International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 6, No. 2, p. 30-35, 2015

RESEARCH PAPER

Silver nanoparticles green synthesis using aqueous extract of *Salvia limbata C.A. Mey*

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Key words: Silver nanoparticles, phytochemical fabrication, herbal extract.

http://dx.doi.org/10.12692/ijb/6.2.30-35 Article published on January 18, 2015

Abstract

Plants and crops as renewable and cheap resources to produce nanomaterial. In recent years the use of plant extracts for nanoparticles production is an alternative to chemical and physical methods. Silver nanoparticles are widely used for health care and it's green synthesis is studied in this research. Phytochemical fabrication of silver nanoparticles from $AgNO_3$ solution was done using aqueous extract of *Salvia limbata*, an endemic herb of Iran. The plant was dried at ambient temperature. The extraction was performed with deionized boiled water. Synthesized silver nanoparticles were characterized by means of ultraviolet-visible spectroscopy, infra-red spectroscopy and X-ray diffraction spectroscopy and transmission electron microscopy. To confirm the formation of silver nanoparticles, the ultraviolet- visible absorption spectrum was obtained. Surface plasma resonance absorption at 420-450 nm showed that silver nanoparticles were formed. The average size of obtained silver nanoparticles was found to be 2-25 nm in face center cubic crystalline structure.

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Introduction

Synthesis of noble metal (e.g. Ag, Au, Pt and Pd) nanoparticles, usually ranging between 1-100 nm, is one of the most important fields of study in modern material science due to their high specific surface which enable them special physicochemical activity (Saxena *et al*, 2010). Because of numerous potential application in material science and biology of Ag, nanoparticles of silver (Ag-NPs) are used widely in optics(Kamat PV. 2002), anti-HIV (Elechiguerra *et al.* 2005), chemical reaction catalyst (Jiang *et al.* 2005), medical application (Jain *et al.* 2009), absorption of solar energy (Moulin *et al.* 2008) and anti-bacterial agents (Montleiro *et al.* 2009). Several methods have been used to reduce Ag^+ to Ag^o nanoparticles. Reduction by sodium citrate ($C_6H_7NaO_7$), sodium borohydrate ($NaBH_4$) and hydrazine (N_2H_4) are such methods which using toxic and expensive compounds (Daniel *et al.* 2004). In last decade, phytochemical procedure is used to synthesis of silver nanoparticles due to their eco-friendly, cost effective and non-toxic substances. Kasthuri, J. *et al* was reported that appin (apigenin-7-O-apioglucoside), a flavonoid, can reduce and coated the Ag-NPs (Kasthuri *et al* . 2009).

The genus of *salvia* which belong to *lamiaceae* family, have 58 species in Iran among the 900 species all over the world[10]. As reported, salvia species have been used in folk medicine as antibacterial, tonic, antirheumatoid and carminative agent (Yinrong. 2002). *S. limbata*, an aromatic plant, grows in Iran, Afghanistan and Turkey(Hedge *et al.* 1983, Guner. 2000). As it was investigated, *s. limbata* are rich in flavonoid compounds. Six flavones including ladanein (1), salvigenin (2), luteolin 7-methyl ether (3), cirsiliol (4), eupatorin (5) and luteolin 7-O-glucoside (6) were characterized in *S.limbata* (Gohari *et al.* 2010) (Figure 1).

In this paper we report an eco-friendly method to synthesis silver nanoparticles using the aqueous extract of areal parts of *S.limbata*, a herb that rich in flavonoid compounds, for phytochemical fabrication and characterization of Ag-NPs due to reductive and capping power of the flavonoieds.

Material and methods

Plant material

The aerial parts (leaves, stem and flowers) of *S.limbata* were harvested in flowering stage from Tehran altitude (latitude 35.793055°, longitude 51.312928°), province of Tehran, Iran, in May 2013. The authentication of plant species was done by Dr. V. Mozaffarian. Voucher specimens (code: 114017048) have been deposited at the Herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran.

