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Original Article

Study of the role of some *Scutellaria Iscandaria L*. extract's flavonoids on nanosilver synthesis

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ABSTRACT

Silver nanoparticles are synthesized with the method of "Green synthesis" using Scutellaria Iscandaria extract. The synthesized silver nanoparticles were studied by HPLS and Atomic Weight microscopies (AWM). Silver nanoparticles, like other nanoparticles, are characterized by unique properties associated with a high ratio of their surface to volume, which determines the high efficiency of their action. Much attention is paid to the functional activity of silver nanoparticles in terms of imparting both bactericidal and bacteriostatic properties to various materials and products. Firstly, this will allow a hundred-fold decrease in the metal concentration while maintaining all its bactericidal properties, and secondly, due to the large specific surface of the nanoparticles, the contact area of silver with bacteria or viruses increases, significantly improving its bactericidal action [1]. In accordance with the results of the analysis, the content of flavonoids in the suspension samples with silver nanoparticles shows the complete absence of apigenin and quercetin, and the peaks characterizing Luteolin (-4.3 min), Rutin (-2.6 minutes) remain, although their content decreased to reading 0 , 05 mg / g and 0.0044 mg / g, respectively. The high activity of flavonoids such as luteolin, apegenin, quercetin and rutin has been proven. Therefore, Scutellaria Iscandaria L. can be a promising raw material for the synthesis of silver nanoparticles.

Key words: Silver nanoparticles, ScutellariaIscandaria L. extract, Green synthesis, Atomic Weight microscopies (AWM), HPLC, Luteolin, Apegenin, Quersetin, Rutin

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INTRODUCTION

Physical and chemical methods are widely used to obtain silver nanoparticles. These traditional methods for the synthesis of nanoparticles require a significant amount of energy and the use of toxic substances. For this reason, there is an increasing demand around the world for the production of nanoparticles using pure, non-toxic methods that are based on the principles of "green chemistry". For example, the Green Synthesis method of silver nanoparticles using plant extracts is cheap, fast and environmentally friendly. [2]

At the same time, it was shown that in plants flavonoids can include metal ions in the chelate complex and restore them. Flavonoids are an extensive group of polyphenolic compounds in which several classes are distinguished: anthocyanins, isoflavonoids, flavonols, chalcones, flavones and flavanones. Flavonoids contain various functional groups that can cause the formation of nanoparticles. It has been suggested that the tautomeric transformations of flavonoids from the enol form to the ketoform can release a reactive hydrogen atom, which can reduce metal ions to form nanoparticles. [3]

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 a forming nanoparticle. This means that they take part in the initiation stages of nanoparticle formation (nucleation) and further aggregation in addition to the bioreduction stage. In addition, isolated flavonoids and glycosides of flavonoids have the ability to induce the formation of metal nanoparticles. Among the wide variety of flavonoid-containing plants growing on the territory of Uzbekistan, special attention is paid to Scutellaria Iscandaria L. from the family of Labret (Scutellaria Lamiaceae), which along with various flavonoids is rich in other biological active substances, such as glycosides, essential oils, organic acids , macro and micro elements, tannins, resins, etc. [4]

The aim of this work is to study the role of individual flavonoids of the extract of Scleellaria Iscadaria L. (Scutellaria Iscandaria L.) in the synthesis of silver nanoparticles by comparative chromatographic study of the initial extract of Scutellaria Iscandaria L. and suspension after the formation of silver nanoparticles.

EXPERIMENTAL

The aboveground part of Scutellaria Iscandaria L., collected in July in the Pap district of the Namangan Region of the Republic of Uzbekistan, and the source of silver ions, 0.01 M silver nitrate, were used as plant raw materials. The production of silver nanoparticles was carried out by the method of "green synthesis" in two ways:

In the first case, using a dry extract of Scutellaria Iscandaria L. as the reducing agent (obtained by extraction with 40% ethanol and further freeze drying) dissolved in 40% ethanol in a ratio of 1: 5 [5].

In the second case, water recovery of the dried aerial part of Scutellaria Iscandaria L. was used as a reducing agent. For this, 1 g of ground raw material was placed in a container, 100 ml of purified water was poured, it was placed in a steam bath for 15 minutes, the obtained extract was cooled to room temperature, left for 24 hours and filtered [6].

