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SYNTHESIS OF NANOPARTICLES IN BIOLOGICAL SYSTEMS AND THEIR PHYSICAL CHEMICAL CHARACTERISTICS - GREEN SYNTHESIS

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Abstract. In the paper the synthesis of Ag and Au nanoparticles was carried out using an extract of wormwood (*Artemisia absinthium*), pomegranate (*Punicagranatum*), ordinary onion (*Allium cepa*), saffron (lat. Crocus), cardamom (lat. Amomum) and Baikal skullcap (*Scutellaria baicalensis*). Nanoparticles synthesis technology has been comparatively developed using suitable forms of the "Green" synthesis technology. The results of experiments on FT-İR, SEM, UV-vis analysis of the size of Ag and Au nanoparticles synthesized in extracts of plants were analyzed and were studied the anti-bacterial activity of these nanoparticles. The size of the synthesized nanoparticles in the extract from the plant extracts depending on the exposure time, the concentration of the extract and the composition of the extract to be extracted. The size of Ag nanoparticles in the wormwood extract were a range of 15-35 nm, Au nanoparticles 5-10 nm, in the ordinary onion for Ag 30-40 nm, Au 10-20 nm, in the ginger seeds Ag nanoparticles were relatively small and are within a range of 30 to 40 nm, in extract of pomegranate seeds Ag are within the range of 20-25 nm, Au of 40 to 50 nm, in saffron extract Ag is within a range of 60-70 nm, Au is 60-70 nm.The study of the anti-bacterial activity of "Green" synthesized silver nanoparticles by different plant extracts shows that gold nanoparticles decrease the grows of bacteria. The decrease range is strongly in wormwood extract than in extracts of other given plants.

Keywords: *Nanoparticles, plant extracts, silver and gold nanoparticles, green synthesis, anti-bacterial activity.*

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1. Introduction

The use of nanoparticles in medical practice necessitated the production of their environmentally friendly forms. It was found that they can accumulate in the human body, animals and plants and have a toxic effect. Therefore, the effect of nanoparticles on biological systems and their non-toxic forms has been the subject of many studies. The use of nanotechnology in medicine has made this problem even more serious. In this regard, silver nanoparticles with unique optical and mechanical properties have antiseptic properties. Recently, silver (AgNPs) and gold (AuNPs) nanoparticles have gained worldwide popularity. This is due to their unique properties and great practical value. Thus, the chemical, optical, electronic properties of these nanoparticles allow the creation of highly sensitive chains (sensors), nanoparticles and the formation of a wide range of catalytic substances. Ag nanoparticles create high surface reactivity (e.g., adsorption, catalysis). One of the unique properties of Ag nanoparticles is their use in fluorescence and surface plasmon resonance. The surface plasmon phenomenon is associated with the distribution of free electrons in the conducting zone, which is accompanied by the formation of an amplified local electromagnetic field. This creates a high sensitivity to environmental variability. In nanoparticles exposed to electromagnetic waves, electrons distribution in accordance with the wave frequency incident on the surface of the electron, and a resonance event occurs. The spectral characteristics of Ag nanoparticles depend on their size, shape, medium, and distance between particles (Pandey *et al.,* 2012; Jha *et al*., 2009). The spectral properties of Ag nanoparticles allow their use in various sensory methods in the creation of chemical and biological sensors. Detection of antibodies in blood serum using sensors from Ag nanowires (Li *et al*., 2012), laser desorption / ionized mass spectroscopy of peptides (Yulizar *et al.,* 2017), calorimetric sensor for histidine, fibrogenesis of microbial membranes (Xu *et al*., 2004; Carmona *et al*., 2017) , used to diagnose the use of biosensors for the detection of herbicides (Subha *et al*. 2016), in the development of calorimetric sensors for measuring the concentration of ammonia, in the study of transport (Xiong *et al.,* 2008), in enhancing infrared absorption (Huo *et al*., 2006).

In recent years, a number of environmentally friendly methods have been used to synthesize Ag Au nanoparticles. An example of this is the biological synthesis of nanoparticles. These methods mainly use bacteria, fungi, proteins and polypeptides, nucleic acids and, finally, various plant extracts. The most advantageous of these biological methods is the synthesis of Ag nanowires using plant extracts. In the field of research, they used a wide range of herbs, including wormwood extracts, vegetable leaves, extracts of leaves and roots of medicinal plants, extracts of fruit juices and leaves of shrubs. It was found that Ag and Au nanoparticles synthesized by extracts of these plants have different sizes, shapes and properties. The properties and sizes of silver nanoparticles depend on the type of plant, the amount of extract, temperature, pH, and shelf life and, finally, the synthesis method. The plants used for the synthesis of Ag nanowires, Baikal baitens (*Scutellaria baicalensis* Georgi), which applied as a medicinal plant, are of great interest. This plant contains a large number of flavonoids - antioxidants that have been used as medicines since ancient times. These antioxidants can act as a reducing agent and stabilizer in the synthesis of silver nanowires. Therefore, the *Scutellaria baicalensis* can play an important role in the synthesis of environmentally friendly forms of Ag nanoparticles. On the other hand, nanoparticles began to be used as medicines in medical practice. These nanoparticles have a size of at least 5-10 nm and use very pure, non-toxic nanoparticles to increase the effectiveness of combination drugs. Since silver nanoparticles often pay for this demand, their acquisition has become widely studied. When Ag nanowires are synthesized in extracts of medicinal plants, they are more likely to combine the antioxidants contained in the extract to the surface.