Preparation of aqueous extract

The plant material was washed triple by deionized water and dried in shade under air stream in ambient temperature (25°C) for 6 days. Dried plant was powdered mechanically by a domestic blender. 10 g of air dried plant powder was boiled with 100 mL sterile deionized water in a 250 mL Erlenmeyer flask for 5 min. the boiled extract was filtered through a Whatman filter paper (no.1). The filtrate was kept in 4°C (Mallikarjuna *et al.* 2011).

Phytochemical synthesis of silver nanoparticles (Ag-NPs)

Silver nitrate ($AgNO_3$) which used for synthesis of Ag-NPs was purchased from Sigma-Aldrich chemicals. 10 mL of *S.limbata* aqueous extract was added to 90 mL of 1 mM solution of silver nitrate (16.9 mg $AgNO_3$ in 100 ml deionized water) and kept in ambient temperature. After 10 min a brownish solution can be seen due to reduction of Ag^+ cations to Ag^o by *S.Limbata* extract (Jain *et al.* 2009).

Characterization of Ag-NPs

UV-VIS spectral

UV-VIS spectral analysis was used to ascertain the formation of Ag-NPs in aqueous solution based on excitation of surface plasmon resonance (SPR) changes at the wavelength range from 300-700 nm after different period of times (0, 1, 2, 3, 6, 24 and 48 h). The UV-VIS analyses were performed using a Varian Car 300 spectrophotometer. The reaction mixture color was changed because of excitation of surface plasmon vibrations in the Ag-NPs. The brownish color of Ag-NPs is related to Surface Plasmon resonance (SPR) arising due to the group of free conduction electrons induced by an interacting electromagnetic field. The SPR band for Ag-NPs normally appears in the 420-450 nm regions of spectra.

FT-IR Spectroscopy

The Ag-NPs synthesized solution was centrifuged at 18000 rpm for 20 min at ambient temperature. The resulting pellet was redisposed in deionized water. This process was repeated three times. The water extract and Ag-NPs pellet was dried by freeze drier (CHRIST alpha1-4 LD plus- Germany) and subjected to FT-IR spectroscopy (Thermo Nicolet Nexus 870-USA) using potassium bromide (KBr, Sigma-Aldrich) at 400-4000 cm^{-1} at a resolution of 4 cm^{-1}. The Figure 4 shows the FT-IR spectrum of before and after bio reduction of Ag^+ ions.

Transmission Electron Microscopy (TEM)

TEM investigation was performed in order to determine the shape and size of phytochemical synthesized Ag-NPs. A 5 µl of Ag-NPs solution was located on a carbon coated TEM grid and dried in ambient temperature. This grid was subjected to TEM imaging using a Philips EM 208 instrument that operated at 100 kV accelerating voltage.

X-Ray Diffraction (XRD) analysis

XRD spectrum can be used to determine the nature of Ag-NPs. The XRD studies were performed using a Sifert XRD 3003 PD (Germany) Equipped with a Cu-$K\alpha_1$ radiation with λ of 1.54 A° in the region of 2θ from 20° to 90°, using a dried thin film of synthesized Ag-NPs. XRD pattern of phytochemical synthesized Ag-NPs show four peaks at 37.8431°, 45.9587°, 64.1242° and 76.9911°. The size of nanoparticles was calculated through the Debye-Scherer's equation as shown below:

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

Where, K is a dimensionless shape factor, with a value close to unity. The shape factor has a typical value of about 0.9, but varies with the actual shape of the crystallite, D is average size of nanoparticle, λ is wavelength of X-Ray in angstrom (A°), β is the full width at half the maximum intensity (FWHM) in radian and θ is the diffraction Bragg's angle. To elimination of additional instrumental broadening the β value should be corrected according to blow formula using the FWHM from a large grained Si sample which was calculated as 0.15868 A° in our XRD instrument (Ghosh *et al.* 2012).

$$\beta_{\text{corrected}} = [\,(\text{FWHM}_{\text{sample}})^2 - (\text{FWHM}_{\text{Si}})^2]^{0.5}$$

Results and discussion

Figure 2 is shown the UV-VIS spectra of 1 mM AgNO3 solution and *S.limbata* aqueous extract at different reaction time. As shown in figure 2, the Ag-NPs were formed 1 h after adding *S.limbata* extract to $AgNO_3$ solution, the reaction mixture color was changed because of excitation of surface plasmon vibrations in the Ag-NPs. The brownish color of Ag-NPs is related to SPR arising due to the group of free conduction electrons induced by an interacting electromagnetic field. The SPR band for Ag-NPs normally appears in the 420-450 nm regions of spectra.

