Can Tho University Journal of Science
website: ctujs.ctu.edu.vn

DOI: 10.22144/ctu.jen.2022.014

Synthesis of silver nanoparticles using extract of *Citrus maxima* peel

Nguyen Dien Trung[1], Le Nguyen Phuong Tung[2], and Nguyen Thi Hong[2*]

[1]*School of Education, Can Tho University, Viet Nam*

[2]*College of Natural Sciences, Can Tho University, Viet Nam*

**Correspondence: Nguyen Thi Hong (email: nthong43@ctu.edu.vn)*

Article info.

Received 30 Dec 2021
Revised 25 Feb 2022
Accepted 11 Mar 2022

Keywords

Citrus maxima, green synthesis, high purity, pectin, silver nanoparticles

ABSTRACT

In this work, silver nanoparticles (AgNPs) were synthesized by the extract of Citrus maxima (C. maxima) peel with the presence of pectin as a principal reductant and stabilizer. Parameters such as concentration of C. maxima powder, extraction temperature, and extraction time for AgNPs synthesis have been studied. The synthesized AgNPs were characterized by ultraviolet-visible spectroscopy (UV-Vis), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction analysis (XRD), and transmission electron microscopy instrument (TEM). The synthesized AgNPs were quasi-spheres with an average particle size of 17 nm and high purity of silver phases. In addition, synthesized AgNPs exhibited good antibacterial activity against Gram-positive bacteria: Salmonella enterica (S. enterica) and Pseudomonas aeruginosa (P. aeruginosa) while no activity inhibited against Gram-negative bacteria: Staphylococcus aureus (S. aureus) and Lactobacillus fermentum (L. fermentum).

1. INTRODUCTION

Chemical methods have long been considered common methods to synthesize silver nanoparticles in terms of synthesis efficiency and reduction of nanoparticle aggregation (Siddiqi et al., 2018). Chemical reduction, electrochemical, irradiation-assisted chemical, and pyrolysis methods were used to synthesize AgNPs (Zhang et al., 2007). To form nanoparticles, metal precursors, reducing agents, and stabilizing or capping agents should be employed in the synthesis process. Many works have used various commercial chemicals such as ascorbic acid, sodium borohydride, sodium citrate, and hydrazine as reducing agents. Although AgNPs with uniform morphology and small size were obtained, the use of toxic chemicals caused potential environmental and biological hazards (Zhang et al., 2007; Wani et al., 2011; Guzman et al., 2012; Zahran et al., 2014; Siddiqi et al., 2018). Therefore, green and low-cost methods for the synthesis of AgNPs have been paid more attention recently.

Various reductants and stabilizers in the plant extract such as leaf extracts (Jeeva et al., 2014), seed extracts (Jagtap & Bapat, 2013), root extracts (Suresh et al., 2014), flower extracts (Arokiyaraj et al., 2014) were used in the reported literature. It has been known that commercial pectin is mainly extracted from lemon, lime, orange, and *Citrus grandis* peel which were known as reducing agents to synthesize AgNPs (Zhang et al., 2007; Balachandran et al., 2015). Although *Citrus. maxima* (*C. maxima*) peel contained a large content of pectin, little information on the synthesis of AgNPs using its extract has been reported. Therefore, a green method using the extract of *C. maxima* peel was investigated in the present work. Several factors such as concentration of powdered *C. maxima* peel, extraction time, and extraction

temperature which affected the formation of AgNPs were studied in detail. Moreover, the characteristics of the obtained AgNPs were analyzed by UV-Vis, XRD, TEM, and FTIR.

2. MATERIALS AND METHODS

C. maxima fruits were purchased in the Can Tho market. The peel of *C. maxima* was dried at 70°C for 48 h. The dried peel was followed by grinding to a fine powder and stored at 4°C for AgNPs synthesis. Additionally, silver nitrate ($AgNO_3$, Merck) was used as a precursor for process synthesis while distilled water was considered as a solvent for extraction.

