

FT-IR spectroscopy and X-ray diffraction characterization of biosynthesised silver/silver chloride nanoparticles

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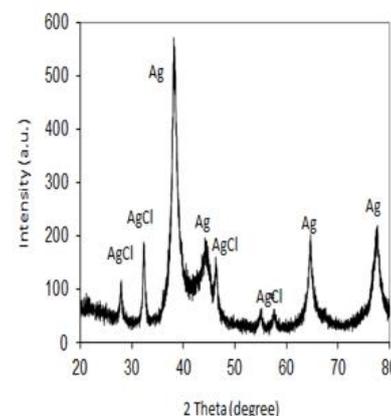
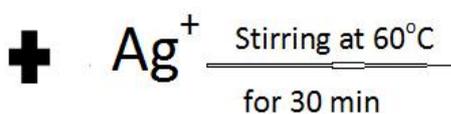
• Novelty and Highlights:

- 1 – New route for biosynthesis of silver/silver chloride nanoparticles.
- 2 – *Ligustrum vulgare* leaf extract acts as a reducing agent and a source for releasing chloride ions.
- 3 – Ag/AgCl nanoparticles evidenced antibacterial activity.

• Graphical Abstract:



Ligustrum vulgare leaves and extract





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Abstract: Biosynthesis of silver/silver chloride nanoparticles (Ag/AgCl-NPs) using aqueous solution of *Ligustrum vulgare* (Lv) leaf extract were confirmed by combining the FT-IR spectroscopy data with the results of X-ray diffraction (XRD). The pathway of Ag/AgCl-NPs formation is by means of reduction silver nitrate (AgNO₃) by *ligustrum vulgare* leaf extract, which acts as both reducing agent and a source to release chlorine ions to react with silver nanoparticles and forming silver chloride nanoparticles. The morphology and structure of such novel product strongly depend on the reaction time, temperature, silver nitrate concentration, and the amount of *Ligustrum vulgare* leaf extract. Ag/AgCl-NPs were found to be nearly spherical in shape and with average crystallite size of ~ 20 nm. UV-vis spectrum displays a single surface Plasmon resonance (SPR) band at 427-440 nm indicating the absence of anisotropic particles. The antibacterial activity of biosynthesized silver/silver chloride nanoparticles showed effective inhibitory activity against water borne pathogens *Listeria* and *Shigella* bacteria.

Keywords: silver/silver chloride nanoparticles; *Ligustrum vulgare* leaf extract; XRD; FT-IR, Antibacterial activity.

Introduction

Ligustrum vulgare (Lv) is a bushy deciduous shrub with dull green, lance-shaped leaves and terminal panicles of small white and unpleasantly scented white flowers in summer and followed by deep purple. The leaves are borne in decussate opposite pairs, sub-shiny green, narrow oval to lanceolate, 2–6 cm long and 0.5–1.5 cm broad. Silver nanoparticles AgNPs have attracted attention in research because of their use in coatings of equipment, bandages and other medical equipment to reduce bacterial transfer, biosensors in diagnostic applications, paints, cosmetics, and conductive inks for attainability of antibacterial activity.

The biosynthesis of silver/silver chloride (Ag/AgCl-NPs) nanoparticles has been proposed as eco-friendly, and cost-effective alternative to chemical and physical methods. Plant extracts for synthesis silver nanoparticles is potentially advantageous over microorganisms due to ease scale-up, biohazards, and elaborate process of cell culture. Recently silver and silver chloride nanoparticles synthesized by different plant extracts, such as seed aqueous extract of *Pistacia atlantica* [1], *Acalypha indicum* leaf extract [2], *Ziziphora tenuior* leaf extract [3], *Vitex negundo* L extract [4], *Lingonberry* and *cranberry* juices [5], *Dalbergia spinosa* leaves [6], European black elderberry fruits extract [7], *Acorous calamus rhizome* extract [8], *Ixora coccinea* leaves extract [9], *Stevia rebaudiana* leaves [10, 11], aqueous extract of *Syzygium aromaticum* [12], Lemon leaves extract [13], *Arbutus unedo* leaf extract [14], *Ficus benghalensis* leaf

extract [15], Loquat leaf extract [16], Carob leaf extract [17], *Coleus aromaticus* leaf extract [18], *Caesalpinia coriaria* leaf extracts [19], *Ixora copccinea* leaves extract [20], *Azadirachta indica* leaf extract [21], *Cynodon dactylon* leaves [22], *Dalbergia sissoo* leaf extract [23], *Cissus quadrangularis* extracts [24, 25], *Onosma dichroantha* Boiss root extract [26], *Morinda citrifolia* leaf extract [27], *Ficus sycomrus* leaf extract [28], Olive leaf [29], black pepper leaf [30], and leaf extract of *Achyranthes aspera* L. [31].

