Short Communication

Green Synthesis of Silver Nanoparticles and Its Effect on Total Proteins in Melia Azedarach Plant

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Abstract:
Silver nano-particles have different biological applications due to their compatibility. Chemical methods usually result in remaining some amounts of toxic reactions on the nano-particles. For this reason, the use of plants as sustainable and accessible resources in preparation of compatible nano-particles has attracted a lot of attention in the recent years. This research investigated the green synthesis of Silver nano-particles using Meliaazedarach plants.The analysis of Ultraviolet–visible spectroscopyshowed the green synthesis of nanoparticles in plants. X-ray diffraction was used to confirm the crystalline nature of the particles. Fourier-transform infrared spectroscopyspectroscopywas performed to analyzethe functional groupin the process of green synthesis, the role of different functional groups such as hydroxyl, amine and alkyl groups were indicated in the synthetic process.Then, proteinscapped on the silver nano-particles were analyzed usingSodium dodecyl sulfate -PAGE. Regarding this, the possibility of extracted protein role in the stabilization of nano-particles requires more analysis.
Keywords: *Green synthesis, Melia azedarach, Silver nano-particles, SDS-PAGE.*

1. INTRODUCTION

Today Silver nano-particles are produced with different chemical and physical methods, but what is important in the case is that in these methods, often high temperature and pressure is needed and different chemical solvents are used in order to do the reactions and stabilize nano-particles [1]. Nano-particles produced biologically are preferred to the ones produced through chemical methods due to lack of organic toxic roots at the surface. Thus, they can have various applications as drug delivery and antimicrobial property in the medical and pharmaceutical industries [2].

Green methods use environment-friendly agents such as sugars, herbal extracts and stabilize Silver nano-particles [3]. The synthesis of nano-particles using plant systems is a useful method that today is focused by many researchers [2,4]. Researchers are turning attention to green

synthesis of nanoparticles because of its eco-friendly, economical, and it provides a single step technique for the biosynthesis process [5] it is cost effective too [6] Control over the shape and size of nanoparticles seems to be very easy with the use of plants [7]. Green method deals with the formation and stabilization of silver nanoparticles using environmentally friendly elements such as sugars and plant [8].

For example, alfalfa can produce and store gold and Silvernano-particles in various forms in the plant tissues [9]. The aggregation of gold nano-particles in the 5-50 nm size range is also reported in Indian mustard [10], the synthesis of nanoparticles using plants such as Chenopodium album [11], Cinnamon [12], Novel sundried Cinnamomumcamphoraleaf [13] Eucalypyus hybrid (Safeda) leaf extract [14] carob leaf extract [15] and the extract of lemon fruit [16] has been fulfilled.

On the other hand, the characteristics of produced nano-particles can be changed by manipulating control parameters such as temperature, substrate density and time of exposure to the substrate [17].

In this study, the green synthesis ability of silver nano-particles in Meliaazedarachplants was investigated. Meliaazedarachplant is from Meliaceae family and is only from the two known species in Iran [18,19].

Meliaceae family is rich in terpenoidsof limonoid types. Some of the chemical compounds such as sulfur, hydrocarbon, fatty acid, di-terpenoids, sterols, phenol, flavonoid, glycosides, lactones, azadirachtin, nimbin, nimboslin, quercetin, escopolnenin, azadiyeron, azadiyeradion,-14 opeksi, azadyeridon, gedonin, nimosinulnimosinolid, nimbandion, salanol, nimbinen, -6 dasti lnimbinen, margozonolid, isomargozonolid, meyanetriol, salanin (its 14 derivatives), feraksinoloz, sinamat meliasin, A and B nimbolinsand limonoid are members of this family [20].

In higher plants, apart from enzymes, other molecules such as polyols, heterocyclic compounds and flavonoids may have a role in the synthesis of nanoparticles. Terpenoids can reduce silver ions to silver while getting transformed to the corresponding carboxylic acids [21].

2. MATERIALS AND METHODS

2.1. Preparation of seed extracts

The Melia azedarach seed was sterilized in NaOCl, washed with deionized water and placed on the seeded culture plate which fully coated the surface of them with moist filter paper and incubated at 25°C. After one week, plant was transferred to a Hoagland environment containing a concentration of 3 mM of silver nitrate. The mentioned Hoagland solution is the modified of one of the main solution and this change is only due to preventing the side effects of Hoagland compounds on the sediment of applied silver nitrate in the planting environment of plant. After 7 days, the ripe plant was brought out from Hoagland solution containing silver nitrate and was washed 3 times with deionized water. Also, device analyses was carried out for identifying the existence of nano-particles.

