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Synthesis of silver nanoparticles using leaves aqueous extract of *Nasturtium Officinale (NO)* and its antibacterial activity

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ABSTRACT

Biogenic reduction of silver ion to base metal is quite rapid, readily conducted at room temperature and pressure, and easily scaled up. Synthesis mediated by plant extracts is environmentally benign. The objective of this study was to synthesis of silver nanoparticles (Ag-NPs) using leaves aqueous extract of Nasturtium Officinale R. Br. (NO) and its antibacterial activity. X-ray diffraction (XRD), transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were performed to determine the formation of Ag-NPs. XRD confirmed the crystalline nature of the nanoparticles of 22 nm size. The XRD peaks at 38ºC, 44ºC, 64ºC and 77ºC 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. Antibacterial activities of Ag-NPs were tested against the growth of Gram-positive (*S.aureus*) using SEM. The inhibition was observed in the Ag-NPs against *S.aureus*. The results of SEM showed that most of S. aureus was damaged and extensively disappeared by the addition of Ag-NPs. The results confirmed that the NO is a very good eco-friendly and nontoxic source for the synthesis of Ag-NPs.

1. Introduction

It has been recently shown that the use of plant extracts for the preparation of metallic nanoparticles is an easy and convenient alternative to chemical and physical methods. Although nanoparticles can be made using various physicochemical methods, their synthesis using nontoxic and environmentally benign biological methods is attractive especially if they are intended for invasive applications in medicine. Several routes have been developed for the biological or biogenic synthesis of nanoparticles from salts of the corresponding metals (Bar et al., 2009; Gan and Li, 2012; Thakkar et al., 2010).

Microorganisms, whole plants, plant tissue and fruits, plant extracts and marine algae have been used to produce nanoparticles.

Biogenic synthesis is useful not only because of its reduced environmental impact (Dahl et al., 2007) compared with some of the physicochemical production methods, but also because it can be used to produce large quantities of nanoparticles that are free of contamination and have a well-defined size and morphology (Hutchison, 2008). Biosynthetic routes can actually provide nanoparticles with a better defined size and morphology than some of the physicochemical methods of production.

The ability of plant extracts to reduce metal ions has been known since the early 1900s, although the nature of the reducing agents involved was not well understood. In view of its

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simplicity, the use of live plants or whole plant extract and plant tissue for reducing metal salts to nanoparticles has attracted considerable attention within the last 30-years (Gan and Li, 2012; Iravani, 2011; Kumar and Yadav, 2009; Park et al., 2011).

Compared with the use of whole plant extracts and plant tissue, the use of plant extracts for making nanoparticles is simpler. Plant extract mediated synthesis is an increasing focus of attention (Ali et al., 2011; Bar et al., 2009; Chandran et al., 2006; Daisy and Saipriya, 2012; Lee et al., 2011; Park et al., 2011).

Processes for making nanoparticles using plant extracts are readily scalable and may be less expensive compared with the relatively expensive methods based on microbial processes and whole plants (Kumar and Yadav, 2009).

Plant extracts may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles (Kumar and Yadav, 2009). The source of the plant extract is known to influence the characteristics of the nanoparticles (Kumar and Yadav, 2009) as different extracts contain different concentrations and combinations of organic reducing agents. Typically, a plant extract-mediated bio-reduction involves mixing the aqueous extract with an aqueous solution of the relevant metal salt. The reaction occurs at room temperature and is generally complete within a few minutes. In view of the number of different chemicals involved, the bioreduction process is relatively complex. We have recently developed a reduction method of converting Ag nanospheres into nanorods (Sadjadi et al., 2008), nanoplates (Sadeghi and Sadjadi, 2009), their antibacterial activity (Sadeghi and Jamali, 2010; Sadeghi and Garmaroudi, 2012), improved an easy synthetic route for silver nanoparticles in poly (diallyldimethylammonium chloride) (PDDA) (Sadeghi and Pourahmad, 2012), synthesis of Gold/HPC hybrid nanocomposite (Sadeghi and Ghammamy, 2011), preparation of ZnO/Ag nanocomposite (Sadeghi, 2014) and comparison of nanosilver particles and nanosilver plates for the oxidation of ascorbic acid (Sadeghi and Meskinfam, 2012). Plants have been traditionally used to treat a variety of common diseases in the world to such a degree that has formed the basis of traditional medicine. But with the rapid development of synthetic drugs in recent years, the use of plants was reduced or abolished, but the appearance of undesirable side effects of synthetic compounds and the lack of compatibility with human nature, once again the attention of researchers to plants base on knowledge of Phytochemistry (Chemical plant) was established. Phytochemistry, including chemistry of natural substances found in plants that have a direct relationship with the chemical structure of these compounds, biosynthesis, extraction and metabolism, distribution of natural and biological activity. In fact, Phytochemistry is an important bridge between chemistry and pharmaceutical sciences like biology.

