizers, so the resulting suspension with nanoparticles can be stored in the form of colloids.

Luteoline reacts with Ag+ as acid through the most reactive hydroxyl groups attached to the carbon atoms of the aromatic ring, which can restore silver ions to nanoparticles and provide stability against agglomeration (Fig. 6).

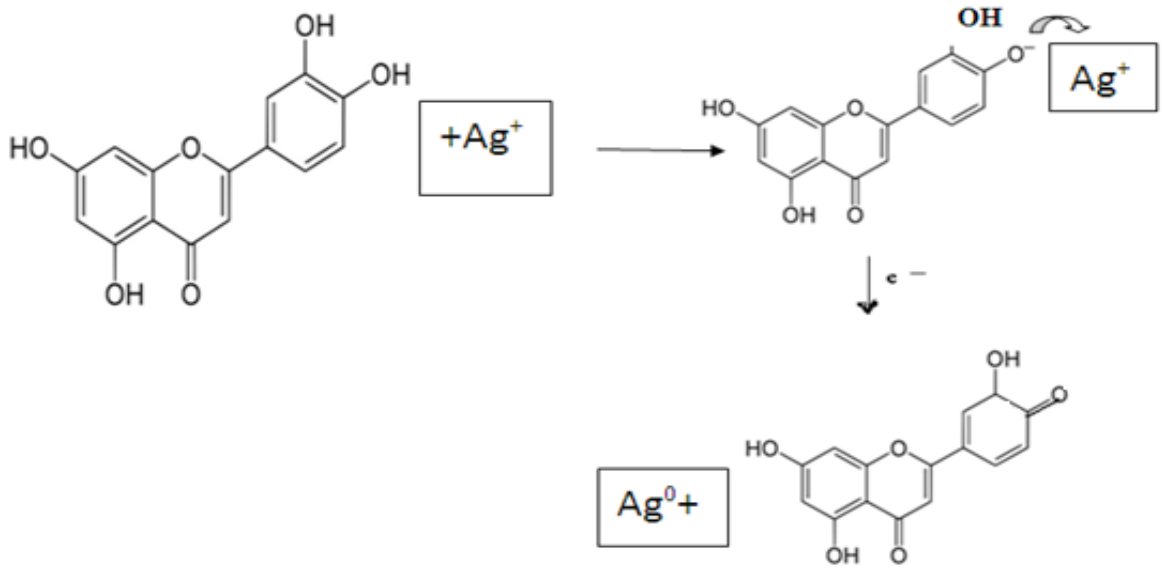

Fig. 6. Approximate mechanism of silver ions reduction to silver nanoparticles by the Luteolin molecule.

From this we can conclude that the formation of silver nanoparticles was due to all flavonoids inherent in the extract of Scutellaria Iscandaria L.

Further, we studied the size and shape of the formed silver nanoparticles by Atomic Force Microscopy.

According to microscopic studies, the morphological characteristics (shape, size, structures) of silver nanoparticles were studied. In particular, the size of silver nanoparticles derived from extracts of medicinal plants Scutellaria Iscandaria L. was 45.7 nm (25% of the total number of particles) with a basic fraction (81%) ranging from 36.6 nm to 64 nm.

Determination of the antimicrobial activity of the preparation by serial dilution makes it possible to determine more precisely the minimum concentration of the preparation that inhibits bacterial growth[27].

The bacteriostatic dose was considered to be the concentration of the preparation, which inhibited the growth of the culture of the test strain, and the bactericidal amount of the preparation, which completely inhibited the growth of microbes. Sensitivity of microorganisms was estimated by a unified method: group 1 was considered highly sensitive, group 2 - sensitive, and group 3 - stable.

Stable - the strain is not suppressed at the concentrations of the drug created in organs and tissues at recommended dosing modes.

Absence of the zone of microbial growth delay around the well indicates that the experimental strain is not sensitive to this experimental drug [28], at a zone with diameter up to 10 mm the strain is considered to be insensitive. In standard conditions of experience by the size of zones formed by preparations, it is possible to judge about the degree of sensitivity of the investigated microorganism.

Based on the results of ICP-MS, the amount of silver was determined to be 0.25%, from these data for analysis of antimicrobial activity in terms of dosage was taken dry powder of silver nanoparticles in 3 different concentrations. 1-0,5%; 2-0,25%; 3-0,125%.

The results of the evaluation of sensitivity and inhibition diameters of growth zones of silver nanoparticles were carried out in Table 2.

Table 2

No	Test strains	Test Results Diameters of microbial growth inhibition zones
1	Staphylococcus aureus ATCC 25923	25-32 mm
2	Bacillus subtilis ATCC 6633	36-40 mm
3	Candida albicans ATCC 885-653	30-38 mm

The results of the conducted studies (Table 2) showed that the greatest efficiency of the studied sample

was observed in relation to Bacillus subtilis ATCC 6633- the diameter of the inhibition zone was 36-40 mm and Candida albicans ATCC 885-653 - the diameter of the inhibition zone was 30-38 mm, also Staphylococcus aureus ATCC 25923 - the diameter of the inhibition zone was 25-32 mm.

