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Phytochemical fabrication and characterization of silver/ silver chloride nanoparticles using *Albizia julibrissin* flowers extract

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ABSTRACT

Silver/ silver chloride (Ag/AgCl-NPs) nanoparticles were synthesized by a simple procedure using *Albizia julibrissin* flowers extract. The flowers of *Albizia julibrissin* extract acts a reducing, chlorinated and capping agent in the formation of Ag/AgCl-NPs. UV-visible spectroscopy was used to monitor the quantitative formation of silver/ silver chloride nanoparticles. For complete conversion of silver ions to silver/ silver chloride nanoparticles, time of reaction is less than 5 min at temperature 80°C and 24h in dark conditions at room temperature. The synthesized Ag/AgCl-NPs nanoparticles were characterized with X-ray diffraction (XRD), UV-vis Spectroscopy, scanning electron microscopy (SEM) and Fourier transforms infrared spectroscopy (FT-IR). UV-visible absorption studies revealed surface plasmon resonance (SPR) peak around 410-430 nm, confirming the presence of Ag/AgCl nanoparticles. The average particle size ranged from 5-20 nm. The particle size could be controlled by changing the flowers extract, silver ion concentration and temperature. FT-IR spectra of flowers extract before and after the synthesis of silver/ silver chloride nanoparticles were determined to allow identification of possible functional groups responsible for the reduction of silver ions to silver/silver chloride nanoparticles. Copyright © 2015 VBRI Press.

Keywords: Green synthesis; silver/silver nanoparticles; *Albizia julibrissin* flowers; characterization.

Introduction

Silver nanoparticles found tremendous applications in antimicrobials and therapeutics, catalysis, micro-electronics, topical ointments and creams. Various approaches were developed for silver and silver chloride nanoparticles synthesis such as electrochemical, chemical reduction, surfactants, photochemical reduction, ionic liquid microemulsion, ultrasound irradiation, hydrolysis and ion-exchange reactions and thermal decomposition **[1-4].** These approaches use hazardous chemicals, low material conversions, high energy requirements and wasteful purification. Therefore, there is a growing need to develop environmentally friendly methods for silver nanoparticles without using hazardous chemicals. Biological approaches using plant extracts for metal nanoparticles synthesis have been suggested as valuable alternatives to chemical methods. The use of plant extract for synthesis of silver nanoparticles could be more advantageous. Recent research reported the synthesis of silver nanoparticles using different plant extracts such as *Caesalpinia coriaria* leaf extracts **[5]**, *Syzygium aromaticum* dried buds [**6**], aqueous extract of *Manilkara zapota* (L.) seeds **[7]**, *Crataegus douglasii* fruit extract **[8]**, *Tribulus terrestris* leaf extract **[9]**, *Tephrosia purpurea* leaf extract **[10]**, *Delphinium denudatum* root extract **[11]**, *Cocos nucifera* coir extract [**12**], *Ixora coccinea* leaves extract **[13]**, *Terminalia chebula* fruit [**14**], seed extract of *Jatropha curcas* [**15**], *Artocarpus heterophyllus* Lam. Seed [**16**], *Cissus quadrangularis* Linn [**17**], *Onosma dichroantha* Boiss. Root extract [**18**] and leaf extracts of *Cycas circinalis*, *Ficus amplissima*, *Commelina benghalensis*, *Lippia nodiflora* [**19**], *Emblica officinalis* fruit extract [**20**], *Lantana camara* leaf extract [**21**], *Onosma dichroantha Boiss*, root extract [**22**], *Beta vulgaris peel* extract [**23**], *Eucalyptus* leaf extract [**24**], *Asiatic pennywort* and *Bryophyllum* leaves extract [**25**], *Passiflora tripartite* fruit extracts [**26**], and *Citrus limon* extract [**27**].

Albizia julibrissin (Persian silk tree, pink silk tree) is a small deciduous tree growing to 5–12 m tall, with a broad crown of level or arching branches. The flowers are produced throughout the summer in dense inflorescences, the individual flowers with small calyx and corola, and a tight cluster of stamens 2–3 cm long, white or pink with a white base, looking like silky threads. They have been observed to be attractive to bees, butterflies and humming birds.

