Research Article Adv. Mat. Lett. 2013, 4(5), 338-342 ADVANCED MATERIALS Letters
www.amlett.com, www.amlett.org, DOI: 10.5185/amlett.2012.11453 Published online by the VBRI press in 2013

Biosynthesis of silver nanoparticles using Loquat leaf extract and its antibacterial activity

Akl M. Awwad[1*], Nidà M. Salem[2], Amany O. Abdeen[1]

[1]*Royal Scientific Society, El Hassan Science City, Amman, Jordan*
[2]*Plant Protection Department, Faculty of Agriculture, University of Jordan, Amman Jordan*

[*]Corresponding author. Tel.: (+962) 6-5344 701; Fax: (+962) 6-5344 806; E-mail: akl.awwad@yahoo.com

Received: 03 November 2012, Revised: 16 December 2012 and Accepted: 05 January 2013

ABSTRACT

This paper reports a rapid and eco-friendly green method for synthesis of silver nanoparticles from silver nitrate solution using loquat leaf extract**.** Effect the amount of leaf extract, reaction time, silver nitrate concentration and temperature were investigated. Biosynthesized silver nanoparticles (AgNPs) were characterized by X-ray diffraction (XRD), atomic absorption spectroscopy (AAS) and Fourier transform infrared spectroscopy (FT-IR). UV-vis spectroscopy showed that the surface plasmon resonance (SPR) at 425 nm. The structural peaks in XRD pattern and average crystalline size around 18 nm clearly illustrates that AgNPs synthesized by our green method were nanocrystalline in nature with face centered cubic geometry. The antibacterial activity of biosynthesized silver nanoparticles showed effective inhibitory activity against water borne pathogens, *Shegella and Listeria* bacteria. Copyright © 2013 VBRI press.

Keywords: Silver nanoparticles; loquat leaf extract; biosynthesis; antibacterial activity.

Akl M. Awwad is professor of physical chemistry at Baghdad University and Senior Scientist at Ministry of Science and Technology, Iraq (1983-2005). He is currently visiting senior Scientist at Royal Scientific Society, El Hassan Science City, Jordan from 2006. He received his Ph.D. degree in Physical Chemistry from University of Strathclyde, Glasgow, U.K. He has published over 90 peer reviewed articles, 7 books and holds 5 patents. His current research interests include the green synthesis of the advanced Nano materials, thermodynamics of liquid mixtures and water treatment. He received several awards for his research.

Introduction

New applications of nanoparticles and nanomaterials are emerging rapidly due to their new properties based on specific characteristic such as size, distribution and morphology of the particles. Silver nanoparticles found tremendous applications in antimicrobials and therapeutics, catalysis, micro-electronics, topical ointments and creams. Various approaches were developed for silver nanoparticles synthesis such as chemical, electro- and photochemical reduction, heat evaporation, sonoelectrochemical, microwave assisted process [**1-4**]. These approaches use hazardous chemicals, low material conversions, high energy requirements and wasteful purification. Therefore, there is a growing need to develop environmentally friendly methods for silver nanoparticles without using hazardous chemicals. Biological approaches using microorganisms [**5-7**] and plant leaf extract [**8-23**] for metal nanoparticles synthesis have been suggested as valuable alternatives to chemical methods. The use of plant leaf extract for synthesis of silver nanoparticles could be more advantageous. Recent research reported the synthesis of silver nanoparticles using different plant leaf extract such as *Ficus benghalensis* [**8**], *Rosa rugosa* [**9**], *Chenopodium album* [**10**], *Nicotiana tobaccum* [**11**], *Stevia rebaudiana* [**12,13**], *Acalypha indica* [**14**], Murraya koenigii [**15**], *Ocimum sanctum* [**16**], *Catharanthus roseus* [**17**], *Capsicum annuum* [**18**], *Coriandrum sativum* [**19**], *Azadirachta indica* [**20**], *Piper iongum* [**21**], *Arbutus unedo* [**22**] and *Dalbergia sissoo* [**23**].

