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Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity

Akl M Awwad^{1*}, Nidá M Salem² and Amany O Abdeen¹

Abstract

Background: This paper describes a rapid and eco-friendly method for green synthesis of silver nanoparticles from aqueous solution of silver nitrate using carob leaf extract (*Ceratonia siliqua*) in a single-pot process.

Results: Formation of stable silver nanoparticles at different concentrations of AgNO₃ gave mostly spherical particles with a diameter ranging from 5 to 40 nm. It was observed that the use of carob leaf extract makes a fast and convenient method for the synthesis of silver nanoparticles and can reduce silver ions into silver nanoparticles within 2 min of reaction time without using any severe conditions. Green synthesis of silver nanoparticles (AgNPs) was characterized by UV-visible (UV-vis) spectroscopy, scanning electron microscopy, atomic absorption spectroscopy, Fourier transform infrared spectroscopy, and X-ray diffraction (XRD). The UV-vis spectra gave surface plasmon resonance for synthesized silver nanoparticles at 420 nm. The XRD analysis showed that the AgNPs are crystalline in nature and have face-centered cubic geometry.

Conclusion: Further, the AgNPs showed an effective antibacterial activity toward *Escherichia coli* pathogen.

Keywords: Biosynthesis, Silver nanoparticles, Carob leaf extract, Characterization, Antibacterial activity

Background

Silver nanoparticles (AgNPs) have become the focus of intensive research owing to their wide range of applications in areas such as catalysis, optics, antimicrobials, and biomaterial production. Silver nanoparticles exhibit new or improved properties depending upon their size, morphology, and distribution. Various approaches using plant extract have been used for the synthesis of metal nanoparticles. These approaches have many advantages over chemical, physical, and microbial syntheses [1-10] because there is no need of the elaborated process of culturing and maintaining the cell, hazardous chemicals, high-energy requirements, and wasteful purifications. Many research papers reported the synthesis of silver nanoparticles using plant extracts such as *Helianthus annuus*, *Basella alba*, *Oryza sativa*, *Saccharum officinarum*, *Sorghum bicolor*, and *Zea mays* [11]; pine, persimmon, ginkgo, magnolia, and platanus leaves [12]; *Jatropha curcas* seeds [13]; *Acalypha indica* leaf [14]; banana peel [15]; *Chenopodium album* leaf [16]; *Rosa rugosa* [17]; *Trianthema decandra* roots [18]; *Ocimum*

sanctum stems and roots [19]; *Sesuvium portulacastrum* leaves [20]; *Murraya koenigii* (curry) leaf [21]; *Macrotyloma uniflorum* seeds [22]; *Ocimum sanctum* (Tulsi) leaf [23]; *Garcinia mangostana* (mangosteen) leaf [24]; *Stevia rebaudiana* leaves [25]; *Nicotiana tobaccum* leaf [26]; *Ocimum tenuiflorum*, *Solanum trilobatum*, *Syzygium cumini*, *Centella asiatica*, and *Citrus sinensis* leaves [27]; *Arbutus unedo* leaf [28]; *Ficus benghalensis* leaf [29]; mulberry leaves [30]; and *Olea europaea* leaves [31].

In this study, we have synthesized silver nanoparticles using carob leaf extract for reduction of Ag⁺ ions to Ag⁰ nanoparticles from silver nitrate solution within 2 min of reaction time at ambient temperature. It was also shown that the average size of silver nanoparticles can be controlled to 5 to 40 nm by varying the concentration of silver nitrate and the volume of leaf extract. Further, biosynthesized silver nanoparticles are found to be highly effective against *Escherichia coli* bacteria.

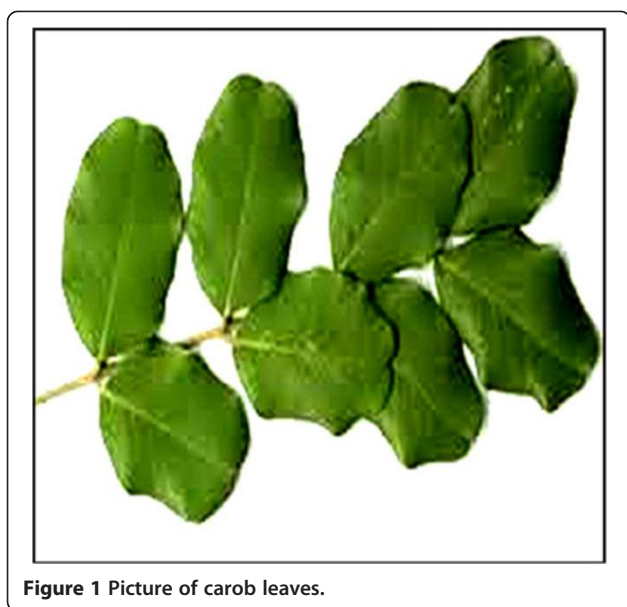
Methods

Preparation of carob leaf extract

Carob leaves (Figure 1) were collected from carob trees at the campus of the Royal Scientific Society, Amman,

