SYNTHESIS OF SILVER NANOPARTICLES BY USING EXTRACT OF OLIVE LEAVES

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Abstract. In this study, silver nanoparticles were synthesized by green synthesis method, using olive leaf extract, which was involved as reducing reluctant. The results of these experiments confirm that extract of olive leaves may be used as a reluctant for the synthesis of silver nanoparticles. The effect of the ratio extract:AgNO3 salt on the synthesis of nanoparticles was studied. The silver nanoparticles is best synthesized in a ratio of 1:3. During biosynthesized of AgNPs are occurs color change of solution. The UV-Vis spectroscopic analysis shows that by absorption peak in the interval 405 nm – 425 nm nanoparticles quantitatively was monitored. Further characterization with SEM analysis shows that synsized AgNPs have a spherical form, are polydisperse and size ranging from 7.12 nm to 18.8 nm with an average size of 11.35 nm.

Keywords: *silver nanoparticles, olive leaves, extract concentration.*

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1. Introduction

Recently, attempts have been made to create various methods and technologies for obtaining non-toxic forms of nanoparticles with the requirement of biocompatibility. Given this point of view, in order to obtain non-toxic, environmentally friendly forms of precious metal nanoparticles, there is currently a great interest in the synthesis by biological methods. When synthesizing nanoparticles by biological methods, high pressures and energies, temperatures, and pesticides are not used. For the synthesis of nanoparticles by the biological method, microorganisms are used (Chandran *et al*., 2006; Nair & Pradeep, 2002; Konishi & Uruga, 2007), bacteria and fungi (Vigneshwaran *et al*., 2007) and extracts prepared from various plant organs (Chandran *et al*., 2006; Jae & Beom, 2009; Ponarulselvam *et al*., 2012). The biological synthesis of nanoparticles using plant extracts in many cases outperforms other methods that allow eliminating technologically complex processes (Ganbarov *et al*., 2016). The use of nanoparticles in medical practice has made this issue even more relevant. In this regard, silver nanoparticles (AgNPs), which have unique optical and mechanical properties, as well as antiseptic properties, are of particular importance. There are reports that 1-4 nm AgNPs can directly cross the cell membrane. Many Ag nanoparticles of such sizes and spherical shape are widely used in the problems of treatment, diagnostics, and sensors. Therefore, the biological synthesis of Ag nanoparticles of this size is one of the main

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tasks (Pramanik *et al.*, 2015). AgNP nanoparticles are of particular importance in the preparation of nanomedical preparations, in the creation of coatings for absorbing solar energy, materials for electric batteries and sensors, as well as optical receptors for biomarking (Bonsak *et al.*, 2011; McFarland & van Duyne, 2003). Since Ag nanoparticles have high surface energy, they cause strong surface reactivity (eg adsorption, catalysis). Another interesting property of Ag nanoparticles is their fluorescence and surface plasmon resonance. The spectral properties of Ag nanoparticles make it possible to use them in various detection methods and in the creation of chemical and biological sensors.

Therefore, the production of non-toxic and antiseptic Ag nanoparticles with unique optical and mechanical properties using various plant organs has become of particular practical importance. In the synthesis of nanoparticles in plant extracts, three different stages can be distinguished (Rodríguez-León E *et al*., 2013). At the first stage, the induction stage, there is a rapid reduction of the metal ion and the formation of a metal nucleus. At the second stage, small crystals self-assemble and become large aggregates. At the third and final stage, the size and shape of the nanoparticles are corrected. At this stage, specific biomolecules act as capping agents and stabilize the nanoparticles. This process is similar to the process of bio mineralization, but the nature of the formation of nanoparticles in plant extracts has not yet been fully elucidated (Skinner & Jahren, 2003). Now Ag nanoparticles are synthesized using extracts and homogenates of various plant species, including wormwood leaves, vegetable plants, leaves and roots of medicinal plants, fruit juices, leaves of trees and shrubs. It has been established that Ag nanoparticles synthesized by extracts of these plants have different sizes, shapes, and properties. The properties and sizes of Ag nanoparticles depend on the plant species, the amount of extract, temperature, pH, exposure time, and, finally, the method of synthesis. In the present article, Ag nanoparticles were synthesized in an olive leaf extract.