The present study was aimed to rapidly green synthesis of silver/silver chloride Ag/AgClNPs nanoparticles, using *Ligustrum vulgare* leaf extract to investigate the biomolecules responsible for reduction of silver ions to silver particles and stabilizing the Ag/AgCl nanoparticles and determining the antibacterial activity. To the best of our knowledge, for the first time, *Ligustrum vulgare* leaf extract is used for the green synthesis of silver nanoparticles.

Experimental

Materials

Silver nitrate (AgNO₃) is purchased from Sigma-Aldrich Chemicals. All glassware is washed with nitric acid (HNO₃) and distilled water and dried in oven at 105 °C. Deionized and distilled water was used in all experiments.

Preparation of *Ligustrum vulgare* leaf extract

Fresh *Ligustrum vulgare* leaves were collected in and around the campus of the Royal Scientific Society, El Hassan Science City, Jordan. Leaves were washed several times with water to remove dust particles and then sun dried to remove

the residual moisture. *L. vulgare* leaf extract was prepared by placing 20 g of dried fine cut in 500 ml glass beaker along with 400 ml of sterile distilled water. The mixture was then boiled for 10 minutes until the colour of aqueous solution changed from watery to brown. Then the mixture was cooled to room temperature and filtered with Whatman No. 1 filter paper before centrifuging at 1200 rpm for 2 minutes to remove biomaterials. The extract was stored at room temperature in order to be used for further experiments. Fig. 1 shows a photograph of *Ligustrum. vulgare* leaves and their aqueous extract.



Fig.1. Photograph shows the *L. vulgare* leaves and their aqueous brown extract.

Synthesis of silver/silver chloride nanoparticles In a typical reaction procedure, *Ligustrum vulgare* leaf extract was used for synthesis silver/silver chloride nanoparticles. 1-10 mL of *Ligustrum vulgare* leaf extract was added dropwise into 400 mL of 0.4 molar aqueous solution of AgNO_3 with constant stirring at temperature 30–60°C for 10-30 minutes. As soon as, *Ligustrum vulgare* leaf extract was mixed in aqueous solution of silver ions, it starts to change colour from colourless to light brown, to deep brown and finally to black due to excitation of surface Plasmon resonance which indicated the formation of silver/silver chloride (Ag/AgCl-NPs) nanoparticles. Once the synthesis is complete, the nanoparticles settle down leaving faint brown supernatant at the top. The synthesized nanoparticles by *Ligustrum vulgare* leaf extract were centrifuged at 1200 rpm for 5 minutes and subsequently dispersed in deionized water to get rid of any uncoordinated biological molecules. The dried Ag/AgCl-NPs were scrapped out for further study.

Characterizations Silver/silver chloride nanoparticles synthesized by this green method were characterized by X-ray diffractometer, (XRD-6000, Shimadzu) equipped with Cu K α radiation source ($\lambda = 0.154056$ nm) using Ni as filter at a setting of 30 kV/30mA. All XRD data were collected under the experimental conditions in the angular range $3^\circ \leq 2\theta \leq 50^\circ$. FT-IR spectra of plant leaf extracts and synthesized AgNPs nanoparticles were obtained in the range 4000-400

cm^{-1} with IR-Prestige 21 spectrophotometer (Shimadzu) using KBr pellet method. Scanning electron microscopy (SEM) images were taken using a field emission scanning electron microscopy (Hitachi S4700, 15 kV). UV-vis spectrum of silver nanoparticles was recorded, by taking 0.1 ml of the sample and diluting it with 2 ml deionized water, as a function of time of reaction using a Shimadzu 1601 spectrophotometer in the wavelength region 300 to 700 nm operated at a resolution of 1 nm.