The 3412 cm^{-1} strong peak in FT-IR spectra of aqueous extract of *S.limbata* (Figure 3, A) shows the existence of alcoholic and phenolic O-H groups which disappear after phytochemical reduction of Ag^+ ions due to responsibility of hydroxyl groups in reduction of Ag^+ ions (Figure 3, B). The 2917cm^{-1}, 1614cm^{-1} and 1394 cm^{-1} absorption are related to alkyl C-C stretch vibration, C=O bond with a benzene ring conjugated with a C=C bond and bending vibration of C-OH, respectively.

TEM image (Figure 4) was revealed the formation of Ag-NPs in a spherical shape with the average size of 5 to 25 nm.

XRD spectrum of Ag-NPs (figure 5) synthesized by *S.limbata* aqueous extract shows four intensive peaks at 2θ of 37.8431°, 45.9587°, 64.1242° and 76.9911° related to (111), (200), (311) and (222) HKL values, respectively, which shown the Ag-NPs were appeared in a face center cubic (fcc) lattice system. The average size of Ag-NPs was calculated to 14.1 nm by Debye-Scherer's equation at 2θ of 37.8431° (111) whit 0.6166 as FWHM (rad) value.

In this work, Rapid, eco-friendly and cost effective phytochemical reduction of Ag ions to nano zero Valente Ag using *S.limbata* water extract was established by UV-Vis, XRD and FT-IR and TEM micrograph. Appearance of yellowish brown color 30 min after adding *S.limbata* extract to 1mM $AgNO_3$ solution Related to SPR in the range of 420-450 nm of UV-Vis spectra proved the formation of Ag-NPs. *S.limbata* are rich in water soluble constituents such as flavonoids and phenolic compounds (13) which are responsible to reduction Ag ion to Ag-NPs. This could be seen using FT-IR spectra by disappearing of 3412 cm^{-1} peak (OH groups) and increasing of 1614 cm^{-1} after phytochemical reduction processes which shown the oxidation of hydroxyl to carbonyl groups.

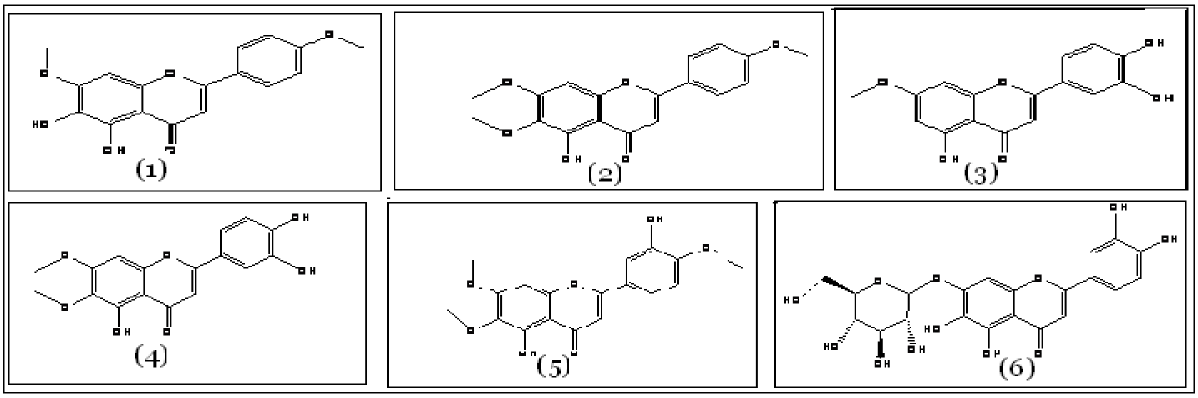

Fig.1. Identified flavonoids from *S.limbata* grown in Iran.

