Original Article / Araştırma Makalesi

ANTIMICROBIAL POTENTIAL OF SILVER NANOPARTICLES PRODUCED BY APRICOT LEAF EXTRACT

Kayısı Yaprak Özütü ile Sentezlenen Gümüş Nanopartiküllerin Antimikrobiyal Potansiyeli

Ayşe BARAN[1] *Özfer YEŞİLADA*[2]
[1]*Institute of Graduate Educational Studies, Department of Biology, Mardin*
[2]*İnönü University, Faculty of Science and Arts, Malatya*

Geliş Tarihi / Received: *19.10.2021* ***Kabul Tarihi / Accepted:*** *20.01.2022*

ABSTRACT

Metallic nanoparticles are important substances in medicine. These particles could be prepared by various ways including green synthesis. The production of silver nanoparticles by plant extracts is widely used because of their environmentally friendly properties. In this study, silver nanoparticles have been synthesized by biological method and Apricot tree (*Prunus armeniaca*) leaf extract was used as reducing agent. The color change and formation of the characteristic absorption peak at 400-500 nm were the first indications of AgNP formation. XRD analysis showed the crystalline particles and obtained AgNPs were generally in spherical shape with the average particle size of 24 nm. Their antimicrobial activity studies against various pathogenic microorganisms showed that they have strong antibacterial activity against Escherichia coli and Staphylococcus aureus and antifungal activity against *Candida albicans*. The minimum inhibitory concentrations for *E. coli, S. aureus* and *C. albicans* were 0.125, 0.125 and 0.250 µg/mL, respectively. The results confirmed that Prunus armeniaca leaf extract could be used to produce AgNPs with efficient antimicrobial activity.

Keywords: Antimicrobial, Apricot, Green synthesis, Prunus armeniaca leaf extract, Silver nanoparticles.

ÖZ

Metalik nanopartiküller tıpta önemli maddelerdir. Bu partiküller yeşil sentez dahil çeşitli yollarla üretilebilirler. Gümüş nanopartiküllerin bitki özütleri ile üretimi çevre dostu özelliklerinden dolayı yaygın olarak kullanılmaktadır. Bu çalışmada gümüş nanopartiküller biyolojik yöntemle sentezlenmiş ve Kayısı ağacı (*Prunus armeniaca*) yaprak özütü redükleyici ajan olarak kullanılmıştır. Renk değişimi ve 400-500 nm'de özgül absorbans piki oluşumu AgNP oluşumunun ilk göstergeleri olmuştur. XRD analizi kristal yapıda partikülleri göstermiştir ve elde edilen AgNP'ler ortalama 24 nm boyutunda sferik şekillidir. Nanopartiküllerin çeşitli patojenik mikroorganizmalar üzerine antimikrobiyal aktivite çalışmaları, bu nanopartiküllerin Escherichia coli ve Staphylococcus aureus üzerine kuvvetli antibakteriyel ve *Candida albicans*'a karşı da kuvvetli antifungal etkisi olduğunu göstermiştir. *E. coli, S. aureus* ve *C. albicans* için minimum inhibe edici konsantrasyonlar sırasıyla 0.125, 0.125 ve 0.250 µg /mL'dir. Sonuçlar, Prunus armeniaca yaprak özütünün etkili antimikrobiyal aktiviteye sahip AgNP'lerin üretiminde kullanılabileceğini ortaya koymuştur.

Anahtar kelimeler: Antimikrobiyal, Gümüş nanopartikül, Kayısı, Prunus armeniaca yaprak özütü, Yeşil sentez.

Özfer YEŞİLADA ✉, *ozfer.yesilada@inonu.edu.tr*
İnönü University, Faculty of Science and Arts, Malatya

Bu makaleye atıf yapmak için(How to cite this article): Baran, A., Yeşilada, Ö. (2022). Antimicrobial Potential of Silver Nanoparticles Produced By Apricot Leaf Extract. İnönü Üniversitesi Sağlık Hizmetleri Meslek Yüksekokulu Dergisi, 10(1), 50-57. doi: 10.33715/inonusaglik.1012011

