

Green Biosynthesis of Silver Nanoparticles Using Aqueous Extract of *citrus sinensis* Peel

Randa Mohammed Dhahi^{1*} Zeena G. Faisel¹ Haitham M. Mikhilif²

¹ Al-Iraqia university/college of Education/department of biology, Baghdad, Iraq

² Al-Mustansiriyah university/college of Science/department of physics, Baghdad, Iraq.

Abstract

Silver nanoparticles (AgNP's) biosynthesis was accomplished using hot aqueous extract of *citrus sinensis* peels as a simple, safe, low cost and ecofriendly reducing agent for silver nitrate ions. This procedure was carried out at 37 °C, the first indication of producing the AgNP's was changing the color of solution from yellow to dark brownish which was confirmed by UV-VIS spectrum, XRD,EDX and SEM analysis. Obtained Ag nanoparticles were evaluated for their antimicrobial activity against the gram-positive and gram-negative bacteria..This work proved the capability of plant mediated silver nanoparticle using the principles of green chemistry as a promising candidate that alternate for physical and chemical synthesis.

Keywords: silver nanoparticles; *citrus sinensis*; green synthesis; XRD; orange peel; active compounds of orange.

التصنيع الحيوي لأنتاج جسيمات الفضة النانوية باستخدام المستخلص المائي لقشور البرتقال

¹ رندة محمد ضاحي ¹ زينة غازي فيصل ² هيثم مولود مخلف

¹ الجامعة العراقية/ كلية التربية/ قسم علوم الحياة/ بغداد/ العراق

² الجامعة المستنصرية/ كلية العلوم/ قسم الفيزياء/ بغداد/ العراق

الخلاصة

تم انتاج جسيمات الفضة النانوية بطريقة الانتاج الحيوي باستخدام مستخلص قشور البرتقال المائي الحار بطريقة تمتاز كونها بسيطة، آمنة، غير مكلفة وصديقة للبيئة من خلال اختزال ايونات الفضة لمحلول نترات الفضة. تم الانتاج بدرجة حرارة 37 °C وتم ملاحظة التغير اللوني من اللون الاصفر الى اللون الاصفر المائل للبيضا باعتباره دليل اولي لأنتاج جسيمات الفضة النانوية، وتم تأكيد الانتاج للجسيمات النانوية اكثر من خلال استخدام عدة تقنيات للتوصيف هي: UV-vis, XRD,EDX و SEM . ايضا تم تقييم فعالية الجسيمات النانوية المنتجة المضادة للبكتريا ضد عزلات مرضية موجبة وسالبة لصبغة غرام وهي: *Escherichia coli* و *Pseudomonas sp.* و *Klebsiella sp.* و *Salmonella sp.* و *streptococcus sp.* و *Staphylococcus*.

الكلمات المفتاحية: دقائق الفضة النانوية، ثمرة البرتقال، التصنيع الحيوي الاخضر، المواد الفعالة لثمرة البرتقال

Introduction

Nanobiotechnology is fast growing as an interdisciplinary eco-friendly research area and used in expansive research section such as biology, chemistry, physics, biomedicine and material engineering [1,2]. It deals with several shapes and size of particles in the range of (1 to 100) nm. From last two decades, top down and bottom-up approaches are used to yield metal nanoparticles with diverse morphologies, compositions and structures. It is known for its antimicrobial, anti-inflammatory activities, bio-labeling and in cancer treatment. Nontoxic and new methods in the field of nano research have been developed that involves microorganism and plants for the synthesis of nano-materials [3].

The Nanoparticles used for all the above purposes are metallic nanoparticles, which is remarkable due to their large surface area to volume ratio. Among all the noble metal nanoparticles. Silver nanoparticles are attractive in the field of nanotechnology due to their unique properties such as chemical stability, good conductivity, catalytic and most important antibacterial, antifungal as well as to anti-inflammatory activities which can be merged into composite fibers, cosmetic products & food industry [4,5]. Silver nanoparticles are of interest because of the unique properties such as: size and shape depending optical, electrical, magnetic properties which can be incorporated into antimicrobial applications, several physical and chemical methods have been used for synthesizing and stabilizing silver nanoparticles. [6]