Results and discussion

UV-vis spectroscopy was used in characterizing the formation and stability of the synthesized silver/silver chloride nanoparticles in aqueous solution, Fig. 2. The colourless silver nitrate solution turned to light brown to deep brown and then to black indicated the formation of Ag/AgCl-NPs. The appearance of the deep brown colour was due to the excitation of the surface Plasmon resonance (SPR). It was observed a deep-brown colour colloidal solution within the first few seconds of reaction of silver nitrate solution with *Lv* leaf extract at 60°C. The spectra display single absorption band at 427 nm was corresponding to the formation of AgNPs. In addition, gradually, longer reaction time periods (10–30 min), increased the corresponding peak intensities, an intense peak at 440 nm was corresponding to the formation of Ag/AgCl-NPs.

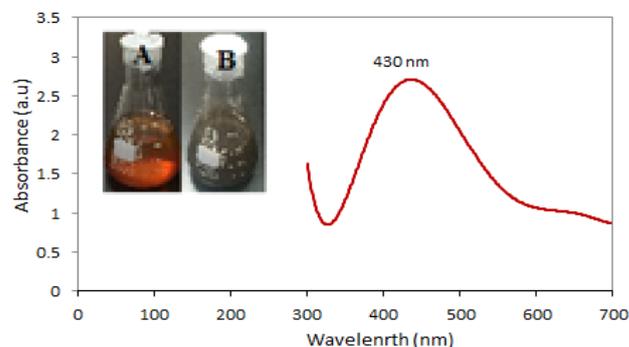


Fig. 2 UV-vis absorbance spectrum of biosynthesized Ag/AgCl-NPs. In set of the figure shows solutions of (A) leaf extract of *Lv* and silver nitrate and (B) resultant Ag/AgCl-NPs

On examination of the synthesis mechanism of Ag/AgCl-NPs, the chlorine ions present in the *Lv* leaf extract which was confirmed by analysis using Dionex ICS-1600 ion chromatography system readily reacts with the formed Ag^0 to form AgCl-NPs. Hence, the chlorine contents of leaf extract could be most attributed source for the formation of AgCl-NPs.

The recorded X-ray diffraction profile for the dried Ag/AgCl-NPs is illustrated in Fig. 3. The XRD pattern indicates four distinct diffraction peaks at 2θ values of 37.94° , 43.62° , 64.16° and 77.14° indexed as (111), (200), (220) and (311) lattice planes of face centred cubic (fcc) structure of metallic silver and is consistency with JCPDS data No. 04-0783. The



broadening and strong signals of pattern evinces that the products are nanosized and well crystallized respectively. The peak corresponding to (111) plane is more intense than other planes suggesting it as a predominant orientation. One can calculate the values of average crystallite size (D) from XRD spectrum using Debye-Scherrer equation [32]

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

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 by using above Debye-Scherrer's formula in range 5-20 nm.

The XRD pattern indicates also another five distinct diffraction peaks at $2\theta = 27.67^\circ$ (111), 32.10° (200), 45.86° (220), 53.88° (311) and 57.52° (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).

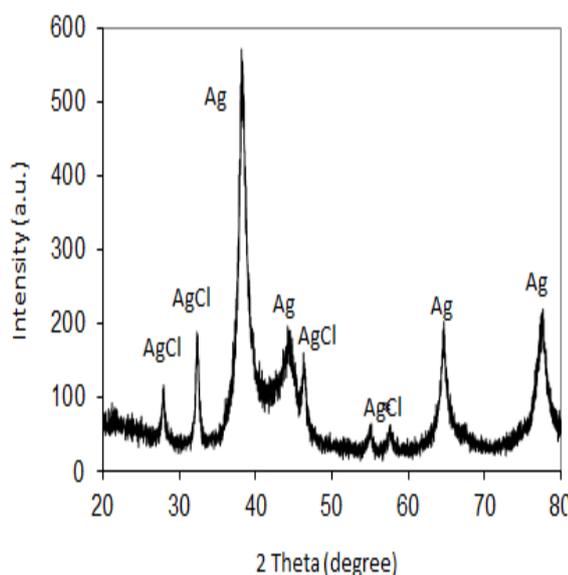


Fig. 3. XRD pattern of biosynthesized silver/silver chloride nanoparticles using *Lv*.

