Short Communication

Green Synthesis of Silver Nanoparticles: Eco-Friendly and Antibacterial

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Abstract:
Silver nanoparticles are one of the most widely applicable particles whose application is increasing in Nano world daily. In this paper the Lens culinaris seed extract was assessed for thegreen synthesis of silvernanoparticlesat temperature of 25°C. The nanoparticles were characterized using Inductively Coupled Plasma spectrometry (ICP), X-ray diffraction (XRD) and Transmission electron microscopy (TEM). Then the antimicrobial activities of these nanoparticles were investigated. P. aeruginosa and S aureus were used as representatives of Gram-negative and Gram-positive bacteria, respectively. Percentage conversion of metal ion to metal nanoparticles were more than 95% after 60 days of the reaction. XRD was used to confirm the crystalline nature of the particles. Silver nanoparticles were mostly spherical with range in size from 5-25 nm. Ag nanoparticles synthesized with extract were exhibited a strong antibacterial activity against bothP.aeruginosa and S.aureus.This process is completely green and eco-friendly compatible.
Keywords: *Silver nanoparticles, ICP, TEM, XRD, Antimicrobial.*

1. INTRODUCTION

Nanoparticle synthesis is executed by different physical and chemical methods that are very expensive and dangerous. Therefore scientists are eagerly expecting for greener methods [2].
Nanoparticles are small particles of 1-100 nm[1]. Recently green synthesis of silver nanoparticles is reported using cycas leaf [2], weed resources [24], *Acalyphaindica* leaf extracts [5], Eucalyptus Hybrida leaf extract [16], Indian gooseberry (*Emblicaofficinalis*) fruit extract [4], *Magnolia kobus* and *Diopyros kaki* [15] Hibiscus leaf extract [7] and black tea leaf extracts [21]. In the past few years, there has been an increasing interest to reducesilver nanoparticles for the antimicrobial characteristics of them [25].
They are even being programmed as future generation antimicrobial factor [18]. Production of nanoparticles under nontoxic green conditions is of vital importance to address growing concerns on the overall toxicity of nanoparticles for medical and technological applications [13,28,23]. Nanosilver is an effective killing agent against a broad spectrum of Gram-negative and Gram-positive bacteria [27,15], including antibiotic-resistant strains [12,32]. Antibiotic-resistant

bacteria include strains such as methicillin-resistant and vancomycin-resistant *Staphylococcus aureus* and *Enterococcus faecium* [19].

The aim of this study was to evaluate the green synthesis of silver nanoparticles. Chosen because of lentil that are high protein in seeds and it is possible that a factor stability of green synthesis of nanoparticles. Legume seeds contain phytochemicals including phenolic compounds, which are mainly present in seed coats [8]. Plant phenolics are the largest class of plant secondary metabolites, which serve in plant defense mechanism to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage [20]. We made use of chemical compounds of *Lens culinaris* seed as reductor agents, reaction mechanism for synthesis of nanoparticles is given by the following equation.

2. MATERIALS AND METHODS

2.1. Preparation of seed extract

The *Lens culinaris* seed was sterilized in NaOCl, then it washed with deionized water. The powder was obtained by grinding. 10 g of powder was mixed with 100 ml of distilled water to make it 10% and stirred at 40°C for 1 h. it centrifuge at 2500 rpm for 20 min were performed. The second cycle of centrifugation is done in the pellet of the first run. The mixture was cooled and separated by filtration with Whatman No. 1 filter paper. The resulting extract was used for further experiments [6].

2.2. Green synthesis of silver nanoparticles

For the synthesis of silver nanoparticles, the source of silver was silver nitrate ($AgNO_3$) from Merck (Darmstadt, Germany) in distilled water. The extract was mixed with $AgNO_3$, to make the final volume concentration of 3 mM aqueous solution. The reaction was carried out at a constant temperature of 25°C for 24 h. This reaction mixture was kept under dark conditions. During the experiment, pH of the solutions was adjusted at 3 using 0.1 N H_3PO_4 solutions.

2.3. Characterization of silver nanoparticles

Inductively coupled plasma spectrometry (ICP) Varian BV ES-700, Australia was executed to decide the remaining concentration of silver ions after equilibrium time. Rate changing of metal ion to nanoparticles is given by the following equation [7,36]:

$$Q = (\frac{C_0 - C_f}{C_0}) \times 100$$

In this equation, the initial and finalconentration of metal ions (mg/L) have been determined with C_0 and C_f and Q is the percentage conversion of silver ion to silver nanoparticles [16]. The morphology of the silver nanoparticlesweredetermined with the Transmission Electron Microscopy (TEM) [33]. TEM images were collected with a LEO912-AB at an accelerating voltage of 80kv. The AgNPs solution obtained was purified by repeating the centrifugation thrice (at 18000 rpm at 25°C) for 20 min followed by redispersion of the pellet in Deionized water [29]. To determine the crystal structure and the size of the silver nanoparticles, the nano powder was subjected to X-ray Diffraction (XRD) analysis. The particle size of silver nanoparticles can be calculated by the Debye–Scherrer equation [26]:

$$D = \frac{K\lambda}{\beta_{1/2} \cos\theta}$$

In this equation, D is the size of crystalline angle, λ is the X-ray wavelength (1.5418 Å), θ is Bragg angle ($30° \leq 2\ \theta \leq 80°$), K is Scherer coefficient between 0.9-1, and β ½ is the maximum peak width by half of its height. XRD was performed using an X-ray diffractometer (PANalitical, X PERTPRO, Holland).