INTRODUCTION

Nanoparticles are substances with particle size between 1-100 nm (Pirtarighat, Ghannadnia, & Baghshahi, 2019). Metallic nanoparticles (MNPs) can be used in various biological and medical applications. These MNPs can be produced by chemical, physical and biological (green synthesis) ways (Chintamani, Salunkhe, & Chavan 2018). However, methods other than biological means have various disadvantages. Therefore, many studies have been focused on biological nanoparticle production. Plant extracts, microorganisms and proteins can be used for MNP production (Bao, Cao, Kang, & Lan, 2019; Khan, Malik, Khan, Cho, & Khan, 2018; Onitsuka, Hamada, & Okamura, 2019; Sharma, Pant, Rai, Yadav, & Dave, 2018). Green synthesis is an ecofriendly way. It is possible to produce various nanoparticles using extracts of various plants. Size and shapes of the nanoparticles are the important factors for their properties and activities. Nanoparticles with various shapes and sizes can be produced by different plant extracts. Therefore, the selection of proper extract for nanoparticle production is important. Silver nanoparticles (AgNPs) that can be produced by plant extracts have broad applications in medicine. AgNPs with different antimicrobial and cytotoxic activities can be obtained by various plant extracts; Spherical AgNPs with 15-20 nm sizes can be produced by using the leaf extract of *Enicostemma axillare* (Lam.) (Raj, Mali, & Trivedi, 2018). Spherical AgNPs were also synthesized with *Mimusops coriacea* leaf extract at room temperature (Lopes & Courrol, 2018). *O. tenuiflorum*, *S. cumini*, *C. sinensis*, *S. tricobatum* and *C. asiatica* extracts were also tested to produce AgNPs and nanoparticles with sizes of 28 nm, 26.5 nm, 65 nm, 22.3 nm and 28.4 nm were synthesized, respectively. These nanoparticles had antimicrobial potential against pathogenic bacteria (Logeswari, Silambarasan, & Abraham, 2015). *Phoenix dactylifera*, *Ferula asafetida*, and *Acacia nilotica* extracts were used as the reductant and stabilizing agents for AgNP production and the obtained AgNPs showed efficient antibacterial activity against *E. coli*, *P. aeruginosa* and *S. aureus* (Mohammed, Al-Qahtani, Al-Mutairi, Al-Shamri, & Aabed, 2018). Spherical AgNPs, about 25 nm in diameter, were produced by *Solanum trilobatum* bark extract and these nanoparticles showed high antimicrobial activity on various bacteria and fungus (Ramanathan et al., 2018). It was reported that AgNPs in spherical shape with bactericidal activity could be prepared by *S. spinosa* extracts (Pirtarighat, Ghannadnia, & Baghshahi, 2019). AgNPs with antimicrobial activity were also produced by tea leaves extracts of *Camellia sinensis* (Onitsuka et al., 2019). AgNP production potential of *Prunus armeniaca* fruit extract has also been reported (Dauthal & Mukhopadhyay, 2013).

In this study, *Prunus armeniaca* leaf extract was used to prepare AgNPs with antibacterial and antifungal activity. Although there exists several studies on AgNP production by plant extracts, according to our literature knowledge, this is the first study about; AgNP production by *Prunus armeniaca* leaf extract and antimicrobial potential of the AgNPs obtained by this extract.

MATERIALS AND METHODS

Extract preparation

The extract obtained from the leaves of *Prunus armeniaca* (apricot) from Malatya/Turkey was used. Following washing, these leaves were dried at 45 °C and then reduced in size. Afterwards, they were soaked in distilled water and heated with continuous stirring (Veerasamy et al., 2011). After cooling, it was filtered and used for AgNP production.

Production of AgNP

In the study, 6 ml of the extract prepared as described above was added to 1 mM 100 ml $AgNO_3$/250 ml flask and the samples were incubated at 150 rpm and 30 °C under light. After incubation, they were centrifuged at 9000 rpm for 10 min. Then, the obtained nanoparticles were washed with distilled water and AgNPs were used after drying.

Characterization of AgNP

AgNP formation was firstly determined with the change of color from light yellow to brown and the characteristic absorption peak at 400-450 nm. Their crystalline natures were determined by x-ray diffractometer and elemental analysis was performed by SEM-EDX spectrometer at IBTAM. Dimension and shape analysis of AgNPs were carried out with HRTEM at METU Central Laboratory.