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Jordan. The leaves were washed several times with distilled water to remove the dust particles and then sun-dried to remove the residual moisture. The dried carob leaves were cut into small pieces and boiled in a 250-ml glass beaker along with 200 ml of sterile distilled water for 10 min. After boiling, the color of the aqueous solution changed from watery to yellow color. The aqueous extract was separated by filtration with Whatman No. 1 filter paper (Maidstone, UK) and then centrifuged at 1,200 rpm for 5 min to remove heavy biomaterials. The carob leaf extract was stored at room temperature to be used for biosynthesis of silver nanoparticles from silver nitrate.

Synthesis of AgNPs

In a typical reaction procedure, 5 ml of carob leaf extract was added to 100 ml of 1×10^{-3} M aqueous AgNO_3 solution, with stirring magnetically at room temperature. The yellow color of the mixture of silver nitrate and carob leaf extract at 0 min of reaction time changed very fast at room temperature after 2 min to a black suspended mixture. This indicated that carob leaf extract speeds up the biosynthesis of silver nanoparticles more than other plant leaves [12-18]. The concentrations of AgNO_3 solution and leaf extract were also varied at 1 to 4 mM and 5% to 10% by volume, respectively. UV-visible (UV-vis) spectra showed strong surface plasmon resonance (SPR) band at 420 nm and thus indicating the formation of silver nanoparticles. The AgNPs obtained by carob leaf extract were centrifuged at 15,000 rpm for 5 min and subsequently dispersed in sterile distilled water to get rid of any uncoordinated biological materials.

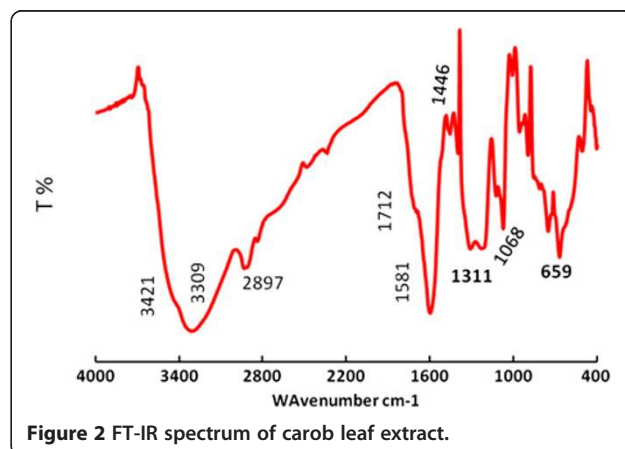
Characterization techniques

UV-vis absorption spectra were measured using a Shimadzu UV-1601 spectrophotometer (Kyoto, Japan). Crystalline metallic silver nanoparticles were examined using an X-ray diffractometer (Shimadzu, XRD-6000) equipped with Cu K α radiation source using Ni as filter at a setting of 30 kV/30 mA. All X-ray diffraction (XRD) data were collected under the experimental conditions in the angular range $3^\circ \leq 2\theta \leq 50^\circ$. Fourier transform infrared (FT-IR) spectra for *Ceratonia siliqua* leaf extract powder and silver nanoparticles was obtained in the range 4,000 to 400 cm^{-1} with an IR-Prestige-21 Shimadzu FT-IR spectrophotometer, by KBr pellet method. Scanning electron microscopy (SEM) analysis of synthesized silver nanoparticles was done using a Hitachi S-4500 SEM machine (Chiyoda-ku, Japan). Concentration of silver ions was analyzed by atomic absorption spectroscopy (AAS; AA-6300, Shimadzu).