2.4. Anti-bacterial assay of silver nanoparticles

LB media was prepared by dissolving 2.5 g of LB-Bouillon (MILLER) and 2 g of agar (agar powder has been used to change liquid LB medium to solid medium) in 100 ml of water, followed by autoclaving under 15 lbs pressure at 121°C for 20 min. The media was poured on sterilized petriplates and allowed to solidify.

The Nanoparticles antibacterial activity were studied by disk method against clinically isolated

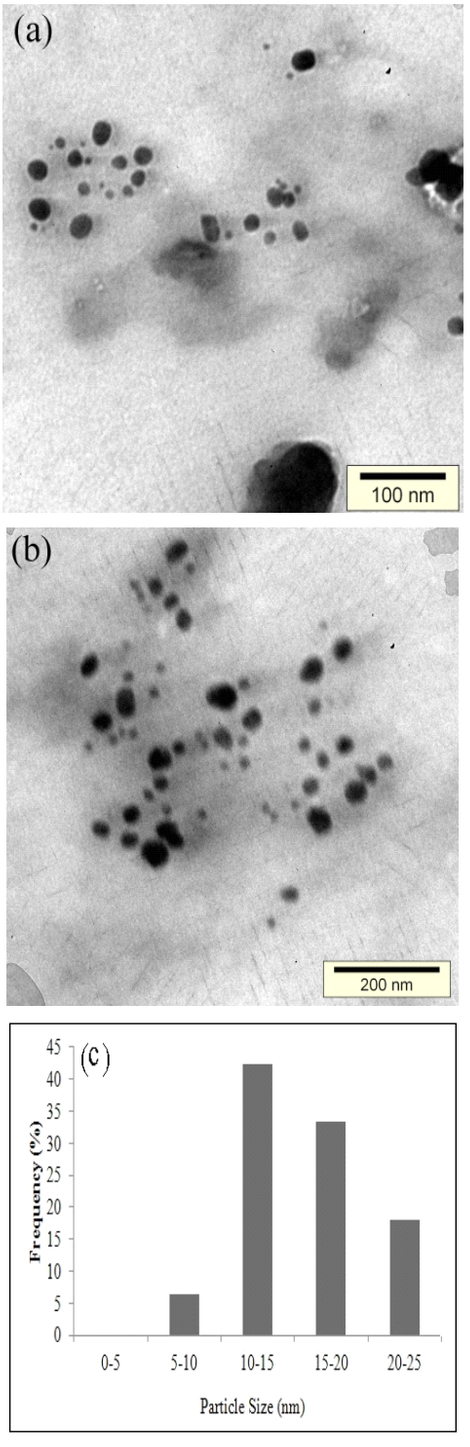

Figure 1.*(a) TEM image of silver nanoparticles synthesized with 10% of Lens seed extract, 3 mM of $AgNO_3$ incubated at 25°C for 24 h at pH 3.0;(a) magnified 40000*×(b) magnified 50000× *(c) Histogram of particle size distribution*

Gram positive (*Staphylococcus aureus* PTU 1431) and Gram negative (*Pseudominosaaeruginosa* PTCC 1430) microorganisms, which fully coat the surface of filter paper with 3 mMconcentration of (I) silver nanoparticles, (II) silver particles and (III) seed extracts of *Lens culinaris* for control. The silver nanoparticles containing discs was placed on the bacterial seeded culture plate. After 24 h of incubation at 37°C, the plates were checked to measure the zone of inhibition.

3. RESULTS AND DISCUSSION

3.2. TEM analysis

The images of *Lens culinaris*seed extractand 3 mM of $AgNO_3$ solution at 25°C after 24 h of synthesis have been shown in Figure 1.a. The nanoparticles were relatively spherical which the size range of Ag nanoparticles were from 5-25.

The TEM image didn't show aggregated nanoparticles (Figure 1.b). variations in the extract content may prevent stabilizing functional groups of silver nanoparticles from agglomeration [17], which suggest that proteins interact with green synthesized nanoparticles and also their secondary structure were not affected during reaction with silver ions or after binding, for example the possible molecules present in cashew leaf which are responsible for reducting Ag^+ [10].

3.2. XRD and ICP analysis

The crystalline nature of silver nanoparticles was confirmed by the analysis of X-ray Diffraction pattern (XRD) as shown in Figure 2. XRD spectrum showed four distinct diffraction peaks at 38.1°, 44.3°, 64.4° and 77.4° that can be indexed to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) reflections of face centered cubic (fcc) crystal structure of silver nanoparticles [9,19,31].

Unassigned peaks were also observed, suggest that the crystallization of bio-organic phase occurs on the surface of the nanoparticles.Similar results were reported in silver nanoparticles synthesized byusing geranium leaf extract [35]. Particle distributions value in this research was consistent with the results obtained by XRD analysis.