Evaluation of Antimicrobial Activity Based on Minimum Inhibitory Concentrations

To determine the MIC of AgNPs 96-well plate microdilution method was used (Apohan et al., 2017). *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC29213 and *Candida albicans* ATCC 90028 were utilized as the pathogen microorganisms to detect the antimicrobial effect of AgNPs. These microorganisms were incubated for 24h and the cell suspensions with proper turbidity were prepared based on the McFarland Standard. Then, 100 µL was inoculated into each well. The plates for bacteria and yeast were incubated for 24 h and 48 h, respectively. Sterility control and growth control wells were also prepared. MIC is the lowest concentration with no growth. Ertapenem, vancomycin and fluconazole antibiotics were used as positive controls.

RESULTS AND DISCUSSION

The agent used for reducing and stabilizing was apricot leaf extract. Color change is the main indicator used to detect the formation of nanoparticles. After 70 min incubation, the color transformation started and the color changed to dark brown after 4h. However, the colors of 1 mM $AgNO_3$ solution without extract and extract-only solution did not change. The reason for the color transformation is the surface plasmon resonance of these particles (Gurunathan, Han, Kwon, & Kim, 2014). The color change resulting from the extract application is shown in Figure 1.

Figure 1. Color Change As The Result Of AgNP Formation

After addition of the extract, UV-Vis spectra of nanoparticles were monitored and characteristic peaks of silver nanoparticles were observed at 422-427 nm (Singh et al., 2015). The intensity of peak increased as a function of reaction time (Figure 2). This situation also shows the increase in the formation of AgNPs (Gurunathan, Raman, Malek, John, & Vikineswary, 2013). This result is consistent with the studies of other researchers (Raj et al., 2018).

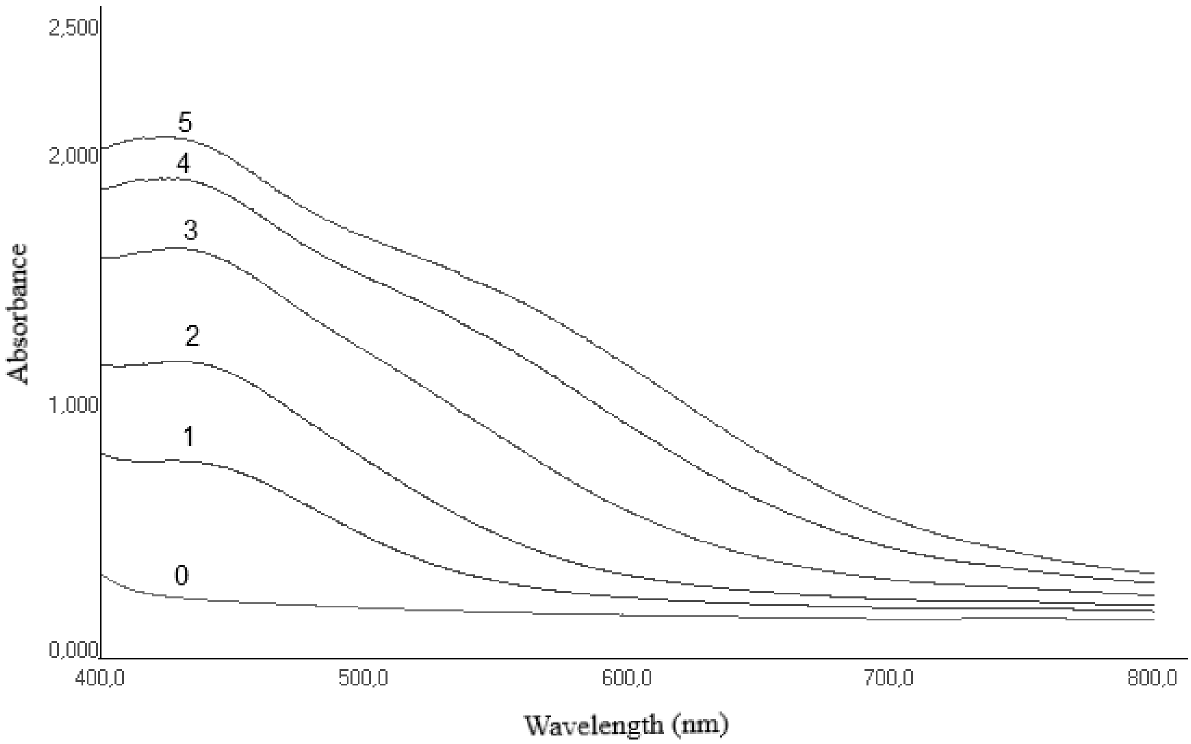

Figure 2. UV Visible Spectra Recorded As A Function Of Reaction Time. 0-5 Show The Reaction Time As Hours

Prior to the investigation of the antimicrobial activity, the obtained AgNPs were characterized. XRD pattern of these nanoparticles were given in Figure 3. The characteristic peaks (2θ= 38.04^o, 44.17^o, 64.43^o and 77.35^o) indicated the crystalline particles (Gopinath et al., 2017). The average crystallite size was calculated using Scherrer equation and average particle size was determined as 12.48 nm. EDX also showed the strong Ag signals indicating that the AgNPs were produced by the extract.

