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Biosynthesis of silver nanoparticles using water extracts of Nepeta cataria L. and Salvia sclarea L., characterization and determination of antibacterial potential

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A B S T R A C T

Benefits of silver nanoparticles (AgNPs) against various pathogens and their other benefits are already well known in medicine. Meanwhile, applications involving nanotechnology have the potential to aid agricultural production. Bacteria, fungi, viruses that cause plant diseases can be controlled with AgNPs. Bacterial infections cause significant loss of yield around the world. AgNPs have shown activity against plant pathogenic bacteria. They have higher antibacterial activity against erwinia cartovora, E. than antibiotics. AgNPs also lave antifungal activity against pathogenic fungi, ie. Fusarium oxysporum, Alternaria Alternaria and Aspergillus flavus. They represent alternative to pesticides, are able to protect plant from pest and enrich soil with nutriants. Their use may reduce demand on chemical fertilizers. It is already used as foliar spray against moulds, rot, fungi and other microbial associated plant diseases . AgNPs can benefit preservation process of food. Their addition to food packaging can improve their barrier, mechanical, and antibacterial properties, and maintain the quality of foods. Biosynthesis is simple, effective, environmentally friendly and inexpensive way for production of silver nanoparticles. Process does not require high temperature and pressure. It is characterized by low energy costs. In given study silver nanoparticles were biosynthesized using water extracts of *Nepeta Cataria L*. (Catnip) and *Salvia Sclarea L*, (Clary sage). Process of biosynthesis was observed and optimal conditions were determined. Morphology of obtained nanoparticles was investigated using TEM method and AgNPS were evaluated for antibacterial activity against ten stains of Gram-negative and Gram-positive bacteria.

Key words: *Nepeta cataria* L., *Salvia sclarea* L., antibacterial potentional

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Introduction

Nanotechnology is a new field of scientific research that has been very well known over the last few decades [1]. It has a wide range of uses in microbiology and biotechnology. It is useful for developing, designing, and manipulating nanostructures with very small dimensions and a large surface area-to-volume ratio. Nanoparticles are attracting the attention of researchers in various fields due to their extraordinary properties. The morphology, size, and chemistry of nanoparticles are used to classify nanoparticles and are therefore classified according to their physical and chemical properties. There are several known classes of nanoparticles, including organic, inorganic, and carbon-based [2].

A bulk material has constant physical properties regardless of their size and shape, but at the nanoscale, the size, morphological substructure of the substance, and shape are the major driving factors for changing their biological, chemical, and physical properties. Because at the nanoscale, the materials behave differently and they emerge with few novel characters in themselves [3].

Metal nanoparticles have size-related properties that are significantly different from bulk materials. These unique properties are ambassadors for potential applications in medicine, catalysis, optics, cosmetics, renewable energy, microelectronics, medical imaging, environmental restoration, and biomedical equipment [4]. Of the wide range of metal nanoparticles, silver nanoparticles (AgNPs) are the most popular due to their unique physical, chemical, and biological properties when compared to their macro-scale equivalents [5].

The effect of silver nanoparticles against various pathogens and other benefits are already well known in medicine. AgNPs can be used in medicine for antimicrobial and antitumor therapy [6]. They exhibit antiviral effect and they were found to be able to combat even SARS-CoV-2 virus [7, 8]. Meanwhile, applications involving nanotechnology have the potential to aid agricultural production. It has already begun to have a significant effect in the main areas of the agriculture and development towards this way continues. Many findings have been made regarding them in agriculture and interest has risen lately. Various bacteria, fungal, viral species can cause plant diseases [9]. While their management is not an easy task AgNPs can be useful to combat microorganisms causing them. Bacterial infections cause significant loss of yield around the world. AgNPs have shown to be active against plant pathogenic bacteria. Research showed that AgNPs have higher antibacterial activity against erwinia cartovora, E. than antibiotics [10]. Antifungal activity of AgNPs was also studied against pathogenic fungi, ie. Fusarium oxysporum, Alternaria Alternaria and Aspergillus flavus [11]. Silver nanoparticles proved themselves as attractive alternative to pesticides. They are able to protect plant from pest and enrich soil with nutriants. Thus, use of chemical fertilizers can be reduced. It is already used as foliar spray against moulds, rot, fungi and other microbial associated plant diseases [12]. AgNPs have good potential for increasing crop yields by promoting seed germination and plant growth, though it has to be mentioned that effect can be also negative [13], but besides, it can benefit preservation process of food which is important in agriculture. The addition of AgNPs into food packaging can improve their barrier, mechanical, and antibacterial properties, as well as maintain the quality of foods [14].