The extract was prepared with a certain amount of powdered peel in 100 mL of distilled water at the required extraction temperature and extraction time. A heterogeneous mixture was filtered by the Whatman paper and centrifuged at 6000 rpm for 30 mins to eliminate insoluble components. The obtained extract of *C. maxima* peel was stored at 4°C for the synthesis of nanoparticles. AgNPs were produced by stirring a suitable amount of $AgNO_3$ in the *C. maxima* extract at room temperature. The color change of the prepared solutions from pale yellow to yellow-brown indicated the formation of AgNPs. The effect of the concentration of powered C. maxima on the AgNPs synthesis was studied at different concentrations (10, 15, 20, 25, and 30 g/L). Moreover, the effect of extraction temperature of *C. maxima* peel on AgNPs synthesis was carried out by alterations at 40, 50, 60, 70, and 80°C. Finally, the effect of the extraction time of *C. maxima* peels on the preparation of AgNPs was studied at 10, 20, 30, 40, and 50 min.

The formation of the synthesized AgNPs was determined by the ultraviolet-visible spectrophotometer (V730, Jasco). The morphology of the obtained AgNPs was characterized by the transmission electron microscopy spectrometer (Jem-1400, Jeol). The crystalline phase of the samples was determined by an X-ray Diffractometer (Empyrean, Malvern Panalytical) with Cu Kα radiation and the diffracted intensities were recorded at a 2θ angle from 10 to 80°. The size distribution of the synthesized AgNPs was calculated by ImageJ software. The average size of AgNPs was calculated by the Gaussian method using origin 9.0 software. The functional groups in *C. maxima* extract and synthesized AgNPs were analyzed by Fourier-transform infrared spectroscopy (Nicolet 6700, Thermo). Furthermore, the synthesized AgNPs were tested for antibacterial effect against *Lactobacillus fermentum* ATCC 14931, *Staphylococcus aureus* ATCC 13709, *Pseudomonas aeruginosa* ATCC 15442, and *Salmonella enterica* ATCC 35664 using a dilution method.

3. RESULTS AND DISCUSSION

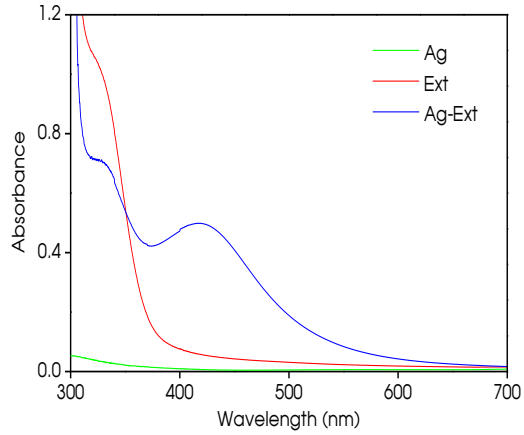

Figure 1. Effect of the extract and LED irradiation on the AgNPs formation: Ag (1 mM $AgNO_3$), Ext (powdered peel of 2.00 g extracted with 100 mL of water), and Ag-Ext (1 mM $AgNO_3$ stirred in the extract)

UV-Vis analysis was used to confirm the AgNPs formation. Figure 1 was illustrated the influence of the pectin-enriched extract of *C. maxima* peel on the AgNPs formation. The surface plasmon resonance (SPR) was observed at 419 nm which is a characteristic absorbance of AgNPs. The AgNPs formation was recognized when Ag^+ solution was mixed with the extract of *C. maxima* peel (Ag-Ext). The intensity of the SPR peak was related to the yield of AgNPs (Yang et al., 2011). Additionally, none of SPR was found in a range of 400-450 nm for a single Ag^+ solution (Ag) and a single extract of C. maxima peel (Ext). The obtained results confirmed that the extract of *C. maxima* peel as a reductant was responsible for AgNPs synthesis.