Regarding the role of green chemistry, it was successfully demonstrated that the size, shape and the antibacterial activity of silver nanoparticles by the reduction of Ag^+ ions with bio-reductants (*Nasturtium Officinale (NO)*) largely depend on the nature of reducing agents, concentration and time of mixing of the reactants (Umesh et al., 2013). The methodology employed here is very simple, easy to perform, inexpensive, and eco-friendly. Moreover, most of these methods entail intricate controls or nonstandard.

2. Materials and Methods

2.1. Materials

Silver nitrate ($AgNO_3$) was obtained from Loba Chemie, India. All other reagents used in the reaction were of analytical grade with maximum purity. *Nasturtium Officinale R. Br. (NO)* 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 *(NO)* leaves were ground to powder and stored for further study. For this experiment, the concentrations of nanoparticles were 0.0976 to100 µg/ml. *S. aureus* (ATCC 51153) was used as a Gram-positive bacterium. For the antimicrobial activity measurement, bacteria cultures were incubated at 38ºC in Luria medium (tryptone 1.5%, yeast extract 0.75 % sodium chloride 1.2%, agar 1%, Difco).

2.2. Synthesis and characterization of silver nanoparticles (Ag-NPs)

In a typical reaction procedure, *Nasturtium Officinale R. Br.(NO)* 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, then the pellet was re-dispersed in the sterile distilled water to get rid of any uncoordinated biological molecules. The color changes upon the formation of silver nanoparticles. The purified pellets were then kept into petri dishes 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.

2.3. Antimicrobial activity studies

S. aureus (ATCC 51153) was used as a Gram-positive bacterium. For the antimicrobial activity measurement, bacteria cultures were incubated at 38°C in Luria medium (tryptone 1.5%, yeast extract 0.75%, sodium chloride 1.2%, agar 1%, Difco). Antimicrobial activities of silver nanoparticles (Ag-NPs) have been investigated against *S. aureus* as the model Gram-positive bacteria. The *in vitro* anti-bacterial activities of silver nanoparticles were examined (Melaiye et al., 2005; Feng et al., 2000; Son et al., 2004). The following microorganism was used: Gram-positive *S.aureus*.

2.4. Characterization of silver nanoparticles (Ag-NPs)

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). 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)).

3. Results

The present study showed an innovative way for synthesizing antimicrobial Ag-NPs using natural products which can be used in various biomedical applications.

3.1. TEM

The morphology and size of the synthesized silver nanoparticles were determined by TEM and they are shown in Fig. 1 (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 10–50 nm in size with the average size of 36. The particles were monodisperse, with only a few particles of different size.

3.2. SEM

SEM micro-graphs show aggregates of silver nanoparticles and the particles are in the range of 25–40 nm and there were not in direct contact even within the aggregates indicating the stabilization of nanoparticles by capping agents (Fig. 2A). 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 *(NO)* (Fig. 2B).

3.3. 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 (Fig. 3) of Ag-NPs obtained were similar to the results reported earlier (Badri et al., 2008). The particle size of the Ag-NPs formed were calculated using Debye–Scherrer equation which

was around 27 nm, were good in agreement with TEM results also.

3.4. Antibacterial activity of silver nanoparticles

We have investigated the use of these *(NO)* mediated silver nanoparticles as possible antibacterial agents. Such *(NO)* mediated silver nanoparticles were immediately tested for antimicrobial activity towards test bacterial strains. Fig. 4A and B show the zones of inhibition that were observed with the *S. aureus*. In all these figures, the black arrows indicate the *S. aureus* colonization.

This is consistent with an earlier report on the antimicrobial activity of silver nanoparticles biosynthesized (Chaloupka et al., 2010), as well as those synthesized chemically (Sadeghi and Jamali, 2010; Sadeghi and Garmaroudi, 2011). In the present study, the nanoparticles thus synthesized could also be applied as selective antibacterial agents. The inhibition was observed in the Ag-NPs against *S.aureus.* The results suggest that the synthesized Ag-NPs act as an effective antibacterial agent.

