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Optimal synthesis and characterization of green synthesized silver nanoparticles by *Lawsonia inermis* extract

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In this study, ultraviolet-visible spectrophotometer (UV-Vis) was used to find the optimum conditions for silver nanoparticles synthesis by using *Lawsonia inermis* extract. Affective parameters such as pH, volume of extract, concentration of salt solution, reaction temperature and time have been optimized for biosynthesis of silver nanoparticle by absorving UV-Vis spectra. The maximum absorbance wavelength of silver nanoparticles was observed at 420 nm and the obtained results from the experiments in this wavelength showed that the best conditions for nanoparticles synthesis are pH of reaction = 8, 4 ml of extract, 0.003 M silver nitrate, reaction temperature= 80 ^{0}C and 70 minutes for reaction time.

The prepared nanoparticles in optimum condition, were characterized by utilizing UV-Vis spectrophotometer, Scanning electron microscope (SEM), transmission electron microscopy(TEM), X-ray diffraction(XRD) and Fourier transform infrared (FTIR) spectroscopy. By results obtained from the devices, synthesis of cubic shaped nanoparticle with an average size of 20 nm confirmed. The results of this study showed that Biological synthesis by using *Lawsonia inermis* extract is a very low cost method without the need for energy.

Keywords: *Lawsonia inermis*, Silver nanoparticles, green synthesis

INTRODUCTION

Over the past two decades, researches and human's life have been influenced by nanotechnology, so researches and development about this science is growing rapidly around the world. Generally, nanotechnology includes a variety of subunits such as nanotubes, nanosensors and nanomaterials, each of them has many uses in industry, medicine, life sciences and agriculture.

Nanomaterials also include subunits that metal nanoparticles are one of the most important and most usable of them. Silver nanoparticles (NPs) are one of the most widely used nanoparticles among metal nanoparticles and it finds more applications in the Nano world every day. Silver (NPs) have been the subject of researchers because of their proper conductivity, chemical stability, catalytic, photonic and optoelectronic due to their special physical and chemical properties [1-3].

There are many methods for nanoparticles synthesis. Many techniques are inefficient in materials and energy consuming. In most chemical methods, a chemical reducing agent, for example, sodium borohydride and a stabilizer, For example, polyvinylpyrrolidone is used to reduce metal ions and control particle growth and prevent accumulation [4]. In addition, in these chemical synthesis methods, particle stability is controversial, and production on a large scale is difficult. For these reasons, there is a demand for nanoparticle production by eco-friendly methods.

In order to nanoparticles synthesis, different physical, biological and chemical methods have been proposed. Among the physical and chemical methods, we could mentioned laser erosion methods, microwave waves, chemical reduction, electrochemical reduction and lithography. The disadvantages of these methods are reduction reagents and energy usage, that can have a potential risk for human health and environment , on the other hand, they are not proper for using on industrial scale production and do not have economic justification. Due to the high costs of chemical and physical methods, it is important to study silver nanoparticles biosynthesis which is an energy efficient and low-cost rout, without using organic solvents compared to the chemical methods. Nowadays biological methods have been developed to produce eco-friendly and inexpensive nanoparticles. Biosynthesis of nanoparticles by plant extracts have been considered as an appropriate substitute for nanoparticles production. Plant pure extracts contain flavonoids, alkaloids, phenolic acid and terpenoids as secondary metabolites, which are constantly involved in bioreduction of metal ions [5-9].

The main richest bioresource of biomolecules such as vitamins, enzymes, amino acids, proteins and polysaccharides are in medicinal plants (dry and

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fresh) extract plant extracts. There is rapid increase in use of various medicinal plants extract for the nanoparticles biosynthesis. The efficient and rapid biosynthesis of silver NPs using aqueous extracts of several plants have been published for example: *Agave tequilana Weber var. azul* , *Euterpe oleracea*, *Laminaria japonica* [10-12].