This study was designed with a simple, rapid, cost-effective and environmentally biosynthesis method of silver nanoparticles (AgNPs) at ambient conditions using a new reducing and stabilizing agent loquat leaf extract. To our best knowledge with all the possible referencing, we state that it is the first study that uses loquat leaves extract as plant source for synthesis of silver nanoparticles. Loquat belongs to order *Rosales*, genus *Eriobotrya*, species *Eriobotrya japonica* and family *Rosaceae* and grown commercially for its yellow fruit. Loquat is a large evergreen shrub or small tree, with a rounded crown, short trunk and woolly new twigs. The tree can grow to 5–10 metres tall, but is often smaller. The leaves are alternate, simple, 10–25 cm long, dark green, tough and leathery in texture.

Experimental

Silver nitrate ($AgNO_3$) was obtained from Aldrich Chemicals. All glassware have been washed with sterile distilled water and dried in an oven before use.

Preparation loquat leaf extract

Freshly loquat leaves, **Fig. 1** were collected from loquat trees at the campus of Royal Scientific Society, Amman Jordan. Leaves were washed several times with distilled water to remove the dust particles and then sun dried to remove the residual moisture. The loquat leaves extract used for the reduction of silver ions (Ag^+) to silver nanoparticles (Ag^o) was prepared by placing 10 g of washed dried fine cut leaves in 250 mL glass beaker along with 200 mL of sterile distilled water. The mixture was then boiled for 5 minutes until the color of the aqueous solution changes from watery to light yellow color. Then the extract was cooled to room temperature and filtered with Whatman No. 1 filter paper before centrifuging at 1500 rpm for 5 minutes to remove the heavy biomaterials. The extract was stored at room temperature in order to be used for further experiments.

Fig. 1. Photograph of loquat leaves and their aqueous extract.

Synthesis of silver nanoparticles (AgNPS)

In a typical reaction procedure, 5 ml of loquat leaves extract was added to 100 mL of $1x10^{-3}$ M aqueous $AgNO_3$ solution, the mixture was heated at 80 ^oC, the resulting solution become reddish in color after 2 minutes of heating. The concentrations of $AgNO_3$ solution and the quantity of loquat leaf extract were also varied at 1–4 mM and 5–10% by volume, respectively. UV-vis spectra showed strong SPR band at 425 nm, thus indicating the formation of silver nanoparticles. The silver nanoparticles (AgNPs) obtained by loquat leaves extract were centrifuged at 15,000 rpm for 5 min and subsequently dispersed in sterile distilled water to get rid of any uncoordinated biological materials.

Characterization techniques

UV-vis absorption spectra were measured using Shimadzu UV-1601 spectrophotometer. Crystalline metallic silver nanoparticles were examined by X-ray diffractometer (Shimadzu XRD-6000) equipped with Cu Kα radiation source using Ni as filter and at a setting of 30 kV/30 mA. All XRD data were collected under the same experimental conditions, in the angular range $3^o \leq 2\theta \leq 50^o$. FTIR Spectra for loquat leaves extract was obtained in the range 4000–400 cm^{-1} with IR-Prestige-21 Shimaduz FTIR spectrophotometer, using KBr pellet method. Scanning electron microscopy (SEM) analysis of silver nanoparticles analysis was done using Hitachi S-4500 SEM machine. Thin films of the silver nanoparticles were prepared on a carbon coated copper grid by just droping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 minutes.

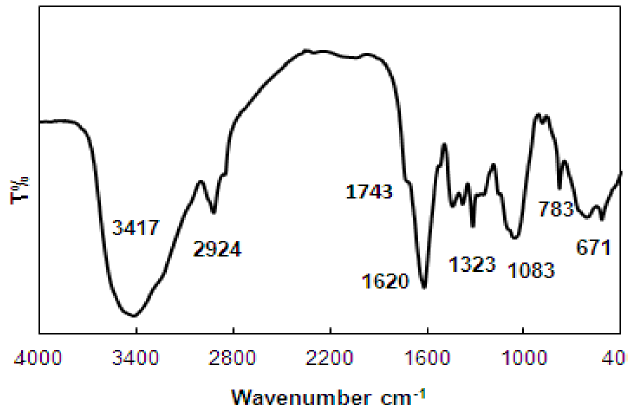

Fig. 2. FT-IR of loquat leaf extract.