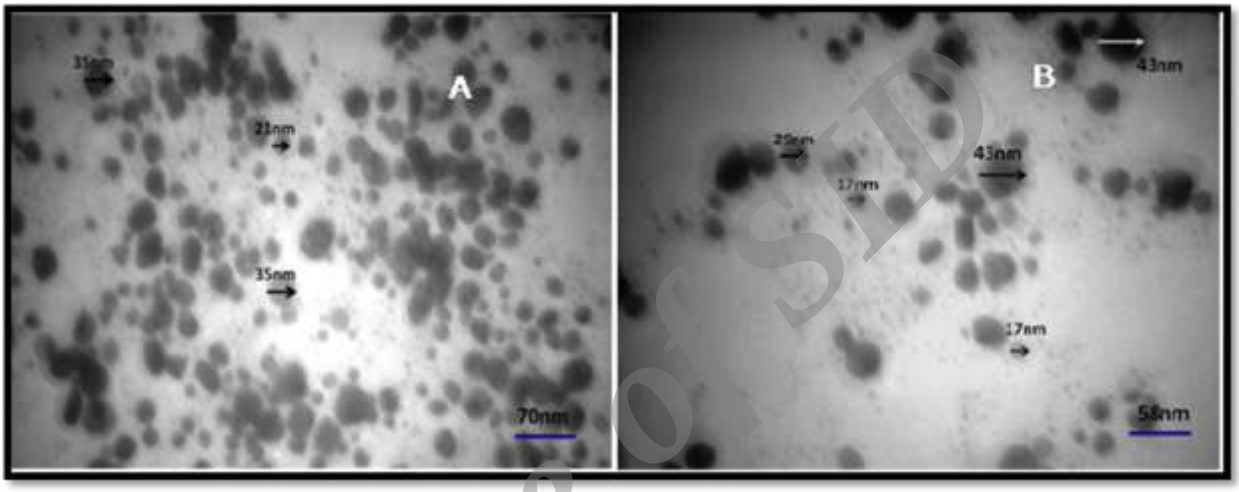

Fig.1. TEM images indicating the presence of spherical silver nanoparticles recorded at various magnifications (A and B).

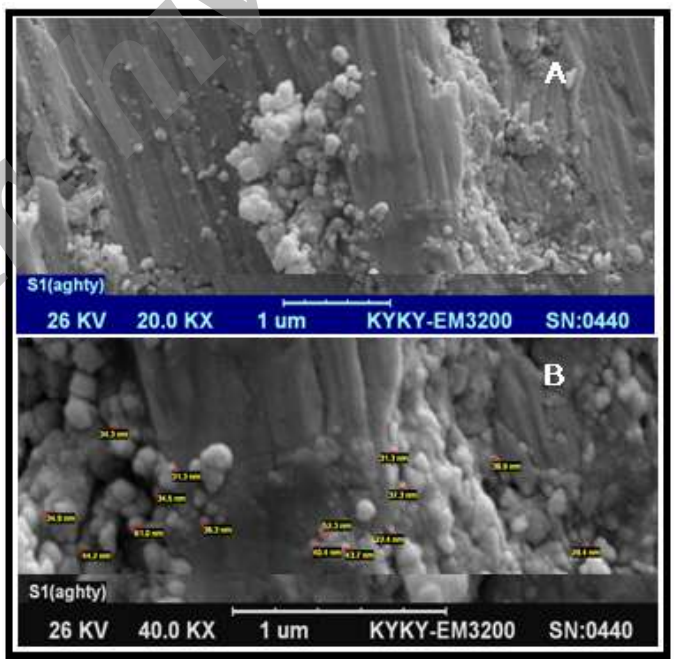

Fig.2. (A, B) SEM images showing the presence of silver nanoparticles and bioorganic components of *Nasturtium Officinale R. Br. (NO).*

