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Green synthesis of silver nanoparticles using seed aqueous extract of *Olea europaea*

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ABSTRACT

Biosynthesis of silver nanoparticles with small size and biostability is very important and used in various biomedical applications. In the present work, we describe the synthesis of silver nanoparticles (Ag-NPs) using seed aqueous extract of *Olea europaea (Oe)* and its antibacterial activity. UV–visible spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), and X-ray energy dispersive spectrophotometer (EDAX) were performed to ascertain the formation of Ag-NPs. It was observed that the growths of Ag-NPs are stopped within 35 min of reaction time. The synthesized Ag-NPs were characterized by a peak at 449 nm in the UV–visible spectrum. XRD confirmed the crystalline nature of the nanoparticles of 34 nm size. The XRD peaks at 38°, 44°, 64° and 77° can be indexed to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) Bragg's reflections of cubic structure of metallic silver, respectively. The results confirmed that the *(Oe)* is a very good eco friendly and nontoxic source for the synthesis of Ag-NPs as compared to the conventional chemical/physical methods.

Keywords: *Silver nanoparticles; Green chemistry; Scanning electron microscopy (SEM); Olea europaea; X-ray diffraction (XRD).*

INTRODUCTION

Utilizing plant extracts for the biosynthesis of silver nanoparticles has gained importance in recent years due to the enhancement of chemical, physical, biological and optoelectronic properties of the particles formed by this green process. Metal and semiconductor nanoparticles are very important due to their unusual size and shape dependant properties. Nanotechnology is a broad interdisciplinary area of research, development and industrial activity which has been growing rapidly worldwide for the past decade. Metallic nanoparticles of specific sizes and morphologies can be readily synthesized using chemical and physical methods [1 – 5].

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Silver nanoparticles have attracted the attention of the researchers in the last two decades due to their wide applications in various fields. The literature is replete with the investigations of the use of plants extracts [6], fungi [7], algae [8], proteins and enzymes [9] as the reductant for carrying out the syntheses of nanoparticles with a variety of shapes and morphologies in high yields, including multi-branched advanced silver and/or gold nanomaterials [10]; but the use of surfactant in the green synthesis of silver sol has been neglected. Most of the methods reported in literature are extremely expensive and they also involve the use of toxic, hazardous chemicals as the stabilizers which may pose potential environmental and biological risks. Because of the increasing environmental concerns by chemical synthesis routes, an environmentally sustainable synthesis process has led to biomimetic approaches, which refers to applying biological principles in materials formation. Bio-reduction is one of the fundamental processes in the biomimetic synthesis. The stability, shape, size, and morphologies of metal nanoparticles strongly depend on the method of preparation, type, nature of reductants, and concentration of stabilizers (polymers, ligands, solid matrix and surfactants) [11]. The surface plasmon resonance and large effective scattering cross section of individual silver nanoparticles make them ideal candidates for molecular labeling where phenomena such as surface enhanced Raman scattering (SERS) can be exploited [12]. In addition, silver nanoparticles play a significant role in the field of biology and medicine due to its attractive physiochemical properties. The strong toxicity of silver against wide range of microorganisms is well known and silver nanoparticles have been recently shown to be a promising antimicrobial material [13 – 17]. Silver nanoparticles have found to posses anti-inflammatory, antiviral, anti-angiogenesis, and anti-platelet activity and cytotoxicity against cancer cells which makes them vital [18 – 20]. However, these methods employ toxic chemicals as reducing agents, or nonbiodegradable stabilizing agents and are therefore potentially dangerous to the environment and biological systems [21]. Moreover, most of these methods entail intricate controls or nonstandard. We have recently developed a reduction method of converting Ag nanospheres into nanorods [22], nanoplates [23], their antibacterial activity [24, 25], an improved an easy synthetic route for silver nanoparticles in poly (diallyldimethylammonium chloride**) (**PDDA) [26], synthesis of Gold/HPC hybrid nanocomposite [27], Preparation of ZnO/Ag nanocomposite [28] and comparison nanosilver particles and nanosilver plates for the oxidation of ascorbic acid [29]. Regarding the role of green chemistry, we have successfully demonstrated that size, shape and the antibacterial activity silver nanoparticles by the reduction of Ag^+ ions with bio-reductants (*Olea europaea*) largely depend on the nature of reducing agents, concentration and time of mixing of the reactants [30]. The methodology employed here is very simple, easy to perform, inexpensive, and eco-friendly.

EXPERIMENTAL

Materials

Silver nitrate ($AgNO_3$) was obtained from Loba Chemie, India and used as received. All other reagents used in the reaction were of analytical grade with maximum purity. *Olea europaea (Oe)*leaves were collected from South of IRAN, and were cleaned with double distilled water and shade-dried for a week at room temperature and further *(Oe)*leaves were ground to powder and stored for further study. For this experiment, nanoparticles have concentrations ranging from 0.0976 to100 µg/mL.

