

## THE SYNTHESIS OF THE SILVER NANODRUGS IN THE MEDICINAL PLANT BAIKAL SKULLCAP (*SCUTELLARIA BAICALENSIS GEORGI*) AND THEIR ANTIOXIDANT, ANTIBACTERIAL ACTIVITY

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**Abstract.** During the synthesis process of silver nanoparticles (AgNPs) by the plant extracts, the biological active molecules in their content act in the role of both the reducing agent and the stabilizer for the formation of the nanoparticles. These molecules acting as stabilizer may form the cover on the surface of the nanoparticle, or may be jointed in the branched form. So, the antioxidants containing in the extract of *Scutellaria baicalensis* may cause the formation of the silver nanoparticle – nanomedicine functionalized by connecting in the form of both the cover and the separate molecules in the synthesis process of the silver nanoparticle. It is possible to obtain the silver nanoparticles carrying out the antioxidants in the content of the Baikal skullcap that, it means the formation of the nano - drugs of new type. In this study were developed the practice of synthesis technology of AgNPs by the extracts from *Scutellaria baicalensis*, determined the sizes, forms of the synthesized nanoparticles by SEM, XRD, and UV-vis spectroscopy. By FT-IR analysis determined the connection of baicalin and baicalein molecules of *Scutellaria baicalensis* to the surface of AgNPs. Have been investigated the antioxidant and antibacterial activity of the AgNPs and antioxidants complexes.

**Keywords:** Nanoparticles, *Scutellaria baicalensis*, silver nano-drugs, antioxidants, antibacterial activity.

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### 1. Introduction

Last years one of the most extensive fields of application of the nanotechnology is a medicine. Such intensive application of the nanoparticles require their ecological clean forms. It was determined that, the nanoparticles may cause the certain toxically affects being collected in the human organism. Therefore it is necessary to get the ecological clean types of nanoparticles, which to be applied as nano-drugs (Akbarzadeh *et al.*, 2018; Ahmadkhani *et al.*, 2017). The silver nanoparticles (AgNPs), which have unique properties and great practical importance become more famous in practical medicine. The high surface energy of AgNPs creates the strong surface reactivity (for example, absorption, catalysis) allow them use as drug delivery. The synthesis of ecological clean forms of AgNPs may be realized by using mainly the bacteria, fungus and polypeptides, nucleic acids and at last, the different plant extracts. The AgNPs are the stable nanoparticles in the colloid disperse solution with the electronic and antiseptic features. The AgNPs are obtained from the reduction of Ag<sup>+</sup> ions and are formed as the particles

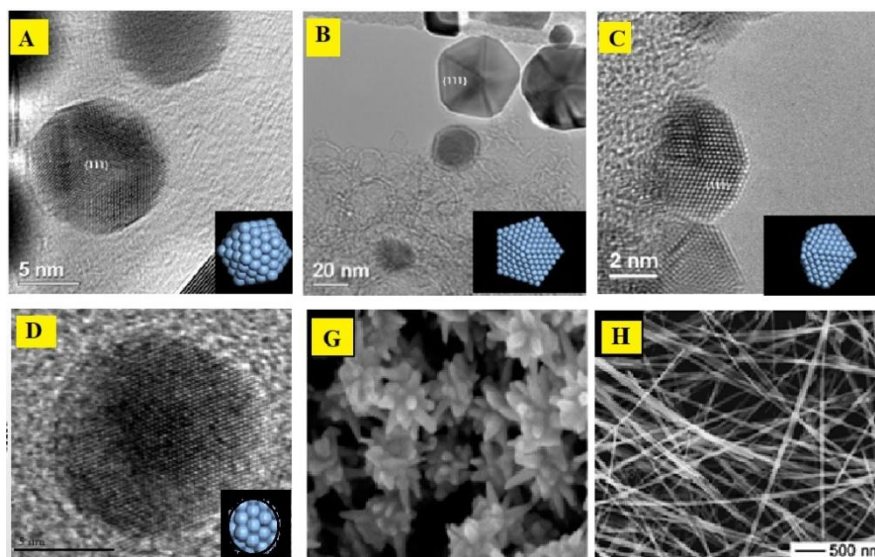
with the different sizes. The stabilization of the AgNPs, the regulation of their sizes and forms depend on nature of oxidation agents, the factors of environments such as pH, temperatures and concentration of extracts and silver nitrate solutions. The synthesized AgNPs by different methods are smallest size of in 2-5 nm, their forms may be spherical, cubic, flower, with lays, triangular, polygonal, irregular and etc. (Fig. 1). The formation of the AgNPs in solution may be determined first of all with the change of the color of the solution. The change of color of the solution is happened due to the formation of surface plasmon effect of nanoparticles (Fig. 2). It was determined that, AgNPs synthesized by the plant extracts have suitable sizes, forms and properties for the functionalization them as drug. The properties and sizes of AgNPs depend on type of plants, the concentration, temperature, pH, exposition period and at last on synthesis method of the extract.