The importance of silver nanoparticles in different applications, such as antimicrobial agents, fertilizers, and insecticides, the development of green methods is highly essential for nanosized silver particles. Biological approach using flowers of *Albizia julibrissin* has been used for the first time as dispersing and stabilizing agent for the synthesis silver nanoparticles at room temperature.

Experimental

Materials

Silver nitrate $AgNO_3$ (99.8%, Merck Chemicals, Darmstadt, Germany) was used without further purification. Fresh flowers of *Albizia julibrissin* have been harvested in July 2014 from in and around Amman City, Jordan. All glassware have been washed with sterile distilled water and dried in an oven before use. Deionized distilled water was used in all experimental work.

Preparation of Albizia julibrissin flowers (Ajf) extract

Freshly *Albizia julibrissin* flowers (*Ajf*), **Fig. 1** were collected from their trees in and around houses in the city of Amman, Jordan. Flowers were washed several times with distilled water to remove the dust particles and then sun dried to remove the residual moisture. The *Albizia julibrissin* flowers extract used was prepared by placing 10 g of washed dried fine cut flowers in 500 mL glass beaker along with 400 mL of sterile distilled water. The mixture was then boiled for 5 minutes until the color of the aqueous solution changes from watery to light brown color. Then the extract was cooled to room temperature and filtered with Whatman No. 1 filter paper before centrifuging at 1500 rpm for 5 minutes to remove the heavy biomaterials. The extract was stored at room temperature in order to be used for further experiments.

Fig. 1. Photograph shows the flowers of *Albizia julibrissin*.

Phytochemical screening of the ajf extract

The aqueous extract of *Albizia julibrissin* flowers extract was subjected to different qualitative chemical tests in order to detect the presence of flower constituents. The phytochemical components of the *Albizia julibrissin* flowers were screened by using standard methods **[28, 29].** The components analyzed are alkaloids, flavonoids, proteins, tannin, steroids, phenolic compounds. This study has revealed the presence of phytochemicals considered as active chemical constituents of *Albizia julibrissin* flowers extract.

Dionex ICS-1600 ion chromatography analysis to *Ajf* aqueous extract indicated that 36 mg/L of chlorine ions is present within the structure of chemical constituents.

Synthesis of silver nanoparticles (Ag/AgCl-NPS)

Two methods were used for synthesis of silver/ silver chloride (Ag/AgCl-NPs) nanoparticles using *Albizia julibrissin* flowers. In the first method, 10 ml of *Albizia julibrissin* flowers extract was added to 100 mL of mM aqueous $AgNO_3$ solution, the mixture was stirred magnetically and heated at 80^o C for few minutes, the resulting solution become deep yellow in color after one minute of heating and then to black. The black suspended particles formed indicated the formation of silver/ silver chloride nanoparticles. In the second method, 20 ml of *Albizia julibrissin* flowers extract was mixed with 100 ml of aqueous solution of silver nitrate ($AgNO_3$) in a 250 ml conical flask and kept in a dark place at room temperature. After 12h, the colorless reaction mixture start changing to deep yellow color and afterwards to black color, indicating the formation of Ag/AgCl-NPs. The silver/ silver chloride nanoparticles obtained by *Albizia julibrissin* flowers extract were centrifuged at 10,000 rpm for 10 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 Shimadzu UV-1601 spectrophotometer. Crystalline metallic silver nanoparticles were examined by X-ray diffractometer (Shimadzu XRD-6000) equipped with Cu Kα radiation source using Ni as filter and at a setting of 30 kV/30 mA. All XRD data were collected under the same experimental conditions, in the angular range $20^o \leq 2\theta \leq 80^o$. FTIR Spectra for *Albizia julibrissin* flowers extract was obtained in the range 4000–400 cm^{-1} with IR-Prestige-21 Shimaduz FTIR spectrophotometer, using KBr pellet method. Scanning electron microscopy (SEM) analysis of silver nanoparticles analysis was done using Hitachi S-4500 SEM machine. Thin films of the silver nanoparticles were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 minutes.