Synthesis and characterization of silver nanoparticles (Ag-NPs)

In a typical reaction procedure, *Olea europaea (Oe)*leaf extract was prepared by taking 2 g of dry leaf powder with 25 mL of distilled water in a conical flask along with 2 mL of methanol (minimum methanol was added in order to initiate the isolation of compounds). The extract was placed in orbital shaker for 1 h and the extract was filtered. For the synthesis of silver nanoparticles various concentrations of leaf extracts were tried and then the extract to be used was optimized to 1 mL. Further, 1 mL of the extract was added to 10 mL of 1 mM silver nitrate ($AgNO_3$) solution and the solution was placed in orbital shaker at room temperature, for reduction of Ag^+ to Ag^0. The broth

containing Ag-NPs was centrifuged at 10,000 rpm for 15 min, following which the pellet was re-dispersed in the sterile distilled water to get rid of any uncoordinated biological molecules. The color change involved in the formation of silver nanoparticles. The purified pellets were then kept into petri plates and left in the oven for drying at 60°C for 24 h. The colorless $AgNO_3$ solution turned yellow to brown or reddish yellow to deep red, indicated the formation of Ag-NPs. The dried Ag-NPs were scrapped out for the further study.

Characterization of silver nanoparticles (Ag-NPs)

The biosynthesis of the Ag-NPs in a solution was monitored by measuring the UV–vis spectra of the solutions (1:4 diluted) of the reaction mixture. UV–vis spectra were recorded on double beam spectrophotometer (Shimazdu, model UV-1800, Kyoto, Japan) from 300 to 800 nm at a resolution of 1 nm. The distilled water was used as a blank. The Ag-NPs synthesized with 8% leaf extracts and 6 mM $AgNO_3$ solution were characterized with the help of scanning electron microscopy (model LEO 440i) equipped with X-ray energy dispersive spectrometer (EDAX) (Bankar et al., 2010). Transmission electron microscopy (TEM) selected area electron diffraction (SAED) images were taken on Zeiss - EM10C - 80 KV operated at accelerating voltages of 40 and 200 kV. The observed reflection planes corresponding to fcc Ag-NPs (~27 nm) in XRD diffraction pattern (Seisert Argon 3003 PTC using nickel filtered XD-3Cu Ka radiations (k = 1.5418 A)).

RESULTS AND DISCUSSION

UV–vis spectral studies

UV–vis spectroscopy was ascertained to check the formation and stability of Ag-NPs in aqueous solution (Figure 1). The colorless $AgNO_3$ solution turned yellow to brown or reddish yellow to deep red, indicated the formation of Ag-NPs. The appearance of the brown color was due to the excitation of the surface plasmon resonance (SPR), typical of Ag-NPs having λ_{max} values which were reported earlier in the visible range of 450–500 nm [31-34] (Figure 1). The SPR absorbance was extremely sensitive to the nature, size and shape of the particles formed, their inter particle.

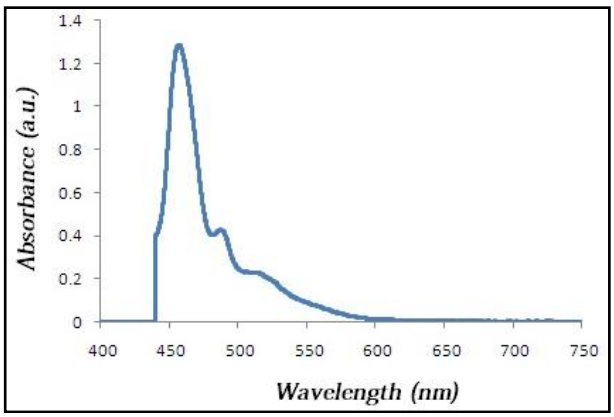

Fig. 1. UV–vis spectra of an aqueous solution of *Olea europaea (Oe)* leaf extract in presence of Ag^+ ions at 32 °C. Reaction conditions: $[Ag^+] = 10.0 \times 10^{-5}$ mol dm^{-3}.

TEM

The morphology and size of the synthesized silver nanoparticles were determined by TEM images and they are shown in Figure 2 (A and B). The particles formed were spherical in shape. The nanospherical formed where shown to have high surface area. The nanoparticles formed were in the range of 20–40 nm in size with 36 nm average size. The particles were monodisperse, with only a few particles of different size.

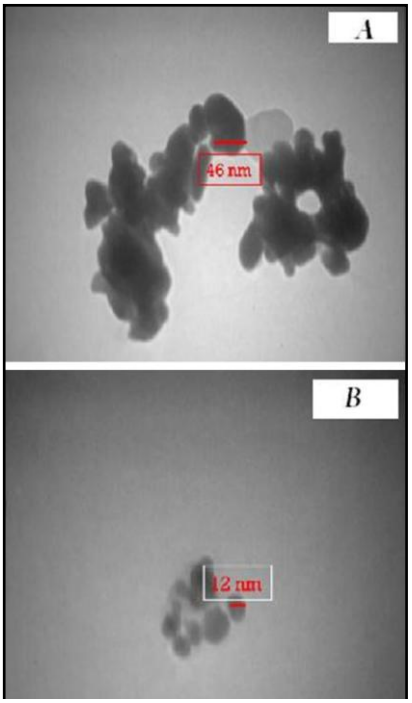

Fig. 2. TEM images indicating the presence of spherical silver nanoparticles recorded at various magnifications (A and B).