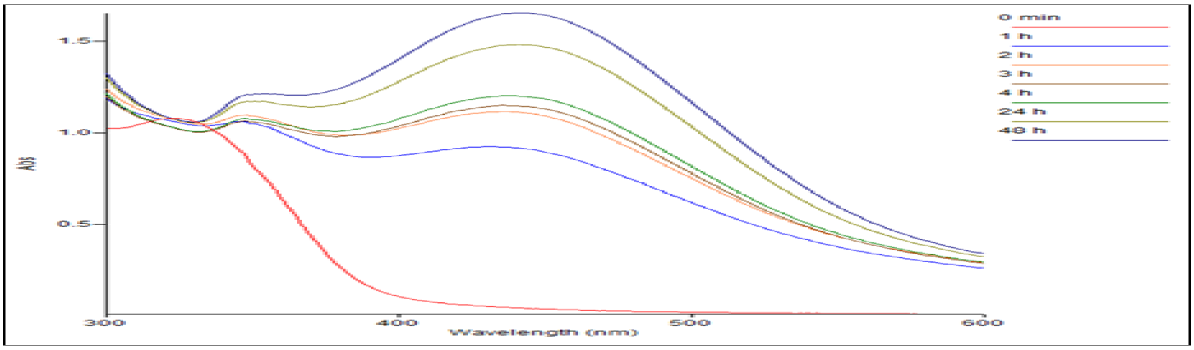

Fig. 2. UV- Vis spectrum of Ag-NPs/ *S.limbata* extract in period of times (0-48 h).

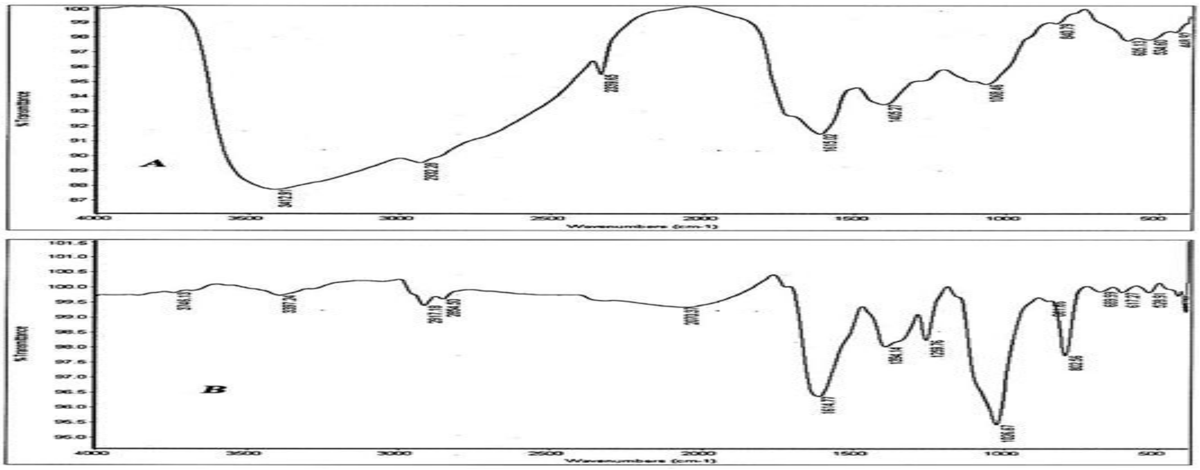

Fig. 3. FT-IR spectra of *s.limbata* Aqueous Extract before (A) and after (B) reduction of Ag^+ ions.