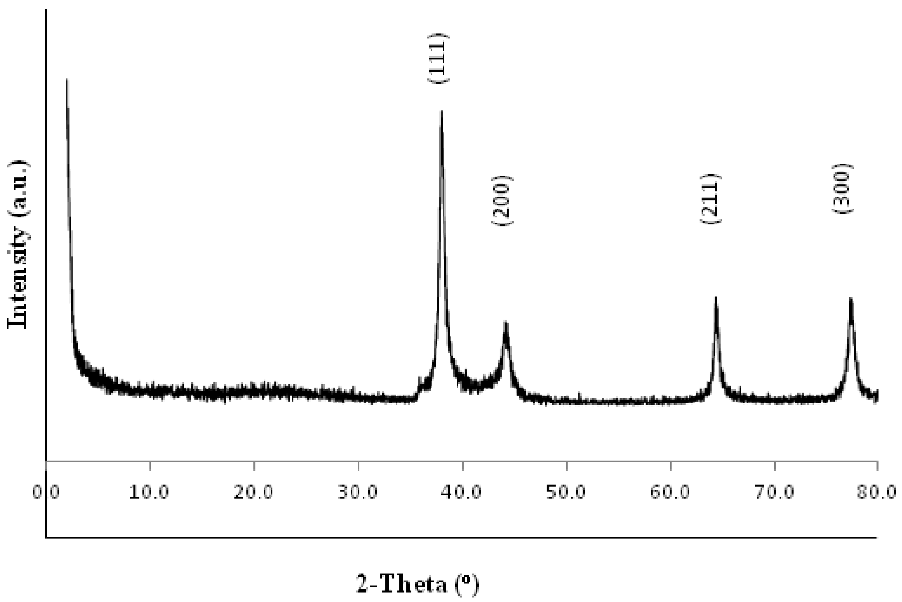

Figure 3. XRD Pattern Of AgNPs

HRTEM images showed generally spherical shaped AgNPs in the range of 7-45 nm. The average size is about 24 nm (Figure 4). Venugopal et al., 2017 stated that spherical AgNPs with particle size in the range of 5-40 nm could be obtained using *Syzygium aromaticum* extract.