Table 1. *Modified Hoagland solution*

Molecular formula	Concentration (mM)
KNO_3	5
$Ca(NO_3)_2.4H_2O$	2
$MgSO_4.7H_2O$	2
KH_2PO_4	0.1
NaFe(III)-HEDTA	0.05
H_3BO_3	0.01
$MnCl_2.4H_2O$	0.002
$ZnSO_4.7H_2O$	0.003
Na_2MoO_3	0.0001
$CuSO_4.5H_2O$	0.0005

2.2. Characterization of silver nanoparticles

Some metals such as gold and silver have absorption capacity called surface plasmon resonance absorption and the absorption is particular to the metal nano-particles. In order to identify silver nano-particles, spectrum spectrophotometer of Ultraviolet–visible (UV-Visible) spectroscopy model of scan drop 250-211FO75 made by Analyticgena Company in German was used. For this purpose, 1 g of powdered plant sample was weighted, and then it was placed in 10 ml of 65% acid nitric for 24 hours. The sample was dehydrated at a temperature of 85 degrees and then 1 ml of 30%

oxygenized water (Kimia materials, Iran) was added to it and was heated until all the brown fumes got out. After cooling of the digested sample, some amounts of distilled water were added to the samples and it was flatten and transferred to 50 ml Volumetric flask and it was reached to the volume with two times distilled water. Optical density (OD) of the sample was read with three replications in order to detect Silver nano-particles using the machine [22]. X-ray diffraction (XRD) was performed using an X-ray diffractometer (PANalitical, X PERTPRO, Holland) with CuKα radiation λ=1.5405 A° over a wide range of Bragg angles (30°≤2θ≤80°). For fouriertransform infrared (FTIR) spectroscopy (TENSOR27, BRUKER, Germany) measurement, the Agnano-particles after 24 h of reaction were centrifuged at 10000 rpm for 10 min, dry powder of the nanoparticle were obtained.

2.3. Quantity andquality of total protein inMeliaazedarachplant

The extraction of total protein in order to determine the quantity value and compare the pattern of the treated protein bands was conducted versus control plant. 1 g of young leaf of the Meliaazedarach samples was powdered in a mortar cooled with liquid Nitrogen. For per gram of leaf, 3 ml extracted buffer (aqueous solution with density 100 mM of Tris-HCI pH=8, 10 mM of EDTA pH=8, half percent of beta-mercaptoethanol, 50 micromollar of cooled PMSF is added to it and it was placed on ice. The sample was centrifuged in the cycle of 11000 g for 20 minutes at 4°C. Supernatant phase was separated and stored. The determination of the quantity was done using Bradford method.

Based on the equation of regression line, standard bovine protein serum albuminy=0.0045x+0.1214 was obtained. In this equation, y is the amount of absorption at the waves of 595 nm based a.u. and x density based µg/ml [23]. The examination of electrophoretic pattern of protein of the previous plant and after the synthesis of nano-particle using polyacrylamide gels inSodium dodecyl sulfate PAGE (SDS-PAGE) system was carried out. The marker of used protein is Spectra Multicolor Broad Range (Fermentas, Germany). In this method, 12% separating gel and 5% compactor gel were used. After the preparation of the gel, 30 ml of protein sample (Mixed with sample buffer) was transferred to each well. Voltage of 100 to 120 volts was established that in this state, the current is 30 mA [24]. After completing electrophoresis, in order to emerge protein bands, We used staining solution consisting 1% of coomassie blue, 50% of methanol and 10% of acetic acid and then it was placed in color-clearing solution containing 50% of methanol and 10% of acetic acid for 2 to 24 hours.

3. RESULTS AND DISCUSSION

3.1. Visual observations and UV–vis absorbance studies

In the green synthesis of Silver nano-particles using Meliaazedarach, the reduction of silver ions was followed by color change from yellow to dark brown in Meliaazedarach root to induce the surfaceplasmon vibrations in Silver nano-particles (Figure1) [25].