2. Material and methods

Wormwood -*Artemisia absinthium*. Worms are spread wild in the roadside, along the river and lake shores. In dry conditions it is used as spices. It contains 0.026-0.2 percent of essential oils. Wormwood’s medicinal, nutritious, essential oily, dye and decoration-suitable species are cultivated in most countries cultured form. There are 13.6% of ash, 15.6% protein, 5.1% fat, 34.1% cellulose, and 31.6% non-nitrogenous substances in the composition of ordinary wormwood.

Figure 1. *Artemisia absinthium* dried (A) and wet (B) forms

Pomegranate- *Punica granatum*. Pomegranate is from punicaceae family and is heat-loving plant. Pomegranate is used for tanning of leather and as dying matter since it has 1.13% of tannic matters in composition. Pomegranate contains 8-19% fructose, 1% sucrose, 0.2% - 2% citric acid in sweet varieties, 2-3% in sour and sweet varieties and 3-7% in sour varieties. In addition, pomegranate juice contains 0.53% mineral substances, 0.50% nitrous substance, 7 mg% vitamin C, 11.85% tannic substance, 9.07% cellulose, 5.38% pectin, 14.1% reducing sugar, 1% acid.

Figure 2. Pomegranate flower and fruit

Ordinary onion- ***Allium cepa*** is the perennial plant (if grown- biennial) of the onion species of onion family. It has been cultivated as the main agricultural plant for more than 5,000 years. An onion contains sugars (fructose, sucrose, maltose, polysaccharides), protein (1.5-2%), vitamins (ascorbic acid), flavonoids, quercetin, enzymes, saponins, mineral salts, potassium, phosphorus, iron and other phytonides The green leaves of the onion are also rich in sugars, proteins and ascorbic acid.

Figure 3. Usual onion and her blossoms

Saffron (lat. Crocus) is a herb that belongs to the of the "*Dicotyledones*" class of the "*Asparagales*" line of the " *Angiospermae*" division of *Iridaceaefamiliy*. Saffron is planted in Absheron in July and August. Its main feature is the good solubility in water. Chemical composition of saffron has recently been studied well. It has been established that there are up to 40-50 chemical active ingredients in it. Crocins give a color to saffron and the carotenoids ate well-soluble in water.

Figure 4. Saffron Blossom & Dried Form

Cardamom (lat. Amomum) is a perennial herb of *Zingiberaceae* family. The basis of essential oil in the hilin seed of ginger consists of alpha terpineol, delta limonene, cineol, terpenylacetate. Essential oil is less (about 0.1 to 0.7%) and cellulose is more (approximately 28-31%) in the peel of ginger fruit. Ginger seeds contain nitrouts substances - 11-15%, oil - 1-2%, essential oils - 3.5%, carbohydrates - 35-60%, sugar - 0.5-0.8%, starch - 30-50%, pentosanes - 4-7 %, pectinic substances - 0.5%, cellulose - 11-19%, mineral substances - 2-10%.

Figure 5. Cardamom blossom and dried form

Baikal skullcap (*Scutellaria baicalensis*) There are 300 types of Baikal skullcap that are widely used in folk medicine. *S. Baicalensis Georgi* is rich in flavonoids, especially baykaline and baykalen. Flavonoids are widely used in pharmacological practice as antioxidants and bioactive agents. In traditional medicine mainly extracts from its roots are produced, because there are more baykaline and baykalen in the roots.

Figure 6. *Scutellaria baicalensis* dried root and blossom

Preparation of extracts. In our experiments, the synthesis of nanoparticles in plants was carried out in two ways: by preparing their homogenates or extracts. To prepare the plant homogenate, 1.6 g of the plant is weighed, washed 3 times in common, 3 times in distilled water, and then dried with paper filter. Then it is cut and crushed. It is solved in 50 ml of distilled water, and 1 ml of $AgNO_3$ or $HauCl_4$ 10^{-1}M salt solution is added on it. It is then heated to a temperature of 70^0C for 10 minutes, stored in a magnetic mixer. To prepare the plant extract, the leaves or roots of the plants were first cut into small pieces after being washed in distilled water. 50 g of crushed leaf or root is boiled for 10-15 minutes in 500 ml of distilled water. The resulting solution was filtered and its particles were settled in centrifuge. When the temperature of the solution was 70-80^0C, $AgNO_3$ or $HauCl_4$ was added in 10 ml of 10^{-1}M density in obtained extract. The solution was stored at room temperature for 24 hours in regular light. After 24 hours

UV-vis spectrum was applied. As pomegranate is granular plant, its homogenate's preparation is somewhat different.