To obtain silver nanoparticles, 10 ml of silver nitrate solution (1 * 10-3 mol / L) were separately added to 1 ml of the obtained extracts.

Identification and quantification of flavonoids in Scutellaria Iscandaria L. was performed by HPLC with photometric detection on an Agilent 1100 instrument. Luteolin, Apigenin, Rutin, Quercetin standards (ICC lnc, USA Indofine) were used. To prepare a standard sample with a concentration of 0.05 mg / cm3, 5 mg of the standard is placed in a volumetric flask with a capacity of 100 cm3, bring the volume to the mark with methanol. To prepare the sample, add 2 ml of hydrochloric acid to a suspension with silver nanoparticles and heat it in a water bath under reflux for 1 hour 30 minutes. The solution was quantitatively transferred to a 100 cm3 volumetric flask and adjusted to the mark with 70% ethanol. Then the flask is placed for 5 minutes in an ultrasonic bath.
Next, a sample for HPLC analysis is filtered through a membrane filter and used for analysis. Chromatographic analysis conditions: column: 5 μm, 250 × 4.6 (for example, Agilent 5 μm C18 (2)); mobile phase: acetonitrile - 0.1% trifluoroacetic acid solution, pH 2.6 (40:60); mobile phase velocity: 1.0 cm3 / min; Column temperature: 30 ° C; Detection: UV, λ = 254 nm. The volume of the introduced sample: 10 mm3.

The calculation of the content of indicator components is carried out according to the calibration schedule or according to the formula:
$X = C \times S1 \times V\ S2 \times m$,
where: C is the concentration of the corresponding standard solution, mg / cm3;

S1 is the area of the peak of the determined component in the analyzed sample;

S2 is the peak area of the determined component in the standard sample;
V is the total volume of dilution of the sample, cm3;
m is the mass of the sample, g.

A detailed picture of the formed silver nanoparticles was visualized by the microscopic method - atomic force microscopy (AFM NtegraPrima ZAO NT-MDT, Russia).

RESULTS AND DISCUSSIONS

The synthesis of nanoparticles was carried out at room temperature with continuous stirring until the color changes. So, in the process of research, we observed that the color of the observed suspensions changed from light yellow to red-brown during 60 minutes of incubation at room temperature, which indicated the formation of nanoparticles. Given that Scutellaria Iscandaria L. contains flavonoid compounds that are involved in the formation of silver nanoparticles, we performed a comparative chromatographic study of the alcohol extract and aqueous extraction of Scutellaria Iscandaria L. and a suspension with silver nanoparticles. For starters, chromatograms of Luteonin standards (Fig. 1), quercetin, rutin, apigenin (Fig. 2.) Were taken.

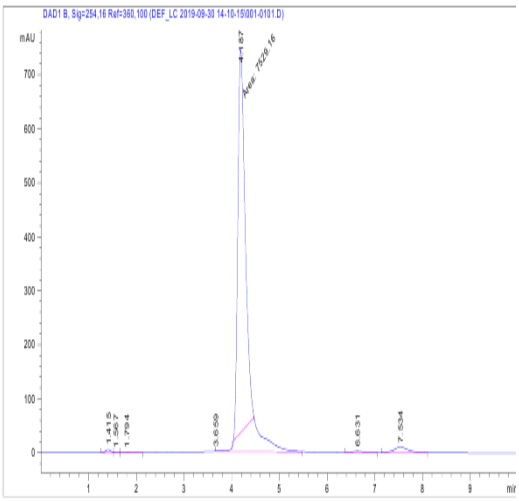

Fig 1. Chromatogram of the Luteolin standard.

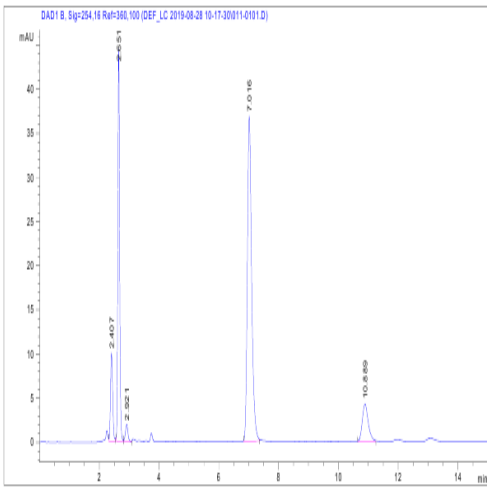

Figure 2. Chromatogram of Rutin, Quercetin, Apigenin standards.