3.1. Effect of concentration of powdered *C. maxima* peel

The effect of *C. maxima* powder on the synthesis of AgNPs was evaluated in the present study. The reduction of Ag^+ ions in the extract was monitored by the concentration of powdered peel. Variation in the intensity of absorption peak with powdered peel was shown in Figure 2. With the increasing powdered peel of *C. maxima*, the intensity of the characteristic SPR peak of AgNPs increased, but the

position of the absorption peak was unchanged. It has been reported that SPR of AgNPs at a higher wavelength corresponded to an increase in particle size while the AgNPs formation with smaller size appeared at a shorter wavelength (Khan et al., 2013). Therefore, the size of AgNPs in our experimental conditions was unaffected by the content of *C. maxima* peel. Besides, there was an insignificant increase in SPR intensity at 30 g/L in comparison to 25 g/L indicating the reduction completely occurred at 25 g/L of powdered peel.

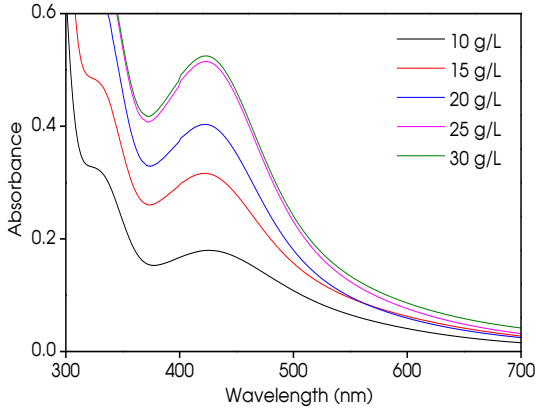

Figure 2. Effect of concentration of powdered *C. maxima* peel on the AgNPs formation at experimental conditions: 1 mM $AgNO_3$ and powdered peel of 1.00-3.00 g extracted with 100 mL of water at temperature 60ºC for 30 mins

3.2. Effect of extraction temperature

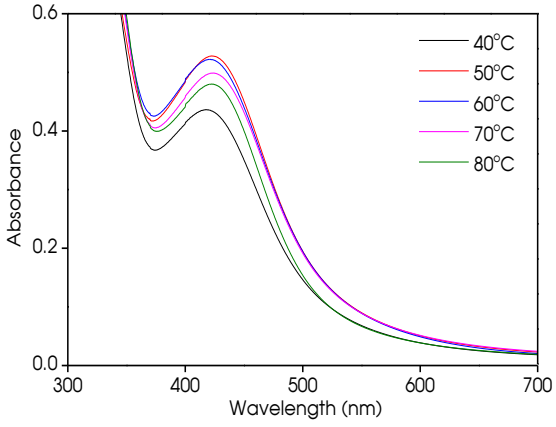

Figure 3. Effect of extraction temperature on the AgNPs formation at experimental conditions: 1 mM $AgNO_3$ and powdered peel of 2.50 g extracted with 100 mL of water at temperature 40-80ºC for 30 mins

Temperature is an essential factor in the synthesis of nanoparticles due to its influence on the chemical content of the extract (Nguyen et al., 2021). Therefore, the effect of the extraction temperature of the powdered peel on the AgNPs formation was studied by varying the temperatures from 40 to 80ºC. The obtained results were shown in Figure 3. The synthesis efficiency of AgNPs increased when reaction temperature increased from 40 to 50ºC. There was a slight decrease in the concentration of AgNPs with a further increase in temperature for extraction. It has been reported that pectin was slowly water-soluble at low temperatures and the solubility of pectin in water was unfavorable at high temperatures (Rubaiyi et al., 2016). This was a possible reason why the intensity of the absorption peak of AgNPs decreased at a temperature higher than 50ºC in the present study. From the obtained results, the extraction temperature of 50ºC was chosen as an optimum condition to synthesize AgNPs in further experiments.