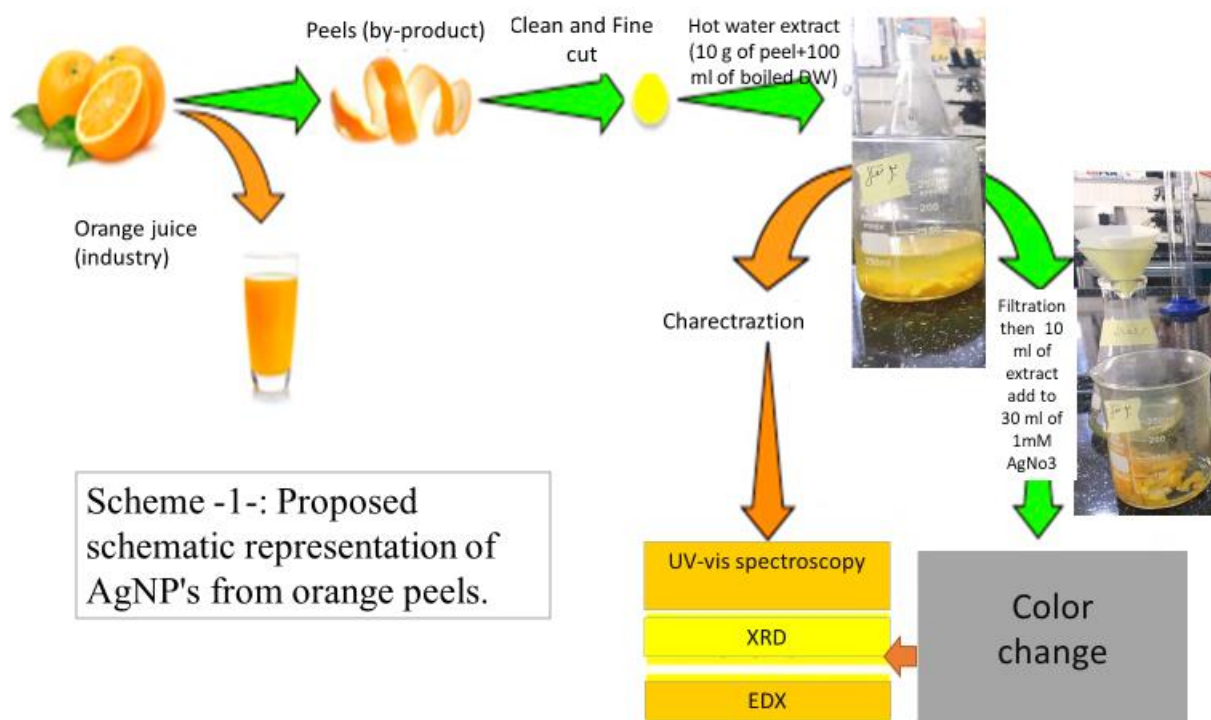
A number of synthetic methods for the synthesis of silver-based nanoparticles involving physical, chemical and biochemical techniques [7]. Chemical-based synthesis techniques are often discouraged as they involve the use of noxious reducing and toxic solvents [8]. Synthesis of nanoparticles through biochemical routes, using plant extracts as reducing and capping agents, has received special attention among others, due to maintaining an aseptic environment during the

process [9]. Therefore, medicinal plants having well established therapeutic importance are being widely used for the size- and shape-controlled synthesis of silver nanoparticles [10].

Recently, nanoparticle synthesis is most interesting scientific areas for attention to produce nanoparticles using environmentally friendly methods (green chemistry) [11] for the search of benign methods for the development nanoparticles and searching antibacterial, antioxidant, and antitumor activity of natural products, in this study, we have proven that AgNP's prepared from peel extract approach appears fast, cost efficient, eco-friendly and alternative for conventional methods. In this study, we have synthesized silver nanoparticles using orange peel. The AgNP's formation were confirmed and analyzed by various physicochemical techniques such as color changing, UV-Visible spectroscopy, x-ray diffraction (XRD) energy dispersion x-ray diffraction (EDX) then the green synthesized silver nanoparticles were evaluated for their antimicrobial activity against: *Escherichia coli*, *Pseudomonas* sp. *Klebsiella* sp., *Salmonella* sp. *streptococcus* sp. and *Staphylococcus* sp.