Silver has a wide range of antibacterial activity, including antibiotic-resistant gram-positive and gram-negative strains. There is a direct correlation between silver's concentration, particle size and its antimicrobial action. The higher the concentration and smaller the particle size, the more effective silver is at inhibiting bacterial growth. The treatment of infection caused by microorganisms in this category is usually effective when tested preparations are used in recommended doses. The sensitive ones were Staphylococcus aureus, Candida albicans with a growth delay zone around wells, which was 30-40 mm. Category-induced infection treatment may be effective when the test subject is administered at elevated doses or when the focus of infection is localized in those organs or tissues in which elevated drug concentrations are created due to physiological features. In addition, after observing the results, we kept the cups in the thermostat for another 1 week and observed microbial growth. During 1 week no microorganism growth was found in the culture.

Wasteless Technology: On the basis of the above data, water was vaporized in the process of obtaining a thick extract. As silver nanoparticles are considered to be small metal particles, they were not only in the dense suspension but also in the water part. On this basis, we found it interesting to study the antimicrobial activity of the evaporated water part.

First, we studied the amount of silver in water using the ICP-MS method. The amount of silver in the tested samples was 0.023 mg/l.

Table 3

No	Test strains	Test results Diameters of microbial growth inhibition zones
1	Staphylococcus aureus ATCC 25923	-
2	Bacillus subtilis ATCC 6633	36-38-40 mm
3	Candida albicans ATCC 885-653	-

During the antimicrobial activity study, water with nano-silver was also taken at 3 x concentrations.

The results of the sensitivity and inhibition diameters of microbial growth zones of water with silver nanoparticles are given in Table -3.

No activity was detected on Staphylococcus aureus and Candida albicans during the studies. The absence of a microbial growth delay zone around the well indicates that the test strain is not sensitive to this test drug. Only Bacillus subtilis ATCC 6633 was considered sensitive, with a growth inhibition zone diameter of 36-38-40 mm.

Conclusions

For the first time, a dry substance of silver nanoparticles was obtained by the "Green Synthesis" method using an extract from a plant of the Common Skullcap. The role of Luteolin flavonoid contained in Scutellaria Iscandaria L. extract in the synthesis of silver nanoparticles as a reducing and stabilizing agent is shown, the possible mechanism of nanoparticle formation is given. Synthesized silver nanoparticles have been studied by IR-spectroscopy, Mass Spectrometry (ICP-MS), High Performance Liquid Chromatography (HPLC). The size and shape of the obtained nanoparticles were characterized by the Atomic Force Microscopy (AFM) method. Their antimicrobial activity was also studied on the test strains: St.aureusATCC 25923, B.subtilis ATCC 6633, Candida alb. 885-653.

IR spectroscopy data suggest that hydroxyl groups of phenolic compounds in the extract take part in the reduction of silver ions and the formation of nanoparticles.

It follows that the Scutellaria Iscandaria L. extract contains flavonoids that participate in the formation of nanoparticles. We have investigated the role of flavonoids, in particular, Luteolin method of HPLC, contained in the extract of Scutellaria Iscandaria L. in the reduction of silver ions in the solution with the formation of silver nanoparticles.

It was found that Luteolin is also actively involved in the restoration of silver nanoparticles, so its content in the biosynthesis process is reduced by half. The formation of silver nanoparticles is confirmed by the appearance of the color of the resulting ash, as well as the results of atomic force microscopy, which allowed to obtain an image of silver nanoparticles. The AFM image allowed us to establish that silver nanoparticles obtained using Scutellaria Iscandaria L. extract have a size of 45.7 nm (25% of the total number of particles)

with a basic fraction spread (81%) of 36.6 nm to 64 nm. Consequently, Scutellaria Iscandaria L. may be a promising raw material for the synthesis of silver nanoparticles.

Silver nanoparticles obtained with the use of Common Skullcap extracts have been shown to exhibit strong antimicrobial activity; they have bactericidal effect on Bacillus subtilis, Staphylococcus aureus, Candida alb.

Based on previous data, it can be concluded that the silver nanoparticles obtained with the use of Common Skullcap extract can be used to treat inflammatory processes. The results of the study confirmed that the bactericidal dose of the drug was higher than the bacteriostatic dose.

Comparative data on the antimicrobial activity of silver nanoparticles powder and water with silver nanoparticles allow concluding that not only the concentration of nanoparticles, but also the biological active substances contained in the dry powder with silver nanoparticles play a major role.