FT-IR analysis is used to identify and get approximate ideas of the possible biomolecules that are responsible for reducing silver ions, capping and stabilization of the synthesized Ag/AgCl-NPs with the *Lv* leaf extract. The spectrum obtained for *Lv* showed bands at 3394, 2916, 2847, 1632, 1435, 1234, 1122, 1068, 806, and 513 cm^{-1} , Fig. 4. A strong peak at 3394 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 in *Lv*. This peak shifted to higher field at 3429 cm^{-1} in the *Lv*-Ag/AgCl-NPs, Fig. 5. The bands at 2916 cm^{-1} and 2847 cm^{-1} are assigned to $-\text{CH}_2$ and C-H stretching mode in

alkanes. The shoulder peak at 1725 cm^{-1} in *Lv* leaf extract could be attributed to C=C stretching vibrations about C=O amide conjugated C=O of the proteins that are responsible for reducing and capping Ag/AgCl-NPs.

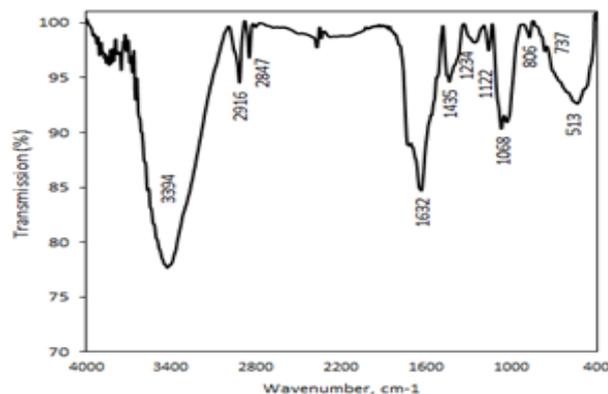


Fig. 4. FT-IR spectrum of *Lv* leaf aqueous extract

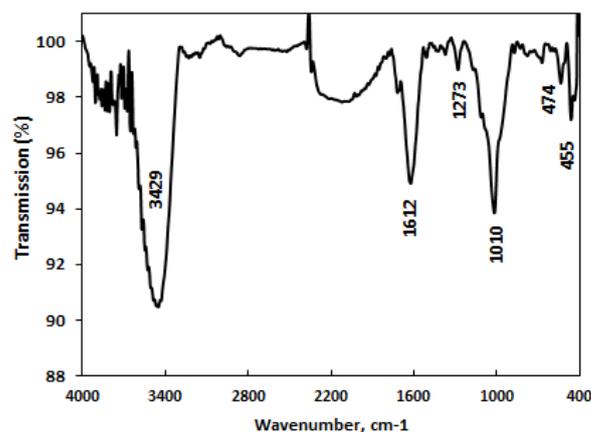


Fig. 5. FT-IR spectrum of *Lv*-Ag/AgCl-NPs

The peaks at 1632, 1435, and 1234 cm^{-1} represent the carbonyl stretching of $-\text{C}=\text{O}$, aromatic stretching of $-\text{C}-\text{N}$ and $-\text{O}-\text{C}-\text{O}$, respectively. The bands at 806 and 513 cm^{-1} indicated the presence of R-CH group and C-H bending. The shifting in the stretching observed in *Lv*-Ag/AgCl-NPs at 3429, 1612, 1273, and 1010 cm^{-1} indicated that the functional groups including phenol, aromatic amine and carbonyl groups from *Lv* mediated the reduction and capping of *Lv*-Ag/AgCl-NPs. Overall the results of microscopy and spectroscopy suggest that proteins in *Lv* leaf extract can act as reducing and capping agent and thus protect the nanoparticles from agglomeration in the aqueous medium. Comparison between spectra of *Lv* leaf extract sample and *Lv*-Ag/AgCl-NPs sample reveal changes in the positions as well as on the magnitude of the biosorption bands.

Green synthesis of Ag/AgCl-NPs is subjected to scanning electron microscopy (SEM) analysis in order the particle size and surface morphology. Fig. 6 shows the SEM photograph, indicating a high density of Ag/AgCl-NPs synthesized using *Ligustrum vulgare* leaf extract. The average particle size was found to be 20 nm.