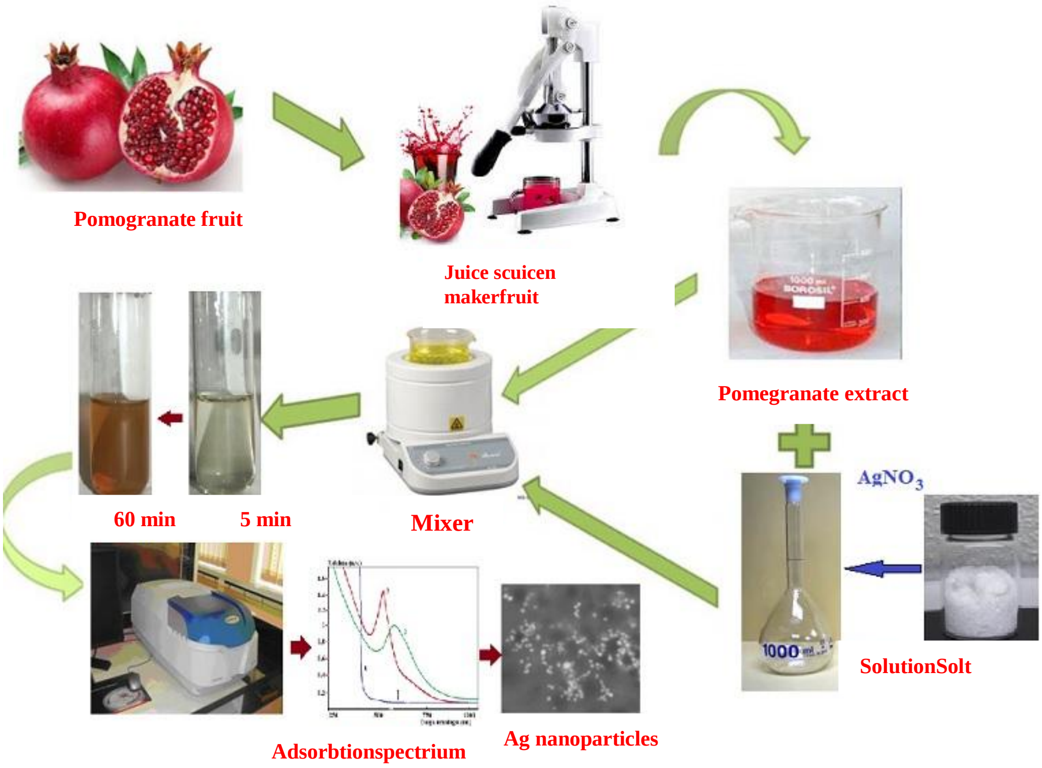

Figure 7. A synthesis procedure of silver nanoparticles in the pomegranate seeds have been given as example

UV-Vis Spectroscopy. One of the most commonly used methods for the observation of synthesis of nanoparticles is UV-Vis absorption spectroscopy. UV-vis spectroscopy is used to obtain the absorption spectrum of solids or complex solutions. In the UV-vis section, the electromagnetic spectrum of the energy is 1.5-6.2 eV, which corresponds to the values of 200-800 nm of the wavelength. The Beer-Lambert law, which is expressed in the equation $A = \mathcal{E}bc$ forms the base of the absorption spectrum. A-absorption for single wavelength, Ɛ- is ability to absorb of the molecule or mixture, b is the length of the cuvette samples and c is the concentration.

FTIR analysis was performed using the Varian 3600 FT-IR spectrometer. Centrifuged and dried silver nanoparticles (exemplary) and dried plant extracts form base on KBr and then pelletized. These granules were exposed to the Fourier infrared wavelength range from 400 to 4000 cm^{-1}.

Scanning Electron Microscope (SEM). With SEM, which is able to zoom 1,000,000 times, it is possible to obtain high-quality images of various nano-dimensional materials, nanoparticles, nanopolymer and nanocomposites, to define the dimensions and to carry out elemental analysis of the sample.

3. Experiments

Synthesis of Ag nanoparticles in the extract of wormwood. In the experiments, both dried and wetted forms of wormwoods were used. The dried form was obtained from the pharmacy, and the wet form was brought from the regions during the initial development of the wormwood. Changing the color of the solution show when forming Ag nanoparticles (Fig.8).The formation of Ag nanoparticles depends on the exposure time (Fig.9) and on the concentrations of $AgNO_3$ (Fig.10)

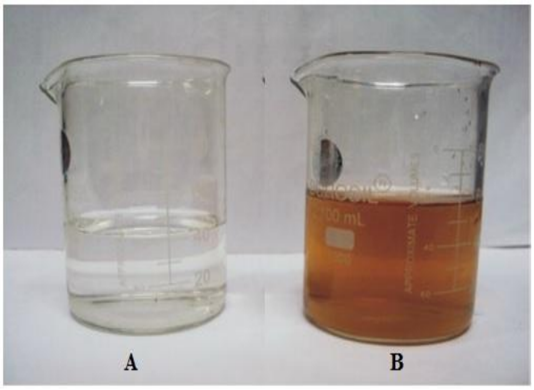

Figure 8. Changing the color of the solution when forming Ag nanoparticles: $AgNO_3$ before adding A-wormwood extract, after adding B-extract

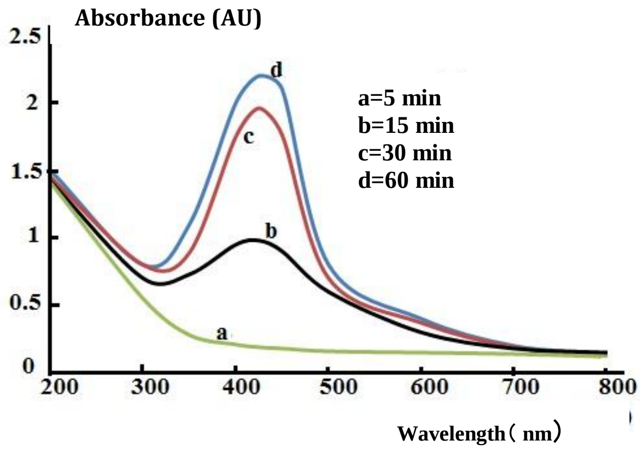

Figure 9. Dependence of intensity of UV-vis spectra on wormwood extract of Ag nanoparticles on exposure time