In recent years, biological synthesis of silver nanoparticles has been carried out using extracts and homogenates made from many different organs of plants (roots, stems, leaves, seeds, fruits, etc.). For example, extract of wormwood (*Artemisia absinthium*), pomegranate (*Punica granatum*), ordinary onion (*Allium cepa*), saffron (lat. *Crocus*), cardamom (lat. *Amomum*) and Baikal skullcap (*Scutellaria baicalensis*) (Ahmadov *et al.*, 2019a; Ahmadov *et al.*, 2019b; Nasibova *et al.*, 2016), leaf extract of *Belosynapsis kewensis* (Bhuvaneshwari R. *et al.*, 2016), aqueous leaf extract of *Azadirachta indica* (Ahmed *et al.*, 2016), root extract of *Helicteres isora* (Bhakya *et al.*, 2015), leaf extract of *Chrysanthemum indicum* (Arokiyaraj *et al.*, 2014), leaf extracts of *Ocimum Sanctum* (Tulsi), (Jain *et al.*, 2017.) the aqueous extract of *Citrullus lanatus* fruit rind (Ndikau *et al.*, 2017), extract of *Origanum vulgare L* (Shaik *et al.*, 2018), the fruit extracts of *Momordica charantia* (Malaikozhundan *et al.*, 2016), Extracts of a diverse range of *Ziziphora tenuior* (Sadeghi *et al.*, 2015). The nanoparticles obtained in "green" synthesis are stable, different sizes (5-80 nm) and mainly spherical. It is possible to come to a conclusion that, it is possible to obtain the silver nanoparticles in the different measure and forms through the extracts prepared from the different parts of the majority of plants. The characterization of the colloid solutions obtained through these extracts, the cleaning of the silver nanoparticles from these colloid solutions requires a special technology. The important works have begun in this field already.

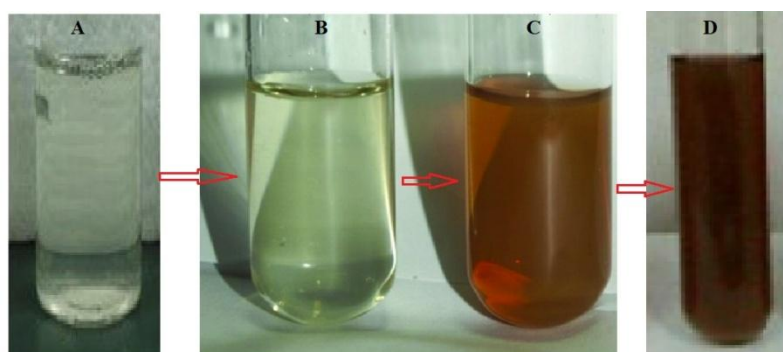
An analysis of the scientific literature in this field has shown that the synthesis of nanoparticles using the plant Baikal skullcap (*Scutellaria baicalensis Georgi*) has been little studied. The *S. baicalensis* is one of the most widely used medicinal plants, and last years has created greater interest among the plants used for the synthesis of AgNPs. In the experiments of Ahmadov and his colleagues AgNPs was synthesized using extracts of from root and aerial part of *S. baicalensis* and obtained spherical forms nanoparticles with size ranging from 7.12 nm to 18.8 nm (Ahmadov *et al.*, 2019). Yulizar and his colleagues synthesized Au nanoparticles using an extract from the leaves of the *Polyscias scutellaria* plant and used them as a catalytic agent in the decolorization of methylene blue dye. The dimensions of the obtained Au nanoparticles ranged from 5 to 20 nm (Yulizar *et al.*, 2017). An another study is the experiments of Chen and his colleagues. They achieved the synthesis of ZnO nanoparticles through an extract from the root of the plant *S. baicalensis*. They studied the catalytic properties of the synthesized Zn nanoparticles, their physical characteristics (Chen *et al.*, 2019). Apart from these studies, there are no experiments in the scientific literature on the synthesis of nanoparticles by the plant *S. baicalensis*. The content of this plant consists a

great number of flavonoids - antioxidants which used as medicine since the ancient times in the content of this plant. These antioxidants may act in the role of both oxidant and stabilizer in the synthesis of silver nanoparticles. During the synthesis process of AgNPs the molecules of the antioxidants in the content of the Baikal skullcap may connected to the surface of the nanoparticles cause their functionalization as nano-drugs.

The rich antioxidants in the Baikal skullcap and the use of molecules in the medicine are important in both the scientific and practical view point. The use of antioxidants as medicines requires the obtaining of the clear forms of these molecules. The nanobiotechnological methods offer an opportunity for obtaining of the antioxidants in the molecular level. The probability of combination of the antioxidant molecules on surface of Ag nanoparticles synthesized in the extract of Baikal skullcap gives opportunity for synthesis of the nanoparticle-antioxidant as nano-drugs in the experiment. The separation of the antioxidants through Ag nanoparticles from the content of the Baikal skullcap are the first levels carried out in this field, and the realization of these opportunities in the project have been tried. The synthesis of silver nanoparticles in the extracts of Baikal skullcap is an innovation in the researches carried out in the project, and it is characterized with its urgency.



**Fig. 1.** The TEM images of different forms of silver nanoparticles



**Fig. 2.** Discoloration of silver nitrate solution during the formation of AgNPs: A- silver nitrate solution; B - after the addition of extract of *S. baicalensis*; C- through 24 hours; D - after 72 hours

## 2. Materials and methods

**Baikal skullcap (*Scutellaria baicalensis* Georgi).** The Baikal skullcap known as a medicinal plant is a genus of flowering plants in the *Labiatae* family. *S. baicalensis* is a perennial growing to 0.3 m. It is in flower in from June to August, and the seeds ripen in September (Kim *et al.*, 2014). The fruits are in the form of walnut, and after being ripen the seeds are spread. It is said that, the Baikal skullcap has about 300 types and sometimes 400 types. The natural spreading areal of the Baikal skullcap is very wide. It is possible to meet this plant in the Eurasia, Northern America, Caucasus, Siberian zone of Russia, and especially around the lake Baikal. Some of its types are growing in the regions of Azerbaijan such as Aghsu, Shamakhi, Goychay and Aghdash. It was found for the first time around the lake Baikal, and its name is connected with the name of the lake (Fig. 3).