2. Materials and methods

A leaves of the olive tree was used for the experimental extract. Olive, (*Olea europaea*), subtropical broad-leaved evergreen tree (family *Oleaceae*) and its edible fruit. The olive fruit and its oil are key elements in the cuisine of the Mediterranean and are popular in the world. The tree’s beauty has been extolled for thousands of years. The edible olive was grown since ancient time in the island of Crete about 3500 BCE. Later, olive growing spread to all the countries bordering the Mediterranean, and the tree is also planted as an ornamental in suitable climates. The olive tree ranges in height from 3 to 12 meters (10 to 40 feet) or more and has numerous branches. Its leaves, leathery and lance-shaped, are dark green above and silvery on the underside and are paired opposite each other on the twig. Olive trees bloom in late spring; small, whitish flowers are borne in loose clusters in the axils of the leaves. The olive is wind-pollinated. Fruit setting in the olive is often erratic. In some areas, especially where irrigation and fertilization are not practiced, bearing in alternate years is the rule. The trees may set a heavy crop one year and not even bloom the next (https://www.britannica.com/plant/olive-plant).

Preparation of the olive leaf extracts. The 50 g leaves of the tree of the Olive were first washed with tap water, then distilled water and dried at room temperature, then cut into small pieces and ground into a blender. Small pieces of leaves were boiled at 100°C with for 10 minutes keeping the volume in balance and cooled in a refrigerator for 24 hours. For the synthesize AgNPs, a solution of 5.10-3M AgNO3 was used. Have been taken a 50 ml olive leaves extract as reluctant and added to 450 ml of AgNO3 solution volume. The solution was kept for 3 hours at 90 C. After 3 hours was filtered and this solution for 12 hours was kept at 4C temperature. The color of the solution became dark brown, indicating that silver nanoparticles were synthesized.

Figure 1. Green olive tree (A) and olive fruit (B)

Synthesis of Silver Nanoparticles. For the synthesis of Ag nanoparticles from each samples of extracts which are made in different composition have been taken 50 ml and was mixed with 450 ml 5.10^{-3} M aqueous $AgNO_3$. After addition of extract of leaf to the silver nitrate solution the white color of solution was turned into dark brown an occurs the formation of AgNPs from AgNO3.

Identification of Ag nanoparticles. Formation of AgNPs was initially characterized by standard protocols such as UV–Visible spectra (Analytic Jena Specord-250 plus spectrophotometer). In UV-vis spectrometer the light was absorbed and scattered by the sample in the interval of 190 nm to 800 nm. For the FTIR analysis have been used Varian 3600 FT-IR spectrometer. The solution of silver nanoparticles (sample) firstly was centrifuged and then dried in water bath and were ground with KBr and then made into pellets. These pellets were analyzed by Fourier infrared wavelength varying from 400 to 4000 cm-1. The morphology of dried AgNPs was characterized by Scanning Electron Microscope (SEM) - JSM 7600F, JEOL. The sample was placed on the metal disc with vacuum pressure. In SEM analysis have been made element analysis also.

3. Results and discussion

After the adding leaf extract of olive to the aqueous solutions of silver nitrate (AgNO3) the formation of AgNPs occurs by inducing the reduction of Ag2+ ions into Ag0 which followed change color of solution. A few hours after the addition of the leaf extract to the solution of AgNO3 , the color of the solution changes and it becomes dark brown due to the surface plasmon resonance phenomenon. In this experiments the reduction was completed after 24 hours with the appearance of brownish - black color which confirms the formation of silver nanoparticles. One of the most important factors influencing the reaction rate, size and shape of the formed nanoparticles in the biological approach to the synthesis of nanoparticles depends on the concentration of the extract and the ratio of $AgNO_3$ salt addition to the solution. In experiments with the extract of plants, the dependence of the extract on the concentration of the nanoparticle formation reaction is understood as the ratio of its addition to the $AgNO_3$ salt solution. The main issue in the synthesis of nanoparticles with extracts from plants is to determine the optimal value of the ratio of extract: salt solution. Considering this point in our experiments, the volume ratio of 10^{-3} M $AgNO_3$ salt of olive leaf extract was determined.

