

Green Synthesis of Silver Nanoparticles Using Sugar Beet Leaf Extracts and Its Antibacterial Activity

Shahryar Pashaei^{*1}, Samaneh Safari¹, Soleyman Hosseinzadeh²

1. Department of Chemistry, Payame Noor University, Tehran, Iran

2. Department of Engineering, Payame Noor University, Tehran, Iran

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Abstract

In the present study, synthesis of silver nanoparticles and its antibacterial activity were investigated. Silver nanoparticles were rapidly synthesized using leaf extract of beet sugar leaf the formation of nanoparticles was observed within 1 hr. The results recorded from UV-vis spectrum, Transmission electron microscopy (TEM) and X-ray diffraction (XRD) support the biosynthesis and characterization of silver nanoparticles. The UV-Visible spectrophotometer was indicated absorbance peak in range of 435-440 nm. From high resolution transmission electron microscopy (HRTEM) analysis, the size of the silver nanoparticles was measured 35-40nm. Further, the antibacterial activity of synthesized silver nanoparticles showed effective inhibitory. It showed that antibacterial activity increased by addition concentration of silver nano particle. The 0.008 molar concentrations of AgNPs, antibacterial activity was higher than other concentrations. Results confirmed this protocol as simple, rapid, one step, and eco-friendly, nontoxic and alternative conventional physical/chemical methods. Nanoparticle synthesis is a novel research are to search for an eco-friendly manner and green materials for potential applications in the fields of medicine and drug delivery.

Keywords

Beet Sugar Leaf; Green Synthesis; Silver Nanoparticles; Bactericidal Activity; Quercetin.

1. INTRODUCTION

The 'green' environment friendly processes in chemistry and chemical technologies are becoming increasingly popular and are much needed as a result of worldwide problems associated with environmental concerns [1]. Green chemical engineering is a way to not only improve the environment but positively impact the client's bottom line. Avoiding the generation of waste (including energy) or pollutants can often be more cost-effective than controlling or disposing of pollutants once formed. Greener syntheses of nanoparticles also provide advancement over other methods as they are simple, one step, cost-effective, environment friendly and relatively reproducible and often results in more stable materials [2]. In the past decade, several research groups have developed metal oxide nanoparticles using savvy routes. Among them, a significant category of silver nanoparticles (AgNPs) have gained importance since few years. There has been much recent interest in using silver nanoparticles (AgNPs) in new technologies owing to their drastically enhanced properties over bulk silver, especially particles of diameters 30 nm and smaller [3]. These NPs are increasingly being incorporated into

consumer products despite rising evidence suggesting AgNPs have toxic effects on humans and experimental animal models meant to mimic human bio- and neurochemistry such as mice, rats, and Drosophila. [4]. There are techniques for the syntheses of silver nanoparticles like ion sputtering, chemical reduction, sol gel, etc [5]. The techniques for obtaining nanoparticles using naturally occurring reagents such as sugars, biodegradable polymers (chitosan, etc.), plant extracts, and microorganisms as reductants and capping agents could be considered attractive for nanotechnology. Microorganisms can also be utilized to produce nanoparticles but the rate of syntheses is slow compared to routes involving plants mediated synthesis [6]. The preparation of AgNPs using plant-based extracts is widely growing in popularity; recently proposed syntheses use reagents such as many types of leaf extract, [7-13] including menthol, [14] aloe vera, [15] clove extract, [16] edible mushroom extract, [17] and extracts from coffee and tea [18]. AgNP synthesis using the extract of the navel orange (*Citrus sinensis*) was proposed by Kaviya [19]. AgNPs were both reduced from silver nitrate (AgNO_3) and capped by the compounds present in the orange peel extract. The objective of the work here was to use AgNO_3 and aqueous extracts of sugar beet leaf to prepare

*Corresponding Author: shahryarpashaei@pnu.ac.ir

AgNPs in a phytochemical synthesis. The nontoxic environmentally friendly synthesis proposed here produced AgNPs of an appropriately small size distribution using the sugar beet leaf extracts.

2. EXPERIMENTAL

Typically, a plant extract-mediated bio reduction involves mixing the aqueous extract with an aqueous solution of the appropriate metal salt. The synthesis of nanoparticle occurs at room temperature and completes within a few minutes.