3.3. Effect of extraction time

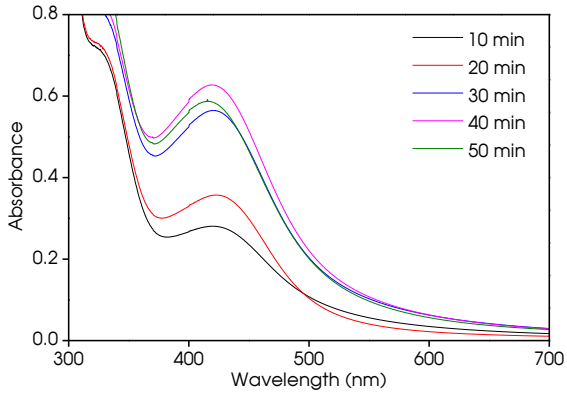

Figure 4. Effect of extraction time on the AgNPs formation at experimental conditions: 1 mM $AgNO_3$ and powdered peel of 2.50 g extracted with 100 mL of water at temperature 50ºC for 10-50 mins

The effect of extraction time of powdered peel on the formation of AgNPs was also studied in the time range of 10-50 mins. In these experiments, 25 g/L of powdered peel was mixed with 1 mM $AgNO_3$ at 50ºC. With the increase of extraction time from 10 to 40 mins, the adsorption peak intensity of AgNPs significantly increased (Figure 4). When the extraction time of powdered peel was higher than 40 mins, there was a decreasing trend in the adsorption peak intensity of AgNPs. The degradation of pectin in the *C. maxima* peel extract in a longer duration might lead to a decrease in the formation of AgNPs

(Fakayode & Abobi, 2018). Therefore, the synthesis of AgNPs using the *C. maxima* peel extract as a reducing agent was recommended to carry out at 40 mins.

3.4. Characterization of the synthesized AgNPs

The XRD pattern of AgNPs synthesized by the peel extract of *C. maxima* at the optimum parameters was shown in Figure 5. It has the formation of pure silver phases on the synthesized AgNPs. Characteristic peaks at 2θ = 38.1, 44.3, 64.5, and 77.3° can be related to the (111), (200), (220), and (311) planes of silver (JCPDS 04-0783).