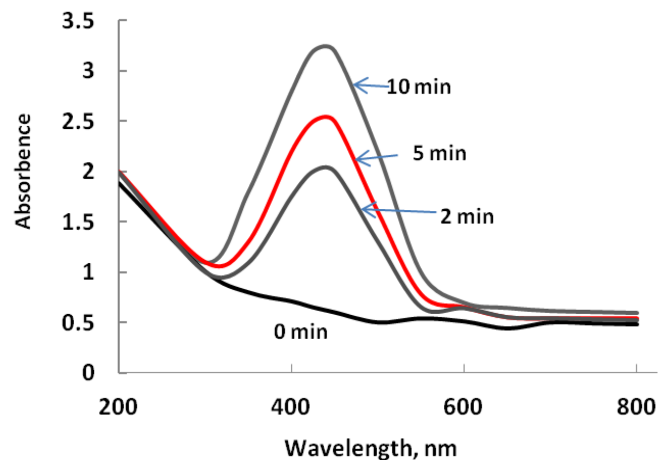

Fig. 3. UV-vis spectra showing absorption of 10^{-3} M aqueous solution of silver nitrate with loquat leaf extract as a function of time.

Results and discussion

FT-IR spectrum

To investigate the functional groups of loquat leaves extract, a FT-IR study was carried out and the spectra are shown in **Fig. 2**. The loquat leaves extract displays a number of absorption peaks, reflecting its complex nature. A peak at 3417 cm^{-1} results due to the stretching of the N–H bond of amino groups and indicative of bonded hydroxyl (-OH) group. The strong absorption peak at 2924 cm^{-1} could be assigned to –CH stretching vibrations of $–CH_3$ and $–CH_2$ functional groups. The shoulder peak at 1743 cm^{-1} assigned for C=O group of carboxylic acids. The peak at 1620 cm^{-1} indicates the fingerprint region of CO, C–O and O–H groups, which exists as functional groups of loquat leaves extract. The absorption peaks at 1555-1323 cm^{-1} could be attributed to the presence of C–O stretching in carboxyl. The intense band at 1083 cm^{-1} can be assigned to the C-N stretching vibrations of aliphatic amines. FTIR study indicates that the carboxyl (-C=O), hydroxyl (-OH) and amine (N-H) groups of mulberry leaves extract are mainly involved in reduction of Ag^+ to Ag^o nanoparticles.

Visual observation and UV-vis spectral study

Formation and stability of AgNPs in sterile distilled water is confirmed using UV-vis spectrophotometer in a range of wavelength from 200 to 800 nm. As soon as, loquat leaves extract was mixed in aqueous solution of silver ion complex, the reduction of pure Ag^+ ions to Ag^0 was monitored by measuring UV–vis spectrum of the reaction media at regular intervals. UV–vis spectra were recorded as function of reaction time. We observe that there is no peak showing no sign for the synthesis of silver nanoparticles but after 5 min the surface plasmon resonance of silver occur at 425 nm and steadily increasing with the time of reaction without much change in the peak wavelength, **Fig. 3**. After 10 min, the increase in the number and size of the AgNPs came to an end, may be due to the depletion of the silver ions (Ag^+) in the loquat leaves extract.

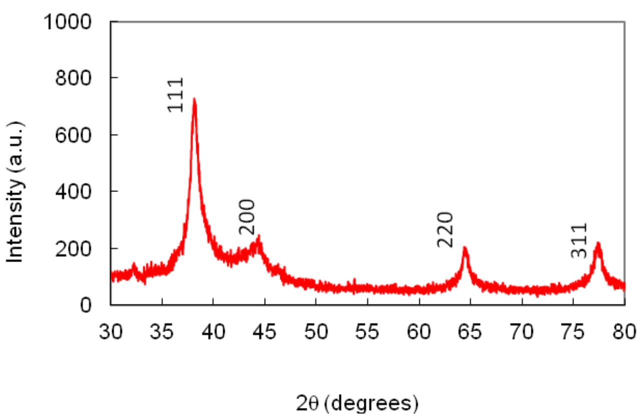

Fig. 4. XRD pattern of silver nanoparticles synthesized using loquat leaf extract.