Several main methods are available for obtaining silver nanoparticle, though currently most interesting, promising for synthesis of silver nanoparticles is so-called green synthesis. Scientists working in this field focuse on the development of efficient and environmentally friendly methods for synthesizing metal nanoparticles using green chemistry. Emerging interest is caused by several reasons. Biosynthesis of silver nanoparticles has significant advantages over chemical and physical methods [15]. Biosynthesis is simple, effective, environmentally friendly and inexpensive way for production of silver nanoparticles [16]. Process does not require high temperature and pressure. It is characterized by low energy costs [17]. Process involves use of biological resources which serve as reducing, stabilizing and capping agent at the same time. Method enables us to obtain relatively safe AgNPs, as the surface of nanoparticles contains little or no toxic materials. Very important finding is that nanoparticles obtained through biosynthesis are primarily biocompatible and can be used for biomedical applications [18,19]. In the scientific literature, biosynthesis of silver nanoparticles is described using plants, bacteria,[20] fungi [21], algae [22] and even with honey [23]. Green synthesis of silver nanoparticles using plant extracts is the most attractive out of listed above. Plants contain various compounds that serve as the reducing agent of metal cations including silver [24].

In given study silver nanoparticles were biosynthesized using water extracts of *Nepeta Cataria L*. (Catnip) and *Salvia Sclarea L*, (Clary sage). Obtained nanoparticles were evaluated for antibacterial activity against ten stains of Gram-negative and Gram-positive bacteria.

Objectives and Methods

Plant extracts used for biosynthesis of silver nanoparticles

The shoots of the raw plants were used to prepare the plant extract. Plant material was washed with distilled water. After drying, they were cut into 2 cm long pieces. To obtain the extract, 35 g of each finely chopped raw material were placed in a beaker. 400 ml of distilled water was added to each of them. Beakers were later placed in a Hyundai microwave for 10 minutes. The mass was heated by the dielectric heat and then was left cool down at room temperature for one hour. On the next step the mass was drained onto cotton to remove the finely chopped plant waste. Finally, to obtain the extract, the liquid was filtered into the filter paper produced by MELIOR XXI Ltd. (ashless filter d = 150 mm).

Biosynthesis of Silver Nanoparticles

For biosynthesis pre-prepared extracts and 0.1N solution of silver nitrate were used. A series of samples with different ratios of AgNO3 and extracts were prepared to determine the optimal conditions for the silver nanoparticle biosynthesis process. To determine the effect of temperature on the reaction, part of the samples were kept at a room temperature and the rest in the refrigerator. The influence of the pH on the process was also observed. Measurements were performed using pH meter, firstly of water extracts and afterwards when the silver nitrate was added to the extract. Later the pH of the reaction mixture was changed using 20% NaOH solution. The biosynthesis process was observed in time dependent manner. After mixing the plant extract and silver nitrate solution in 1, 24 and 48 hour intervals samples were photographed to detect visually noticeable changes.

UV-Vis spectroscopy

In order to prove and evaluate formation of silver nanoparticles in the prepared solutions, UV-Vis spectroscopy was performed after 1, 24 and 48 hour intervals. The research was conducted on the i9 UV-VIS spectrophotometer (Hanon Instruments) owned by the Department of Pharmaceutical Technology of Tbilisi State Medical University. This method is used to detect plasmon resonance characteristic for silver nanoparticles [5]. Mostly silver nanoparticles have absorption peak in the range of 400-450 nm. In present case the absorption spectrum was recorded in the range of 330-800 nm.