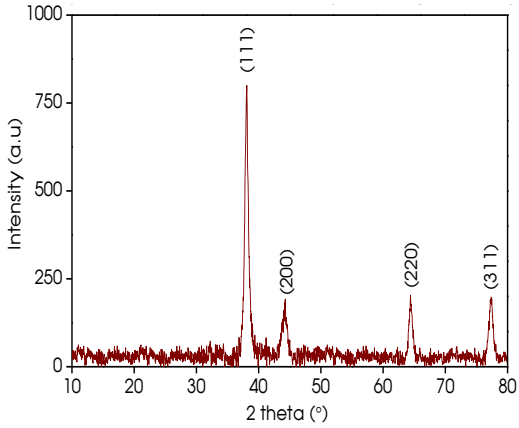

Figure 5. XRD pattern of the synthesized AgNPs

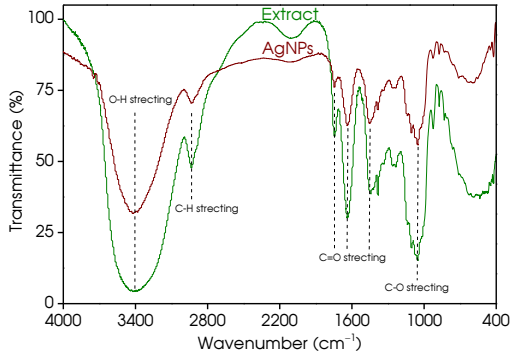

Figure 6. FTIR spectra of extract of the *C. maxima* peel and synthesized AgNPs

The characteristic peaks of functional groups on the extract of the *C. maxima* peel and synthesized AgNPs were presented on the FTIR spectra (Figure 6). A strong band near 3405 cm^{-1} was assigned to O-H stretching vibration and C-H stretching vibration appeared at 2934 cm^{-1}. C=O stretching of carboxy groups (-COOH and -$COOCH_3$) related to a peak at 1745 cm^{-1} while a couple of bands at 1638 and 1447 cm^{-1} were recognized as asymmetric and symmetric stretching of the COO^- ions (Košťálová et al., 2013). The peak at 1051 cm^{-1} was due to the stretching vibration of C-OH of alcoholic groups and carboxylic acids (Guibaud et al., 2003). The obtained peaks of extract of the *C. maxima* peel and synthesized AgNPs attributed to the free pectin, indicating pectin was involved in the synthesis and stabilization of AgNPs.

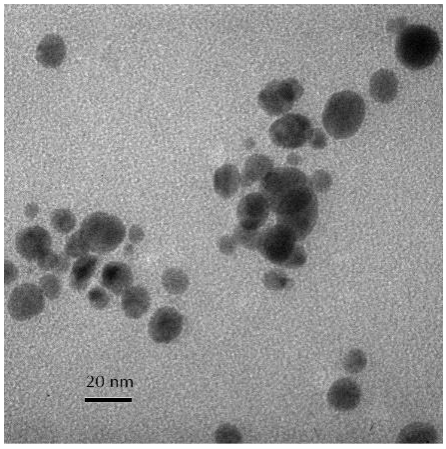

Figure 7. TEM image of the synthesized AgNPs

The size and morphology of AgNPs were determined by transmission electron microscopy (TEM) images. Figure 7 showed that most of AgNPs were spherical with a moderate variation in particle size. According to the size distribution in Figure 8, nanoparticle sizes ranged from 6 to 30 nm and the average size was estimated at 17.2 ± 2.9 nm. The size of AgNPs in the present study was similar to that in the reported literature which has used commercial pectin (Pallavicini et al., 2017; Zhang et al., 2017; Hileuskaya et al., 2020). It can be concluded that the use of a direct pectin-rich extract from a by-product of C. *maxima* peel was simple and possible to synthesize AgNPs.

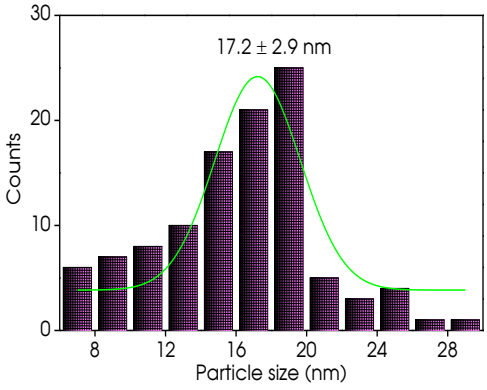

Figure 8. Distribution size of the synthesized AgNPs

3.5. Antibacterial activity of the synthesized AgNPs

The antibacterial activity of synthesized AgNPs was investigated against the Gram-positive and Gram-negative bacteria. The antibacterial activity at different concentrations of AgNPs was shown in Figure 9. In general, synthesized AgNPs have good activity against Gram-positive bacteria *(S. enterica* and *P. aeruginosa)*. However, synthesized AgNPs were virtually inactive against Gram-negative bacteria *(S. aureus* and *L. fermentum)*. The best activity was found on *P. aeruginosa* with half-maximal inhibitory concentrations of 7.9 pM.

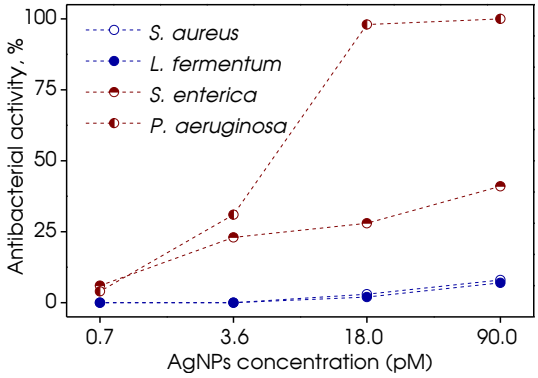

Figure 9. Antibacterial activity of the synthesized AgNPs at different concentrations of AgNPs