In this study, *Lawsonia inermis* extract was employed for silver nanoparticles synthesis as a reducing agent. Several batch experiments were carried out to investigate the influence of pH, volume of extract, silver nitrate solution, temperature and time on silver nanoparticles formation. In addition, characterization of silver nanoparticles synthesized were investigated using UV-VIS, SEM, TEM, XRD and FT-IR.

MATERIALS AND METHODS

Chemicals such as silver nitrate, hydrochloric acid, sodium hydroxide, were analytical grade purchased from Sigma-Aldrich or Merck and *Lawsonia inermis* leaves (Fig.1) were collected from Khozestan, Iran.

To prepare the *Lawsonia inermis* extract 20 gram of dried leaves powder boiled in 200 ml double distilled water at 59 ºC for 30 min at shaker incubator. After cooling, the extract was filtered through Whatman No.1 filter paper, then centrifuged at 5000 rpm for 15 minutes and the copper color supernatant carefully decanted (Fig . 1) and the final volume of *Lawsonia inermis* extracts was made up to 100 mL by adding double distilled water and stored at 4 ºC for further nanoparticles biosynthesis process.

Fig.1. Lawsonia inermis extract solution

Biosynthesis of silver NPs

By adding the 5 ml of *Lawsonia inermis* extracts into 95 ml of 1 mM silver nitrate aqueous solution and incubated at room temperature, the color changing to brown was indicated synthesis of silver nanoparticles (Fig. 2).

To determination the optimum parameters for silver nanoparticles biosynthesis, the experiments were carried out at different pH (2, 4, 6, 8 and 10), volume of *Lawsonia inermis* extract (2,4,6 and 8 ml), silver ion concentration (0.001, 0.003, 0.005 and

Fig.2. Color of the Lawsonia inermis extract solution after reaction with $AgNO_3$.

0.01 mM), temperature (25, 35, 65, 80 and 100 ºC) and time (10, 25, 35, 55, 75min). In order to investigate the effect of these parameters on silver nanoparticles synthesis UV-Vis spectrophotometer was used (the UV-Vis Agilent – 8541 has been setup at wavelength of 330-800 nm).

Effect of pH on silver NPs biosynthesis

5 ml *Lawsonia inermis* extract were added to 5 Erlenmeyer flask containing 95 mL of 1m M silver nitrate solution. The pH of each Erlenmeyer flask was adjusted to 2, 4, 6, 8 and 10 by using 0.1 M sodium hydroxide and 0.1 M hydrochloric acid and then stirred with the stirring rate of 150 rpm for 30 min at room temperature. By adding the metallic salt solution, the color of solution obviously changed from yellow (pH=2) to earthy dark brown within few minutes which is indicated the formation of silver NPs (Fig. 3). The solutions were further characterized using UV-Vis in the range of 330-800 nm, and the best pH for this biosynthesis has been determined(Fig. 4). pH were adjusted to this value for further experiments.

Fig.3. Effect of pH on silver nanoparticles synthesis by *Lawsonia inermis* extract

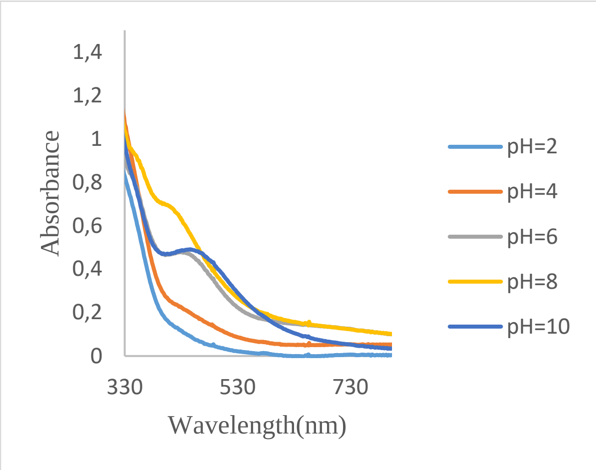


Fig.4. Effect of pH on Silver nanoparticles synthesis by *Lawsonia inermis* (5 ml of extract solution,95 ml of 0.001 M Silver nitrate, time: 30 min, stirring rate 150 rpm)