Transmission electron microscopy - TEM

TEM microscopy was used to determine the size of formed silver nanoparticles and to study their morphology and distribution. Microscopy was performed at the George Eliava Institute of Bacteriophage, Microbiology and Virology using a JEOL JEM-100SX transmitting electron microscope. Samples were prepared according to regime required by TEM analysis, negatives were taken, and finally the exposed images were obtained at 120,000 magnification. Particle sizes were measured following it.

Antibacterial activity assay

The study of antibacterial activity was performed at the Giorgi Eliava Institute of Bacteriophage, Microbiology and Virology. The study of the antibacterial activity of biosynthesized silver nanoparticles was conducted using so-called Spot Test method on the following strains: Klebsiella spp, E-coli, Pseudomonas aeruginosa, Streptococcus spp, Enterococcus spp, Shigella spp, Salmonella spp, Enterobacter spp, Proteus spp, Staphylococcus aureus. For this on a petri dish containing 2% BH agar bacterial lawns were formed: 1 ml of bacterial culture was applied and distributed over the entire cup. 10 µl test objects were applied after drying. Plates were placed in a thermostat for 18-24 h incubation. Solutions of biosynthesized silver nanoparticles from both catnip (N1) and clary (N2) represented research objects, but for control antibacterial activity of blank extracts were also determined in order to evaluate action in relative aspect (*Nepeta Cataria L.* extract N3, *Salvia sclarea L.* N4)

Results and Analysis

Obtained UV-vis absorption spectra proves formation of silver nanoparticles. The absorption measure increased with time, indicating enhanced plasmonic resonance which on its side is a sign that number of formed nanoparticles increased as time passed. For silver nanoparticles biosynthesized with Catnip extract, the peak of absorption after one hour was observed at 460 nm and reached 0.7. After 24 hours, the peak on the spectrum was recorded at 462 nm and reached 1.0. And after 48 hours the peak was observed again at 462 nm, the absorption maximum increased to 1.3. In case of silver nanoparticles biosynthesized with Clary sage extract, the peak of absorption after one hour was observed at 484 nm and reached 1.6. After 24 hours, the peak on the spectrum was recorded at 488 nm and reached 2.2. And after 48 hours the peak was observed again at 504 nm with absorption maximum 2.5. (Fig.1). According to the literature we have reviewed, the change in wavelength is due to the clustering of particles and the change in size [2]. The ongoing process of biosynthesis was reflected by a change in the color intensity of the study objects. The water extract of Nepeta before addition of silver nitrate solution, and color change dynamics caused by biosynthesis are shown in Figure N2.

Regarding the effect of temperature on biosynthesized silver nanoparticles, the results described above were obtained on a samples stored in refrigerator (+5^{o} C). At room temperature (with constant temperature 23^{o} C), after 24-hour observation strong, visible precipitation took place. From the above we can conclude that the process is more intense at room temperature than it is in refrigerated conditions. However, nanoparticles had tendency to agglomerate, the system was less stable, and the resulted in much stronger precipitation than that of the sample stored under refrigeration conditions.

The study of pH influence on the biosynthesis process of silver nanoparticles showed that process is more intense in alkaline medium. The pH of Catnip water extract was 5.7 and after mixing with silver nitrate equaled 5.1. Addition of sodium alkali solution rose the pH of the mixture up to 10.0. The color of mixture changed from a light brown to a dark green, almost black in few minutes, which proves once again, as it was described in scientific works multiple times, that biosynthesis process in such conditions runs with higher rate.

With conducted TEM x120,000 magnification images were obtained. The size of formed silver nanoparticles was to determined, their morphology and distribution was investigated. Silver nanoparticles biosynthesized by *Nepeta Cataria L.* water extract are represented as agglomerates. On image several such groups are spread and they are very similar to one another. In all of them many individual small particles are defined, however, many of them are united. It should be noted that visually no precipitation was observed in the solution at this time. As for the shape, it varies and particles are capped as transparent membrane is observed which surrounds dark content. Particle diameters range from 4 to 80 nm.