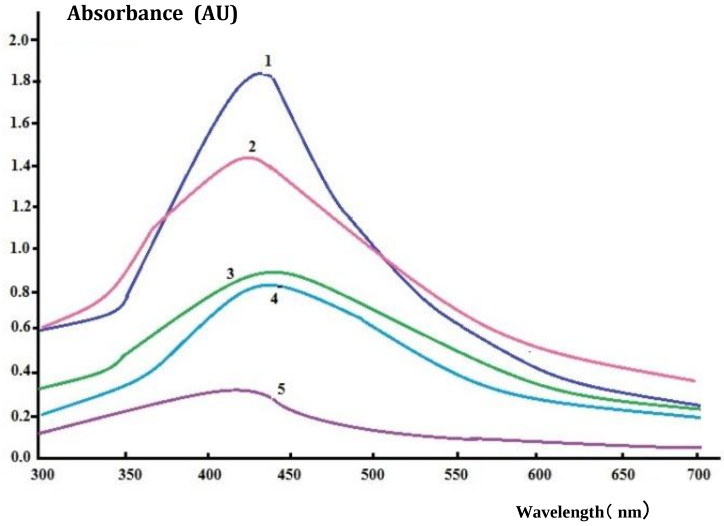

Figure 10. UV-vis spectra of Ag nanoparticles synthesized in wormwood extract at different concentrations of $AgNO_3$ salt: 1- 1 mM; 2 - 2 mM; 3 - 3 mM; 4 - 4 mM; 5 - 5 mM

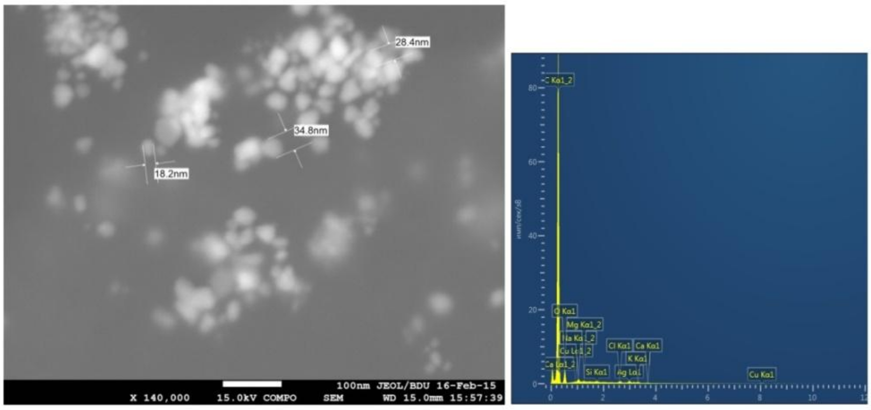

Figure 11. Histogram of SEM image (A) and elemental analysis (B) of silver nanoparticles synthesized in wormwood extracts

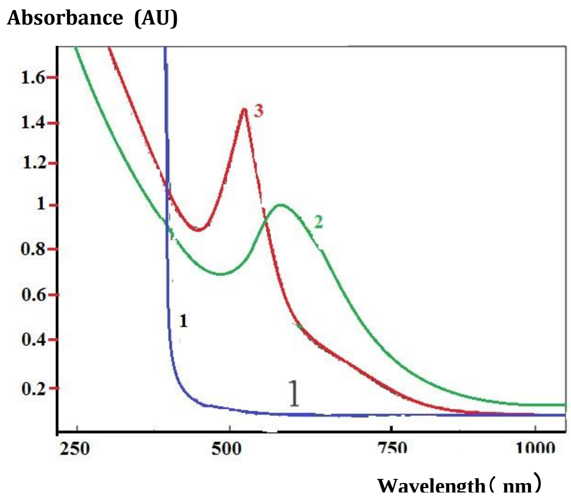

Figure 12. UV-vis spectra of gold nanoparticles synthesized in wormwood extract (1), in homogenate (3), in the extract (2)

Synthesis of silver and gold nanoparticles on extract of onion plant. Both a bulb of onion and its leaves were used to investigate the synthesis of silver nanoparticles on the ordinary onion plant.In the fig.13 was shown the UV-vis spectra of Ag and Au nanoparticles that are synthesized in the extract of ordinary onion.