The flavonoids - antioxidants are the important medicines that contain this plant. These antioxidants are very rich in the root part of the plant. The flavonoids such as the baicalin, baicalein, steroids, wogonin, saponins, kumarin, chrysenes, pirokatekhins are obtained from the root of the Baikal skullcap, as well as, the starch, essential oils, abrasives, resin, micro and macroelements are rich in the roots of this plant (Long *et al.*, 2015; Gasiorowski *et al.*, 2011; Horvath *et al.*, 2005; Hassanpour *et al.*, 2018). The biodiversity of its content gives opportunity for the sedative, hypotensive, antiseptic, anti-inflammatory, hemostatic, corrosive, vasodilator, anthelmintic, anticonvulsant, anti-sclerotic affects. Besides, the plant is a good antioxidant, hypoallergenic and the immunomodulator (Heo *et al.*, 2004; Hwang *et al.*, 2005; Nasibova *et al.*, 2017).



**Fig. 3.** The roots (in the left), the body with flowers and leaves (in the right) of the Baikal skullcap

**The chemical content of the Baikal skullcap.** The extract of the Baikal skullcap has been used till the modern development of the biotechnologies in the national medicine. The root of the plant is used more cases in order to obtain these extract. The extracts from the roots used in the form of solution as medicine. But the flavonoids, antioxidants, as well as, some interesting organic combinations are obtained by applying the modern methods for obtaining the affecting molecules in its content. It was possible to separate in clean form several flavonoids from the roots. Here belongs the baicalin, baicaline, oroxylin A, wogonin, skutelarin, chrysenes and others. The caustic matters,

steroids, kumarins, resin and essential oils may be shown as other combinations. Currently, about 100 flavonoid combinations are separated from the root of these plants.

**Baicalin (C<sub>21</sub>H<sub>18</sub>O<sub>11</sub>).** Recent studies have stated that Baicalin is used as an active drug in vitro and in vivo circumstance against influenza viruses (Ding *et al.*, 20014; Zhu *et al.*, 2014), dengue viruses (Zandi *et al.*, 2012), Enteroviruses (Moghaddam *et al.*, 2014), and Japanese encephalitis viruses (Johari *et al.*, 2012). Baicalin is used as an active flavonoid against some tumor diseases. Due to its effect, the cell cycle is blocked and the growth and breaking of the tumor cells are inhibited. It plays a photoprotector role against UV rays and photosynthesis caused by oxidative stress (Fig. 4).

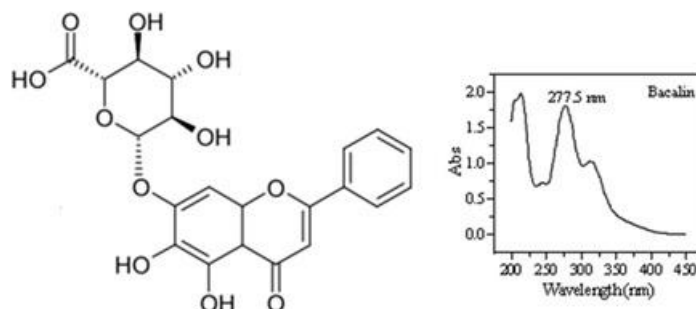


Fig. 4. The chemical structure of the Baicalin and its absorption spectra

**Baicalein (C<sub>15</sub>H<sub>10</sub>O<sub>5</sub>).** Baicalein is isolated from the roots of the Baikal skullcap as one of the flavonoids. Baicalin is an active ingredient of Sho-Saiko-To the Chinese grass addition, it is considered that, this matter helps the liver to remain healthy. It has been determined that this molecule is an antagonist to the estrogen and antiestrogen receptors. The experiments have stated that, this flavonoid inhibits the certain types of lipoxygenases and is used against the cold. The baicalein is an aglycon of the baicalin and blocks completely the proliferation of the cells (60 and 90 μmol) in the high thicknesses, decreases G<sub>0</sub>-G<sub>1</sub> phases of the cell cycle during 24 hours, but increases the period of G<sub>2</sub>-M phases (Fig. 5).

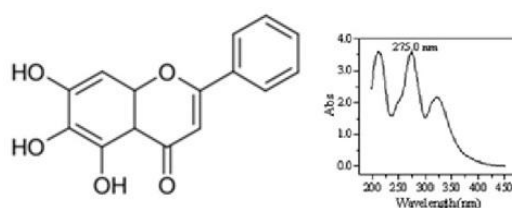


Fig. 5. The chemical structure of the Baicalein and its absorption spectra

**Wogonin (C<sub>16</sub>H<sub>12</sub>O<sub>5</sub>).** Wogonin (5,7-Dihydroxy-8-methoxyflavone) has been separated from the content of the chemical combinations of flavonoid type and Baical skullcap (Fig. 6). It was determined that, wogonin shows the sedatives in 7,5 – 30 mg/kg doses in the mouse having the anxiolytic feature. Besides, it has been detected that, the opportunity of using of wogonin against the tumor diseases during the research of the pharmacological effects. Even it was offered to use them against the convulsions. It has anti-oxidant effects on eye inflammation (Xiao *et al.*, 2014)