2.1. Preparation of plant extract

Sugar beet leaf extract was used to prepare silver nanoparticles on the basis of cost effectiveness, ease of availability and its medicinal property. Fresh leaves were collected from sugar beet farmer in Naghadeh city of Iran. They were surface cleaned with running tap water to remove debris, followed by double distilled water and air dried at room temperature and then further cut into small pieces to make powder by hand. About 10 g of finely powder sugar beet leaf were kept in a beaker containing 100 mL double distilled water during one day and then boiled for 2 hrs. The extract was cooled down and filtered with Whatman filter paper and extract was stored at 4 °C. Finally, the extract was used for the synthesis of silver nanoparticles.

2.2. Preparation of silver nanoparticles

Solutions of silver nitrate were prepared at different concentrations of AgNO_3 in an Erlenmeyer flask. Then, 5 mL of plant extract was added separately to 20 mL of silver nitrate solution and incubated in a dark chamber to minimize photo-activation of silver nitrate at 45 °C. Silver nanoparticles have been synthesized by varying concentration of AgNO_3 (1 mM, 4 mM, 8 mM and 10 mM). Reduction of Ag^+ to Ag^0 was confirmed by the colour change of solution from yellow to dark. Its formation was confirmed by using UV-Visible spectroscopy. Acetone was added to aqueous samples in a ratio of 1:2 to encourage precipitation of AgNPs. After allowing the AgNPs to precipitate, the samples were centrifuged for 10 min at 4500 rpm. Then, it dried at 60 °C for 3 hr for further characterization. For antimicrobial applications, the AgNPs were redispersed in distilled water without drying. The chemical structures of the compounds from alcoholic leaves extract of sugar beet have been indicated in Fig. 1 [20].

2.3. Characterization of synthesized silver nanoparticles

2.3.1. UV-Visible Spectroscopy

UV-vis spectra were collected in an optical-quality quartz cuvette with a 1 cm path length,

requiring approximately 2 mL of solution to fill past the light path of the instrument (Agilent 8453 system, Santa Clara, CA). Spectra were collected at room temperature using the appropriate aqueous sugar beet leaf extracts as the blank, always taken with the same cuvette as used for analysis. Solutions were diluted immediately before analysis, if required, in order to normalize absorbance to approximately 2 AU. Spectra were collected from 300 to 700 nm.

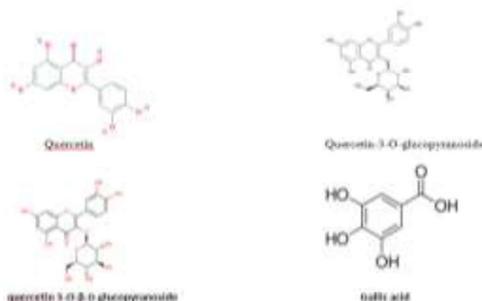


Fig. 1. Chemical structure of the compounds from alcoholic leaves extract of sugar beet [20]

2.3.2. Powder X-ray Diffraction

X-ray diffraction was performed on a Scintag X-2 advanced diffraction system (Cupertino, CA) equipped with Cu K α radiation ($\lambda = 1.54 \text{ \AA}$) using a drop-cast sample of the AgNPs on a zero background Si plate sample holder (30 mm \times 30 mm \times 2.5 mm SiO_2 single crystal plate; MTI Corporation, Richmond, CA). AgNP samples were concentrated via centrifugation as described above prior to being drop-casted.

2.3.3. Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) analysis of the sample was done using PHILIPS- CM 200 instrument operated at an accelerating voltage of 200 kV with resolution of 0.23 nm. A drop of the solution was placed on carbon coated copper grid and later exposed to infrared light (45 minutes) for solvent evaporation.

3. RESULT AND DISCUSSION

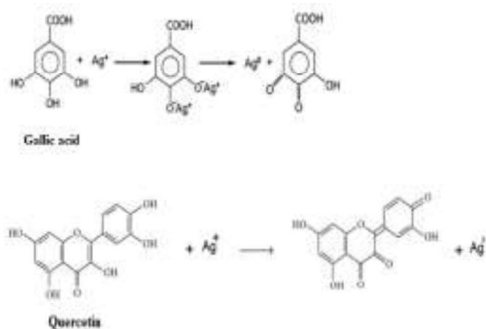
3.1. UV-vis absorption spectroscopy analysis

In addition of sugar beet leaf extracts into the beaker containing aqueous solution of silver nitrate led to the change in the colour of the solution to a dark brown (shown in Fig. 2). The dark brown color showed the formation of silver nanoparticles. Reduction of the Ag^+ ions was monitored with respect to time using UV-visible spectral analysis [21]. The aqueous sugar beet leaf extract involves main metabolites along with gallic acid, alkaloids, quercetin, quercetin 3-O- β -D-glucopyranoside, tannins, phytosterols, etc [22].