In the case of silver nanoparticles prepared by *Salvia sclarea L.* extract, the extremely strong aggregation is observed. In the image it appears in the form of large spots, few small particles are also presented though with an obvious tendency to coalesce. Unlike the first sample, a membrane-like formation can't be found and it indicates that AgNPs weren't capped which eventually could cause agglomeration. Size distribution is between 4-175 nm. Images are given on Figure N3.

Antibacterial activity

As it was already mentioned above, the study of antibacterial activity of biosynthesized silver nanoparticles was conducted using so-called Spot Test method. List of used bacterial strains and results are given in Table N1.

A sample containing silver nanoparticles (N1) biosynthesized by *Nepeta cataria L.* extract showed good antibacterial activity. Out of ten bacterial strains tested, it wasn't able to affect only Staphylococcus aureus, while test blank extract (N3) affected only Enterococcus spp. Similar effect was not shown by silver nanoparticles prepared from *Salvia sclarea L.* extract (N2). Only three strains were sensitive to it, moreover, blank extract showed the same effect on two of them (N4).

Conclusion

Based on conducted experimental research we are able to conclude that Silver nanoparticles of optimal characteristics can be biosynthesized using water extract of *Nepeta Cataria L.* Appropriate conditions of biosynthesis were determined and as it was expected, process proved to be easy and has low costs. Obtained AgNPs have potential to be used as antibacterial agent.

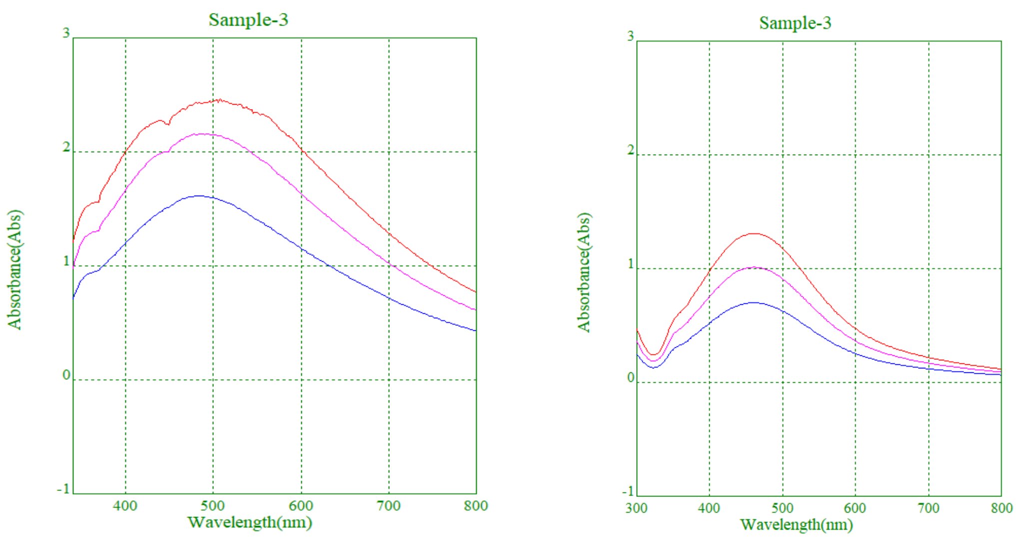

Figure N1. UV-Vis absorption spectra of silver nanoparticles biosynthesized with N.cataria (left) and S.sclarea (right) extracts