Material and methods

The method for production and characterization of silver nanoparticles was summarized in the following scheme:



Preparation of orange peel extract

In this experiment fully ripened, clean and free of disease orange was collected from a local market in the month of February 2018. The Samples were bought to the laboratory in polythene covers and cleaned thoroughly using distilled water and running water for one hour to remove dust particles may be adhering the surface of these fruit. Each peel of 10 g was cut into smaller pieces macerated in 100 ml of boiled distilled water and stirred for two min to obtain the peel solution. This aqueous peel solution was filtrated through Whatman's filter paper 0.45 μ M for further purification, then used for the preparation of SNP [10]. Silver nitrate (AgNO_3 , analytical grade) was purchased from Sigma-Aldrich.

Preparation of AgNP's

We have synthesized AgNP's using about 10ml of orange was added dropwise to 30 ml of 1mM solution of silver nitrate and mix the solution well under dark condition, rapidly the color of the solution has turned which indicates that the reduction was completed within a period (two hours) at

room temperature [11] with the appearance of yellowish brown color which confirms the formation of AgNP's (figure 1). The obtained SNPs were analyzed by using UV-Visible spectroscopy, XRD.

Analysis of the antibacterial activity of silver nanoparticles

The Ag nanoparticles antibacterial activity was assessed by the agar diffusion method against different pathogenic bacteria: *Escherichia coli*, *Staphylococcus* sp. and *Pseudomonas* sp., *Salmonella* sp. *streptococcus* sp. and *Klebsiella* sp. were used for this analysis. These bacteria were seeded in agar plates by the pour plate technique. Four cavities were made using a cork borer (about 7 mm diameter) at an equal distance and were filled with the Ag nanoparticles solution (100 μ L) and then incubated at 37⁰ C. The formation of a clear zone (restricted bacterial growth) around the cavity is an indication of antibacterial activity.

Characterization Techniques

By color change: The color change in the reaction mixture was recorded through visual observation. The color change from yellow to yellowish brown indicated that the silver nanoparticles were synthesized

UV-Vis Spectroscopy

The UV-Vis absorption spectra of the AgNP's were recorded using Pharmaspec UV-1700 (Shimadzu Corporation, Tokyo, Japan) UV-Visible spectrophotometer at room temperature. The scanning range for the samples was 300–800 nm with a resolution of 1 nm at a scan speed of 300 nm/min. The X-ray diffraction analysis was conducted on Bruker D8 Discover Powder Diffractometer (Berlin, Germany) using monochromatic Cu K α radiation ($\theta = 1.5406 \text{ \AA}$) operating at a voltage of at 40 kV and a current of 30 mA at room temperature. The intensity data for the lyophilized nanosilver powder were collected over a 2θ range of 10°–80°. To obtain information about sub-structure and topography of the nanoparticles, AFM measurements were carried out in a Scanning Probe Microscope SPM-9500 J3 (Shimadzu Corporation, Tokyo, Japan) under normal

atmospheric conditions. The sample was observed within an area of $5.0 \times 5.0 \mu\text{m}$ using contact mode. A freshly prepared sample was deposited on fine metal surfaces, air dried in a dust-free environment and the resultant smooth surfaces were subjected to AFM analysis.

X-ray Diffraction Analysis

The X-ray diffraction analysis was conducted on Bruker D8 Discover Powder Diffractometer (Berlin, Germany) using monochromatic Cu K α radiation ($\theta = 1.5406 \text{ \AA}$) operating at a voltage of at 40 kV and a current of 30 mA at room temperature. The intensity data for the lyophilized nanosilver powder were collected over a 2θ range of 10° - 80° . To obtain information about sub-structure and topography of the nanoparticles, AFM measurements were carried out in a Scanning Probe Microscope SPM-9500 J3 (Shimadzu Corporation, Tokyo, Japan) under normal atmospheric conditions. The sample was observed within an area of $5.0 \times 5.0 \mu\text{m}$ using contact mode. A freshly prepared sample was deposited on fine metal surfaces, air dried in a dust-free environment and the resultant smooth surfaces were subjected to SEM and EDX analysis.