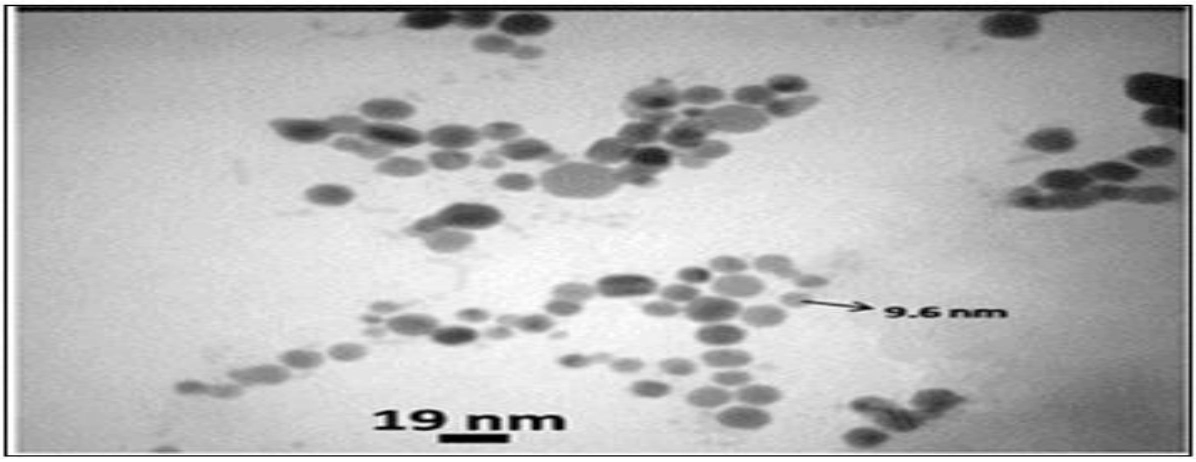

Fig. 4. TEM image of phytochemical synthesized Ag-NPs using *S.limbata* water extract.

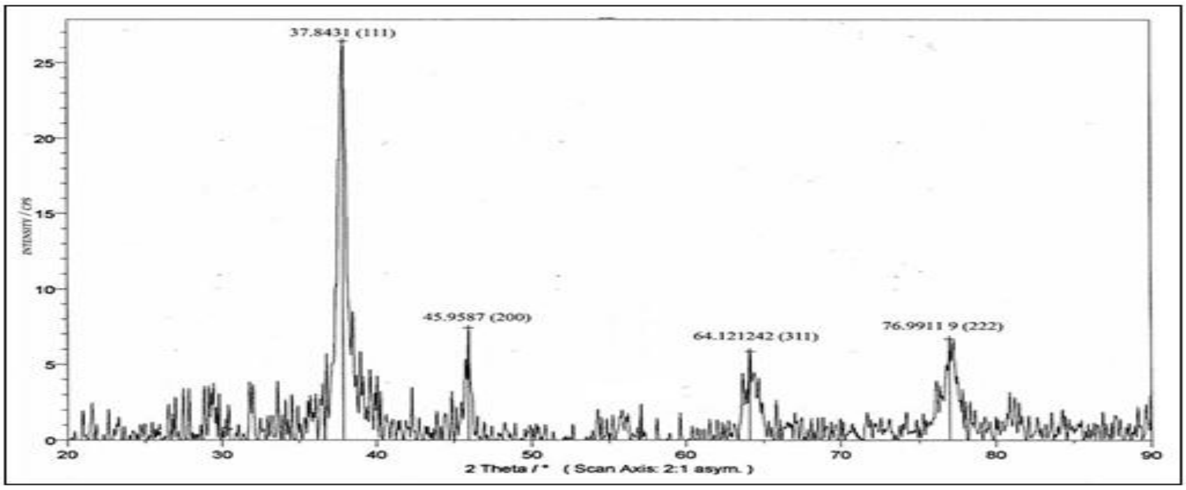

Fig. 5. XRD pattern of phytochemical synthesized Ag-NPs.

Conclusion

In this work. Silver nanoparticles were synthesis using water extract of *Salvia limbata*, a *lamiaceae* family, in to replace hazardous chemical reducing agent with an eco- friendly one. The synthesized Ag nanoparticles were characterized by analytical instruments such as UV-VIS, FT-IR, XRD and TEM. The obtained data were revealed that these nanoparticles are in face center cubic (fcc) lattice system with the average size between 5-25 nm.

Acknowledgments

The authors are grateful to Dr. Mozzafarian (TARI) for his help for identification the plant materials and Mr. H. Pourhadgi (Science and Research Branch, Islamic Azad University) for XRD analyses and Mr. Norani (Science and Research Branch, Islamic Azad University) for TEM images.

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