Results and discussion

UV–visible spectroscopy is a significant technique to ascertain the formation and stability of metal nanoparticles in aqueous solution. The size and shape of metal nanoparticles determine the spectral position of Plasmon band absorption as well as its width. The UV–Visible absorption of silver nanoparticles is exhibit maximum in the range of 400-440 nm due to this property. The mixture of *Albizia julibrissin* flowers extract and silver nitrate solution was subjected to UV-vis spectra, based on the color change and the absorbance of the reaction medium was noted. **Fig. 2** showed SPR bands of the colloidal silver nanoparticles were centered at around 430 nm for the silver nanoparticles synthesized by heating and dark method.

In the present study, the absorption spectra of silver/silver chloride nanoparticles synthesized using *Albizia julibrissin* flowers extract reveals the conversion of silver ions to silver/silver chloride nanoparticles with almost 100% bioreduction of metal ions as evidenced by qualitative testing of supernatant after the purification of silver/silver chloride nanoparticles. The difference in the rate of bioreduction observed may be assign to the differences in the activities of the aminoacids and flavonoids present in *Albizia julibrissin* flowers extract. The entire reaction mixture is turned to black color, and exhibit an absorbance peak around 430 nm characteristic of Ag/AgCl-NPs nanoparticle, due to its surface plasmon resonance absorption band. In present investigation, the reaction mixtures showed a single SPR band revealing spherical shape of silver/silver chloride nanoparticles, which was further confirmed by SEM images.

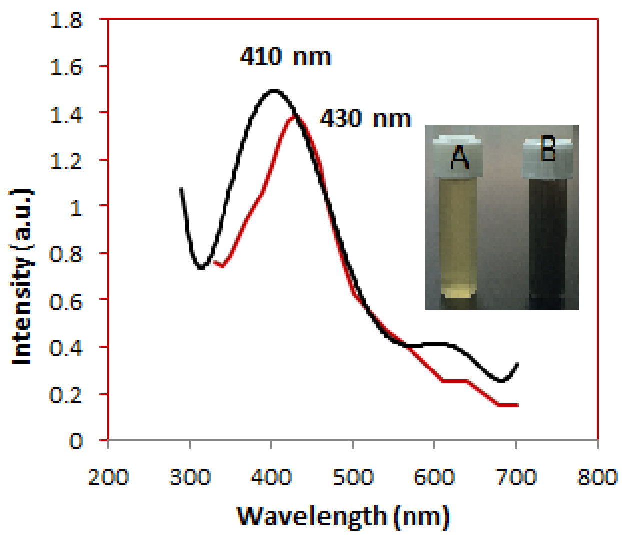


Fig. 2. UV-vis absorption spectra of silver/silver chloride nanoparticles using *Ajf* by two methods. In set of the figure shows solutions of A, flowers extract and silver ions and B resultant Ag/AgCl-NPs.

The crystalline nature of silver/silver chloride nanoparticles was confirmed from the analysis of X-ray diffraction (XRD) pattern, **Fig. 3** and **4**. The XRD pattern indicates four distinct diffraction peaks at 2θ values of 38.43°, 44.53°, 64.67° and 77.49° indexed as (111), (200), (220) and (311) lattice planes of face centered cubic (fcc) structure of metallic silver and is consistence with JCPDS data No. 87-0720. Also another five distinct diffraction peaks at 2θ = 28.13 (111), 32.10° (200), 46.50° (220), 55.06° (311) and 57.75° (222) indexed as (111), (200), (220), (311), and (222) lattice planes of face centred cubic (fcc) structure matched to silver chloride nanoparticles (JCPDS file No.: 85-1355). The broadening and strong signals of pattern evinces that the products are nanosized and well crystallized respectively. One can calculate the values of average crystallite size (D) from XRD spectrum using Debye-Scherrer equation [**30**]:

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

Where K denotes the Scherrer's constant, λ is the X-ray wavelength, β the full-width at half-maximum of diffraction line in radian and θ is half diffraction angle. The size of the silver/silver chloride nanocrystallites was calculated in range of 5-20 nm. XRD data showed that Ag/AgCl-NPs synthesized in dark method at room temperature gave smaller average size than that synthesized by heating at 80 °C.