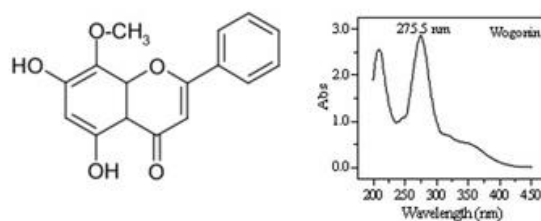


Fig. 6. The chemical structure of the Wogonin and its absorption spectra

**Chrysin**( $C_{15}H_{10}O_4$ ). The chrysin is a pigment of several flowering plants from the flavon family. The main biological function of this pigment is a involvement of the insects in the pollination of the flowers. Its main source is flowers belonging to the family of Passiflora. There is also baicalin in the seeds of Oroxilin along with the chrysene. The extracts obtained from the Oroxyline are used widely in the national medicine of India and Southern-Eastern Asia. This extract is used in order to remove the diseases of the respiratory system, cough and snoring. The sportsmen use the chrysene in order to keep the hormonal balance. It decreases the amount of estrogens in the blood. The chrysene has been detected about in 50 plants, sometimes it is found in small amount in the honeycombs. It has been determined during the effect of the chrysene to the animal organisms that, the oxidative metabolisms is not happened.

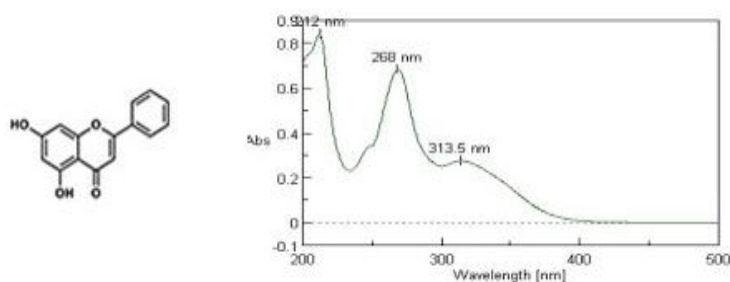


Fig. 7. The chemical structure of the chrysene and its absorption spectra

**Characterization of silver nanoparticles.** The absorption spectrum of AgNPs solution was taken in UV-Vis spectrum (SPECORD 250 PLUS, Analytic Jena, Germany). The AgNPs were powdered and characterized by Scanning Electron Microscopy (SEM, JEOL-MODEL 7600F) and Fourier Transform Infra-Red spectroscopy (FT-IR, Varian 3600).

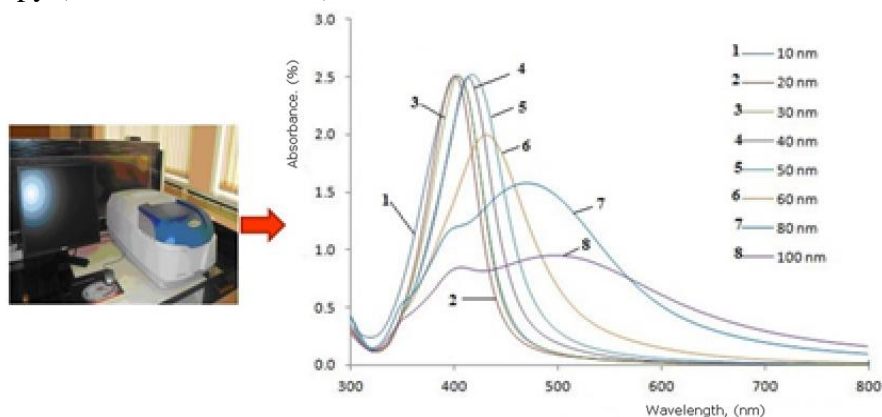
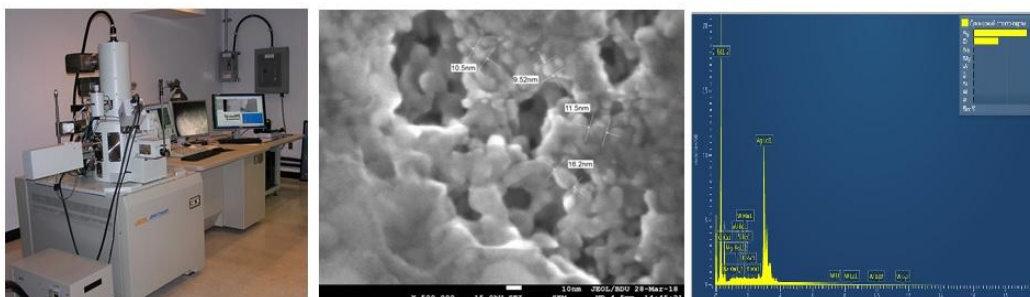
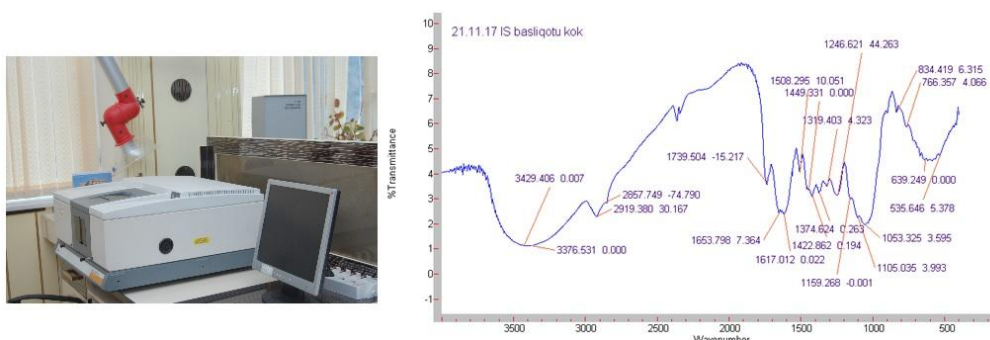


Fig. 8. UV-vis spectrum and absorption spectra of the pattern with the AgNPs



**Fig. 9.** Modern Scan Electron Microscope – JSM 7600F, images of the nanoparticles and the histogram of the element analysis



**Fig. 10.** Modern FT-IR spectrometer and spectrum of AgNPs

**Preparation of extracts.** *S. baicalensis* plants were gathered from different regions of Azerbaijan. The roots, leaves and flowers were dried and powdered using a grinder. The aqueous extract of *S.baicalensis* prepared using 500 ml Erlenmeyer flask containing 1,6 g powder and 100 ml deionized water and heated at 70<sup>0</sup> C using a hot plate for 2 h. The extract was obtained by centrifuge the mixture at 5 000 rpm for 3 min followed the filtration using whatman no.1 filter paper. These filtrates were stored in the refrigerator for 24 hours.