Effect of Lawsonia inermis extract volume on silver nps biosynthesis:

5 ml *Lawsonia inermis* extract 95 mL of 1m M silver nitrate solution were added to 4 Erlenmeyer flask and 2, 4, 6 and 8 ml of *Lawsonia inermis* extract solution was added to them. Then the pH of all of them were adjusted to the optimized pH and stirred with the stirring rate of 150 rpm for 30 min at room temperature, like the past step, centrifuged, and UV spectrums were recorded(Fig. 5). Thus the best volume of extract has been determined and used for further experiments.

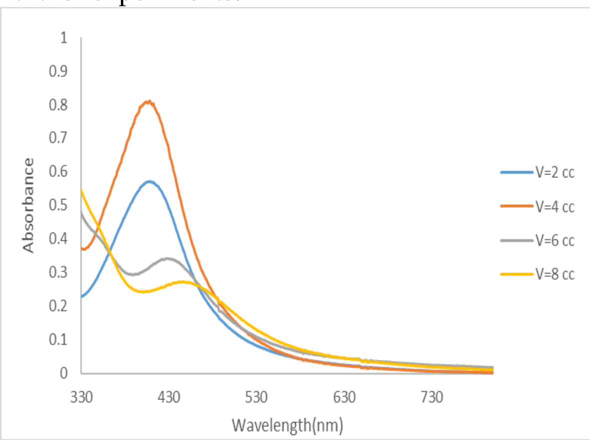


Fig.5. Effect of *Lawsonia inermis* extract solution on Silver nanoparticles synthesis (pH of the solution was 8, 95 ml of 0.001 M Silver nitrate, time: 30 min, stirring rate 150 rpm)

Effect of metal salt concentration on silver NPs biosynthesis:

In order to optimize the concentration of the metal salt solution, 4 ml of *Lawsonia inermis* extract was poured into 4 Erlenmeyer flasks, then 5 ml of 0.001, 0.003, 0.005 and 0.01 M silver nitrate was added to them. The pH of all Erlenmeyer flasks were adjusted to the optimized pH and after these solutions stirred with the stirring rate of 150 rpm for 30 min at room temperature, after UV spectrum was taken from them in the range of 330-800 nm (Fig. 6) and then the best concentration was determined and used for next experiments.

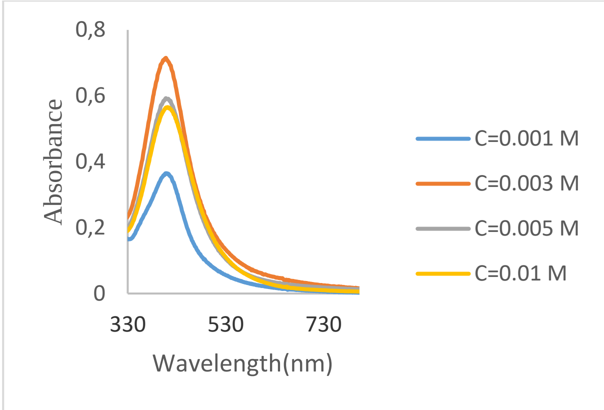


Fig.6. Effect of nitrate solution concentration on Silver nanoparticles synthesis by *Lawsonia inermis* extract (pH of the solution was 8, 4 ml of the *Lawsonia inermis* extract, time: 30 min, stirring rate 150 rpm)

Effect of temperature on silver NPs biosynthesis:

According to the prior steps the optimal temperature was measured. 4 ml of *Lawsonia inermis* extract and 95 ml silver nitrate(0.003 M) were poured into 5 Erlenmeyer flasks and pH was set at 8.then all Erlenmeyer flasks were placed in the oven with temperatures 25, 35, 65, 80 and100 ˚c for 1 hour. After that all samples were centrifuged and UV spectrums were recorded with the same conditions as before. So the optimal temperature was obtained (Fig. 7).