In the first experiment, the volume of $AgNO_3$ salt solution is kept constant and the volume of the extract is changed. In the second experiment, the volume of the extract is kept constant and the volume of $AgNO_3$ salt solution is varied. Both practices are adopted in obtaining nanoparticles using green synthesis. The main issue in the synthesis of nanoparticles with extracts from plants is to determine the optimal value of the ratio of extract: salt solution. Considering this point in our experiments, the volume ratio of 10^{-3} M AgNO salt of olive leaf extract was determined. Therefore, in one embodiment of the experiments, 1:1,1:3,1:5; 1:7 and 1:9 of extract:$AgNO_3$ salt solution (50 mM extract: 50mM $AgNO_3$ solution; 50 mM:150 mM; 50 mM:250 mM; 50 mM:350 mM mM; 50 mM:450 mM mM) ratios, in another variant, on the contrary, 1:1, 3:1, 5:1; 7:1 and 9:1 ratios were taken. One of the most widely used method for the identification of nanoparticle formation is UV–Vis spectroscopy which is simple and sensitive techniques. In order to identification of AgNPs, the absorption spectra of synthesized AgNPs were recorded. The UV – vis analysis was studied after through the time duration of 24 hours. Based on the absorption spectra, the biosynthesized Ag nanoparticles by root extract showed surface plasmon resonance (Klaus et. al., 2014) bands at about 414 nm – 461 nm interval as shown in Figure 3. İt is interesting that on increasing the reaction time, the amplitude of absorption peak also increases. The maximum of absorption wavelength of metall based nanoparticles depends on different factors such as particle size, shape and distribution. It also dependences on the dielectric constant of medium. In this case, absorption peaks indicate the formation of Ag nanoparticles in spherical shape with 7 – 20 nm of average diameter. (Klaus et al., 2014). In Fig .2 shown the UV-vis spectra of Ag nanoparticles synthesized by the leaf extracts of olive tree which was prepared into the first variant methods.

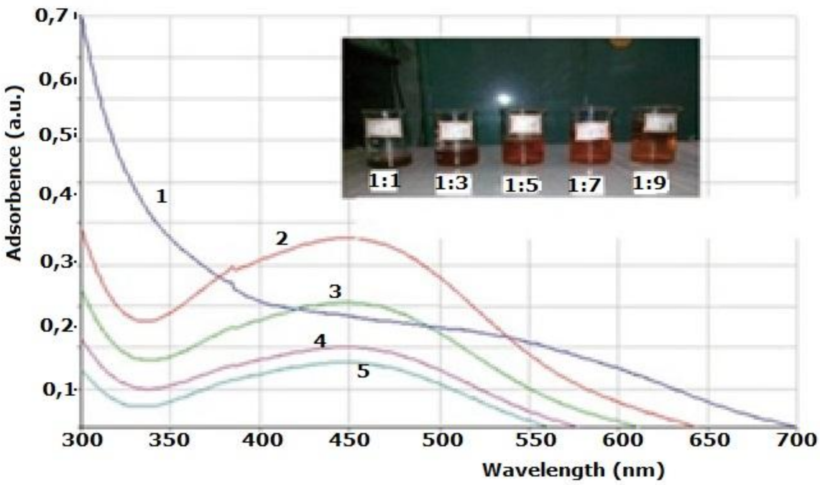

Figure 2. UV-vis spectra of Ag nanoparticles synthesized from olive leaf extract. Absorption spectra change when changing the ratio of and extract: $AgNO_3$ salt **1**-1:1; **2** - 1:3; **3**-1:5; **4** - 1:7; **5**-1:9

By taking different ratio of extract: $AgNO_3$ salt solution, we experimentally determined the optimal price of synthesis of Ag nanoparticles with olive leaf extract. As a result, we determined that the ratio of Ag nanoparticles 50 mM extract: 150 mM $AgNO_3$ solution is more optimal. It was found that the size distribution, quantity and shape of Ag nanoparticles at 1:3 ratios were more efficient than other ratios. Figure 3 shows the UV-vis spectrum of Ag nanoparticles synthesized in this ratio.