SEM

SEM micro-graphs show aggregates of silver nanoparticles and the particles are in the range of 15–30 nm and there are not in direct contact even within the aggregates indicating the stabilization of nanoparticles by capping agents (Figure 3a). In EDAX strong signals were observed from the silver atoms in the nanoparticles and weaker signals for carbon, oxygen, potassium and chloride were provenients from biomolecules of *(Oe)* (Figure 3b) [35].

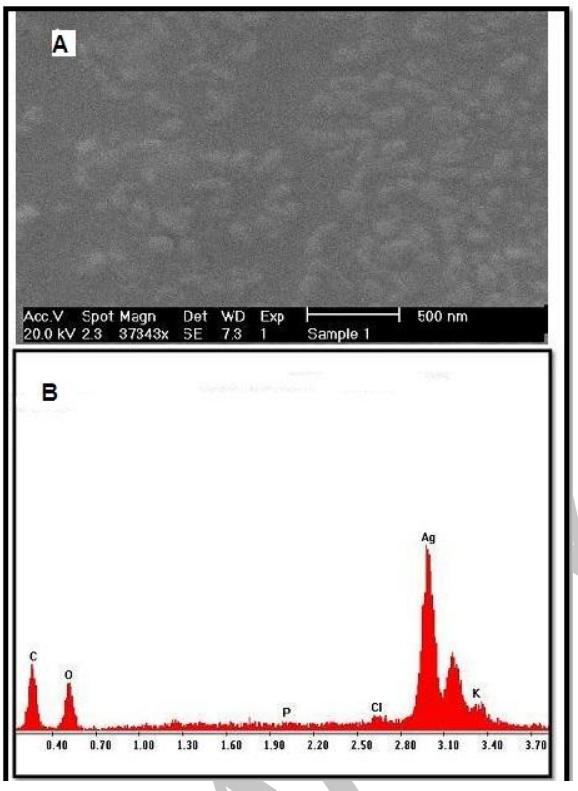

Fig. 3. (A) SEM and (B) EDAX images showing the presence of silver nanoparticles and bioorganic components of *Olea europaea (Oe)*.

XRD

The crystalline nature of Ag-NPs was carried out using XRD where three diffraction peaks were observed in the 2θ range of 30–80°, which can be indexed as (1 1 1), (2 0 0), (2 2 0), (311) reflections of fcc structure metallic silver respectively similar to Joint Committee on Powder Diffraction Standards (JCPDS) file no: 04- 0784 revealing that synthesized Ag-NPs are of pure crystalline silver. The XRD patterns in (Figure 4) of Ag-NPs obtained were similar to the results reported earlier [36-40] The particle size of the Ag-NPs formed were calculated using Debye–Scherrer equation which was around 38 nm, were good in agreement with TEM results also.

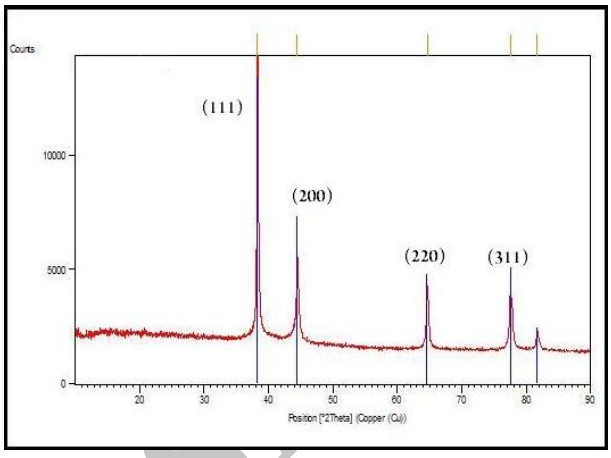

Fig. 4. XRD pattern of silver nanoparticles obtained using *Olea europaea (Oe)*.

CONCLUSIONS

To conclude, the Ag-NPs were produced by the use of the extract of *Olea europaea (Oe)*as reducing and capping agent. In this study, it was observed that the reaction is rapid and is completed within 30 min at room temperature. We have demonstrated an eco-friendly, rapid green chemistry approach for the synthesis of Ag-NPs by using *(Qe)*, which provides a simple, cost effective and efficient way for the synthesis of Ag-NPs. Therefore, this reaction pathway satisfies all the conditions of a 100% green chemical process. The amount of plant material was found to play a critical role in controlling the size and size dispersity of nanoparticles in such a way that smaller silver nanoparticles and narrow size distribution are produced when more *(Oe)* extract is added in the reaction medium. The present study showed an innovative way for synthesizing antimicrobial Ag-NPs using natural products which can be used in various biomedical applications. The results confirmed that the *(Oe)* is a very good eco friendly and nontoxic source for the synthesis of Ag-NPs as compared to the conventional chemical/physical methods.

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