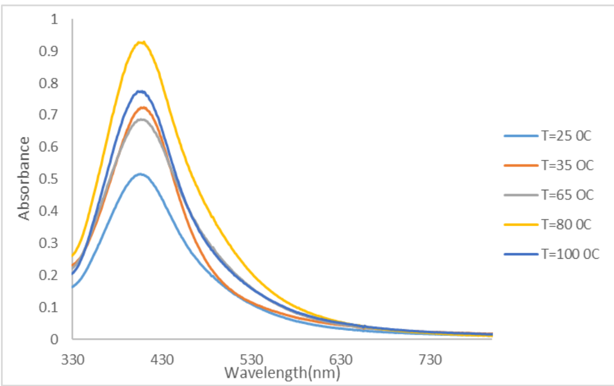


Fig.7. Effect of temperature on Silver nanoparticles synthesis by *Lawsonia inermis* extract (pH of the solution was 8, 4 ml of the *Lawsonia inermis* extract, 0.003 M Silver nitrate, time: 30 min, stirring rate 150 rpm)

Effect of time on silver nanoparticle synthesis using by Lawsonia inermis extract

Determining the appropriate time for silver nanoparticles synthesis is an important step because metal ions increase and release at specific time, then the surface plasmon resonance would be decreased. 4 ml of the *Lawsonia inermis* extract and 95 ml silver nitrate(0.003 M) were poured into 7 Erlenmeyer flasks and the pH was set at 10 and the experiments were performed 80 °C then took the UV spectrum after (10, 25, 35, 55, 75 min, respectively. According to the results, the best time for nanoparticle synthesis was determined (Fig. 8).

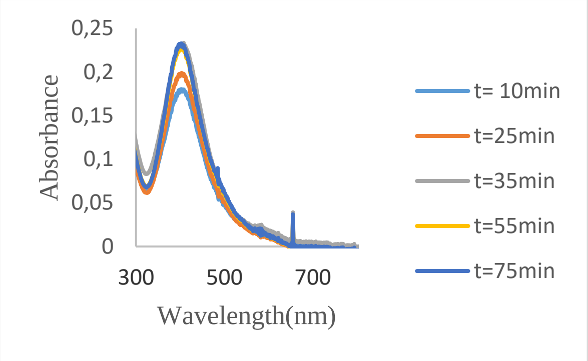


Fig.8. Effect of time on Silver nanoparticles synthesis by *Lawsonia inermis* extract (pH of the solution was 8, 4 ml of the *Lawsonia inermis* extract, 0.003 M Silver nitrate, temperature 80˚C, stirring rate 150 rpm)

Characterization of the green synthesized silver nanoparticles

It has been reported that the nanoparticles size and shape mainly depends on different factors like concentration of leaf extract, salt solution, pH and temperature [13, 14]. In order to characterize silver nanoparticles by SEM, TEM, XRD and FTIR devices, according to obtained results from the UV spectra for the studied optimal parameters(figures 4-8) , silver nanoparticles were synthesized again at conditions: pH=8, *Lawsonia inermis* extract valume = 4 ml, salt solution concentration= 0.003 M, temperature= 80 ˚C, time= 75 min. After a suitable time, in order to separate the metallic nanoparticles sediment from solution, sample was centrifuged. Then metallic nanoparticles were characterized by above devices after washing and drying.

RESULTS AND DISCUSSION

Uv-Vis analysis

The UV-Vis spectra shows effect of the different optimal parameters which is affecting on silver nanoparticles synthesis by *Lawsonia inermis* extract. Metal-based nanoparticles have many free electrons that move by conduction and balance bands which is caused surface plasmon resonance (SPR) when the UV light collision to them, This spectrum records the vibrations of the free electrons nanoparticle. In this work a high peak which is appeared in the visible area also confirms the formation of silver nanoparticles and SPR absorption band was observed around at 420 nm wavelength region due to the presence of brown coloured silver nanoparticles synthesized (figures 4-8). Similar results obtained were reported by Muhammad Amin and et al [15].