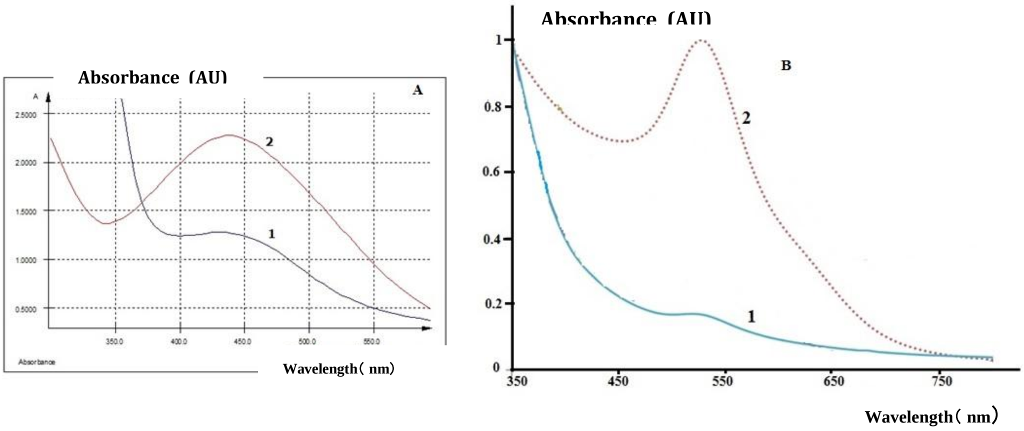

Figure 13. UV-vis spectra of Ag (A) and Au (B) nanoparticles that are synthesized in the extract of ordinary onion: 1. Extract from the onion leaves, 2- in extract from bulb

Synthesis of silver and gold nanoparticles in homogenate of pomegranate seeds. Homogenate of pomegranate seeds taken from pomegranate is selected for preparation and washed in distilled water. The seed are crushed until the juice is squeezed after being dried. 25 ml of the obtained solution is taken and 1 ml of $AgNO^3$ 10^{-1} M silver salt solution is added to the thermostatic container. It is then heated to a temperature of 60^0C for 10 minutes, mixed in a magnetic mixer.In the fig.14 was shown UV-vis spectrum of Ag and Au nanoparticles synthesized in pomegranate homogenate.

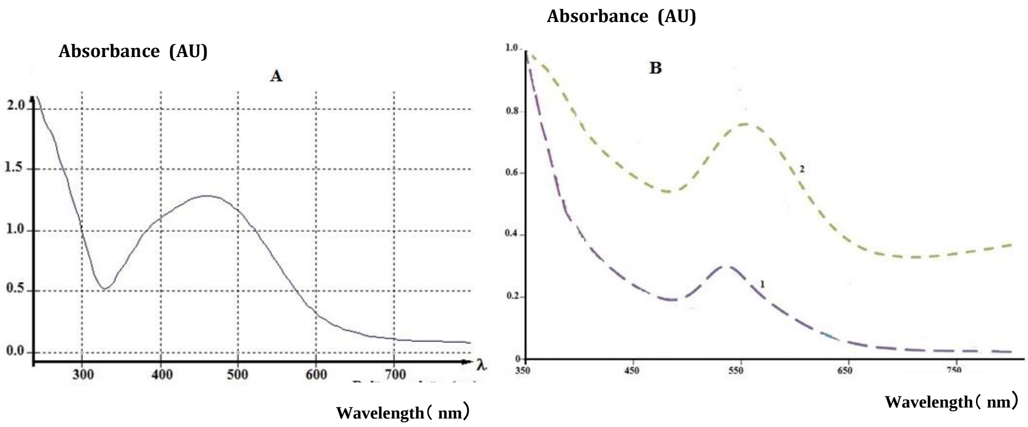

Figure 14. UV-vis spectrum of Ag (A) and Au (B) nanoparticles synthesized in pomegranate homogenate

Synthesis of silver and gold nanoparticles in the extract of saffron. In our experiments, one of the specific plants of Azerbaijan - saffron was used. Synthesis of nanoparticles in saffron plant, especially precious metals - silver, gold and so on are scientifically interesting. Since most of the active compounds in saffron are reductive agents, it is possible to synthesize nanoparticles through them. The dimensions of silver nanoparticles were basically within a range of 60-70 nm. Gold nanoparticles are formed, but their dimensions are considerably larger. In the fig.15 was shown UV-vis spectra of Ag nanoparticles synthesized in extract of saffron plant and Au nanoparticles synthesized in saffron extract.

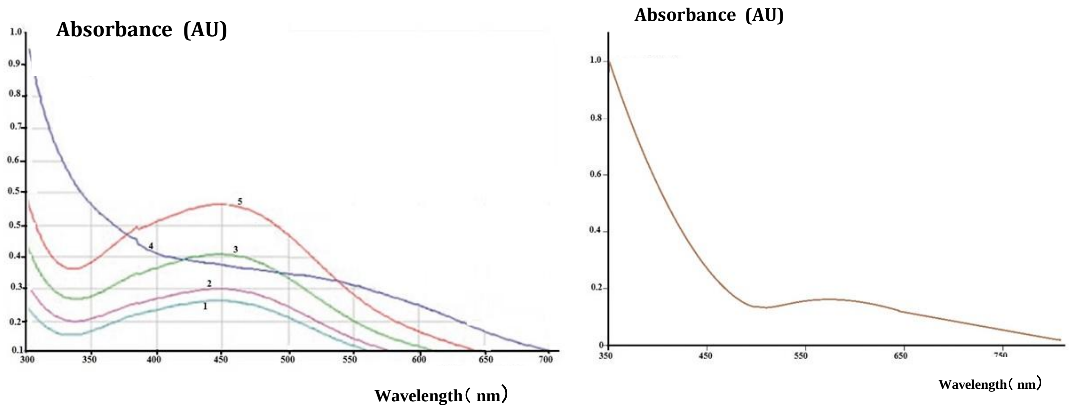

Figure 15. UV-vis spectra of Ag nanoparticles synthesized in extract of saffron plant (in the left): 1 - 1: 9; 2 - 1: 7; 3 - 1: 5; 4 - 1: 3; 5 - 1: 1;UV-vis spectrum of Au nanoparticles synthesized in saffron extract (in the right)