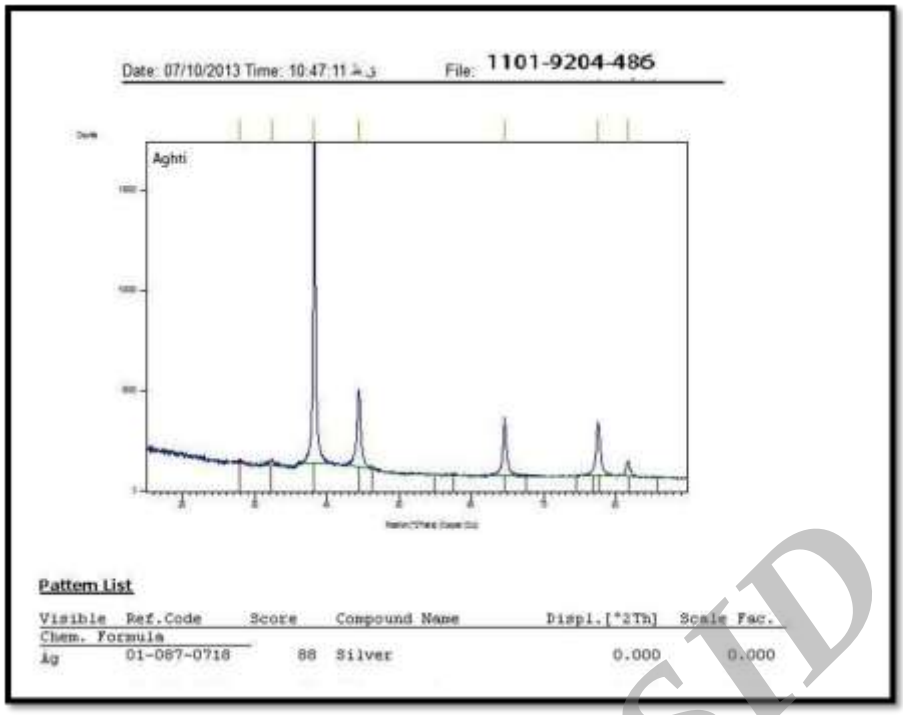

Fig.3. XRD pattern of silver nanoparticles obtained using *Nasturtium Officinale R. Br. (NO).*

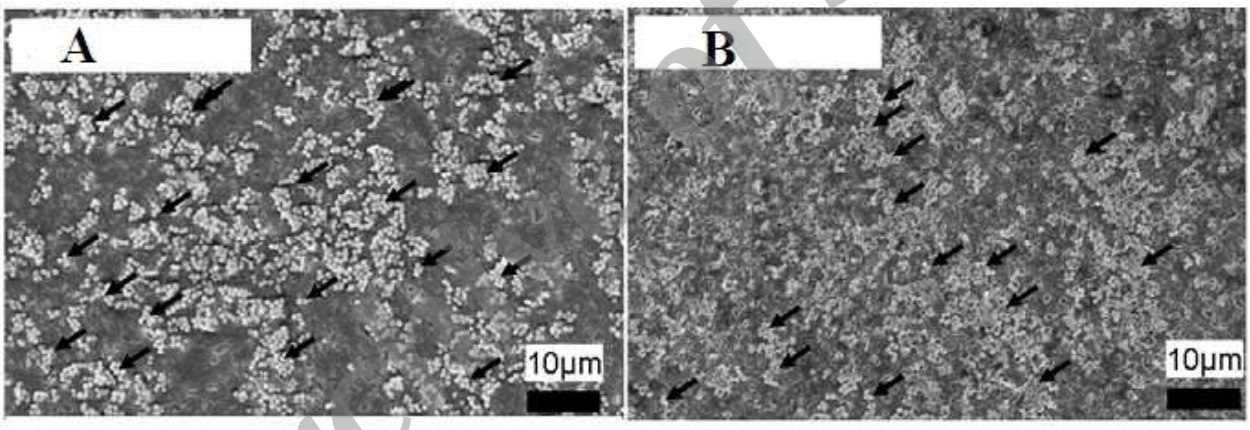

Fig.4 Representative SEM images showing reduced *S.aureus* colonization on Ag-Nps/extract (B) compared to extract (A). Arrows show bacteria.

4. Discussion

In conclusion, we have demonstrated an eco-friendly, rapid green chemistry approach for the synthesis of Ag-NPs by using *(NO)*, which provides a simple, cost effective and efficient way for the synthesis of Ag-NPs. Ag-NPs were produced by the use of the extract of *Nasturtium Officinale R. Br.(NO)* as reducing and capping agent. 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 disparity of nanoparticles in such a way that smaller silver nanoparticles and narrow size distribution are produced when more *(NO)* extract is added in the reaction medium. The inhibition was observed in the Ag-NPs against *S. aureus*. The present study showed an innovative way for synthesizing antimicrobial Ag-NPs using natural products which can be used in various biomedical applications. Thus, the synthesized Ag-NPs may have a high potential for use in biological applications. The results confirmed that the *(NO)* 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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