Results and discussion

FT-IR spectrum

The FT-IR spectrum obtained for carob leaf extract (Figure 2) displays a number of absorption peaks, reflecting its complex nature. Strong absorption peaks at 3,309 and 3,421 cm^{-1} result from stretching of the -NH band of amino groups or is indicative of bonded -OH hydroxyl group. The absorption peaks at about 2,897 cm^{-1} could be assigned to stretching vibrations of -CH₂ and CH₃ functional groups. The peaks at 1,712 and 1,581 cm^{-1} indicate the fingerprint region of CO, C-O, and O-H groups. The intense band at 1,068 cm^{-1} could be assigned to the C-N stretching vibrations of aliphatic amines. The FT-IR spectrum also shows bands at 1,581 and 1,446 cm^{-1} identified as amide I and amide II which arise due to carbonyl (C=O) and amine (-NH) stretching vibrations in the amide linkages of the proteins, respectively. The absorption band at 1,446 cm^{-1} could be attributed to methylene scissoring vibrations from the proteins. FT-IR study indicates that the carboxyl (-C=O), hydroxyl



(-OH), and amine (N-H) groups in carob leaf extract are mainly involved in reduction of Ag^+ ions to Ag^0 nanoparticles. The FT-IR spectroscopic study also confirmed that the protein present in carob leaf extract acts as a reducing agent and stabilizer for the silver nanoparticles and prevents agglomeration. The carbonyl group of amino acid residues has a strong binding ability with metal, suggesting the formation of a layer covering silver nanoparticles and acting as a stabilizing agent to prevent agglomeration in the aqueous medium.

UV-vis absorbance study

The addition of carob leaf extract to silver nitrate (AgNO_3) solution resulted in color change of the solution from transparent to brown due to the production of silver nanoparticles. The color changes arise from the excitation of surface plasmon vibrations with the silver nanoparticles. The SPR of silver nanoparticles produced a peak centered near 420 nm. UV-vis absorbance of the reaction mixture was taken from 0 till 2 min (Figure 3). It was observed that the absorbance peak was centered near 420 nm, indicating the reduction of silver nitrate into silver nanoparticles. It was also observed that the reduction of silver ions into silver nanoparticles started at the start of reaction and reduction was completed at almost 2 min at room temperature, indicating rapid biosynthesis of silver nanoparticles.

XRD studies

Analysis through X-ray diffraction was carried out to confirm the crystalline nature of the silver nanoparticles. The XRD pattern showed numbers of Bragg reflections that may be indexed on the basis of the face-centered cubic structure of silver. A comparison of our XRD spectrum with the standard confirmed that the silver particles formed in our experiments were in the form of nanocrystals, as evidenced by the peaks at 2θ values of 38.28°, 44.04°, 64.34°, and 77.28° corresponding to (111),

(200), (220), and (311) Bragg reflections, respectively, which may be indexed based on the face-centered cubic structure of silver. X-ray diffraction results clearly show that the silver nanoparticles formed by the reduction of Ag^+ ions by the carob leaf extract are crystalline in nature. The unassigned peaks at $2\theta = 27.96^\circ$, 32.28° , and 46.18° denoted by (*) in Figure 4 are thought to be related to crystalline and amorphous organic phases. It was found that the average size from XRD data and using the Debye-Scherrer equation was approximately 18 nm. The presence of structural peaks in XRD patterns and the average crystalline size around 18 nm clearly illustrate that the AgNPs synthesized by our green method were nanocrystalline in nature. The average particle size of silver nanoparticles synthesized by the present green method can be calculated using the Debye-Scherrer equation [19,22]:

$$D = K\lambda/\beta \cos\theta,$$

where D is the crystallite size of AgNPs, λ is the wavelength of the X-ray source (0.1541 nm) used in XRD, β is the full width at half maximum of the diffraction peak, K is the Scherrer constant with a value from 0.9 to 1, and θ is the Bragg angle.

FT-IR spectra of biosynthesized silver nanoparticles

Results of the FT-IR study of biosynthesized AgNPs showed sharp absorption peaks located 3,410, 1,608, 1,558, and 1,408 cm^{-1} (Figure 5). The absorption peak at 1,608 cm^{-1} may be assigned to the amide I bond of proteins arising from carbonyl stretching in proteins, and the peak at 3,410 cm^{-1} is assigned to OH stretching in alcohols and phenolic compounds. The absorption peak at 1,608 cm^{-1} is close to that reported for native proteins, which suggests that proteins are interacting with biosynthesized silver nanoparticles and also their secondary structure was not affected during reaction

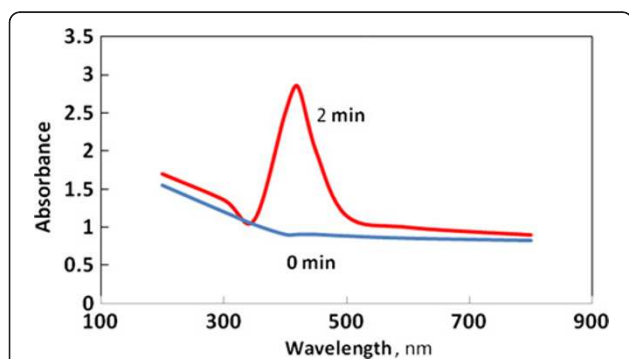


Figure 3 UV-vis spectrum. Showing absorption of 10^{-3} M aqueous solution of silver nitrate with carob leaf extract as a function of time.