Synthesis of silver and gold nanoparticles in the extracts from the seeds of the ginger plant. 1.6 g of the crushed ginger seeds were weighed and boiled for 10 minutes in 250 ml of distilled water in a 500 ml thermostatic tube. Then the extract was cooled to 60^{0}C. Various varieties of cold extracts were added to various amounts of $AgNO_3$ salt. In the fig.16 was shown the UV-vis spectra of Ag and Au nanoparticles synthesized in the extract of ginger seeds dependence the amount of extract.

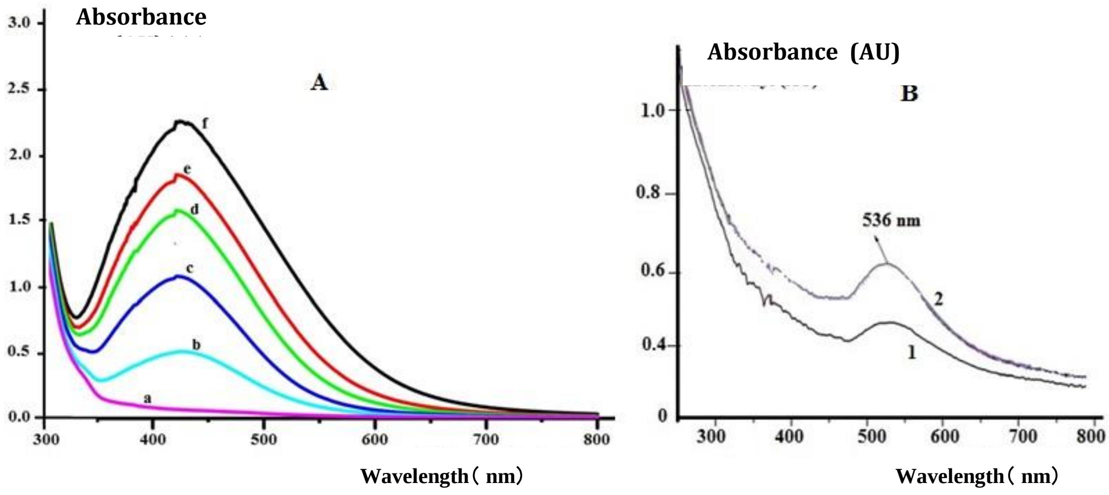

Figure 16. Dependence of the UV-vis spectra of Ag (A) and Au (B) nanoparticles synthesized in the extract of ginger seeds on the amount of extract: a - extract solution; b - 1 ml; c - 5 ml; d - 7 ml; e - 10 ml; f - 15 ml, 1 extract was added. 1 - 1 ml of 10-1 M $HauCl_4$, 2 - 2 ml of 10-1 M $HauCl_4$ were added to the green ginger extract.

Synthesis of silver nanoparticles in the extract from the root of *Scutellaria baicalensis* plant. In the experiments, 1,6 g of root powder was extracted from the *Scutellaria baicalensis* and dissolved in 100 ml of distilled water. 5.10-3 M AgNO3 solution was used for the synthesis of silver nanoparticles. 50 ml of extracts were added to 450 ml of $AgNO_3$ solution for synthesis and heated with mixer at 60-70^oC. The resulting solution is kept at room temperature for 24 hours, and then its characteristics are studied.In the fig.17 was shown UV-vis spectra of Ag nanoparticles synthesized in extracts of root of the *Scutellaria baicalensis*.

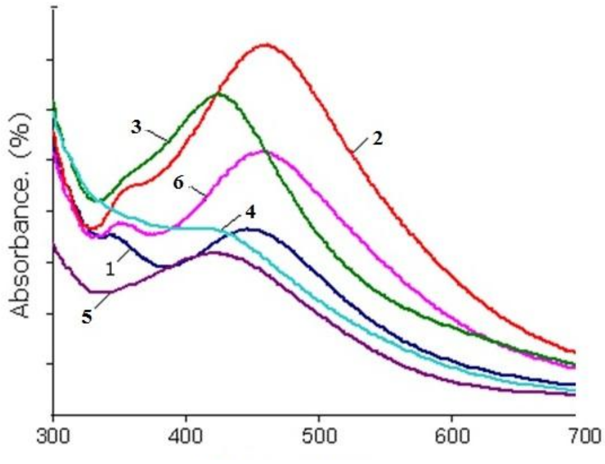

Figure 17. UV-vis spectra of Ag nanoparticles synthesized in extracts of root of the *Scutellaria baicalensis* 1- After 24 hours; 2- after 15 days; 3- after 1 month