It can be seen from the chromatogram that the peak retention time for the Luteolin standard is 4.1 minutes, for Rutin standards - 2.6 minutes, Quercetin - 7.01 minutes, Apigenin - 10.6 minutes. On the abscissa axis, retention time (min), on the ordinate axis, optical density (mAU). Next, a chromatogram was taken of a solution of the initial extract of Scutellaria Iscandaria L. (Fig. 3). As can be seen from the presented figure, peaks in the 254 nm region are observed on the chromatogram, the retention time (min) that characterize the presence of Luteolin - 4.1 minutes, Rutin - 2.59 minutes, Quercetin - 7.2 minutes, Apegenin - 10.1 minutes.

According to the results of the analysis, the content of flavonoids in the samples of the extract of Scutellaria Iscandaria L. is: Apigenin-0.035708 mg / g, Quercetin -0.014557 mg / g, Rutin-0.237127 mg / g, Luteolin -0.131709 mg / column

It was also of interest to us to perform a chromatographic analysis of a suspension of an extract of Scutellaria Iscandaria L. with silver nanoparticles already formed in it. The results of the study are shown in Fig. 4.

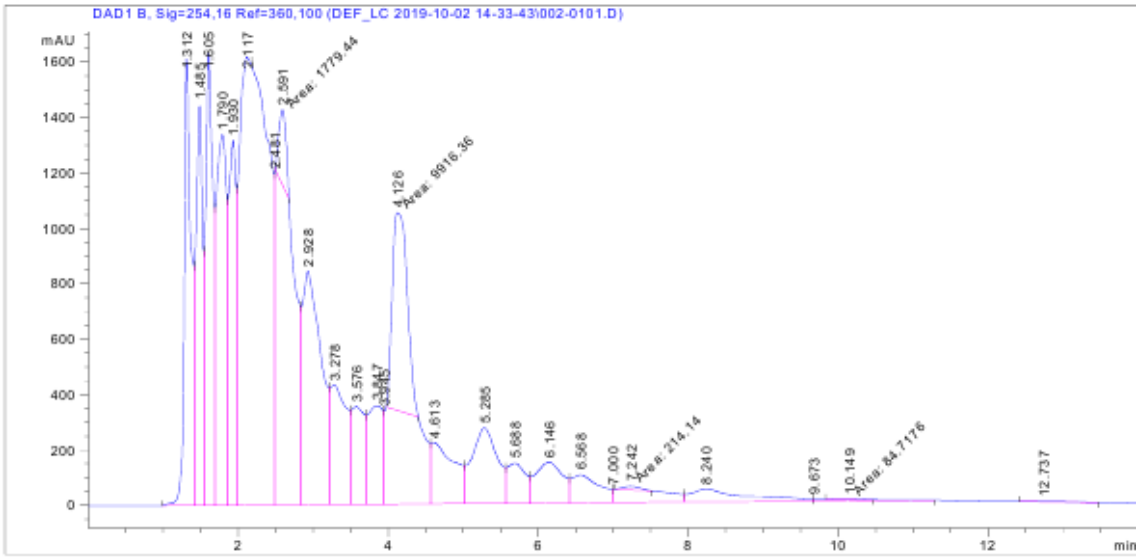

Figure 3. Chromatogram of an alcoholic extract of Scutellaria Iscandaria L. (Definition of flavonoids: Rutin, Luteolin, Quercetin, Apegenin).