The flavonoids are strong reducing agents and are contributed to the reduction of Ag^+ ions to nanoparticles. However, flavonoids are powerful reducing agents and they may also directly scavenge molecular species of active oxygen, this antioxidant activity of flavonoids emanates from their ability to donate electrons or hydrogen atoms. The reasonable mechanism of AgNPs formation may be suggested as the flavonoids are oxidized during the reduction of Ag^+ to AgNPs. Chemistry behind the nanoparticles formation is signified in Equation 1, which states that flavonoids are responsible for the reduction of Ag^+ ions and AgNPs are stabilized through negatively charged carboxylate groups of proteins. Therefore, reduction and capping processes by the biomolecules present in the leaf extract could be accountable for the length stability.



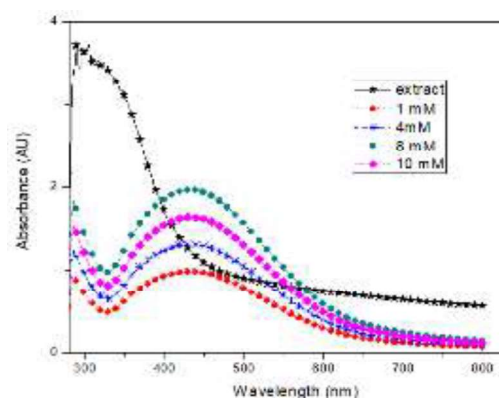
Fig. 2. Digital optical images of (a), AgNO_3 (10 mM), (b) leaf beet sugar extracted and (c) nanosilver solution



Equation. 1. Indicate the possible silver nanoparticles formation mechanism in the leaf sugar beet extract.

The characteristic absorption peak at 435 nm in UV-vis spectrum (Fig. 3) confirmed the formation of silver nanoparticles. Surface Plasmon Resonance (SPR) patterns, characteristics of metal nanoparticles strongly depend on particle size, stabilizing molecules or the surface adsorbed particles and the dielectric constant of the medium. The single SPR band in the early stages of synthesis corresponds to the absorption spectra of spherical nanoparticles. Many SPR bands resulted later, with increase in the incubation period and two such bands were prominent with 4 h incubation, it indicates the formation of anisotropic molecules that later stabilized in the medium. In all

experiments, by adding sugar beet leaf extract into the beakers containing aqueous solution of silver nitrate led to the change in the colour of the solution to yellow to dark (shown in Fig. 3) within reaction duration due to excitation of surface Plasmon vibrations in silver nanoparticles [23]. The appearance of the brown colour was due to the excitation of the Surface Plasmon Resonance (SPR), typical of silver nanoparticles having absorbance values which were reported earlier in the visible range of 435-440 nm [24]. The UV-vis spectra recorded, implied that most rapid bio reduction was achieved using sugar beet leaf extract as reducing agent. The UV-vis spectra and visual observation revealed that formation of silver nanoparticles occurred rapidly within 30 min. As shown in Fig. 3, the synthesized AgNPs exhibited a broad absorbance at $\lambda_{\text{max}} = 435$ nm for AgNPs by sugar beet extract, owing to the surface plasmon resonance of the AgNPs [25]. This Plasmon resonance is an intrinsic property of AgNPs and arises from the coupling between the electron clouds on the AgNP surface with the incident electromagnetic radiation [26-29] and typically occurs between 380 to 420 nm depending on



the size of the AgNPs analyzed.

Fig. 3. UV-vis absorbance spectrum of synthesized AgNPs from 250–800 nm is shown, with the absorbance maximum occurring at $\lambda_{\text{max}} = 435$ nm for AgNPs fabricated with different concentrations of AgNO_3 keeping fixed the volume of sugar beet extract and $\lambda_{\text{max}} = 310$ nm (leaf sugar beet extract).