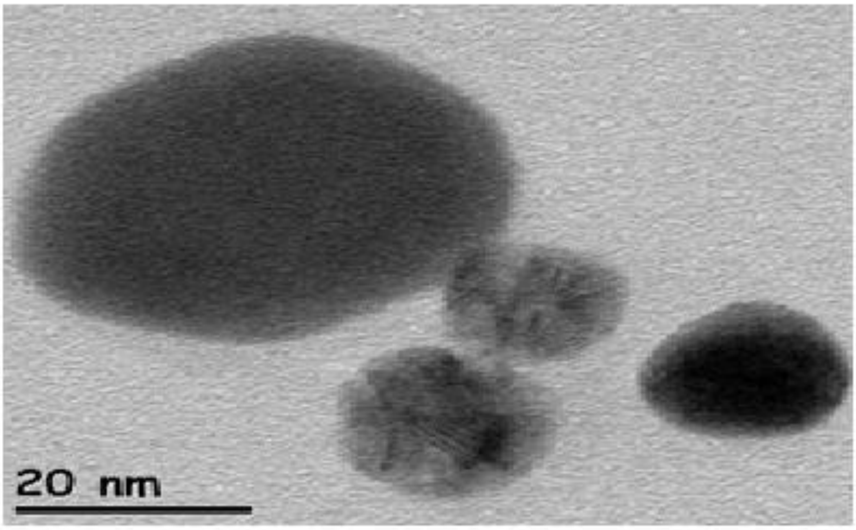

Figure 4. HRTEM Image Of AgNPs

After the characterization, their antimicrobial activities against pathogen bacteria and yeast were investigated. The AgNPs obtained sing *P. armeniaca* leaf extract showed high antimicrobial activity against the microorganisms used. The minimum inhibitory concentration values for *E. coli*, *S. aureus* and *C. albicans* were 0.125, 0.125 and 0.250 µg/ml, respectively (Table 1). The antimicrobial activities of AgNPs on gram-positive bacteria, gram negative bacteria and yeast were highly similar. It was reported that AgNPs synthesized by using apple extract have antimicrobial activity on various bacteria (Ali, Yahya, Sekaran, & Puteh, 2016). The antibacterial potential of green synthesized AgNPs on *E. coli* and *S. aureus* have also been reported (Wang, Zhang, Zheng, & Zhu 2017). AgNPs obtained with *Millettia pinnata* flower extracts had different levels of antibacterial activity on *E. coli*, *K. pneumoniae, P. aeruginosa*, *S. aureus* and *P. vulgaris* (Rajakumar et al., 2017). However, there are some studies reporting the low antimicrobial potential of AgNPs. While the green synthesized AgNPs showed desirable antimicrobial activity on *B. subtilis* (12 mm) and *P. aeruginosa* (11 mm), the zone of inhibition for *S. aureus, C. tropicalis* and *E. coli* was 8 mm and no inhibition was observed on *A. flavus* (Velammal, Devi, & Amaladhas, 2016).

Table 1. MIC Values (μg/mL) Of AgNP, $AgNO_3$ And Antibiotics On Microorganisms (μg/mL)

Substances	***E. coli***	***S. aureus***	***C. albicans***
AgNP	0.125	0.125	0.250
$AgNO_3$	1.000	0.500	0.500
Ertapenem	0.125	-	-
Vancomycin	-	0.500	-
Fluconazole	-	-	0.0625

Our results confirmed that *P. armeniaca* leaf extract can successfully be used to produce AgNPs and these AgNPs were efficient antimicrobial agents. AgNP production by this extract is an ecofriendly and easy way for green synthesis of nanoparticles that have antimicrobial potential.

Acknowledgement

This work was supported by Inonu University Scientific Research Projects Coordination Unit (Grant No: 2016/111)

Note: This research was presented; Ayşe Baran, Özfer Yeşilada (2019). Yeşil sentezle gümüş nanopartikül sentezi ve antimikrobiyal aktiviteleri. 2nd International Eyrision Conference on Biological and Chemical Sciences (EurasianBioChem 2019) 28-29 June 2019, Ankara, Turkey

REFERENCES

Ali, Z. A., Yahya, R., Sekaran, S. D., Puteh, R. (2016). Green synthesis of silver nanoparticles using apple extract and its antibacterial properties. Advances in Materials Science and Engineering, Volume 2016, Article ID 4102196, 1-6.

Apohan, E., Yilmaz, U., Yilmaz, O., Serindag, A., Kucukbay, H., Yesilada, O., Baran, Y. (2017). Synthesis, cytotoxic and antimicrobial activities of novel cobalt and zinc complexes of benzimidazole derivatives, Journal of Organometallic Chemistry. 828, 52-58.

Bao, Z. Q., Cao, J. H., Kang, G. B., Lan, C. Q. (2019). Effects of reaction conditions on light-dependent silver nanoparticle biosynthesis mediated by cell extract of green alga Neochloris oleoabundans. Environmental Science and Pollution Research, 26, 2873-2881.

Chintamani, R. B., Salunkhe, K. S., Chavan, M. J. (2018). Emerging use of green synthesis silver nanoparticle: An updated review. International Journal of Pharmeceutical Sciences and Research, 9, 4029-4055.

Dauthal, P., Mukhopadhyay, M. (2013). In-vitro free radical scavenging activity of biosynthesized gold and silver nanoparticles using Prunus armeniaca (apricot) fruit extract. Journal of Nanoparticle Research, 15, 1-11.

Gopinath, K., Devi, N. P., Govindarajan, M., Bhakyaraj, K., Kumaraguru, S., Arumugam, A., Alharbi, N. S., Kadaikunnan, S., Benelli, G. (2017). One-Pot green synthesis of silver nanoparticles using the orchid leaf extracts of Anoectochilus elatus: Growth inhibition activity on seven microbial pathogens. Journal of Cluster Science, 28, 1541-1550.

Gurunathan, S., Han, J. W., Kwon, D. N., Kim, J. H. (2014). Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against Gram-negative and Gram-positive bacteria. Nanoscale Research Letters, 9, 1-17.