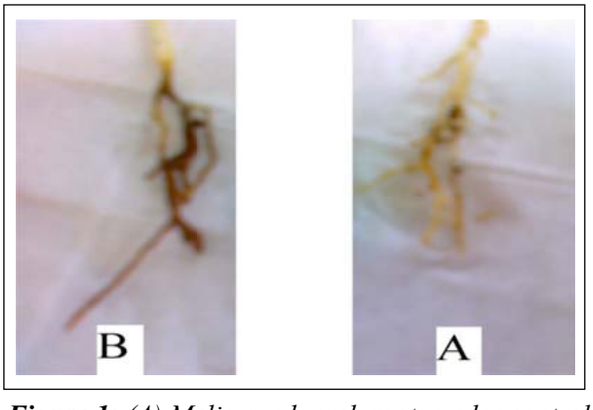

Figure 1: *(A) Melia azedarach roots under control condition (0 mM $AgNO_3$) (B)Initial observations after exposure to the silver nitrate solution and the Melia azedarach root color changed to the brown.*

Figure 2 shows the UV–vis absorption spectra of silver synthesized nano-particles after 7 days. It is observed that absorbance of Ag nano-particles occurs at 425 nm.The 450 nm is the wave length of silver and fluctuation is seen in absorption spectrum depending on the size and the form of particle in this range [26]. No absorption peak was observed in

UV–vis spectrum of Ag+ solution before reduction.

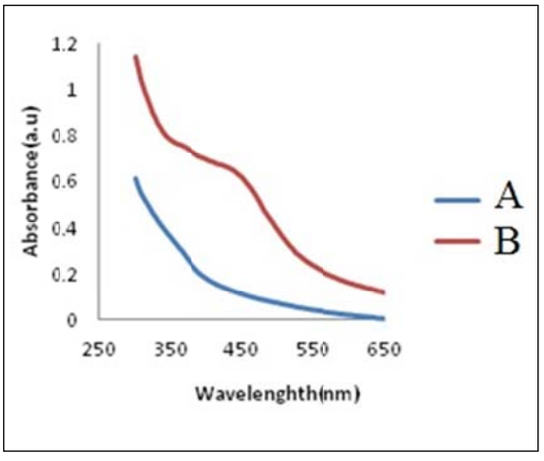

Figure 2: *Absorption spectra of (A) control plant (B) Silver nano-particles in Melia azedarach plant*

3.2. XRD and FTIR analysis

The XRD analysis showed diffraction peaks at 38.1◦, 44.3◦, 64.4◦ and 77.4◦ (Figure 3.a), The lattice plane value was observed at 111, 200, 220 and 311 of Silver nano-particles [27]. Extra peaks are related to other compounds which have existed in the extract of the plant and have crystal structure [28].

The FTIR measurements of green synthesized Silver nano-particles were executed to identify the possible interaction between protein and Silver nano-particles. The non-treated sample shows absorption bands about at 3406, 1605, 1404 and 1027 cm^{-1} (Figure 3.b.A) and 3418, 1532, 1153 and 1031 cm^{-1} (Figure 3.b.B).

Absorption peaks at 1000-1260 cm^{-1} and 1620-1680 cm^{-1} were attributed to the –C–O–C stretching vibrations and amide I band, respectively [22] (Figure3.b). Macdonald and smith (1996) reported the absorption peak at 1620-1680 cm^{-1} is close for native proteins.

Also, secondary structure of proteins was not affected during reaction with Ag ions or after binding with Ag nano-particles [30]. The FTIR peak in 2850-3000 cm-1shows alkyl groups in the extract and the peak in 3100-3500 cm^{-1} was attributed to the O–H stretching vibrations while the non-treated sample shows stronger absorption and minor shift on hydroxyl peak. Comparison between spectra of non-treated (A) and treated (B) samples discloses only little changes in the positions on the wave numbers and absorption bands.

These IR spectroscopic studies confirmed that carbonyl group of amino acid residues have strong binding ability with metal suggesting the formation of layer covering metal nano-particles and acting as capping agent to prevent agglomeration and providing stability to the medium [31].