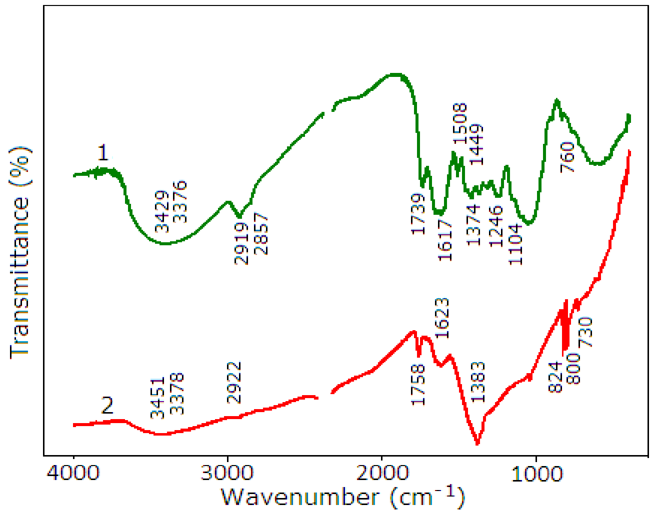

Figure 18. FT-IR spectra of Ag nanoparticles synthesized in extracts of root of the *Scutellaria baicalensis*: 1- root extract; 2- of Ag nanoparticles' synthesized solution

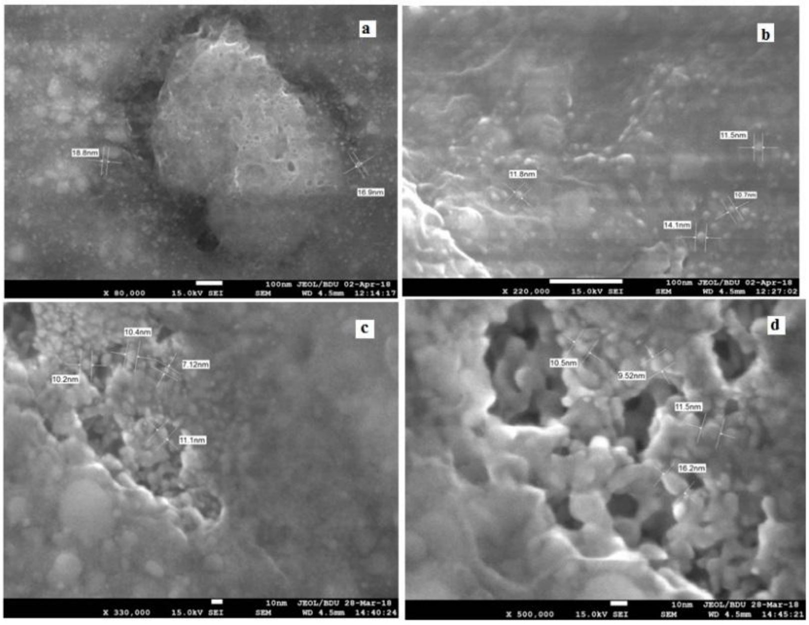

Figure 19. SEM images of Ag nanoparticles synthesized in root extract of *Scutellaria baicalensis*

The study of antibacterial activity of "Green" synthesized silver nanoparticles. For the study of the antibacterial activity of silver nanoparticle synthesized by the plant extracts to the Bacterial cultures (*Candida albicans*) we used two methods: diffusion and the measure of optic density of cultural suspension of bacteria. Firstly prepared Nutrient agar medium for the growth of bacteria in test tubes. Then for single colony isolation, cells were streaked on a Petri dish containing Nutrient agar. The antibacterial activity of silver nanoparticle suspension, bacteria were allowed to grow in Luria broth medium (10 ml) in its presence and absence at 370C with continuous shaking (150 rpm) for 20 hrs. Growth of bacteria was monitored by measuring the optical density of inoculated growth media at regular time interval (4 hrs) by an UV-Vis Spectroscope at 600 nm. During experiments a fresh colony of each strain was picked up from the Petri plate and suspended evenly in separate tubes containing 10 ml Luria broth. The tubes were incubated for an hour; then 1 ml of each culture was inoculated in separate flasks (control and experimental) containing 10 ml Luria broth. To the experimental flasks, 1 ml of nanoparticle suspension was added; in the corresponding control, deionized water was added. A few volume from each culture was withdrawn at regular intervals and the optical density was measured. The OD values of each culture were put against time to draw the growth curves of bacterial strains.

Figure 20 shows that in absence of silver nanoparticles the optical density (at λ = 600 nm) of bacterial culture increased steadily up to 16 hrs. indicating rapid bacterial growth, while in presence of silver nanoparticles there was a distinct reduction in the growth of *Candida albicans*. This confirmed the antibacterial effect of 'Green' silver nanoparticles on both Gram-positive *Candida albicans.*