X-ray diffraction (XRD) studies

Analysis through X-ray diffraction was carried out to confirm the crystalline nature of the particles, and the XRD pattern showed numbers of Braggs reflections that may be indexed on the basis of the face centered cubic structure of silver. A comparison of our XRD spectrum with the standard confirmed that the silver particles formed in our experiments were in the form of nanocrystals, as evidenced by the peaks at 2θ values of 38.2, 44.1, and 64.1, and 77.6 θ, corresponding to (111), (200), (220) and (311), respectively Bragg reflections of silver, The X-ray diffraction results clearly show that the silver nanoparticles formed by the reduction of Ag+ ions by the loquat leaves extract are crystalline in nature. As mentioned in the method section, the silver nanoparticles once formed were repeatedly centrifuged and redispersed in sterile distilled water prior to XRD analysis, thus ruling out the presence of any free biological material that might independently crystallize and giving rise to Bragg reflections. It was found that the average size from XRD data and using Debye-Scherer equation was 18 ± 2 nm. The presence of structural peaks in XRD patterns and average crystalline size around 18 nm clearly illustrates that AgNPs synthesized by our green method were nanocrystalline in nature. The XRD pattern of the dried AgNPs obtained by loquat leaves extract is shown in **Fig. 4**.

The average particle size of silver nanoparticles synthesized by the present green method calculated using Debye-Scherrer equation [**8**]:

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

where, D = the crystallite size of AgNPs particles, λ = the wavelength of x-ray source (0.1541 nm) used in XRD, β = the full width at half maximum of the diffraction peak, *K* = the Scherrer constant with value from 0.9 to 1, and θ = the Bragg angle.

It was observed that the silver nanoparticles synthesized are extremely stable for nearly two weeks with very little aggregation of silver particles in the solution by this method. A FT-IR spectrum of synthesized silver nanoparticles by this green method is shown in **Fig. 5**. A number of absorption peaks at 3410 cm^{-1}, 1608 cm^{-1}, 1558 cm^{-1} and 1408 cm^{-1} indicating the biomaterial bind to the silver nanoparticles through amine and C=O of amide I and amid II of the protein. Thus indicating loquat leaves extract act as a reducing and capping agent for silver particles.

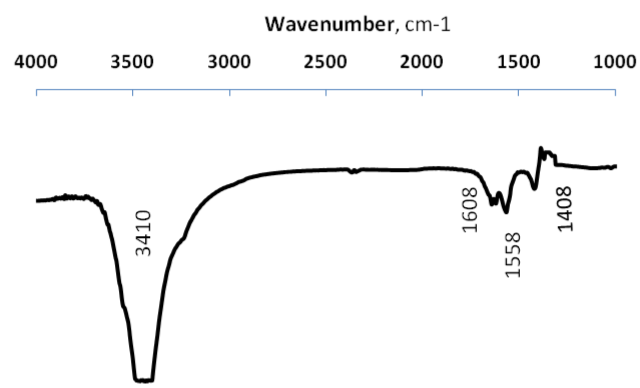

Fig. 5. FT-IR spectrum of silver nanoparticles synthesized by loquat leaves extract.

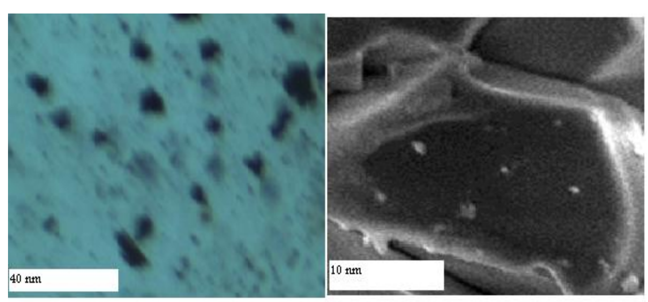

Fig. 6. SEM image of biosynthesized silver nanoparticles.

SEM analysis of silver nanoparticles

The suspended silver nanoparticles in sterile distilled water were used for scanning electron microscope analysis by fabricating a drop of suspension onto a clean electric stubs and allowing water to completely evaporate. **Fig. 6** shows the SEM images of the silver nanoparticles synthesized by different concentrations of silver nitrate and loquat leaf extract are homogenously dispersed and ranges approximately from 5-40 nm. The shape of the silver nanoparticles is spherical with few exceptional as ellipsoidal. The larger silver particles may be due to the aggregation of the smaller ones, due to the SEM measurements. It was found that decreasing the amount of loquat leaf extract in the reaction mixture leads to decrease the particle size of AgNPS and their agglomeration tendency.