Gurunathan, S., Raman, J., Malek, N. A., John, P. A., Vikineswary, S. (2013). Green synthesis of silver nanoparticles using Ganoderma neo-japonicum Imazeki: a potential cytotoxic agent against breast cancer cells. International Journal of Nanomedicine, 8, 4399-4413.

Khan, A. U., Malik, N., Khan, M., Cho, M., Khan, M. M. M. (2018). Fungi-assisted silver nanoparticle synthesis and their applications. Bioprocess and Biosystem Engineering, 41, 1-20.

Logeswari, P., Silambarasan, S., Abraham, J. (2015). Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property. Journal of Saudi Chemical Society, 19, 311-317.

Lopes, C. R. B., Courrol, L. C. (2018). Green synthesis of silver nanoparticles with extract of Mimusops coriacea and light. Journal of Luminescence, 199, 183-187.

Mohammed, A. E., Al-Qahtani, A., Al-Mutairi, A., Al-Shamri, B., Aabed, K. (2018). Antibacterial and cytotoxic potential of biosynthesized silver nanoparticles by some plant extracts. Nanomaterials-Basel. 8, 1-15.

Onitsuka, S., Hamada, T., Okamura, H. (2019). Preparation of antimicrobial gold and silver nanoparticles from tea leaf extracts. Colloids and Surfaces B, 173, 242-248.

Pirtarighat, S., Ghannadnia, M., Baghshahi, S. (2019). Green synthesis of silver nanoparticles using the plant extract of Salvia spinosa grown in vitro and their antibacterial activity assessment. Journal of Nanostructure in Chemistry, 9, 1-9.

Raj, S., Mali, S. C., Trivedi, R. (2018). Green synthesis and characterization of silver nanoparticles using Enicostemma axillare (Lam.) leaf extract. Biochemical and Biophysical Reserach Communications, 503, 2814-2819.

Rajakumar, G., Gomathi, T., Thiruvengadam, M., Rajeswari, V. D., Kalpana, V. N., Chung, I. M. (2017). Evaluation of anti-cholinesterase, antibacterial and cytotoxic activities of green synthesized silver nanoparticles using from Millettia pinnata flower extract. Microbial Pathogenesis, 103, 123-128.

Ramanathan, S., Gopinath, S. C. B., Anbu, P., Lakshmipriya, T., Kasim, F. H., Lee, C. G. (2018). Eco-friendly synthesis of Solanum trilobatum extract-capped silver nanoparticles is compatible with good antimicrobial activities. Journal of Molecular Structure, 1160, 80-91.

Sharma, P., Pant, S., Rai, S., Yadav, R. B., Dave, V. (2018). Green Synthesis of silver nanoparticle capped with Allium cepa and their catalytic reduction of textile dyes: An ecofriendly approach. Journal of Polymers and The Environonment, 26, 1795-1803.

Singh, P., Kim, Y. J., Singh, H., Wang, C., Hwang, K. H., Farh, M. E. A., Yang, D. C. (2015). Biosynthesis, characterization and antimicrobial applications of silver nanoparticles. International Journal of Nanomedicine, 10, 2567-2577.

Veerasamy, R., Xin, T. Z., Gunasagaran, S., Xiang, T. F. W., Yang, E. F. C., Jeyakumar, N., Dhanaraj, S. A. (2011). Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. Journal of Saudi Chemical Society, 15, 113-120.

Velammal, S. P., Devi, T. A., Amaladhas, T. P. (2016). Antioxidant, antimicrobial and cytotoxic activities of silver and gold nanoparticles synthesized using Plumbago zeylanica bark. Journal of Nanostructure in Chemistry, 6, 247-260.

Venugopal, K., Rather, H. A., Rajagopal, K., Shanthi, M. P., Sheriff, K., Illiyas, M., Rather, R. A., Manikandan, E., Uvarajan, S., Bhaskar, M., Maaza, M. (2017). Synthesis of silver nanoparticles (AgNPs) for anticancer activities (MCF 7 breast and A549 lung cell lines) of the crude extract of Syzygium aromaticum. Journal of Photochemistry and Photobiology B: Biology, 167, 282-289.

Wang, M. M., Zhang, W. J., Zheng, X. S., Zhu, P. Z. (2017). Antibacterial and catalytic activities of biosynthesized silver nanoparticles prepared by using an aqueous extract of green coffee bean as a reducing agent. Rsc Advances, 7, 12144-12149.