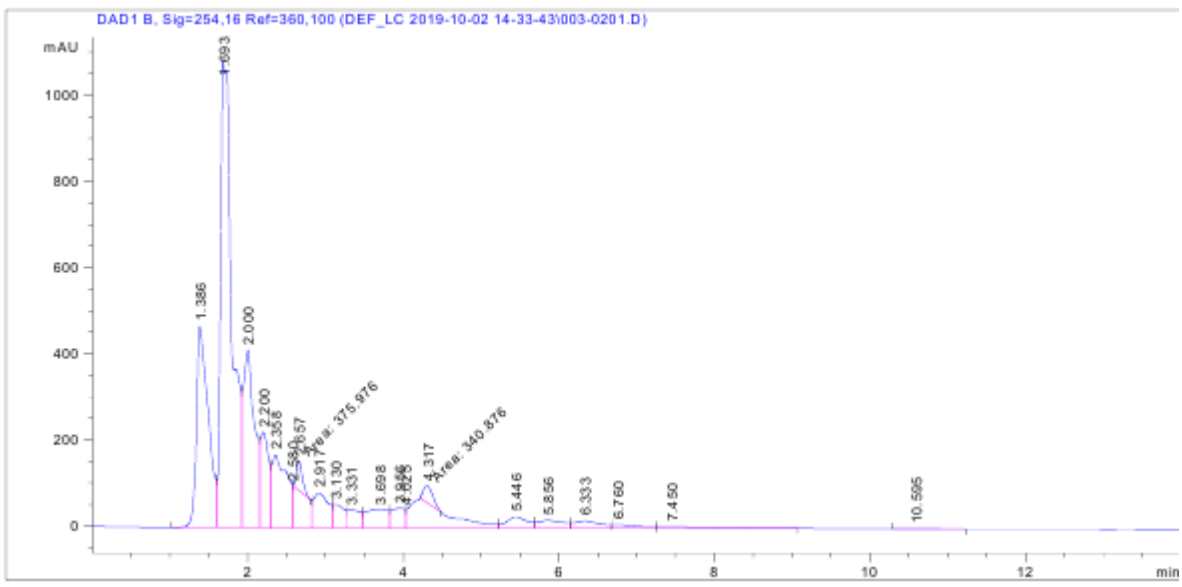

Figure 4. HPLC chromatogram of an alcoholic extract of Scutellaria Iscandaria L. with silver nanoparticles.

In accordance with the results of the analysis, the content of flavonoids in the suspension samples with silver nanoparticles shows the complete absence of apigenin and quercetin, and the peaks characterizing Luteolin (-4.3 min), Rutin (-2.6 minutes) remain, although their content decreased to reading 0 , 05 mg / g and 0.0044 mg / g, respectively.

Further, a chromatogram of water extraction of Scutellaria Iscandaria L. was taken (Fig. 5). As can be seen from the presented figure, peaks in the region of 254 nm are also observed on the chromatogram, the retention time (min) of which characterizes the presence of Luteolin alone - 4.1 minutes.

According to the results of the analysis, the Luteolin content in the samples of Scutellaria Iscandaria L. extract is 0.0062 mg / g (Fig. 5), and in suspension with the formation of silver nanoparticles, 0.0039 mg / g (Fig. 6).