**Antioxidant activity. 2,2-diphenyl-1-picrylhydrazyl (DPPH) method.** The DPPH method was used to measure the antioxidant activity of AgNPs (Kim *et al.*, 2014). Briefly, a solution of 23 mg/ml DPPH was dissolved in ethanol, and then the optical density of the solution was measured at 517 nm. In this test, DPPH, as a free radical, was reduced to diphenylpicrylhydrazine after exposure to antioxidants. The ability to absorb radicals was calculated by the formula below.

$$\text{Percent Radical Purification DPPH} = \frac{A_c - A_s}{A_c} \cdot 100\%$$

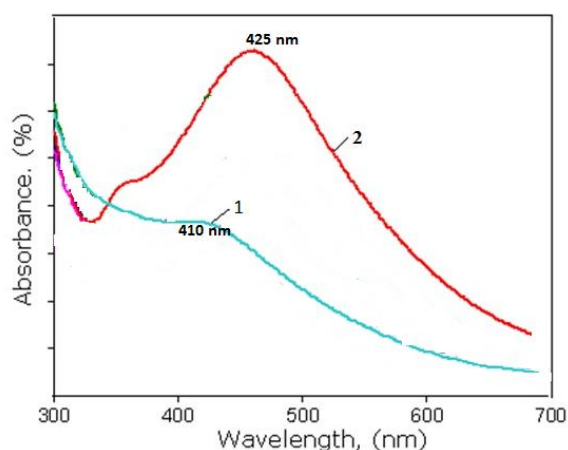
where  $A_c$  and  $A_s$  denote the absorption of the control sample and the sample, respectively. A control solution was prepared by mixing ethanol and a solution of DPPH radicals.

**Antibacterial activity.** For the study of the antibacterial activity of silver nanoparticle synthesized by the plant extracts to the Bacterial cultures (*B. subtilis* and *E. coli*) we used two methods: diffusion and the mesaure 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.

### 3. Experiments

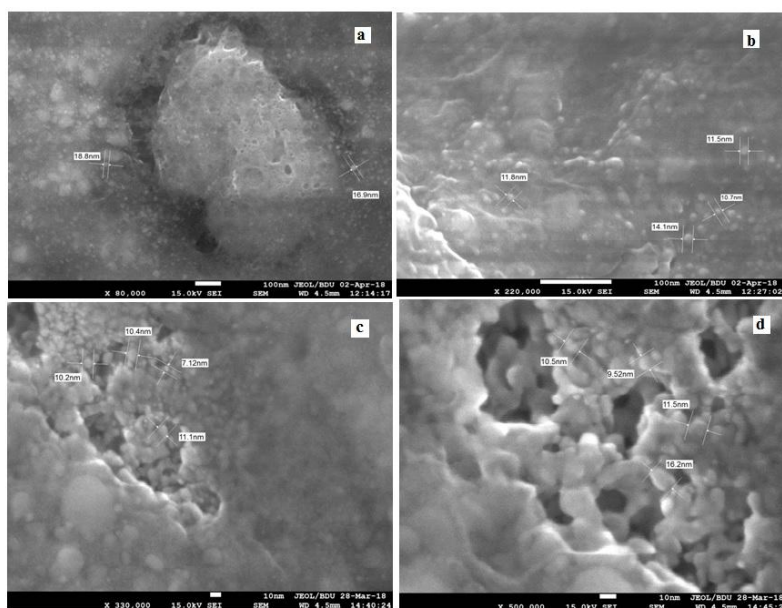
**“Green” synthesis of Ag nanoparticles.** Recently the results of the experiments of the scientists are more by archiving the synthesis of the silver nanoparticles in the plants. It is possible to come to a conclusion that, it is possible to obtain the silver nanoparticles in the different measure and forms through the extracts prepared from the different parts of the majority of plants. The characterization of the colloid solutions obtained through these extracts, the cleaning of the silver nanoparticles from these colloid solutions requires a special technology. The stabilization of the silver nanoparticles, the regulation of their sizes and forms depend on nature of reducing agent, the factors of environments such as pH, temperature and concentration extracts and solutions. The formation of the silver nanoparticles may be determined first of all with the change of the color of the solution. The change of color of the solution is happened due to the formation of surface plasmon effect on silver nanoparticles (Fig. 2). For the synthesis of AgNPs we have take the solution 5.10-3M AgNO<sub>3</sub> and 50 ml extract which was prepared from roots and from aerial parts of *Scutellaria baicalensis*. 50 ml the solution of extract has been added to 200 ml silver nitrate solution (5.10-3M) in 500 ml volumetric flask, heated 10 minutes at 70 C with stirred using a magnetic stirrer at room temperature in the flask of 500 ml. The change in color of the solution after 10-30 minutes indicated the reduction of silver nitrate. The solution achieved is stored under the room temperature for 24 hours, and its UV-vis analysis has been performed. The result of this experiment has been given in the Fig.11. It is seen from the wave length of the picks in UV-vis spectrum that, the sizes of the silver nanoparticles synthesized in the extract achieved from the aerial parts of the plant is less than the sizes of the nanoparticles synthesized by the extract of the root, but the experiments have stated that, the concentration of the nanoparticles synthesized by the root extract may vary depending on the period of the exposition period, extract concentration and the content of the solution of which the extract prepared.