4. CONCLUSIONS

A simple green method was used to synthesize AgNPs using the extract of *C. maxima* peel as a reducing and stabilizing agent. The UV-vis absorption spectra and TEM images indicated that the synthesized Ag NPs had a spherical shape with a size of about 17 nm. The extraction condition of *C. maxima* peel including *C. maxima* powder, extraction time and temperature influenced the formation of AgNPs. The optimum conditions for the synthesis of AgNPs were 25 g/L of powdered peel, extraction temperature of 50^{o}C, and extraction time of 40 mins. XRD indicated the formation of pure silver phases and FTIR spectroscopy investigated the role of pectin content in the AgNPs formation. The obtained AgNPs have good inhibitory against *P. aeruginosa*. The present work suggested the use of a by-product of *C. maxima* peel, a suitable way for the synthesis of AgNPs.

ACKNOWLEDGMENT

This work was supported by a grant from Can Tho University (No. TSV2021-51). The authors would like to thank Can Tho University for the financial support.

REFERENCES

Arokiyaraj, S., Arasu, M. V., Vincent, S., Prakash, N. U., Choi, S. H., Oh, Y. K., Choi, K. C., & Kim, K. H., (2014). Rapid green synthesis of silver nanoparticles from Chrysanthemum indicum L and its antibacterial and cytotoxic effects: an in vitro study. *International Journal of Nanomedicine, 9*, 379-388. https://doi.org/10.2147/IJN.S53546

Balachandran, Y. L., Panarin, A. Y., Khodasevich, I. A., Terekhov, S. N., Gutleb, A. C., & Girijaa, S., (2015). Environmentally friendly preparation of gold and silver nanoparticles for SERS applications using biopolymer pectin. *Journal of Applied Spectroscopy, 81(6)*, 962-968. https://doi.org/10.1007/s10812-015-0036-9

Fakayode, O. A. & Abobi, K. E., (2018). Optimization of oil and pectin extraction from orange (Citrus sinensis) peels: a response surface approach. *Journal of Analytical Science and Technology, 9(1)*, 20. https://doi.org/10.1186/s40543-018-0151-3

Guibaud, G., Tixier, N., Bouju, A., & Baudu, M., (2003). Relation between extracellular polymers' composition and its ability to complex Cd, Cu and Pb. *Chemosphere, 52(10)*, 1701-1710. https://doi.org/10.1016/s0045-6535(03)00355-2

Guzman, M., Dille, J., & Godet, S., (2012). Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria. *Nanomedicine, 8(1)*, 37-45. https://doi.org/10.1016/j.nano.2011.05.007

Hileuskaya, K., Ladutska, A., Kulikouskaya, V., Kraskouski, A., Novik, G., Kozerozhets, I., Kozlovskiy, A., & Agabekov, V., (2020). 'Green' approach for obtaining stable pectin-capped silver nanoparticles: Physico-chemical characterization and antibacterial activity. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 585*, 124141. https://doi.org/10.1016/j.colsurfa.2019.124141

Jagtap, U. B. & Bapat, V. A., (2013). Green synthesis of silver nanoparticles using *Artocarpus heterophyllus* Lam. seed extract and its antibacterial activity. *Industrial Crops and Products, 46*, 132-137. https://doi.org/10.1016/j.indcrop.2013.01.019

Jeeva, K., Thiyagarajan, M., Elangovan, V., Geetha, N., & Venkatachalam, P., (2014). *Caesalpinia coriaria* leaf extracts mediated biosynthesis of metallic silver nanoparticles and their antibacterial activity against clinically isolated pathogens. *Industrial Crops and*