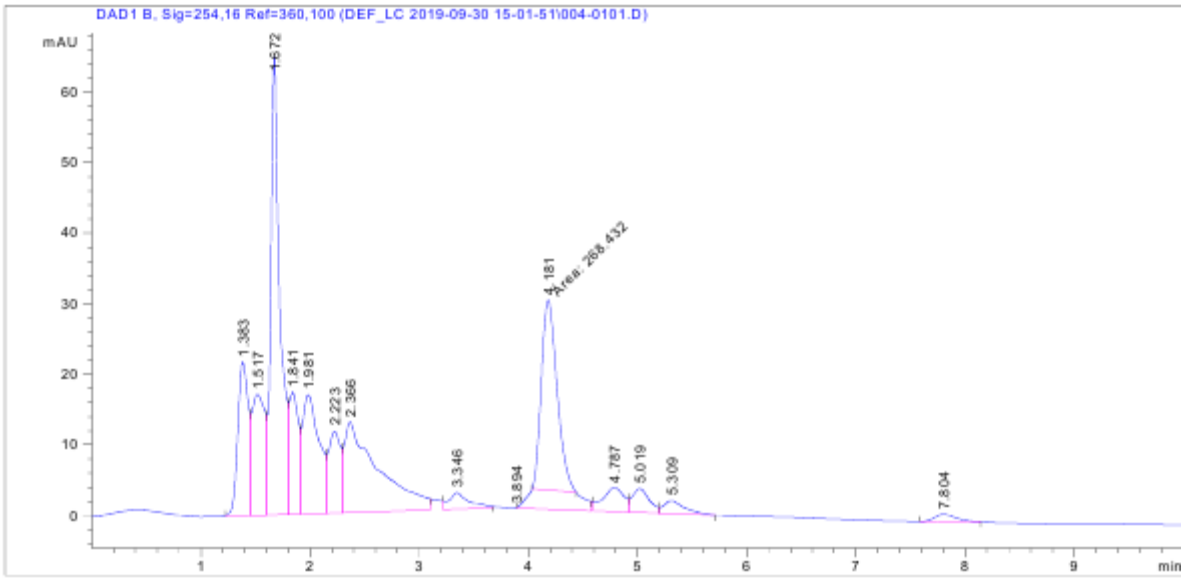

Figure 5. Chromatogram of water extraction of Scutellaria Iscandaria L. (Determination of flavonoids: Luteolin).

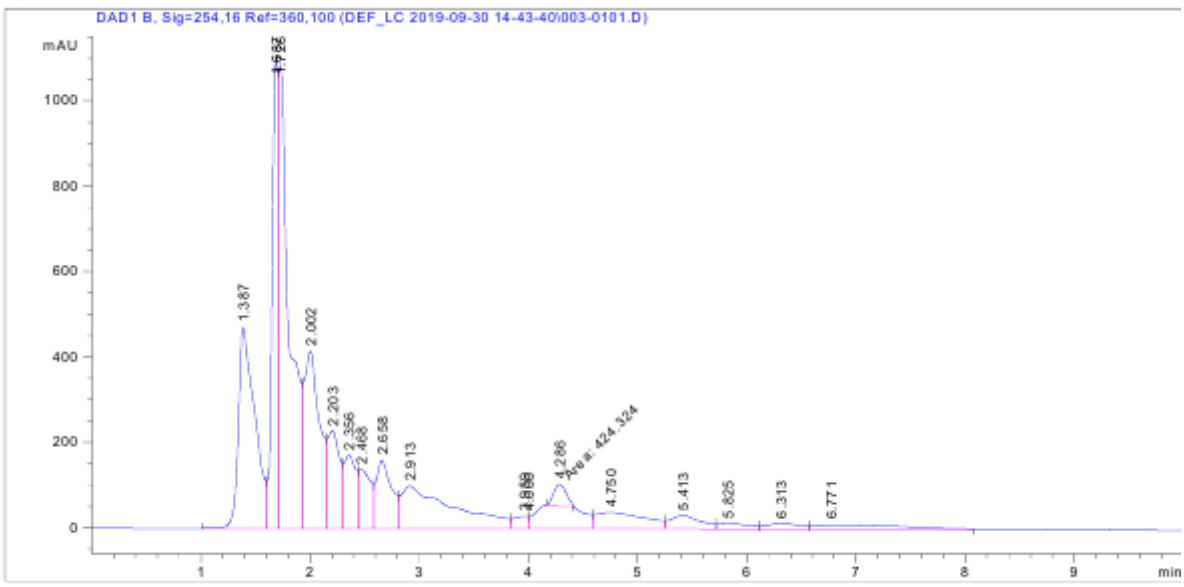

Figure 6. HPLC chromatogram of water extraction of Scutellaria Iscandaria L. with silver nanoparticles.
Summarized results of chromatographic analysis are presented in table 1.

Table 1.

Standard Names	Content of Standards 40% alcohol extract of Scutellaria Iscandaria L.	Content of Standards alcohol extract of ScutellariaIscandaria L. with silver nanoparticles.	ContentofStandards water extraction of ScutellariaIscandaria L.	ContentofStandards water extraction of ScutellariaIscandaria L. with silver nanoparticles.
	Mg / g concentration			
Apigenin	0,035708	0	0	0
Quercetin	0,014557	0	0	0
Rutin	0,237127	0,05	0	0
Luteolin	0,131709	0,0044	0,0062	0,0039

As can be seen from the data presented, all flavonoids are actively involved in the formation of silver nanoparticles. Moreover, in suspension with silver nanoparticles, there is a complete absence of apigenin and quercetin, as well as 100% participation of these flavonoids in the formation of nanoparticles. And the content of Rutin in the suspension is 20%, 3%, respectively, which also indicates a high 80% and 97% participation of these flavone compounds in the formation of nanoparticles.