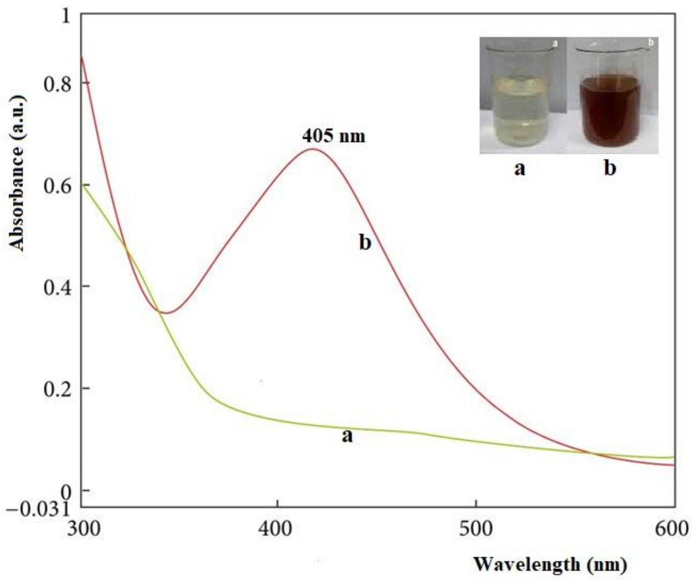

Figure 3. UV-vis absorption spectra of extract of olive leaf (a) and absorbtion spectra of Ag nanoparticles synthesized in the ratio extract: $AgNO_3$ salt 1:3 (b)

It was obtained that the peak of absorption spectra of Ag nanoparticles synthesized in the ratio extract: $AgNO_3$ salt 1:3 is 405 nm shows that the size of Ag nanoparticles are about 7.12 nm.

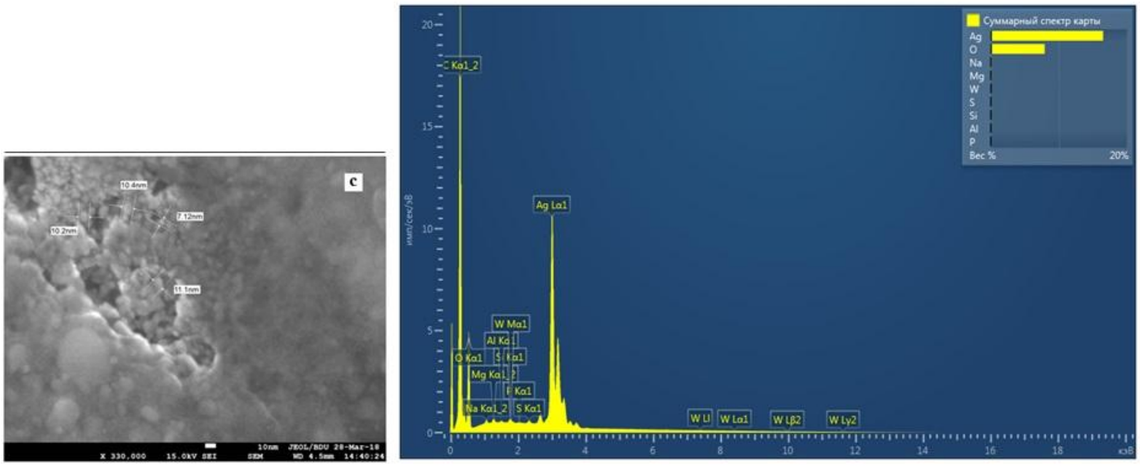

Figure 4. The SEM pictures of Ag nanoparticles synthesized by extract of olive leavs and thier EDAX spectrum

Figure 4 shows typical results the studies the sizes, forms and element analysis of Ag nanoparticles synthesized in the ratio extract: AgNO3 salt 1:3 (b) by means of SEM. The figure represents the view of the sample at 500 000× magnification. SEM images showed that most of the silver nanoparticles are predominately spherical in shape having smooth surface and well dispersed with close compact arrangement. The smallest size of nanoparticle was found around 7.12 nm – 18.8 nm. The SEM images shows that nanoparticles may direct contact even within the aggregates, indicating that stabilizer agents act (Priya *et al*., 2011). During the aggregations the small nanoparticles become larger.

4. Conclusion

In conclusion, there has been a great increasing interest in green synthesis of Ag nanoparticles. In this study, Ag nanoparticles were synthesized by an ecofriendly and convenient method using the extract of olive leaves at ambient temperature. The results of this experiments confirm that extract of olive leaves may use as a reluctant for the synthesis of silver nanoparticles. The effect of the ratio extract: AgNO3 salt on the synthesis of nanoparticles was studied. The synthesis of silver nanoparticles is best synthesized in a ratio of 1:3. During biosynthesized of AgNPs are occurs color change of solution,the UV-Vis spectroscopic analysis shows that by absorbtion peak at 405 nm – 425 nm interval nanoparticles quantitatively was monitored. Further characterization with SEM analysis shows that synsized AgNPs have a the spherical form, are polydisperse and size ranging from 7.12 nm to 18.8 nm with an average size of 11.35 nm.

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