Later, when planning the production of dry substance with silver nanoparticles, we can use the water obtained in the process of evaporation to obtain a thick extract in various areas, such as in cosmetology, meal grass for addition to feed, because in the process of extraction was not used organic substances. On this basis, we can conclude that the selected technology for the phytosynthesis of nanoparticles will relate to the wasteless technology.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

References

1. Arora S. RJM, PKM. Nanotoxicology and in vitro studies - the need of the hour. Toxicology and applied pharmacology. 2012; 5(258): p. 161.
2. D R. Nanotechnology and consumer products. 2009.
3. Mansour G.-M. RH-, MK, MR, KS. “Green synthesis of silver nanoparticles using plant extracts”. Korean J. Chem. Eng. 2014; 31: p. 548.
4. Poulose S. PT, NPP, TT. Biosynthesis of silver nanoparticles. J. Nanosci. Nanotechnol. 2014; 14: p. 2038.
5. Mansour G.-M. RH-, MK, MR, KS. Green synthesis of silver nanoparticles using plant extracts. Korean J. Chem. Eng. 2014; 31: p. 548.
6. Firdhouse M.J. PLP.. Biosynthesis of silver nanoparticles and its applications. J. Nanotechnol. 2015; 2015: p. 1.
7. Rauwel P. KS, FS, RE. A review on the green synthesis of silver nanoparticles and their morphologies studied via TEM. Adv. Mater. Sci. Eng. 2015; 2015: p. 1.
8. Srikar S.K. GDD, PDB, MPK, USNGsosnar. Green Sustain. Chem. 2016; 6: p. 34.
9. Sankar N.S. DP, NH, ea. Green synthesis of silver nanoparticles using fresh water green alga Pithophora oedogonia (Mont.) Wittrock and evaluation of their antibacterial activity. Appl. Nanosci. 2015; 5: p. 703-709.
10. Song J.Y. KBS. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst. Eng. 2009; 32(1): p. 79-84.
11. Sheny D, MJ & PD. Phytosynthesis of Au, Ag and Au–Ag bimetallic nanoparticles using aqueous extract and dried leaf of Anacardium occidentale. Spectrochim.. Acta A Mol. Biomol. Spectrosc. 2011; 79: p. 54-62.
12. Singh P., Kim Y.J., Singh H., et al. Biosynthesis, characterization, and antimicrobial applications of silver nanoparticles. Int. J. Nanomedicine. 2015; 10: p. 2567–2577.
13. Khan M. ASFTMN, TW, AHZ, AWA, SMR. Green synthesis of silver nanoparticles mediated by Pulicaria glutinosa extract. Int. J.Nanomedicine. 2013; 8: p. 1507-1516.
14. Egorova EM, KSIaKAA. Toxicity of silver nanoparticles obtained by bioreduction as studied on malignant cells: is it possible to create new generation of anticancer remedies, (A review). In: Applications of Nanobiomaterials (Ed. by A.M. Grumezescu). Elsevier, North Holland. 2016; 7: p. 505-542.
15. Marambio-Jones C. HEMV. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. Journal of Nanoparticle Research. 2010; 12: p. 1531.

16. Ismailova PL, Ismailova MG. "Spectrophotometric determination of the total flavonoid content in Scutellaria Iscanderia L. grass". Kharkov, National University of Pharmacy. 2019;: p. 262-112.
17. Rai, M.K. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria/ M.K. Rai, S.D. Deshmukh, A.P. Ingle, A.K. Gade. Journal of Applied Microbiology. 2012; 112(5): p. 841-852.
18. M.G. Ismailova IBS. "Biosynthesis of silver nanoparticles from flavonoid-containing extracts and their IR-spectroscopy study". Tashkent Pharmaceutics Institute "Pharmacy Journal". 2018;: p. 52-56.
19. S.Iravani. "Green synthesis of metal nanoparticles using plants". Green Chem. 2011; 13: p. 2638–2650.
20. I.B. Shermatov MGI"oSNbAFM. Kharkiv Materials II International Scientific and Practical Conference Centre. 2019; 2: p. 14-15.
21. B.R. Tausarova AJKMSSANM. Antibacterial Properties of Silver Nanoparticles: Achievements and Prospects. 2014; 1: p. 76.
22. al. BHPe. Extract: A Promising Biomatrix for Ecofriendly, Controlled Synthesis of Silver Nanoparticles. Applied Biochemistry and Biotechnology. 2014;(173): p. 1-29.
23. Yugal K. M., Sujogya K. P., Rasu J., et al. Antimicrobial, antioxidant and cytotoxic activity of silver nanoparticles synthesized by leaf extract of Erythrina suberosa (Roxb.). Front Mol Biosci. 2017; 4(14).
24. Krishnaraj C. JEG, RSea. Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. Colloids and Surfaces B: Biointerfaces. 2010; 76(1): p. 50.