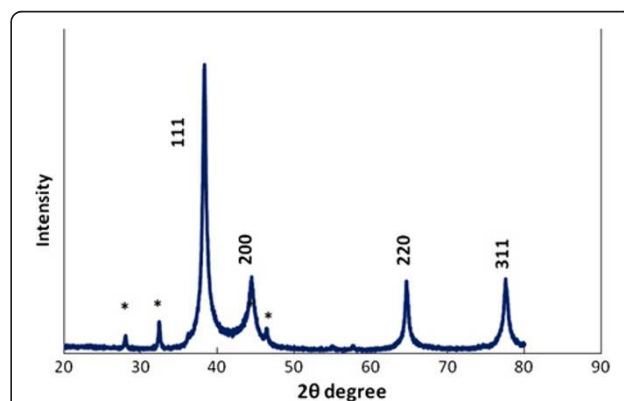
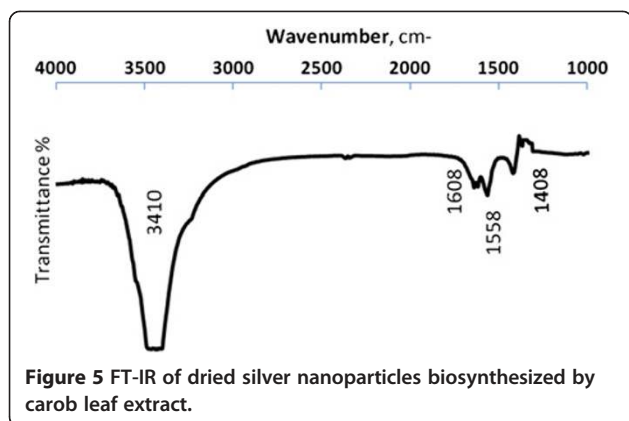


Figure 4 XRD pattern of silver nanoparticles biosynthesized by treating silver nitrate with carob leaf extract.



with Ag^+ ions or after binding with Ag^0 nanoparticles. This FT-IR spectroscopic study confirmed that the carbonyl group of amino acid residues has a strong binding ability with silver, suggesting the formation of a layer covering silver nanoparticles and acting as a capping agent to prevent agglomeration and provide stability to the medium. These results confirm the presence of possible proteins acting as reducing and stabilizing agents.

Atomic absorption spectroscopy analysis

Silver ion concentration was analyzed by AAS which showed the conversion of Ag^+ ions into Ag^0 nanoparticles. Initially, a standard solution of 10 ppm of AgNO_3 was prepared and analyzed with AAS at 0 min. The Ag^+ ion concentration in the reaction solution, after adding carob leaf extract, was monitored at different time intervals. The result showed a decrease in concentration of Ag^+ ions with reaction time, indicating the complete conversion of Ag^+ ions into Ag^0 nanoparticles at 2 min of reaction time (Figure 6).

SEM analysis of silver nanoparticles

SEM analysis shows uniformly distributed silver nanoparticles on the surfaces of the cells (Figure 7). The

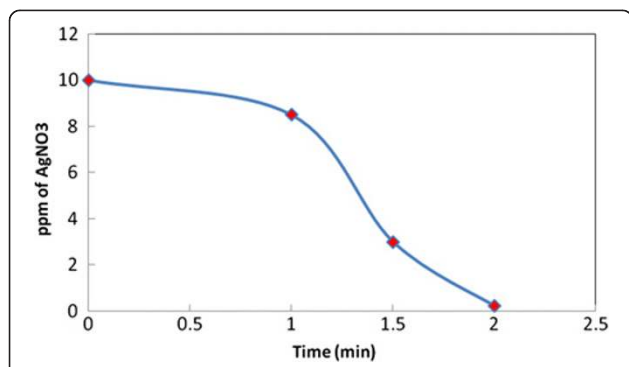
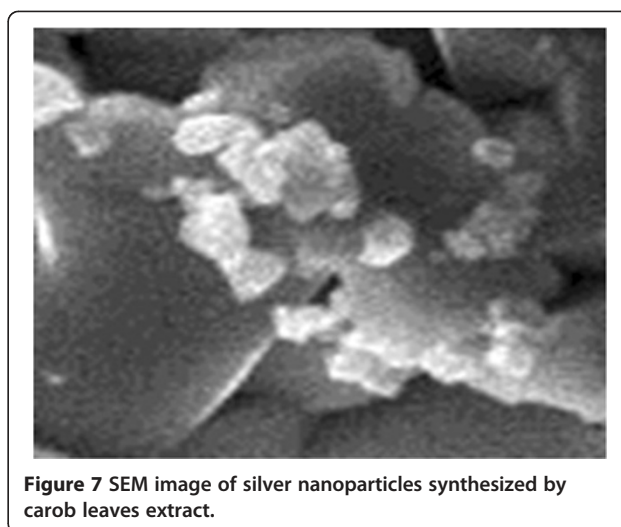


