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Green synthesis of silver nanoparticles using *Taxus baccata* Leaves extract and identify its specifications

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- ✓ Infrared spectroscopy.

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1. Introduction

Abstract

In this research, green synthesis of silver nanoparticles from the *Taxus baccata* leaves extract was investigated. The morphology of synthesized Ag-NPs was confirmed by field emission scanning electron microscopy (FE-SEM), UV spectra and Fourier transform infrared spectroscopy (FT-IR). Also, the results of X-ray diffraction (XRD) was confirmed that the size of Ag-NPs are about 15-50nm. This green procedure has high yields and easy operations without application of toxic reagents or surfactant template. Environmental compatibility and low cost and easy synthesis are the advantages of this method to comparing with other reported methods.

Nowadays, nanotechnology was focused in various fields such as electronics, chemicals, biotechnology, bio analytics and other industries [1]. Recent achievements in the field of nanotechnology, such as the capability to prepare of highly ordered nanoparticles of any size and shape, have led to the development of new bioactive agents. Metal nanoparticles have been used widely in recent studies due to their unique electronic, optical, mechanical, magnetic and chemical properties which greatly differ from the bulk substances [2]. These special and unique properties could be related to their small dimensions and large surface areas. Among the different metal nanoparticles, Ag NPs more have been paid attention during the past few years due to their potential. The recent studies for the preparation of silver nanoparticles with a controlled size will valuable in many methods such as: Chemical reduction of silver ions generally in the presence of stabilizing agents [3,4], Thermal decomposition in organic solvents [5,6], Reversed micelle processes [7,8], photo Reduction[9], ultrasonic radiation[10,11], and microwave irradiation [12]. It is notable that the most of these methods have potential hazards to health and environment. But by reduction potential of plant extract, we could be able to reduce the metal ions. Therefore, the potential of plants as biological materials for the synthesis of nanoparticles still should be explored. Green synthesis of NPs using plants extract has several advantages over chemical synthesis, such as simplicity, cost effectiveness as well as compatibility for biomedical and pharmaceutical applications [13-16]. Since medicinal plants have antioxidant compounds, can change metal ions into zero-capacity metal. For the plant of Taxus baccata L, this tree is from European or English yew (Taxus baccata L.) and native to most of Europe. It is an extremely long-living tree, with reports of some specimens of up to 5 000 years old [17]. However, since it is very difficult to determine age accurately as the oldest specimens are almost always hollow [18]. Making tree ring-based age estimation impractical, opinion is divided about the exact age of the oldest specimens [19] (Figure 1). Taxus baccata is one of the valuable and species source for taxol or paclitaxel extraction [20]. These compounds were used in the preparation of anti-cancer drugs and other medical uses in Ayurveda and Tibetan medicine [21]. The ideal altitudinal zone that the *Taxus baccata* tree grows is between 2000 to 2500 m [22].



Figure 1. Taxus baccata plant from left to right: whole of tree, leave and fruit

Moreover, only 0.01 to 0.03% of the dry weight of plant is taxol, yet as much as 2 g of purified taxol is required for anti-tumor treatment [23]. Taxol is an alkaloid diterpenoid that belongs to a group of diterpens called Taxanes [24]. Here in, in this research the synthesis of silver nanoparticles was done using *Taxus baccata* leaves extract as an oxidation and reduction agent. Environmental compatibility and low cost and easy synthesis are the advantages of this method compared to other reported methods.

2. Material and Methods

2.1. Material

All chemicals used in this work are prepared from Fluka (Buchs, Switzerland) and employed without further purification. Silver nitrate (AgNO₃) was prepared from Aldrich Company. The *Taxus baccata* leaves used in this study originated from Zarin Gol region of Iran. Distillated water was used during this research. IR spectra are measured on a Shimadzu IR-460 spectrometer. The morphology of Ag nanoparticles was characterized by SEM using a Holland Philips XL30 microscope. The structure of AgNPs was characterized by XRD analysis at room temperature using a Holland Philips Xpert X-ray powder diffractometer. The scanning rate was 0.5 °/min in the 20 range from 10° to 80°. The element analyses (C, O, and N) were obtained from a SAMX analyzer. Field Emission scanning electron microscopy (FE-SEM) images were recorded on a FE-SEM (TESCAN Company, Czech Republic). UV–visible spectral analysis was recorded on a double-beam spectrophotometer (JENWAY, 6705 UV, Germany) to ensure the formation of nanoparticles.