Results and Discussion

In the present work, AgNP's have been synthesized by the reduction of aqueous silver ions using *C. sinensis* aqueous extract was studied. The checking of formation of AgNP's was primary recognized by the color change from yellow to yellowish brown (Figure 1) while adding *C. sinensis* extract into silver nitrate solution. The color formation occurred within 20 minutes. This change in color due to the excitation of free electrons in nanoparticles [12] which gives the surface plasmon resonance (SPR) absorption band by the combined vibration of electrons of metal NPs in resonance with light wave [13]. Color changes of the solutions also may be due to some chemical compound

such as alkaloids, flavonoids, saponins, steroids, and color present in plant extract acts as a reducing agent that reduced silver ions (Ag^+) to a silver atom (Ag^0). Metal nanoparticles exhibit different colors in solution due to their optical properties [14]. The solution was stored at room temperature for 24 hours for the complete settlement of nanoparticles. After 24 hours the reaction mixture was centrifuged at 15000 rpm for 15 min and pellets were collected followed by washing with deionized water then freeze-dried, powdered and used for XRD. The presence of silver nanoparticles in the solution was observed by UV-Visible.

Most important properties of silver nanoparticles are their optical properties which change with altering the size, the shape of the particle. The surface plasmon resonance of nanoparticles is responsible for the unique and beneficial optical properties of nanoparticles which depend on the size, shape of the nanoparticles, their distance from each other and the refractive index changes. UV-Visible spectrum was carried out to monitor the reduction process when *C. sinensis* extract was added into silver nitrate (1 mM) solution resulted in color change of the solution from yellowish to yellowish brown due to excitation of surface plasmon vibrations of the silver nanoparticles, also indicating the formation of silver nanoparticles in the solution and got a peak centered near about 340 nm (Figure 2 A). It was also noticed that the reduction process of silver ions into silver atom was very rapid. Generally, most of the papers reported the wavelength range of AgNPs absorption peak is in the range of 300-360 nm [15]. In our work, solution showed absorbance peak near at 340 nm as shown in Figure 2 A which was confirmed from the reported specified range of Ag nanoparticles. XRD analysis of dried green synthesized AgNP's by *C. sinensis* aqueous extract was achieved to find the crystalline nature and structure of silver nanoparticles. Pattern showed numbers of diffraction peaks at 2θ such as: (38.1312), (38.6149), (20.0028) (Figure 3) The spectrum showed three distinct separate peaks at 2θ that could be indexed to reflection planes of face centered cubic structure of silver respectively. XRD spectrum visibly

shows that the silver nanoparticles formed by a green approach using *C. sinensis* extract through reduction process are crystalline in nature.

Average size of AgNPs from XRD data was 26.76 nm which calculated by Debye Scherrer's equation:

$$D = 0.9 \lambda / \beta \cos \theta$$

Where 'D' is AgNPs crystal size, β is the full-width half maximum (FWHM) of diffraction peaks θ is Bragg diffraction angle and λ is the wavelength of CuK α and found to be 0.15406 nm.

The presence of silver atoms in the silver nanoparticles was further confirmed by energy dispersive x-ray spectroscopy (EDX) figure (4), The optical absorption peak was observed at energy between 0.3 and 3 keV, which is typical for silver nanocrystallite absorption as reported in many researches. SEM studies revealed the spherical nature of particles produced from Ag metal, figure (3,B).

The spherical shape of bio-synthesized AgNP's was well dispersed in solution with an average size of roughly 27 nm as evidenced by XRD analysis. The antibacterial activity of silver species has been well known since the ancient times, moreover, it has been demonstrated that, in low concentrations, silver is non toxic to human cells [16]. The zone of inhibition was very slight for some bacteria while it is not produced for other that verify the bacteria develop resistance toward the nanoparticles. The citrus peel used did not show any efficacy against pathogenic bacteria this could be related to the concentration of the active ingredient in the extract may not sufficient to show the desired effect, also the storage for long time was the effective factors in the inhibitory action. The reason for the resistance of pathogenic bacteria to nanoparticles is that the molecules may not be composed of the plant extracts of the studied samples and depending on the diagnostic methods used in the present study. The actual bactericide mechanism of silver nanoparticles is not clear. Several studies have investigated the interaction of the nano-Ag with bacteria [17], so in this

article, it was emphasized the production of nanosilvers as mention before but the future study for bacteriacidal activity need more assessment.