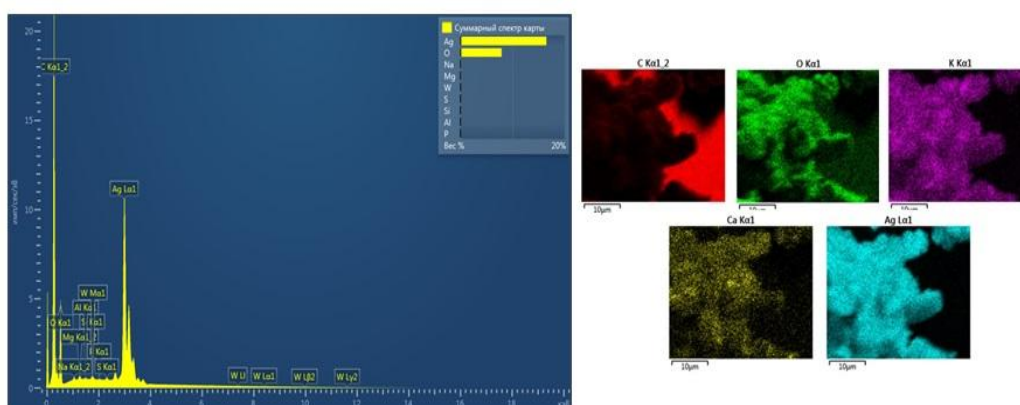
**Fig. 11.** UV-vis spectra of the AgNPs synthesized in the extracts of the aerial part (1) and extracts of the roots of *Scutellaria baicalensis*



**SEM analysis of AgNPs.** The information of surface morphology, on sizes, forms and basic composition of the synthesized AgNPs were investigated using SEM analyses. The AgNPs were spread evenly on the sample holder with the help of a carbon tape and sputter-coated with platinum, by means of an ion coater machine for 50 s before observation under SEM. After obtaining SEM images, the elemental composition of AgNPs also was investigated. It is seen from the images achieved by enlarging 500000 times in the electron microscope that, the sizes and forms of the AgNPs are different (Fig.12 and 13). Here we can see the silver nanoparticles with the measures of 9.52 nm, 10.5 nm and 11.5 nm, 16.2 nm. So, by electing correctly the parameters of the reducing agent and stabilizer (extract of plants) we can achieve the AgNPs in the sizes required.



**Fig.12.** SEM images of the AgNPs synthesized by the extract prepared from the roots of the *Scutellaria baicalensis*.



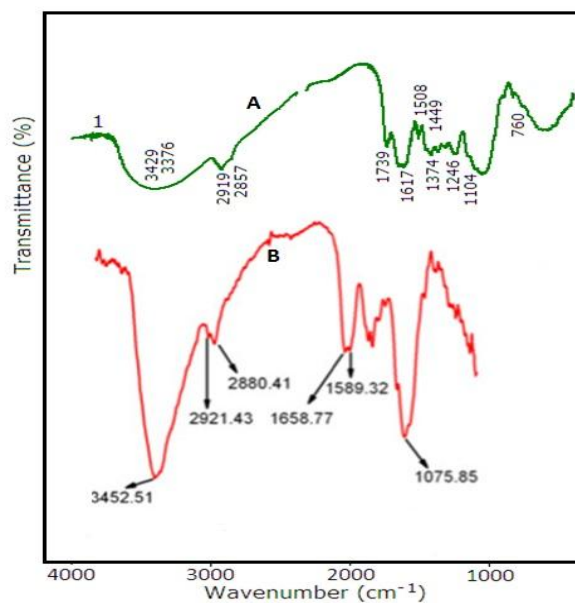
**Fig. 13.** The elemental composition analysis of AgNPs synthesized by the extract from the roots of the *Scutellaria baicalensis*

**FT-IR analysis of AgNPs.** The probability of connection of the antioxidant molecules on the surface of Ag nanoparticles synthesized in the extract of the Baical skullcap gives opportunity for the synthesis of the nanoparticle-antioxidant complex (nano-drugs). An attempt was made to test the release of the antioxidants by Ag nanoparticle from the content of the Baical skullcap. FT-IR analysis method is one of the ways of determination of absorption of the antioxidants on the surface of silver nanoparticles during the synthesis process in the extract and the identification of remaining time stable for a long period. With this purpose, FT-IR spectra in the crystallized patterns of the solution with the AgNPS were drawn and compared with the FT-IR spectra of the clean antioxidants.