SEM and TEM analysis

Microscopic techniques such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are mainly used for morphological studies of nanoparticles. In figure 9, SEM image clearly confirmed the presence of silver nanoparticle with a size of 30- 50 nm. The results obtained from SEM analysis were very similar to the findings of other researchers [16-18]. The TEM images of colloid nanoparticles is shown in figure 10, It is clear that the morphology of nanoparticles is often spherical and cubic with average particle size 20 nm.

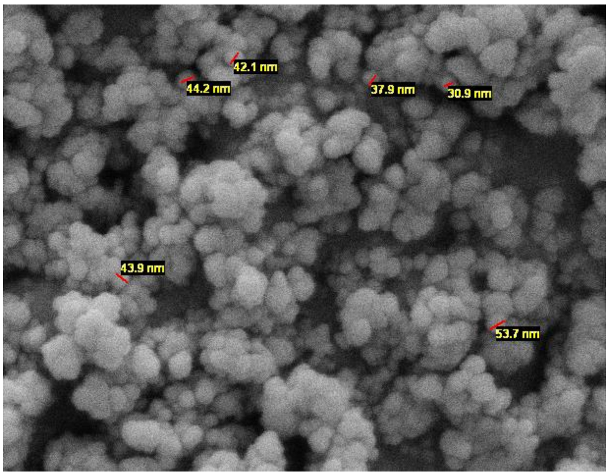


Fig. 9. SEM images of silver nanoparticles synthesized by *Lawsonia inermis* extract

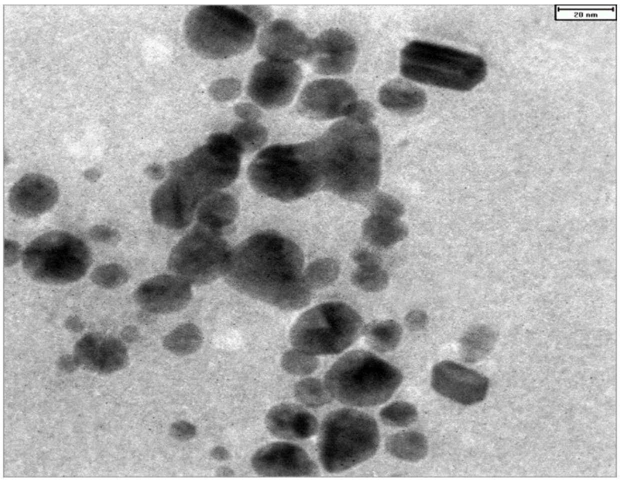


Fig. 10. TEM images of silver nanoparticles synthesized by *Lawsonia inermis* extract

XRD analysis

X-ray diffraction (XRD) analyses were carried out with a GNR MPD 3000 made Italy diffractometer using a Cu anode (λ = 1.54056 Å).

The diffractograms were recorded at 2θ in the range 9˚ - 80˚, counting time is 0.5 secound and with step size of 0.02˚. In figure 11, the XRD pattern clearly shows cubic structure matches the values (38.4) , (44.52), (64.7), (77.73) correspond to the hkl planes (1 1 1), (2 0 0), (2 2 0), (3 1 1), respectively, with an average size ~ 20. All the peaks match well with the standard JCPDS file 87-0717.

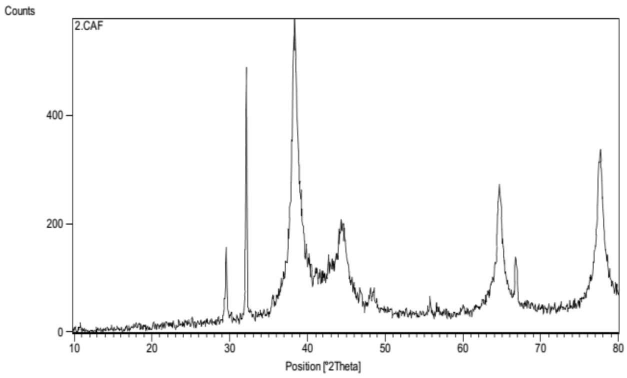


Fig. 11. X-ray diffractogram of silver nanoparticles synthesized by *Lawsonia inermis* extract