3.3. Evaluation of Melia azedarach total protein under silver nitrate stress

Based on the equation of y=0.0045x+0.1214, the density of the charged protein in the control sample was calculated 232 and 268 µg/ml that indicated higher total protein in the treated Melia azedarach

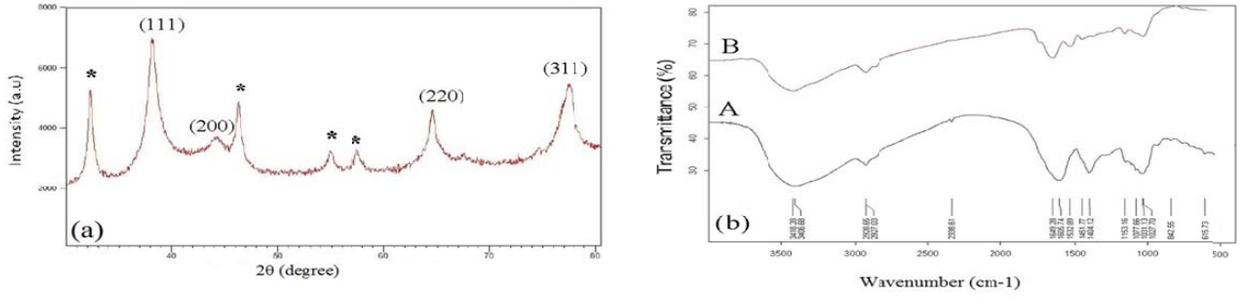

Figure 3: *(a) XRD Pattern of Silver nano-particles (b) Spectrum of FTIR (A) spectra of control plant (B)spectra of Melia azedarach plant under treated with silver nitrate*

plant with the density of 3 mM of silver nitrate in relation to the plant control sample (non-treatment). The high R2=0.9347 shows the justification of more than 90% changes y by x and high confidence level. The analysis of electrophoretic pattern of the Melia azedarach using Alpha Ease FC 4.0 software demonstrated 12 protein bands with the molecule weights of 220, 174, 165, 140, 115, 100, 88, 79, 74, 61, 55, 50 kDa in the control sample and 10 protein bands with the molecule weights 165, 157, 140, 115, 88, 79, 74, 60, 55, and 50 kDa in the treated sample were showed (Figure 4). The absence of protein bands 220, 174, 100 kDa in the sample treated with silver nitrate (C) in comparison with the control Melia azedarach (B) is the result of electrophoretic pattern of the two samples. Protein bands with the molecule weight of 140 and 55 kDa are determined with higher intensity in relation to other bands.

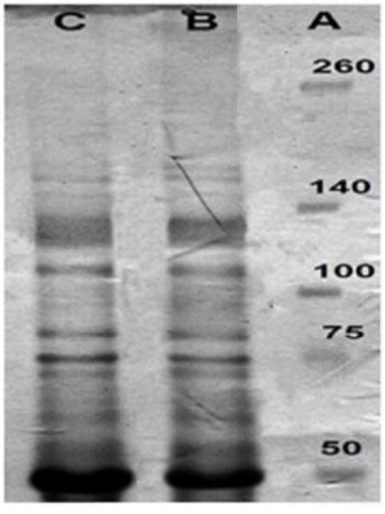

Figure 4: *Electro phoretic pattern of Melia azedarach plants: (Lane A) marker proteins (Lane B) the pattern of control plant (Lane C) the pattern of Melia azedarach plants treated with silver nitrate*

Nikolaj et al (2006) considered the existing proteins in the extract as the stability agents and stated that benefit from a particular polymer such as proteins with suitable solvency properties is related to the possibility of polymer stabilizers to the level of nano-particles. Balaji et al (2009) reported that with analysis of the proteins involved in heavy metals we can find out about metabolic paths that are effective in the synthesis of silver nano-particles. According to the fact that the studied plants in the research are able to synthesize silver nano-particles. Thus, they can be used as selective plants. The examination of biochemical paths involving in the synthesis of nano-particles can be used in the future examinations.

4. CONCLUSION

The growth of the plant in an environment containing heavy elements is a kind of stress on the plant. If the plant is placed in the compound stress such as nitrate, silver, cadmium chloride and so on, the amount of enzymes and proteins that play role in the synthesis path of absorption of heavy metals will be changed.

As these enzymes and proteins are more produced under stress conditions, the objective of increasing greensilver nanoparticles is closer and also Synthesis of silver nanoparticles by plants are more depended to the selecting effective plants. Physical and metabolism examination of plants producing silver particles can provide with us useful information about the suitable planting environment for us. It is suggested among plants for the synthesis silver nanoparticles should be enhancedand the dimensions of nano-particles should become smaller. Furthermore, the analysis of nanoparticles, temperature effect, PH and the density of different salts in the synthesis of different particles should be taken in to consideration.

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