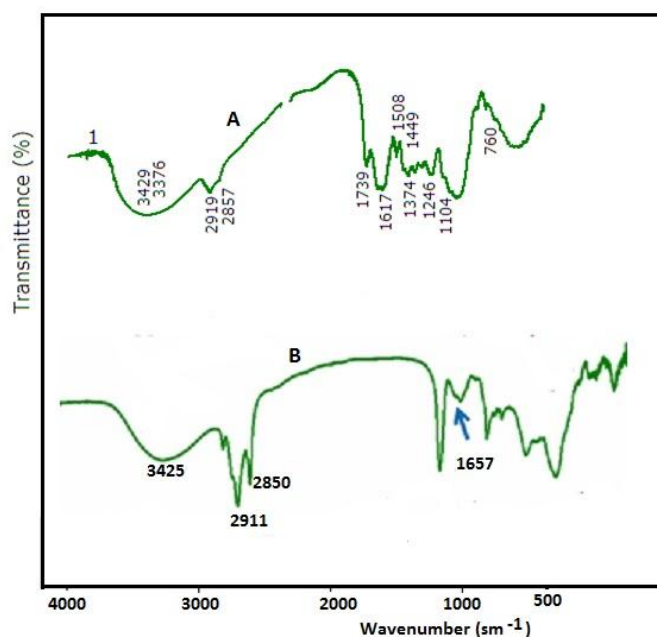
**a) The absorbtion of baicaline on the AgNPs and mechanism of their connection to the nanoparticles.** As we mentioned above the roots of *Scutellaria baicalensis* wery rich the flavonoids such as the baicalin, baicalein, steroids, wogonin, saponins, kumarin, chrysen, pirokatekhins. C.Wu and his colleagues (Wu *et al.*, 2017) have proved that, the flavonoids mentioned in the *Scutellaria baicalensis* are involved in the synthesis of the nanoparticles. The main purpose of our experiments was to determine which of these flavonoids participates most actively and which of them was better integrated into the silver nanoparticle. The experiments have been carried out in the variants given below.

For the FT-IR analysis the AgNPs solution synthesized by the root extract of the *Scutellaria baicalensis* were crystallized and have made FT-IR spectrum. These spectra have been compared with FT-IR spectrum of the clean baicalin powdere obtained from the root o f the plant *Scutellaria baicalensis*. The spectrum A in the Fig. 14 is FTIR spectrum of the crystallized solution of the AgNPs synthesized in the extract gained from the root *Scutellaria baicalensis*, when the spectrum B is the spectrum of clean baykalin powder. 3452 cm<sup>-1</sup> (tension O-H), 2880 cm<sup>-1</sup>, 1617 cm<sup>-1</sup> (C = O) are peaks belonging to the baicalin. The observation of these peaks in the spectrum of the AgNPs shows that baicalin is adsorbed to the surface of the silver nanoparticles.

**b)The absorbtion of baicalein on the AgNPs and mechanism of their connection to the nanoparticles.** In the next experiments the FT-IR spectrum (Fig. 15, B) of the baicalein powder was compared with spectrum AgNPs solution. The wavenumbers crystallized AgNPs synthesized by the extract root of *Scutellaria baicalensis* are 3429 cm<sup>-1</sup> (tension O-H), 2919 cm<sup>-1</sup> (the index of the average valent vibration of the NH group), 2857 cm<sup>-1</sup>, 1617 cm<sup>-1</sup> (C=O). These wavenumbers also are observed in the FT-IR spectrum of baicalein slightly modified form - 3425 cm<sup>-1</sup> (tension O-H), 2911 cm<sup>-1</sup> (the index of the average valent vibration of the NH group), 2850 cm<sup>-1</sup>, 1657 cm<sup>-1</sup> (C=O). It is seen from the FT-IR spectrum (Fig.15, B) of the baicalein powder that, the peaks belonging to the baicalein are also seen in the FT-IR spectrum of the AgNPs solution. It shows that, baicalyin also participates in the synthesis of silver nanoparticles and probably, these molecules may also be connected on surface of the silver nanoparticles.



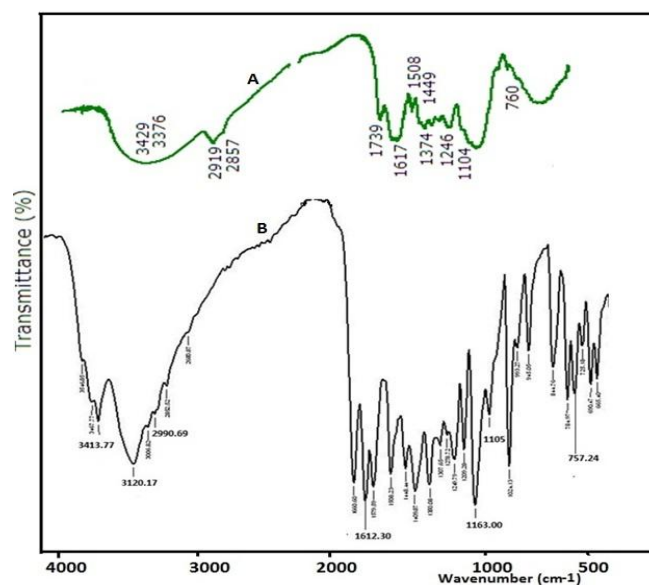
**Fig.14.** FT-IR spectra of the crystallized solution of AgNPs (A) and clean baicaline powder (B)