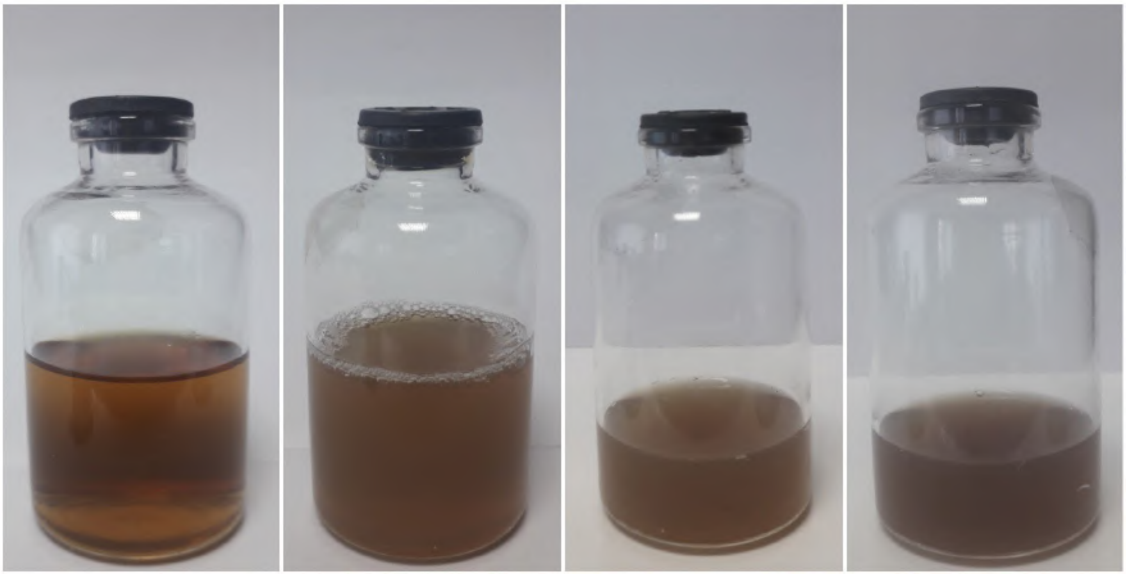

Figure N2. Water extract of Nepeta cataria before adding AgNO3 solution and mixed solutions after launching the biosynthesis in 1, 24, 48 hours (from left to right)

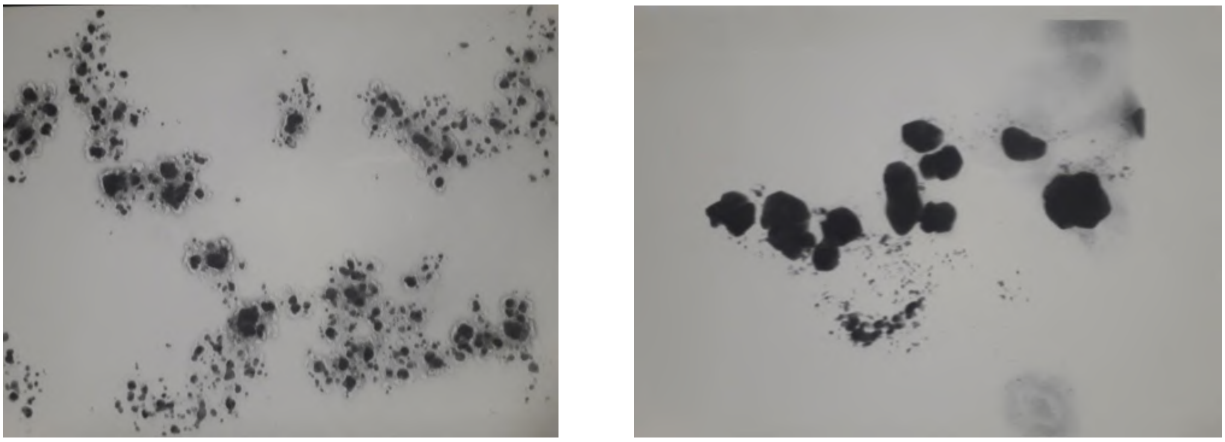

Figure N 3. *a-silver nanoparticles biosynthesized with Nepeta cataria extract; b-silver nanoparticles biosynthesized with Salvia sclarea extract;*

Table N1. *Results of antibacterial activity of biosynthesized silver nanoparticles*

Bacteria	Sample №1	Sample №2	Sample №3	Sample №4
Klebsiella spp	4+	-	-	-
E-coli	1+	-	-	-
Pseudomonas aeruginosa	4+	-	-	-
Streptococcus spp	4+	2+	-	-

Enterococcus spp	2+	2+	2+	2+
Shigella spp	2+	-	-	-
Salmonella spp	4+	-	-	-
Enterobacter spp	3+	2+	-	2+
Proteus spp	2+	-	-	-
Staphylococcus aureus	-	-	-	-

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