ICP analysis revealed complete reduction of Ag ions within 60 days of the reaction and more importantly, it showed that the percentage conversion of metal ion to metal nanoparticles were more than 95% (Table.1).

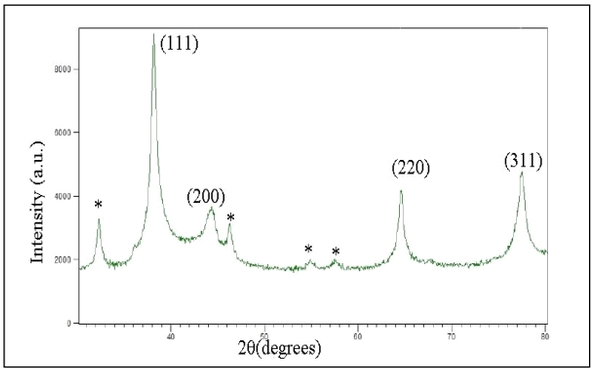

Figure 2. *XRD pattern recorded for the nanaoparticles synthesized from Lens culinaris seed extract (*show unassigned peaks).*

Table. 1. *ICP analysis(temperature: 25°C, contact time: 60 days, n = 3)*

silver		
C_0 (mg/L)	C_f(mg/L)	Q (%)
169.87	6.67	95.48

3.3. Antibacterial efficacies of synthesized nanoparticles

The diameters of the zone of inhibition (ZOI) around areplaced in table 2. The coated disk with silver nanoparticle (Ag^0) formed zone of inhibited (ZOI) equal to 5 and 7 mm while the coated disk with silver ion (Ag^+) formed ZOI of 7 and 11 mm in media culture plates of *S.aureus* and *P.aeruginosa*, respectively. It should be mentioned that the concentration of applied silver ion and nanoparticle was the same. Moreover, the results of investigating *P.aeruginosa* culture medium showed that the antibacterial activity of silver nanoparticles against *P.aeruginosa* PTCC 1430 is more than that against *S.aureus* PTU 1431. This is due to the structural difference in cell wll composition of gram positive and gram negative bacteria. It can be seen that the synthesized silver nanoparticles have the maximum antibacterial efficacy. Whereas no zone of inhibition arounddisk was observed *in Lens* seed extract (Figure 3. a and 3. b). These observations suggest that *Lens* seed extracts may not show antimicrobial potency.

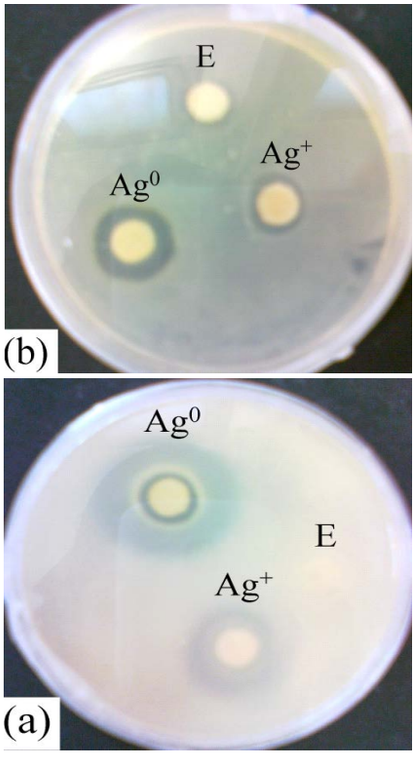

Figure 3. *Antibacterial assay of Lens seed extract (E), Ag nanoparticles (Ag^0) and Ag particles (Ag^+) using disk method against (a) S. aureus PTU1431, (b) P. aeruginosa PTCC 1430*

Shahverdi et al. reported that the silver nanoparticles have an antimicrobial effect on *S. aureus* and *E.coli* [3]. The mechanism of the antimicrobial action of silver ions is closely related to their interaction with thiol (sulfhydryl) groups [30, 14], although other target sites remain a possibility [11]. Enhancement of antibacterial effects of novel silver nanoparticles characterized, nanoparticles would interfere with the bacterial growth signaling pathway by modulating tyrosine phosphorylation of putative peptides substrate critical for cell viability and division and the nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent [22, 34].

Table 2. *Mean zone of inhibition (mm) of Lens seed extract (E), Ag nanoparticles (Ag^0) and Ag ions (Ag^+) against (a) S. aureus PTU 1431 (b) P. aeruginosa PTCC 1430*

	E (3mM)	Ag^+ (3mM)	Ag^0(3mM)
*S. aureus*PTU 1431	0.00	5.00	7.00
*P. aeruginosa*PTCC 1430	0.00	7.00	11.00

4. CONCLUSION

The results demonstrated that the green synthesize of silver nanoparticles using the seed extract of *Lens culinaris*. Lens seed extract play important roles in reduction and stabilization of nanoparticles. The synthesized silver (size range from 5 to 25nm) exhibited a strong antibacterial activity against both *P.aeruginosa* and *S.aureus*. Particles are mostly spherical in shape.

5. ACKNOWLEDGMENT

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