2.2. Preparation of the plant extract

In the extraction processes of *Taxus baccata* leaves, firstly the leaves of *Taxus baccata* collected from the north of Iran, Golestan privence (Afratakhtec zone) and after that the leaves were dried in shadow area out of direction sunlight and powdered in next step by a moulinex mill. Then about 100 g of leaves powder was poured into a Erlenmeyer flask (1000 mL) with 500 mL of distilled water and was boiles for 30 min. The aqueous extract was filtered and stored at 4 °C for next experiment of nano-synthesis reaction.

2.3. Green synthesis of silver nanoparticles using Taxus baccata leaf extract

For the synthesis of Ag NPs, 50 mL of *Taxus baccata* leaf water extract was added dropwise to aqueous solution of AgNO3 (50 mL of 0.03 mol/L, 99.99%) with constant stirring at 80° C (Ultrasonic Device) and the color change of the solution was observed from whitish to yellowish brown (see Fig 2). Reduction of Ag^+ to Ag nanoparticles was completed in 180 s (as monitored by UV–vis spectra). The nanoparticles suspension was diluted 10 times with distilled water to avoid the errors due to high optical density. Finally, the apparent and structural properties of the silver nanoparticles were determined by FT-IR, UV, and XRD, FESEM techniques [25].

3. Results and discussion

3.1. UV-Vis study

The UV spectrum is showed specific peak in the 415 nm region that confirmed the presence of silver nanoparticles. This strong strip corresponds to the surface Plasmon resonance of silver nanoparticles (Figure 3).



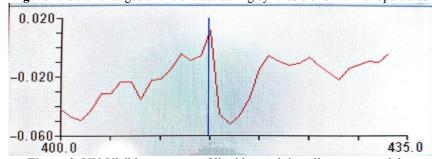


Figure 3. UV-Visible spectrum of liquid containing silver nanoparticles

3.2. Scanning electron microscopy results

The shape and size of the silver nanoparticles synthesized by the green method were determined by scanning electron microscopy (Figure 4). The silver nanoparticles are formed in the form of white cloudy masses between colored gray masses of the extract and the size of the nanoparticle diameter is about 20 to 45 nm.

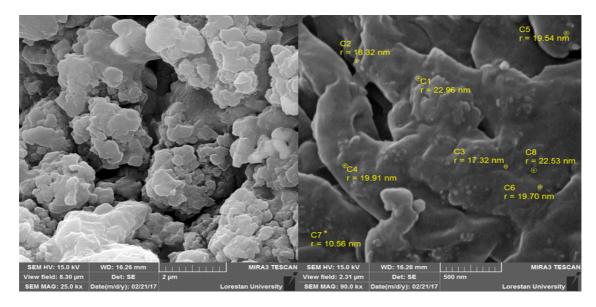


Figure 4. FE-SEM images of silver nanoparticles synthesized with taxus baccata

3.3. X-ray Diffraction Analysis (XRD)

The diffraction pattern display pure silver metal powder. The XRD pattern indicates that the nanoparticles had a face-centered cubic (fcc) structure and topography. The Ag NPs formed by the reduction of Ag^+ ions using the *Taxus baccata* leaves water extract are crystalline in nature. The XRD analysis of synthesized silver nanoparticles has four peaks at 38.36, 44.48, 64.62 and 77.62 angles. Also, at 38.36 = 20, the curve has the highest peak, which was determined according to the standard JCPDS # 001 Ag₀ card (Figure 5).

The crystallite size has been estimated from the XRD pattern using the Scherrer's eqution (Eq. (1)): $d=K\lambda/\beta\cos\theta$ Where k = 0.9 is the shape factor, λ is the X-ray wavelength of Ag K α radiation (1.54 Å), θ is the Bragg diffraction angle, and β is the full width at half maximum (FWHM) of the respective diffraction peak. Based on the scherrer equation, the crystallite size of AgNPS was estimated to be 26.5 nm.