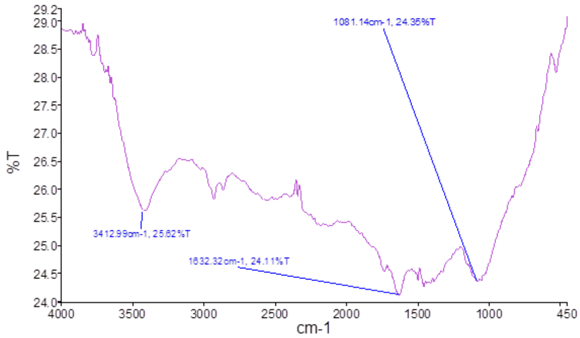
FTIR analysis

Fourier transform infrared (FTIR) spectra of the samples were obtained using Perkin Elmer Spectrum 2,

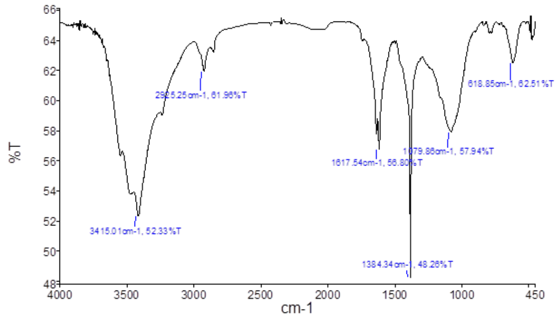
in the diffuse reflectance mode operating at a resolution of 4 cm^{-1} in KBr pellets. FTIR spectroscopy was used to determine the nature of associated molecules of plants or their extracts with nanoparticles.

The FTIR spectra of *Lawsonia inermis* extracts taken before and after synthesis of silver nanoparticles were analyzed that discussed for the possible functional groups for the formation of silver nanoparticles. FTIR spectra were obtained to identify the possible biomolecules in the plant extract responsible for predict their role in nanoparticle synthesis and the reduction of silver ions and also the capping agents responsible for the stability of the biogenic nanoparticle solution. The FTIR spectra indicates various functional groups presence at different positions. Figure 12 a) and b) shows the FTIR spectra of plant extract before and after synthesis which represents prominent absorption band at around 3413, 1632, 1081.14 cm^{-1}.

The peaks in the region 3413 cm^{-1} were assigned for O-H stretching vibration indicating the presence of hydroxyl groups such as alcohol and phenol compounds and aldehyde –C-H- stretching of alkanes and around 1620cm^{-1} C=O stretching vibrations or stretching of alkenes, and absorption band at 1080 cm^{-1} could be assigned to the C–OH stretching vibrations of primary alcohol groups in the *Lawsonia inermis* extracts [19]. The presence of absorption bands in the *Lawsonia inermis* extract spectrum and absence of the same peak in the silver nanoparticle spectrum indicated its involvement in the synthesis of the silver nanoparticles. According to the figures 12 a) and b), the shift in higher to lower wave numbers in comparison of two figures is indicated the facilitation resonance for the binding and reduction of silver nanoparticles surface [20].



(a)



(b)

Fig. 12 FT-IR spectra of (a) *Lawsonia inermis* extract and (b)silver nanoparticles synthesized by *Lawsonia inermis*

CONCLUSION

In this work, biosynthesis of silver nanoparticles by *Lawsonia inermis* extract was studied. In order to achieve optimal synthesis conditions effective parameters such as pH, volume of extract, concentration of salt solution, reaction temperature and time were investigated. In optimum conditions, cubic silver nanoparticles were synthesized with an average size of 20 nm and were characterized by utilizing UV-Vis spectrophotometer, (SEM), (TEM), (XRD) and (FTIR) techniques.

This research showed that the biological synthesis of nanoparticles by using plant extracts is a very low cost and efficient method, and it could be an appropriate alternative to typical chemical methods for the synthesis of nanoparticles.

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