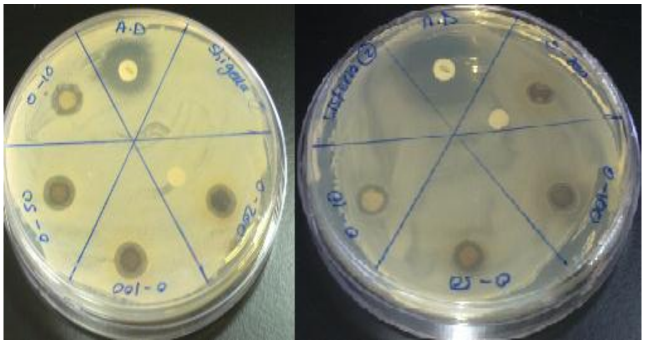

Fig. 7. The antibacterial activity of silver nanoparticles against *Shigellia* and *Listeria* bacteria.

Antibacterial activity study of silver nanoparticles (AgNPs)

Biosynthesized silver nanoparticles by this method were studied for antimicrobial activity against pathogenic bacteria by disc diffusion method; it was observed that silver nanoparticles have antibacterial activities at concentration of 2µg/disc. Chloromphenical was used as a control antimicrobial agent. The silver nanoparticles biosynthesized showed inhibition zone against *Shigella and Listeria monocytogenes* bacteria, **Fig.7.** Maxmium zone of inhibition (MZI) are listed in **Table 1**. It was observed that an increase in AgNPS concentration incrassates the MZI of *Listeria* bacteria but no significant effect in case of *Shigella* bacteria.

Table 1. The antibacterial activity of AgNPs using loquat leaves extract.

AgNPs concentration (µg/L)	*Shigella*	*Listeria*
200	9.5	20
100	10	11
50	9.5	7.5
10	11.5	7
Ref. Drug	17	20

Conclusion

Green chemistry approach towards the synthesis of nanoparticles has many advantages such as, ease with which the process can be scaled up and economic viability. We have developed a fast, eco-friendly and convenient method for the synthesis of silver nanoparticles using loquat leaves extract with a diameter range of 18 nm. These particles are monodispersed and spherical. No chemical reagent or surfactant template was required in this method, which consequently enables the bioprocess with the advantage of being environmental friendly. Color change occurs due to surface plasmon resonance during the reaction with the ingredients present in the plant leaves extract results in the formation of silver nanoparticles which is confirmed by UV–vis, XRD, FT-IR and SEM, having average mean size of 18 nm had fcc structure. The antibacterial activity of biologically synthesized silver nanoparticles was evaluated against *Shigella and Listetia* showing effective bactericidal activity.

Acknowledgements

Authors are thankful to Royal Scientific Society, SABEQ program and Jordan University for financial support and having given feasibilities to carry out the research work.