Products, 52, 714-720. https://doi.org/10.1016/j.indcrop.2013.11.037

Khan, M., Khan, M., Adil, S. F., Tahir, M. N., Tremel, W., Alkhathlan, H. Z., Al-Warthan, A., & Siddiqui, M. R. H., (2013). Green synthesis of silver nanoparticles mediated by Pulicaria glutinosa extract. *International Journal of Nanomedicine, 8*, 1507-1516. https://doi.org/10.2147/IJN.S43309

Košťálová, Z., Hromádková, Z., & Ebringerová, A., (2013). Structural diversity of pectins isolated from the Styrian oil-pumpkin (Cucurbita pepo var. styriaca) fruit. *Carbohydrate Polymers, 93(1)*, 163-171. https://doi.org/10.1016/j.carbpol.2012.05.017

Nguyen, T. D., Nguyen, D. P., Hoang, Y. H., Nguyen, T. T., & Nguyen, H. T., (2021). Rapid synthesis of silver nanoparticles using the extract of Psidium guajava leaf based on light-emitting diodes irradiation. *Chemical Papers, 75(11)*, 5623-5631. https://doi.org/10.1007/s11696-021-01747-z

Pallavicini, P., Arciola, C. R., Bertoglio, F., Curtosi, S., Dacarro, G., D'Agostino, A., Ferrari, F., Merli, D., Milanese, C., Rossi, S., Taglietti, A., Tenci, M., & Visai, L., (2017). Silver nanoparticles synthesized and coated with pectin: An ideal compromise for anti-bacterial and anti-biofilm action combined with wound-healing properties. *Journal of Colloid and Interface Science, 498*, 271-281. https://doi.org/10.1016/j.jcis.2017.03.062

Rubaiyi, M. Z., Zularisam, A. W., & Mimi Sakinah, A. M., (2016). Effect of process parameters on pectin extraction from dragon fruit. *Australian Journal of Basic and Applied Sciences, 10(17)*, 69-74.

Siddiqi, K. S., Husen, A., & Rao, R. A. K., (2018). A review on biosynthesis of silver nanoparticles and their biocidal properties. *Journal of Nanobiotechnology, 16(1)*, 1-28. https://doi.org/10.1186/s12951-018-0334-5

Suresh, G., Gunasekar, P. H., Kokila, D., Prabhu, D., Dinesh, D., Ravichandran, N., Ramesh, B., Koodalingam, A., & Vijaiyan Siva, G., (2014). Green synthesis of silver nanoparticles using Delphinium denudatum root extract exhibits antibacterial and mosquito larvicidal activities. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 127*, 61-66. https://doi.org/10.1016/j.saa.2014.02.030

Wani, I. A., Ganguly, A., Ahmed, J., & Ahmad, T., (2011). Silver nanoparticles: Ultrasonic wave assisted synthesis, optical characterization and surface area studies. *Materials Letters, 65(3)*, 520-522. https://doi.org/10.1016/j.matlet.2010.11.003

Yang, J., Yin, H., Jia, J., & Wei, Y., (2011). Facile synthesis of high-concentration, stable aqueous dispersions of uniform silver nanoparticles using aniline as a reductant. *Langmuir, 27(8)*, 5047-5053. https://doi.org/10.1021/la200013z

Zahran, M. K., Ahmed, H. B., & El-Rafie, M. H., (2014). Facile size-regulated synthesis of silver nanoparticles using pectin. *Carbohydrate Polymers, 111*, 971-978. https://doi.org/10.1016/j.carbpol.2014.05.028

Zhang, W., Qiao, X., & Chen, J., (2007). Synthesis of silver nanoparticles: Effects of concerned parameters in water/oil microemulsion. *Materials Science and Engineering: B, 142(1)*, 1-15. https://doi.org/10.1016/j.mseb.2007.06.014

Zhang, W., Zhao, X. J., Jiang, Y., & Zhou, Z., (2017). Citrus pectin derived silver nanoparticles and their antibacterial activity. *Inorganic and Nano-Metal Chemistry, 47(1)*, 15-20. https://doi.org/10.1080/15533174.2015.1137073