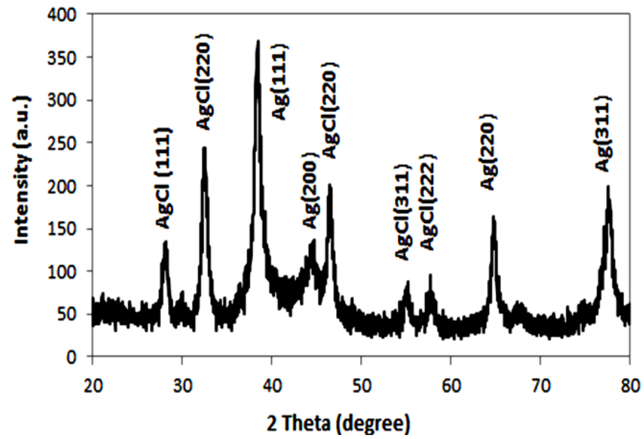


Fig. 3. XRD spectrum of biosynthesized Ag/AgCl-NPs using *Ajf* extract at 80 °C.

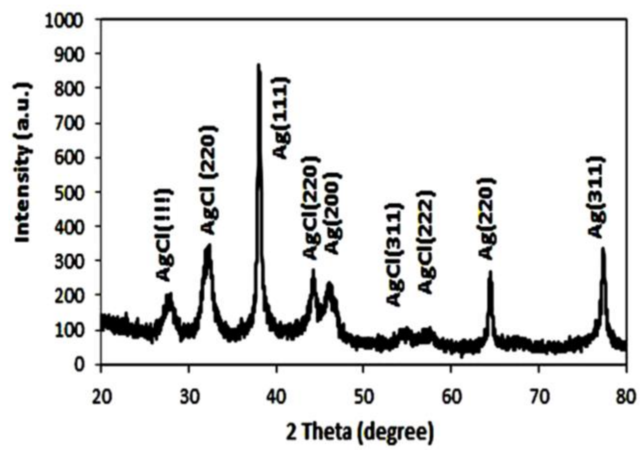


Fig. 4. XRD spectrum of biosynthesized Ag/AgCl-NPs using *Ajf* extract by dark method at room temperature.

Fourier transform infrared spectroscopy is used to identify and get an approximate identification of the possible biomolecules in plant extract are responsible for reducing, capping and stabilization of the Ag/ AgCl-NPs with the *Albizia julibrissin* flowers extract. **Fig. 5** shows strong peak at 3444 cm^{-1} can be attributed hydrogen bonded O-H groups of alcohols and phenols and also to the presence of amines N-H of amide. The –OH is derived from water soluble phenolic compounds in the plant flowers, as qualitative test for phenolic. The bands at 2924 cm^{-1} and 2850 cm^{-1} are assigned to $–CH_2$ and C-H stretching mode in alkanes. The shoulder peak at 1755 cm^{-1} in *Albizia julibrissin* flowers extract could be attributed to C=C stretching vibrations about C=O amide conjugated C=O of the proteins that are responsible for capping and stabilizing of Ag/AgCl-NPs.

The bands at 1647 cm^{-1} can be allocated to the stretching vibration of C-OH bond from proteins (amide I) of the flowers extract, whereas the band at 1543 cm^{-1} is characteristic of amide II, **Fig. 6**. The peak at 1416 cm^{-1} is characteristic to the C-N stretching of aliphatic amines. Peaks in the region 1315 cm^{-1}, 1199 cm^{-1} and 1049 cm^{-1} may be attributed to the presence of the stretching vibrations of carboxylic acids and amino groups. The peaks in the region 652-887 cm^{-1} attributed to alkyl halide stretching.