**Fig. 15.** FT-IR spectra of the crystallized solution of AgNPs (A) and clean baicalein powder (B)

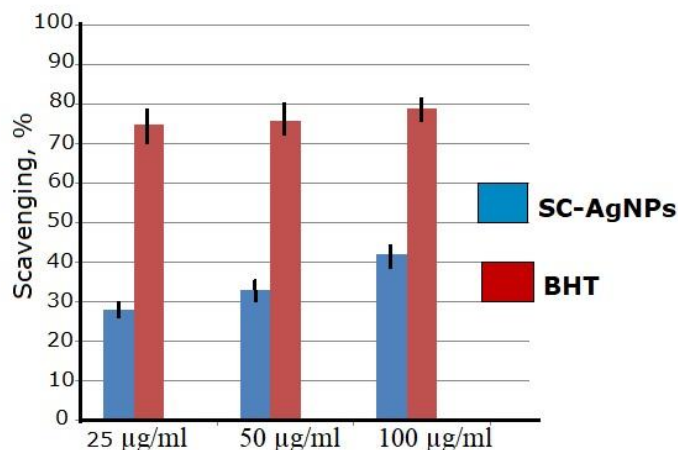
c) **The absorption of wogonin on the AgNPs and mechanism of their connection to the nanoparticles.** In the next experiments the FT-IR spectrum (Fig.16, B) of the wogonin powder was compared with spectrum AgNPs solution. As seen from the Fig. 16, FT-IR spectrum (B) of the clean powder of the wogonin wave number 3413 sm<sup>-1</sup> is a weak pick being in the wogonin powder, and does not conform to the pick of the silver nanoparticles, and the wavenumber 3120 sm<sup>-1</sup> is not observed in the spectrum of AgNPs solution, and the other picks seen in the wogonin are not observed. It shows that, the wogonin molecules do not participate in the synthesis of the silver

nanoparticles, and their connection to the surface of the silver nanoparticles are not supposed.



**Fig.16.** FT-IR spectra of the crystallized solution of AgNPs (A) and clean wogonin powder (B)

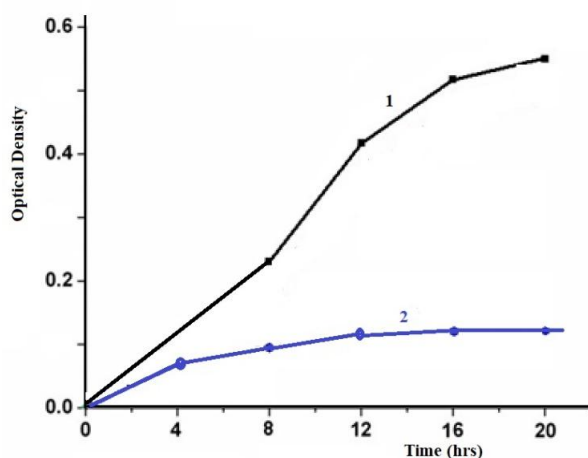
**Antioxidant activity of AgNPs.** The synthesized SC-AgNPs were investigated for their antioxidative potential according to the assays performed in DPPH free radical scavenging. The results are displayed in Fig.17. The DPPH free radical scavenging activity of SC-AgNPs at a concentration of 25–100  $\mu\text{g/mL}$  ranged from 29.13% to 42.31%, whereas that of BHT ranged from 75.14% to 82.21% at the same concentration.



**Fig.17.** Antioxidant scavenging potential of the SC-AgNPs synthesized by root extract of *Scutellaria baicalensis*: DPPH free radical scavenging activity

**Antibacterial activity of AgNPs.** The antibacterial activity of AgNPs synthesized by the root extract of SC was studied by the method of OD (optical density of bacterial grows). 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 volumes from each culture were 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 18 shows that in absence of silver nanoparticles the optical density (at  $\lambda = 600$  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 both *B. subtilis* and *E. coli*. The growth reduction was more prominent in case of Gram-negative bacterium *E. coli*. This confirmed the antibacterial effect of AgNPs on both Gram-positive *B. subtilis* and Gram-negative *E. coli*.



**Fig.18.** Impact of the AgNPs synthesized by root extract of *Scutellaria baicalensis* on the growth of Gram-negative bacterium *E. coli*. 1- in the normal culture; 2- in the culture containing AgNPs

#### 4. Conclusion

In the field of nanobiotechnology for synthesis of metallic nanoparticles it is important the development of reliable and eco-friendly processes. In these experiments we have reported a simple green and low-cost approach for synthesizing of stable silver nanoparticles by reduction of silver nitrate solution with a bioreduction method using the aqueous extracts of root and aerial part of *Scutellaria baicalensis* as the reducing agent. Ag nanoparticles were synthesized by an ecofriendly and convenient method using the extracts of root and aerial part of *Scutellaria baicalensis* at ambient temperature. The results of this experiments, confirm that root extract of *Scutellaria baicalensis* may be used as a reductant for the synthesis of silver nanoparticles. During biosynthesis of AgNPs occurs color change of solution, the UV-Vis spectroscopic analysis shows that by absorption peak at 414 nm – 461 nm interval nanoparticles quantitatively was monitored. Further characterization with SEM analysis shows that synthesized AgNPs have the spherical form, are polydisperse and size ranging from 7.12 nm to 18.8 nm with an average size of 11.35 nm. Interestingly, AgNPs synthesized by extract of roots differ greatly from the aerial part of *Scutellaria baicalensis*. Second interesting fact is that the antioxidants of *Scutellaria baicalensis* participate in the synthesis process AgNPs and may adsorb or connect on surface of AgNPs. By

examining the FT-IR spectra of the synthesized AgNPs we have determined which of these antioxidants are involved in the synthesis of AgNPs and which of them are connected to the surface. The absorption peaks of the wave numbers in the FT-IR spectra show the vibration of the chemical bonds of one or another group of molecules. The weakening and depletion of this vibration determines the bonding of a given group of molecules with the nanoparticles and its adsorption on the surface. The results of the experiments revealed that baicalin and its derivative baicalein are more involved in the green synthesis process. The molecules of these antioxidants are mainly composed of O-H and C = O groups and probably they connected by these groups to the surface of silver nanoparticles. Thus, in the process of green synthesis almost exclusively by *Scutellaria baicalensis* extract, flavonoids are the most important components in the formation and stabilization of silver nanoparticles.

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