3.2. X-ray diffraction analysis (XRD)

The crystalline nature of AgNPs was confirmed from XRD analysis. The nature of AgNPs was identified to be crystalline using XRD analysis with Cu K α target at 20–80 °C. The XRD pattern of the silver nanoparticles is shown in Fig. 4. The average grain size of the silver nanoparticles formed in the bio reduction process was determined using Scherrer's formula, $d =$

$(0.9\lambda \times 180^\circ) / \beta \cos(\Theta) \pi$ and was estimated as 4nm (Fig. 4). The peaks assigned to the diffraction pattern clearly indicate peaks corresponding to face-centered cubic (FCC) silver, with peaks at $2\theta = 38.4^\circ, 44.6^\circ, 64.4^\circ, 77.3^\circ$, and 81.7° corresponding to the (111), (200), (220), (311), and (222) planes, respectively. The broadness of the peaks was indicated the small size of the AgNPs synthesized. The interplanar spacing (d_{hkl}) values (2.349, 2.040, 1.437, 1.239, and 1.176 Å) was obtained from the XRD spectrum of silver nanoparticles. It was in agreement with the standard silver values [30].

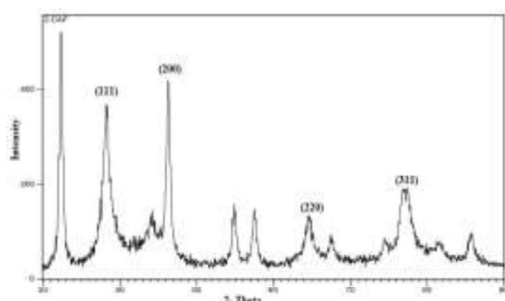


Fig. 4. XRD pattern of synthesized AgNPs by sugar beet extracts.

3.3. TEM Analysis

Transmission electron microscopy (TEM) is used to determine particle size, shape and morphology of nanoparticles. It showed that the silver nanoparticles are well dispersed and predominantly spherical nature in shape, while some of the NPs have been found to be having structures of irregular shape as shown in Fig. 5. The TEM image of a single nanoparticle is revealed crystalline nature of the particles. The nanoparticles are homogeneous and spherical. It conformed to the shape of SPR band in the UV-visible spectrum. From Fig. 4, it was obtained that average particle size about 15 nm. In spite of, some of the silver nanoparticles were cluster and irregular shape.

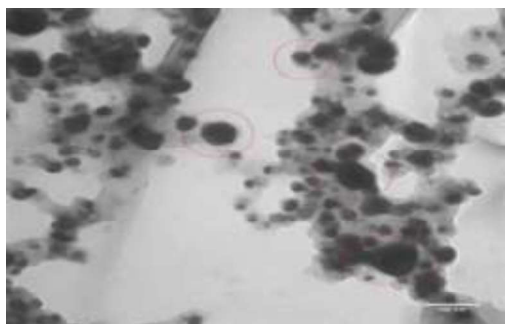


Fig. 4. TEM image of synthesized silver nanoparticles.

3.4. Antimicrobial activity

Silver nanoparticles most widely have been used in the health industry, medicine, textile coatings, food storage, dye reduction, wound dressing, antiseptic creams and a number of environmental applications, because of their antimicrobial properties [31]. We have examined sugar beet extract mediated silver nanoparticles as possible antibacterial agents. The plant extract and those mediated silver nanoparticles were immediately tested for respective antimicrobial activities towards both gram positive (*S. aureus*) and gram negative (*E. coli*) bacterial strains showing the zones of inhibition. The results of antibacterial activities of prepared silver nanoparticles evaluated from the disc diffusion method are given in Fig. 5. The silver nanoparticles showed efficient antimicrobial property compared to other due to their extremely large surface area providing better contact with cell wall of microorganisms [32]. Fig. 5 has been indicated that silver nanoparticle has shown antibacterial activity against all tested microorganism and maximum Zone of inhibition was found against *Bacillus cereus*. It showed that antibacterial activity increased by addition concentration of silver nanoparticle. The 0.008 molar concentrations of AgNPs, antibacterial activity was higher than other concentrations (Fig. 5). Biological molecules extracted from living systems show potential role and have become preferred choice in the synthesis of AgNPs, which are summarized in Table 1.