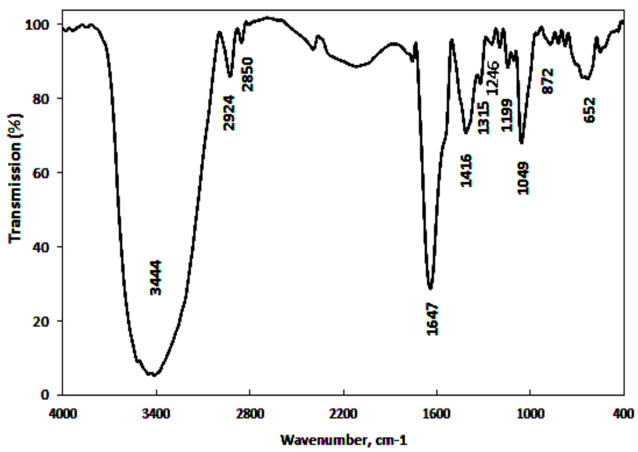


Fig. 5. FT-IR spectra of *Albizia julibrissin* flowers.

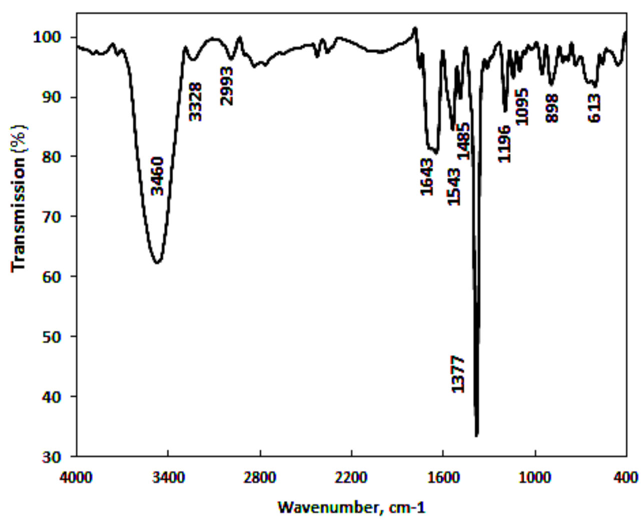


Fig. 6. FT-IR spectra of *Ajf*-Ag/AgCl-NPs.

Phytochemical screening of *Albizia julibrissin* flowers extract by standard methods **[28, 29]** indicated the presence of alkaloids, proteins, glycosides tannin, steroids, phenol, and flavonoids. Dionex ICS-1600 ion chromatography analysis to *Albizia julibrissin* flowers extract indicated that 36 mg/L of chlorine ions are present in *Albizia julibrissin* flowers extract. This suggests that the plant extract release chlorine ions to form silver chloride during the reaction process in addition of the reduction of silver ions to silver nanoparticles. FT-IR analysis of was carried out for biosynthesised silver/ silver chloride nanoparticles by using *Albizia julibrissin* flowers extract to identify the possible biomolecules responsible for the reduction, chlorination, capping, and stabilization of nanoparticles. FT-IR spectrum of Ag/AgCl-NPs is illustrated in **Fig. 6**. Small shifts in band position with *Albizia julibrissin* flowers extract and Ag/AgCl-NPs suggest that the nature of coordination of capping agents on different Ag/AgCl-NPs surface. The band at 1315 cm^{-1} in the spectrum of *Albizia julibrissin* flowers (*Ajf*) extract is characteristics of C-H alkyl halide group shifted to higher field and become sharp and stronger in *Ajf*-Ag/AgCl-NPs indicating that *Ajf* extract release chlorine ions to react with silver ions to form AgCl with silver nanoparticles.

SEM analysis of nanoparticles

The SEM micrograph for as prepared Ag/AgCl-NPs is shown in **Fig. 7**. The SEM micrograph clearly showed rough agglomerations of nanostructural homogeneities with spherical morphologies of Ag/AgCl-NPs. The SEM observation showed the presence of agglomerated nanospheres with an average diameter of 5–20 nm.