As is known from the literature [7], the rich phytochemical composition of the extracts used assumes its complex effect, for example, as reducing, stabilizing agents. The nanoparticle formation mechanism consists mainly of three stages: ion reduction, clustering, and further growth of nanoparticles. The features of each stage depend on the nature of the reducing agent, its concentration, pH, AgNO3: the concentration of the reducing agent. The –OH groups present in flavonoids, such as quercetin, rutin, luteolin, apegenin, are responsible for reduction and play the role of stabilizers, therefore, the suspension with nanoparticles we obtained can be stored in the form of colloids.

Based on this, we can conclude that the formation of silver nanoparticles occurred due to all flavonoids present in the extract of Scutellaria Iscandaria L.

Next, we studied the size and shape of the formed silver nanoparticles by atomic force microscopy.

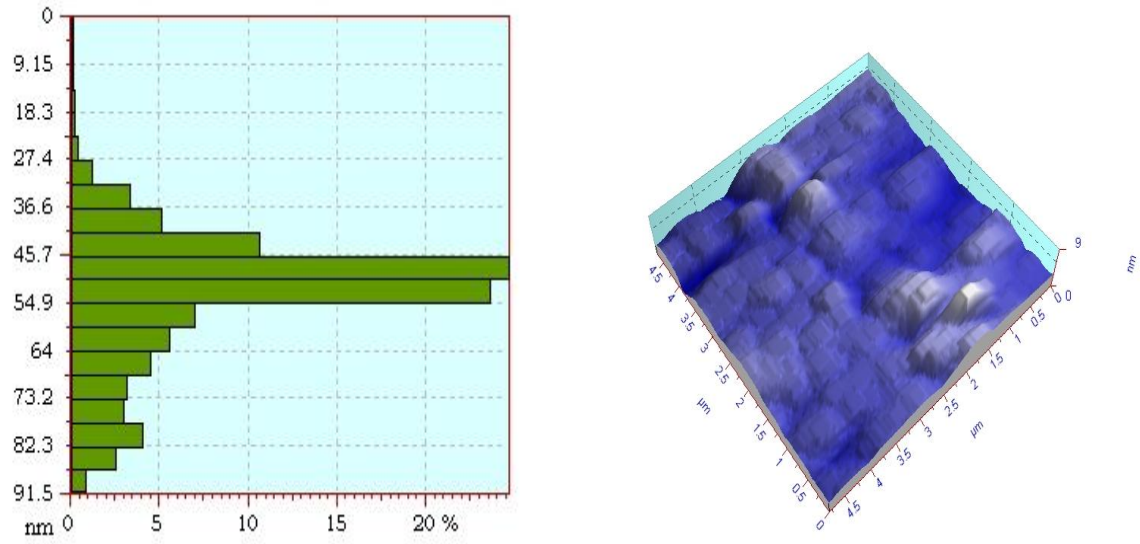

Figure 8. Microscopic study of silver nanoparticles obtained using Scutellaria Iscandaria L. extract by atomic force microscopy.

According to microscopic studies (Fig. 8), the sizes of silver nanoparticles obtained using extracts of medicinal plants Scutellaria Iscandaria L. amounted to 45.7 nm (25% of the total number of particles) with a spread of the main fraction (81%) from 36.6 nm to 64 nm.

CONCLUSIONS

We investigated the ability of biologically active substances contained in the extract of Scutellaria Iscandaria L. to restore silver ions in solution with the formation of silver nanoparticles, the high activity of flavonoids such as luteolin, apegenine, quercetin and rutin was proved.

Consequently, Scutellaria Iscandaria L. may be a promising raw material for the synthesis of silver nanoparticles. Moreover, it was established that such flavanoids as luteolin, apegenin, quercetin, and rutin were actively involved in the restoration of silver nanoparticles.

The formation of silver nanoparticles is confirmed by the appearance of coloration of the formed sols, as well as the image of silver nanoparticles obtained using atomic force microscopy. The AFM image showed that silver nanoparticles obtained using an extract of Scutellaria Iscandaria L. are 45.7 nm in size (25% of the total number of particles) with a scatter of the main fraction (81%) from 36.6 nm to 64 nm. most efficiently providing bactericidal properties present in the extract of Scutellaria Iscandaria L. in the synthesis of silver nanoparticles.

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