Fig. 5. Antibacterial activity of silver nanoparticles synthesized using the leaf extract of sugar beet against *E. coli*.

Table 1. Plant-mediated synthesis of AgNPs, and the phytochemicals involved in reduction Ag⁺ ions.

Reducing agents		Phytochemicals involved	AgNPs characteristics (shape and size)	Reference
Plant name	Plant parts used			
Musa	Flower	proteins	Spherical; 12.6-15.7 nm	33
Prosopis Juliflora	Leaf	Alcoholic, Phenolic, Aromatic Compound	Spherical; 10-20 nm	34
Withania coagulans	Leaf	Amides, flavonoides, phenols, amino acids	Spherical; 14 nm	35
Solanum	Leaf	Phenolic compound	Spherical; 35-50 nm	36
Piper longum L.	Leaf	Hydroxyl, carbonyl, amide, flavonoids, terpenoids,	Spherical; 25-32 nm	37
Aesculus	Leaf	Saponin, protein, alkanes, ester	Spherical; 50 nm	38
Ocimum Sanctum	Leaf	Quercetin	Spherical; 11.35-14.6 nm	39
Oriza sativa	Leaf	Sugars, Proteins, phenolics	Spherical; 3.7-29 nm	40
Sugar beet leaf	Leaf	Quercetin, flavonoids, polyphenoles, Hydroxyl, carboxyl	Spherical; 15 nm	Present work

4. CONCLUSION

The present study demonstrates the use of unreported sugar beet extracts for the quick synthesis of silver nanoparticles from silver nitrate. Variation in reaction conditions affected nanoparticles synthesis where the reaction mixtures displayed typical colors and UV-visible spectra, characteristic of silver nanoparticles. The biosynthesized nanoparticles produced by this novel, cost-effective, non-toxic, environmentally safe protocol were characterized by a variety of standard analytical techniques like XRD, TEM and were further tested against bacterial. Nanoparticle synthesis is a novel research area to search for an eco-friendly manner and green materials for potential applications in the fields of medicine and drug delivery.

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سنتز سبز نانوذرات نقره با استفاده از عصاره برگ چغندر قند و فعالیت ضد باکتریایی آن

شهریار پاشایی^{۱*}، سمانه صفری^۱، سلیمان حسین زاده^۲

۱. گروه شیمی، دانشگاه پیام نور، تهران، ایران

۲. گروه مهندسی شیمی، دانشگاه پیام نور، تهران، ایران

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چکیده

در مطالعه حاضر، سنتز نانوذرات نقره و فعالیت ضد باکتریایی آن مورد بررسی قرار گرفت. نانو ذرات نقره با استفاده از عصاره برگ چغندر به سرعت سنتز و نانوذرات نقره در عرض ۱ ساعت تشکیل شد. نتایج ثبت شده از طیف سنجی UV-Vis، میکروسکوپ الکترون عبوری (TEM) و پراش پرتو ایکس (XRD) خصوصیات نانو ذرات نقره را تعیین می کنند. طیف سنجی UV-Vis پیک جذب نانو ذرات نقره را در محدوده ۴۳۵ – ۴۴۰ نانومتر نشان می دهد. از تجزیه و تحلیل میکروسکوپ الکترون عبوری با وضوح بالا (HRTEM) اندازه نانوذرات نقره را ۳۵ تا ۴۰ نانومتر اندازه گیری شد. علاوه بر این، فعالیت ضد باکتریایی نانو ذرات نقره با افزایش غلظت نانو افزایش می یابد و اثر موثری داشت. غلظت ۰/۰۰۸ مولی نانوذرات نقره، فعالیت ضدباکتریایی بیشتری نسبت به سایر غلظت ها داشت. نتایج این پروتکل را به عنوان روش های فیزیکی/شیمیایی معمولی، ساده، سریع، یک مرحله ای و سازگار با محیط زیست و غیرسمی تأیید نمود. سنتز نانوذرات به روش سنتز سبز، یک تحقیق جدید برای جستجوی روشی سازگار با محیط زیست و مواد سبز و برای کاربردهای بالقوه در زمینه های پزشکی و دارورسانی می باشد.

واژه های کلیدی

برگ چغندر قند؛ بیوسنتز؛ نانو ذرات نقره؛ فعالیت باکتریایی؛ کوئرستین.