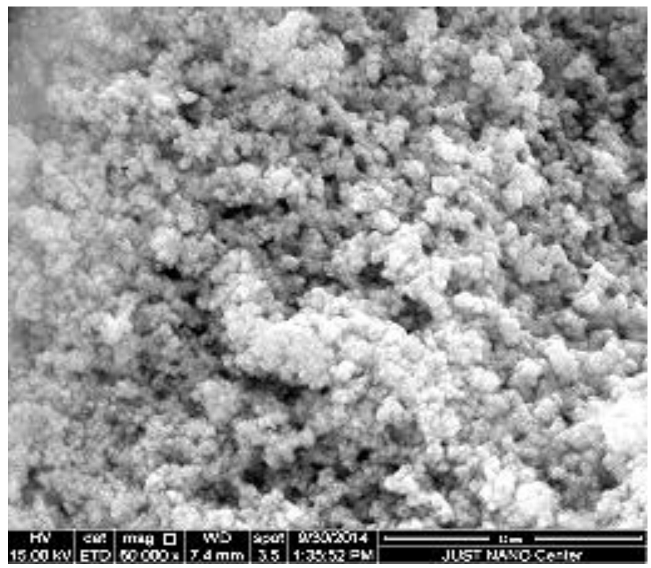
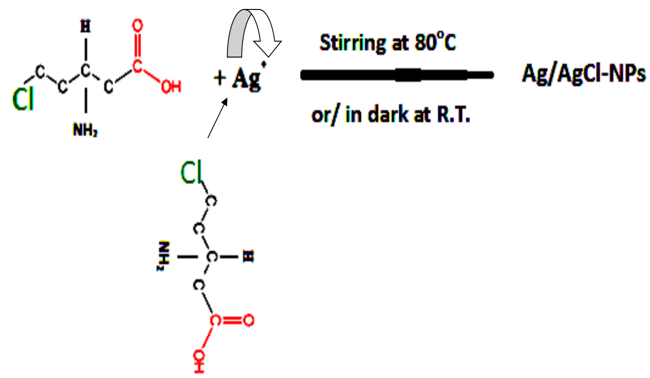


Fig. 7. SEM image of silver/silver chloride nanoparticles synthesized using *Ajf* extract.

Possible mechanism for the synthesis of Ag/AgCl-NPs

The mechanism of biosynthesis silver/silver chloride nanoparticles, proposed, **Scheme 1** on the presence of the bioactive constituents such as flavonoids, alkaloids, tannin, amino acids and phenols in *Albizia julibrissin* flowers.



Scheme 1. The proposed mechanism of biosynthesis of silver/silver chloride nanoparticles.

Dionex ICS-1600 ion chromatography analysis to *Ajf* extract indicated that 36 mg/L of chlorine ions is present. Hence, content of *Ajf* extract contains chlorine ions could be most attributed source for the formation of AgCl-NPs. According to that we proposed the mechanism for the production of silver/silver chloride nanoparticles. The hydroxyl groups of amino acids reduces the Ag^{+} to Ag^{o} and then the chlorine ions present in *Ajf* extract react with Ag^{+} to form AgCl. It has been confirmed by powdered XRD. Hence, content of the extract of *Ajf* contains chlorine could be most attributed source for the formation Ag/AgCl-NPs.

Conclusion

In the present study, we reported for the first time green synthesis of silver/ silver chloride nanoparticles using aqueous flowers extract of *Albizia julibrissin* at 80^{o}C and in dark at room temperature. We found that flowers act as reducing and capping agent and a source for releasing chlorine ions to form silver chloride nanoparticles. The physical property of synthesized Ag/AgCl-NPs was characterized using UV-visible spectroscopy, XRD, scanning electron microscopy, and FT-IR techniques. The crystalline nature of biosynthesized Ag/AgCl-NPs is evident from XRD diffraction peaks. The average crystallite size of nanoparticles deduced from XRD results is found to be 12 nm. Morphological studies showed the formation of spherical nanoparticles. The amount of *albizia julibrissin* flowers extract was found to play a critical role in controlling the shape, size, and size dispersity of silver/ silver chloride nanoparticles. Smaller nanoparticles and narrow size distribution are produced when larger quantiles of the extract is added to the reaction mixture. The average particle size ranged from 5-20 nm.

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