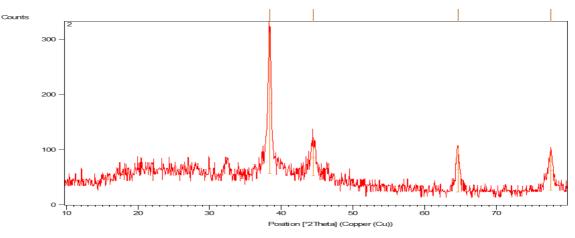


Figure 5. The XRD image of synthesized silver nanoparticles with a valence of zero

3.4. Infrared spectroscopy (FTIR)

The nanoparticles suspension was centrifuged at 10,000 rpm for 20 min and the dried samples evaluated using FT-IR spectroscopy. This technique was used for the characterization of the *Taxus baccata* leaf extract and prepared silver nanoparticles. FTIR technique was used to observe the chemical structure of silver nanoparticles synthesized by *Taxus baccata* leaf extract (Fig.6). The absorption peaks of silver nanoparticles at 3458 cm^{-1} (stretch OH), 2923 cm⁻¹ (CH = stretch), 1064 cm⁻¹(stretch O-C) and the 694 peak for to aromatic ring in the phenolic compounds, which confirms the presence of the phenolic groups present in the extract in the presence of silver nanoparticles.

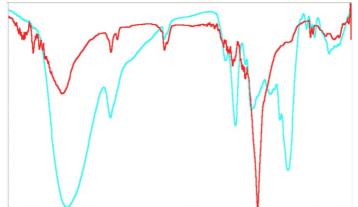


Figure 6. The infrared spectrum associated with the silver nanoparticles synthesized by the green chemistry method

3.5. EDS pattern

The presence of Ag elements in the AgNPS can be confirmed by EDS pattern (Fig7). The signal of carbon and oxygen elements in EDS spectra is because of presence compounds in the *Taxus baccata* extract. Moreover EDS spectrum shows the signal of Magnesium and Potassium elements because of absorption of soil by plant.

3.6. Possible mechanism

The mechanism for the biosynthesis of metal oxide NPs using plant extracts has not been confirmed. However, it was recommended that polar groups are responsible for the synthesis of NPs [26-28]. Possible mechanism for the capping effort of extract is showed in the following (Fig 8). At firstly it appears the lone pair electrons in the polar groups of 2,4-di methoxy phenol is occupy by vacant orbital of Ag^+ [29].

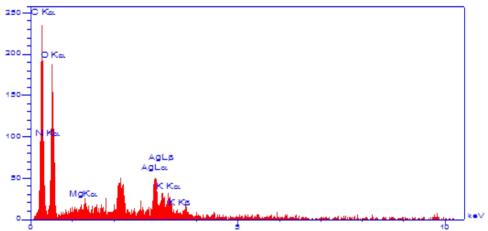


Figure 7. EDS spectrum of silver nanoparticles synthesized of Taxus baccata extract.

Then Ag⁺ is capped with polar groups of extracted organic compound as secondary metabolites form a complex compound. Finally, the reaction could result to AgNPs by calcination in furnace. According to the half reaction of reduction potential, the overall reaction was determined to have a potential of 0.807 V vs Saturated calomel electrode (SCE) which is effective enough to reduce from NO₃⁻ to NO which has the reduction potential of 0.960 V vs. SCE, respectively (Eq. (2)): NO₃⁻ + 4H₃O⁺ + 3e⁻ \rightarrow NO (g) + 6H₂O

Since H^+ was released in the oxidant half reaction Figure 8 and consume in Eq. (2) [30], so the redox process continues properly.

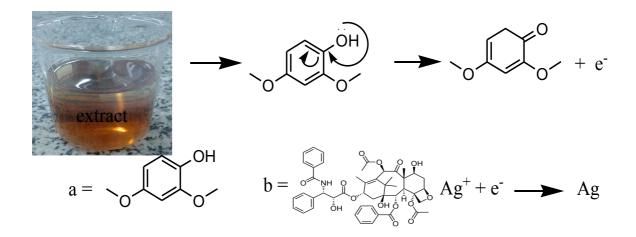


Figure 8. Possible mechanism for the capping effort of taxus baccata extract. a) 2, 4-di methoxy phenol, b) taxol

Conclusion

The Ag NPs were synthesized by *Taxus baccata* leaf water extract as a green reducing and stabilizing agent. Nowadays, one of the most basic needs to the field of nano is its compatibility with the environment and its use in antimicrobials utilization. This study represents a new way of synthesizing silver nanoparticles which has both antimicrobial and environmental compatibility by green chemistry without using the chemical and materials which is previously used to stabilize particles from *Taxus baccata* leaf extract. The analysis on synthesized nanoparticles clearly showed that silver nanoparticles had dimensions from 20 to 45 nm in oxidation state of zero charge. This green procedure has high yields and easy operations without application of toxic reagents or surfactant template. Environmental compatibility and low cost and easy synthesis are the advantages of this method to comparing with other reported methods. The synthesized nanoparticles were characterized by FE-SEM, XRD, FT-IR and UV–visible spectroscopic techniques.

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