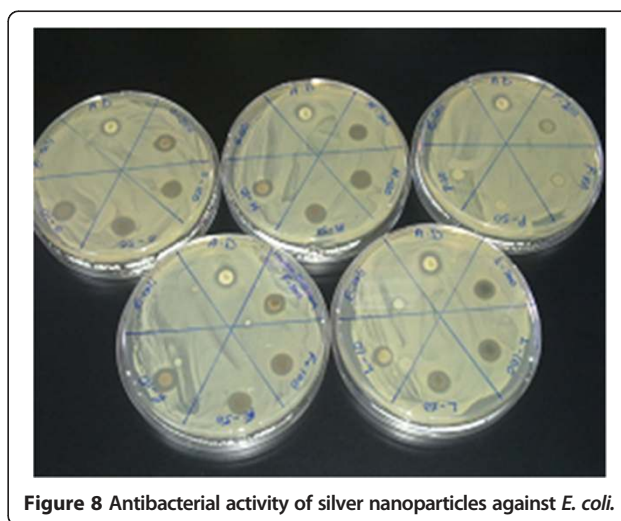
Figure 6 AAS analysis of silver nitrate (ppm) with reaction time (min) using carob leaf extract.



silver nanoparticles were spherical in shape with particle size range from 5 to 40 nm. The larger silver particles may be due to the aggregation of the smaller ones, due to the SEM measurements.

Antibacterial assays

Biosynthesized silver nanoparticles were analyzed for their antimicrobial activity against *E. coli* by disk diffusion method (Figure 8). It was observed that microbial growth of *E. coli* was independent on AgNP concentration. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of silver nanoparticles that inhibited the visible growth of *E. coli*. It was found that the MIC ($\mu\text{g/l}$) for silver nanoparticles is 0.5, for silver nitrate 1.8 mg, and for standard antibiotic 0.6 mg. The zone of inhibition ranged from 8 to 12 mm. These results indicated that the silver nanoparticles



synthesized using carob leaf extract have stronger activity than standard antibiotic.

Experimental

For synthesis of silver nanoparticles, at first, carob leaf extract is prepared by boiling the dried small pieces of carob leaves in sterile distilled water. In a typical reaction procedure, 5 ml of carob leaf extract is added to 100 ml of 1×10^{-3} M aqueous solution of silver nitrate (AgNO_3), with stirring magnetically at room temperature. At this stage, the color of the reaction mixture changes from yellow to black and black suspended particles are produced, indicating the formation of silver nanoparticles. The black mixture is centrifuged, washed three times with distilled water and ethanol, and finally dried at 60°C . Synthesized silver nanoparticles were characterized by UV-vis spectroscopy, SEM, AAS, FT-IR, and XRD. Afterwards the antibacterial activity of silver nanoparticles was studied by diffusion method.

Conclusions

We have developed a fast, eco-friendly, and convenient green method for the synthesis of silver nanoparticles from silver nitrate using carob leaf extract at ambient temperature. Carob leaf extract is found suitable for the green synthesis of silver nanoparticles within 2 min at ambient conditions. Spherical, polydisperse AgNPs of particle sizes ranging from 5 to 40 nm with an average size of 18 nm are obtained. Color changes occur due to surface plasmon resonance during the reaction with the ingredients present in the carob leaf extract resulting in the formation of silver nanoparticles, which is confirmed by XRD, FT-IR, UV-vis spectroscopy, and SEM. FT-IR spectroscopic study confirmed that the carbonyl group of amino acid residues has a strong binding ability with silver, suggesting the formation of a layer covering silver nanoparticles and acting as a capping agent to prevent agglomeration and provide stability to the medium, yet further research is needed in this area to explore the possible biomolecule responsible for the bioreduction process. The antibacterial activity of biologically synthesized silver nanoparticles was evaluated against *E. coli* pathogen.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AMA participated in the design of the study and performed the green synthesis method and characterization of synthesized nanoparticles. NMS participated in the design of the study and carried out the extraction of carob leaf extract and analysis of antibacterial activity results. AOA performed the antibacterial activity. All authors read and approved the final manuscript.

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