However, firstly, this report was aimed for nanoparticles production by eco-friendly process as it is free of any solvent or toxic chemicals, also easily applicable on a large-scale production.

Conclusion

Green technique for fabrication of AgNPs was utilized using pine *C.sineses* aqueous extract appears to be low cost, not toxic. Some natural bio-compounds found in extract that is confirmed by UV-VIS peaks that are responsible for reducing and stabilizing agent. Nanoparticles obtained with green approach were smaller in size than other chemical and physical methods but uniform in size monodispersed in nature and was synthesized very rapidly. This method does not require downstream processing and it may be scaled up to develop a viable technology for the silver nanoparticles synthesis. The explore of biomedical properties for the preparation of biosensors using AgNP's and its application in medicine by using herbal medium is the thrust area for future work.

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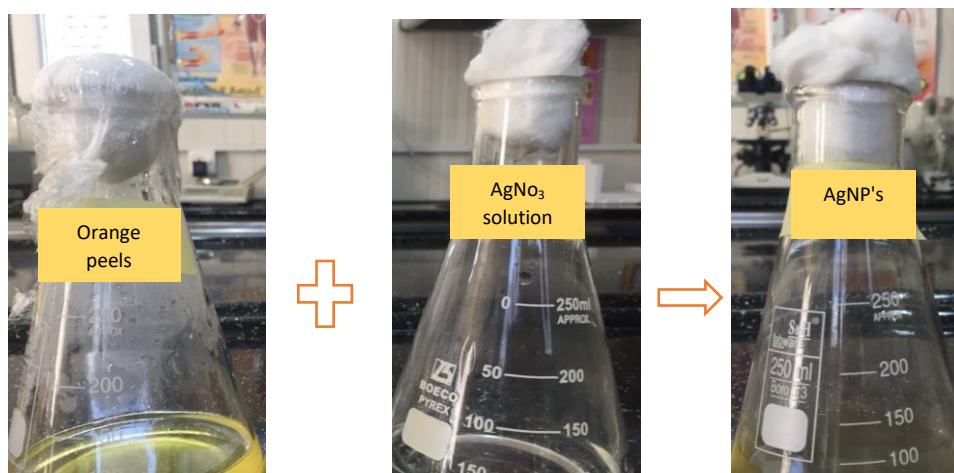


Figure (1): color change as indication of AgNP's production of *C. sinensis* when add AgNo3 solution

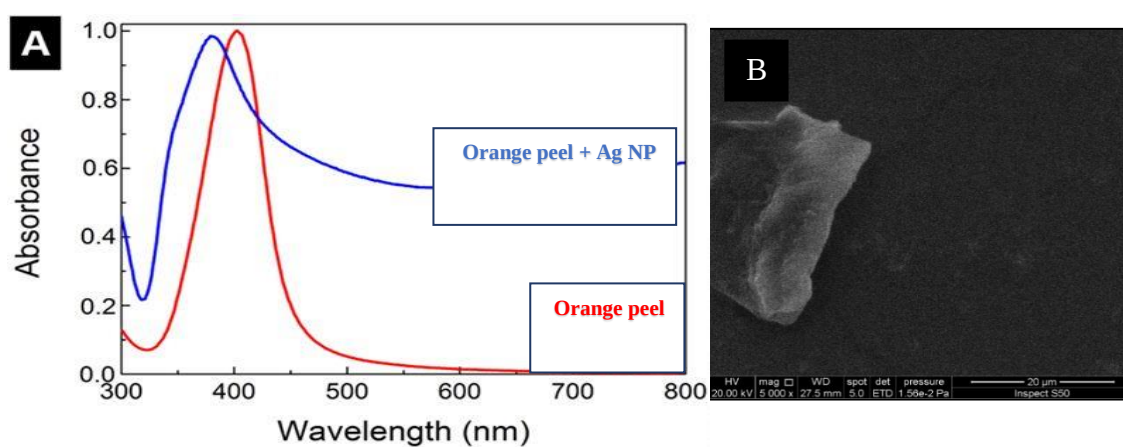


Figure 2: A)UV-vis spectra of AgNPs formation, B) SEM images of silver nanoparticles synthesized by reduction of silver ions using orange peel extract (x 5000)