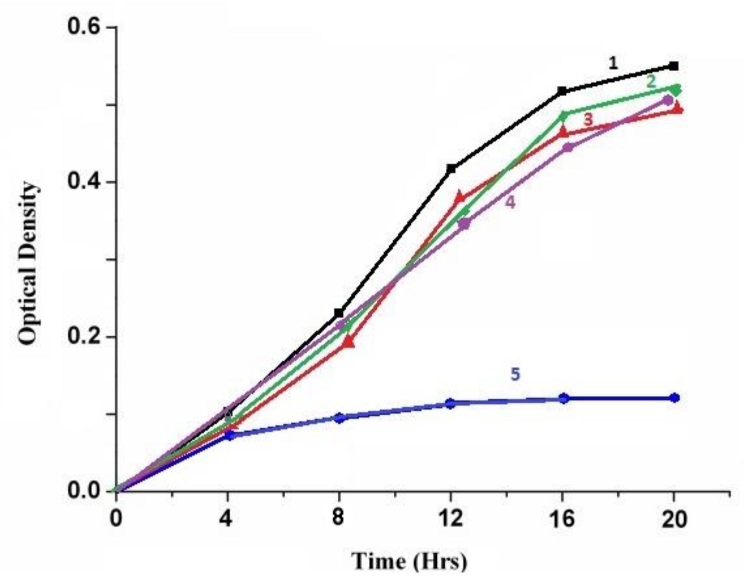

Figure 20. Optical density of bacterial culture in silver nanoparticles "Green" synthesized silver nanoparticles by different plant extracts: 1- control; 2 - bulb of ordinary onion ; 3 - ginger seeds; 4 - pomegranate seeds;5- wormwood;

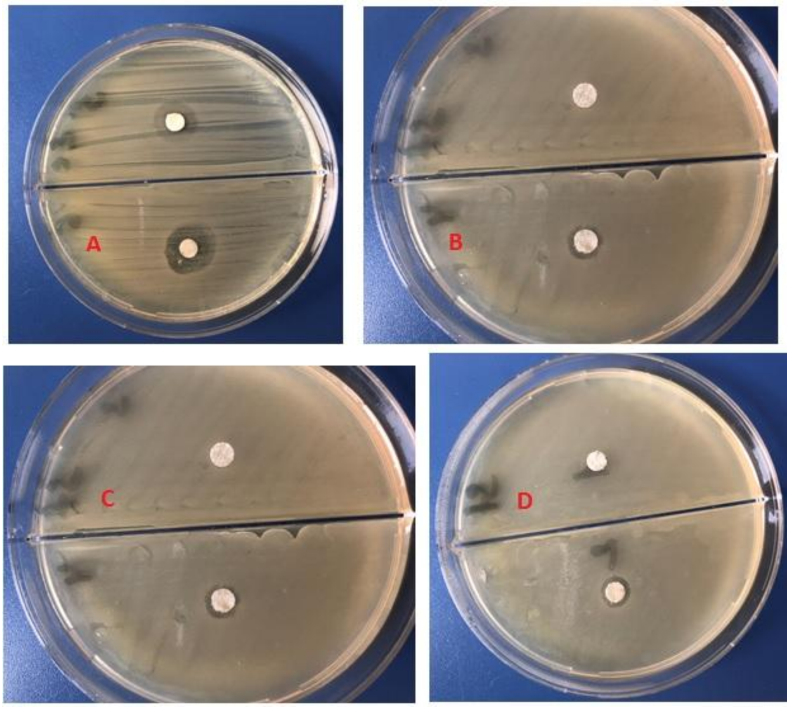

Figure 21. The anti-bacterial activity of aqueous extract of silver nanoparticles in the culture of bacteria *Candida albicans*: A- silver nanoparticles synthesized by wormwood extract; B – by bulb ordinary onion extract; C-by ginger seeds extract; D- by pomegranate seeds.

In the second method for the determination anti-bacterial activity of aqueous extract of silver nanoparticles was used diffusion method. The culture of bacteria *Candida albicans* was inoculated by spread plate method. Gentamycin disc was used as standard control and distilled water was used as control for the extract. The plates were then incubated for 24hours at 37°C.The anti-bacterial activity of aqueous extract of silver nanoparticles in the culture of bacteria Candida albicans was studied nanoparticles synthesized by wormwood extract, by bulb of ordinary onion extract, by ginger seeds extract and by pomegranate seeds. The results of these experiments was shown in the fig.21.

4. Results

- Synthesis of silver nanoparticles through plant extracts depends on the pH of the extract, temperature, density and exposure time. For synthesis of stable and spherical shaped silver nanoparticles, the optimum pH should be 5-8, the temperature should be in the range of 60^0C to 70^0C.
- UV-vis and SEM analysis showed that the dimensions of silver nanoparticles taken from the wormwood extract were a range of 15-35 nm, gold nanoparticles 5-10 nm and are in spherical form.
- Results of experience shows that silver nanoparticles synthesized on the bulb of ordinary onion have relatively small dimensions (30-40 nm), gold nanoparticles is within a range of 10-20 nm and their size distribution is broad. Analysis of FTIR spectra has revealed that OH groups play an important role in the synthesis of silver and gold nanoparticles in the bulb extract.
- Sizes of silver nanoparticles which are synthesized in ginger seeds are relatively small and are within a range of 30 to 40 nm. The distribution according to the sizes is normal and the resulting nanoparticles are homogeneous in size.
- Based on the maximum peak wave lengths of the absorption spectrum in the UV-vis spectrum, it could be stated that the silver nanoparticles synthesized in extract of pomegranate seeds are within the range of 20-25 nm, gold nanoparticles synthesized in the extract of pomegranate peel are within the range of 40 to 50 nm.
- Largest size of nanoparticles is observed in the saffron extract. The size of silver nanoparticles obtained by saffron extract is within a range of 60-70 nm. The size of the gold nanoparticles obtained by the saffron extract is within a range of 60-70 nm.
- The study of the anti-bacterial activity of "Green" synthesized silver nanoparticles by different plant extracts shows that gold nanoparticles decrease the grows of bacteria. The decrease range is strongly in wormwood extract than in extracts of other given plants

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