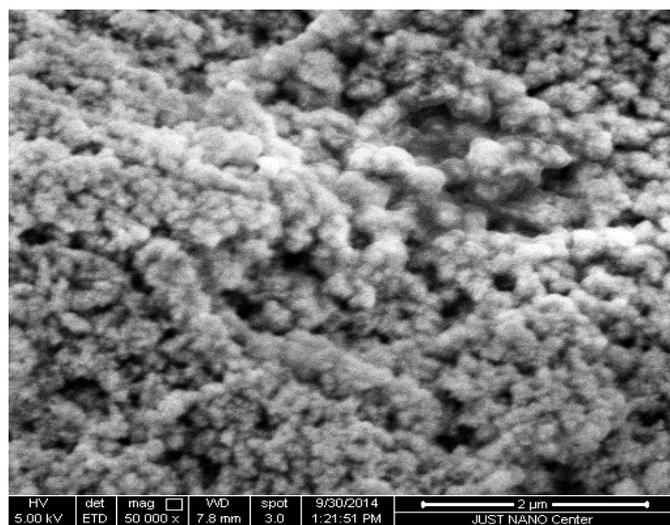
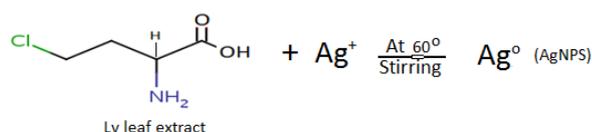


Fig. 6. SEM of biosynthesized silver/silver chloride nanoparticles using *Ligustrum vulgare* leaf extract

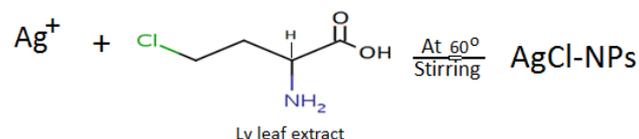
Biosynthetic pathway responsible for the synthesis of Ag/AgCl-NPs using *Ligustrum vulgare* leaf extract may be proposed as follows: (1) The reduction of silver ions (Ag^+) to silver nanoparticles (Ag^0) is due to the presence of the main functional groups $-\text{OH}$, NH_2 , and COO^- of *ligustrum vulgare* leaf extract.



(2) After the formation of silver nanoparticles, the chlorine ion which present in the *Ligustrum vulgare* leaf extract will react with the Ag^+ to form AgCl-NPs. It has been confirmed by XRD analysis. Dionex ICS-1600 ion chromatography system analysis to *Ligustrum vulgare* leaf extract indicated that 200 ppm of chloride ions is present in *Ligustrum vulgare* leaf extract. Hence, content of *Ligustrum vulgare* leaf extract contains chlorine could be most attributed source for the formation of AgCl-NPs. From reactions in steps 1 and 2, the final product is a mixture of silver and silver chloride nanoparticles.

Biosynthesized Ag/AgCl-NPs nanoparticles by this method were studied against pathogenic bacteria by disc diffusion method; it was observed that Ag/AgCl-NPs have antibacterial

activities at concentration of 2 $\mu\text{g}/\text{disc}$.



Chloromphenical was used as a control antimicrobial agent. The silver/silver chloride nanoparticles biosynthesized showed inhibition zone against *Shigella* and *Listeria monocytogenes* bacteria. Maximum zone of inhibition (MZI) are listed in **Table 1**. It was observed that an increase in Ag/AgCl-NPs concentration increases the MZI of *Listeria* and *Shigella* bacteria. *Ligustrum vulgare* leaf extract showed no effect on the bacteria.

Table 1. The antibacterial activity of Ag/AgCl-NPs using *Ligustrum vulgare* (Lv) leaf extract.

Test drug	Conc. (ppm)	MZI(mm)	
		<i>Listeria</i>	<i>Shigella</i>
Lv	50	-	-
	100	-	-
Ag/AgCl	50	11	13
	100	14	16
Drug		17	20

Conclusions

A facile, simple and low cost method was developed for the synthesis of silver/silver chloride nanoparticles using *Ligustrum vulgare* leaf extract. *Ligustrum vulgare* leaf extract acts as an efficient reducing, a source of releasing chlorine ions and stabilizing agent for formation stable Ag/AgCl-NPs from AgNO_3 , without using any hazardous chemicals. The reaction was carried out in water as an environmentally safe solvent. The silver/silver chloride nanoparticles stabilized in Lv. The Ag/AgCl-NPs were synthesized with an average size of 5- 20 nm, with spherical shapes structures. The diameters of the biosynthesis Ag/AgCl-NPs depended on the quantity of *Ligustrum vulgare* leaf extract. Thus, with the increase of the quantity of Lv leaf extract in the reaction mixture, the size of Ag/AgCl-NPs decreases.

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