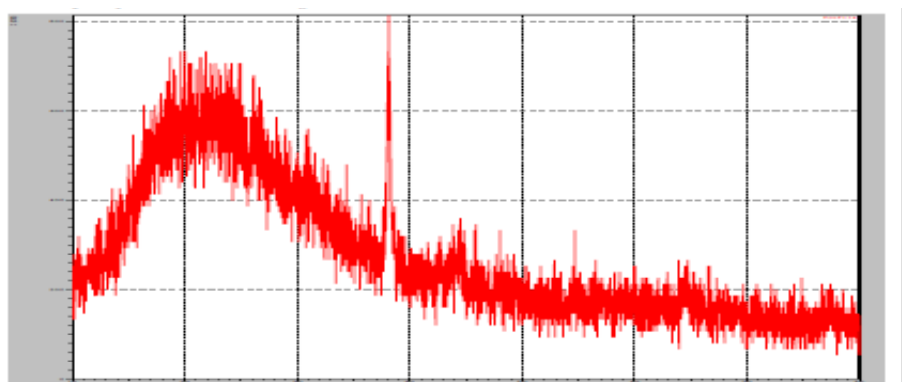


Figure 3: XRD Spectra of synthesized Ag-NPs.

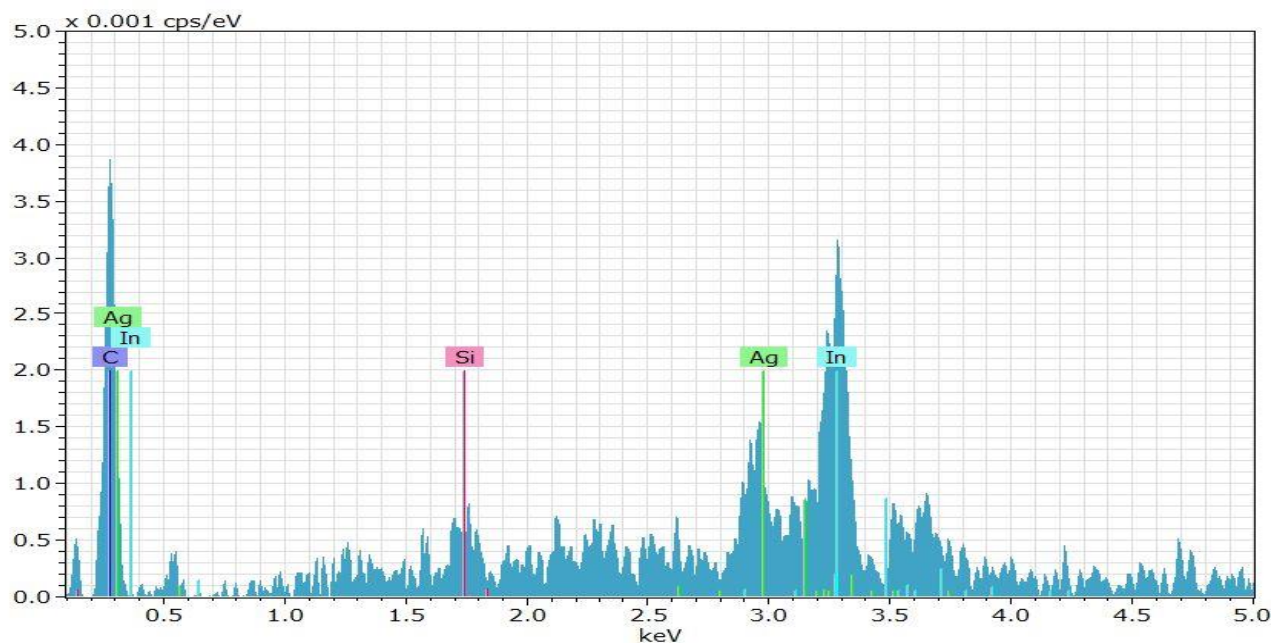


Figure (4) Energy dispersive x-ray spectroscopy of silver nanoparticles synthesized by *citrus sinensis* peel extract

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