Reference

1. Liu, Y.C.; Lin. L.H. *Electrochem. Commun*. **2004**, 6, 78-86. **DOI:** 10.1016/j.elecom.2004.09.010.
2. Vorobyova, S.A.; Lesnikovich, A.I.; Sobal. N.S. *Colloids Surf. A.* **1999**, 152, 375-379. **DOI:** 10. 1016/S0927.7757(98)00861-9.
3. Bae, C.H.; Nam, S.H.; Park. S.M. *Appl. Surf. Sci.* **2002**, 197, 628-634. **DOI:** 10.1016/S0169-4332(02)00430-0.
4. Khan, Z.; Al-Thabsiti S. A.; Obaid, A. Y.; Al-Youbi A.O. *Colloids and Surfaces B; Biointerfaces*. 2011, 82, 513-517. **DOI:** 10.1016/j.colsrfb.2010.10.008.
5. Mandal, D.; Blonder, M.E.; Mukhopadhyay, D.; Sankar, G.; Mukherjea, P. *Appl. Microbiol. Biotechnol*. **2000**, 69, 485-492. **DOI:** 10.1007/s00253-005-0179-3.
6. Basavaraja, S.; Balaji, S. DF.; Lagashetty, A.; Rajasab, A. H.; Venkataraman, A. *Mater. Res. Bull*. **2008**, 43, 1164-1170. **DOI:** 10.1016/j.materresbull.2007.06.020.
7. Kowshik, M.; Ashtaputre, S.; Kharrazi, S.S.; Vogel, W.; Urban, J.; Kulkarni, S. K.; Paknikar, K. M. *Nanotechnology* **2005**, 14, 95-100. **DOI:** 10.1088/0957-4484/14/1/321.
8. Saxena, A.; Tripathi, R. M.; Zafar, F.; Singh, P. *Materials Letters*. **2012**, 67, 91-94. **DOI:** 10.1016/j.matiet.2011.09.038.
9. Dubey, S. B.; Lahtinen, M.; Sillanpää, M. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. **2010**, 364, 34-41. **DOI:** 10.1016/j.coisurfa.2010.04.023.
10. Dwivedi, A. D., Gopal, K. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. **2010**, 369, 27-33. **DOI:** 10.1016/j.coisurfa.2010.07.020.
11. Prasad, K. S.; Pathak, D.; Patel, A.; Dalwadi, P.; Prasad, R.; Patel, P.; Selvara, K. *African Journal of Biotechnology.* **2010**, 10, 8122-8130. **DOI:** 10.5897/AJB11.394.
12. Yilmaz, M.; Turkdemir, H.; Bayram, H. E.; Cicek, A. *Materials Chemistry and Physics*. **2011**, 130, 1195-1202. **DOI:** 10.1016/j.matchemphys.2011.08.068.
13. Ratnika Varshneya, R.; Bhadauriaa, S.; Mulayam S.Gaurb, M.S. *Adv. Mater. Lett*. 2010 ,1, 232-237. **DOI:** 10.5185/amlett.2010.9155.
14. Krishnaraj, C.; Jagan, E. G.; Rajasekar, S.; Kalaichelvan, P. T.; Mohan, N. *Colloids and Surfaces B: Biointerfaces*. **2010**, 70, 50-56. **DOI:** 10.1016/j.coisurfb.2009.10.008.
15. Christensen, L.; Vivekanandhan, S.; Misra, M.; Mohanty, A. K. *Adv. Mater. Lett*. **2011**, 2, 429-434. **DOI:** 10.5185/amiett.2011.4256.
16. Singhal, G.; Bhavesh, R.; Kasariya, K.; R.Sharma, A. R.; Singh, R. P. *J. Nanopart. Res.* 2011, 13, 2981–2988. **DOI:** 10.1007/s1051-010-0193-y.
17. Mukunthan, .; Elumalai, K.; Patel, T. N.; Murty, V. R. *Asian Pacific Journal of Tropical Biomedicine,* **2011**, 1, 270-274. **DOI:** 10.1016/S2221-1691(11)60041-5.
18. Li, S.; Shen, Y.; Xie, A.; Yu, X.; Qiu, L.; .Zhang, L.; Zhang, O. *Green Chem*. 2007, 9, 852–858. **DOI:** 10.1039/B615357G.
19. Sathyavathi,R.; Krishna,M.B.; Rao, S.V.; Aritha, R.; Rao, D.N. *Adv. Sci. Lett.* 3 (2010) 1-6. **DOI:** 10.1166/asl.2010.1099.
20. Shankar, S.S.; Rai, A.; Ahmad, A.; Sastry, M. *J. Colloid Interface Sci*. 275 (2004) 496-502. **DOI:** 10.1016/j.jcis.2004.03.003.

21. Jacob, S.J.B.; Finub, J.S.; Narayanan, A. Colloids and Surfaces B: Biointerfaces 91 (2012) 212-214.
DOI: 10.1016/j.coisurfb.2011.11.001.
22. Kouvaris, P.; Delimittis, A.; Zaspalis, V.; Papadopoulos, D.; Tsipas, S.A.; Michailidis, N. *Materials Letters* 76 (2012) 18-20.
DOI: 10.1016/j.matlet.2012.02.025.
23. Singh, C.; Baboota, R.K.; Naik, P.K.; Singh, H. *Adv. Mater. Lett*. 3 (2012) 279-285.
DOI: 10.5185/amlett.2011.10312.

