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Biosynthesis of Ag Nanoparticle Using Catharanthus roseus Extract and Investigation of its Toxic Effect in Healthy Cell

Catharantus roseus Ekstratı Kullanarak Ag Nanopartikülünün Sentezlenmesi ve Sağlıklı İnsan Hücresinde Toksik Etkisinin İncelenmesi

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ABSTRACT

Biosynthesis of nanoparticles from plant extracts is of great interest because it is inexpensive, environmentally friendly, and suitable for large-scale production compared to other synthesis methods. In this study, it was aimed to perform the synthesis and characterization of Ag nanoparticles (Ag NPs) by using *Catharantus roseus* (*C. roseus*) extract as a reducing and stabilizing agent and to evaluate their toxic effects against a healthy human cell line. The synthesis of Ag NPs were successfully carried out using the extract of *C. roseus*. By using FTIR, XRD, UV-Vis, and TEM analysis, the synthesized Ag NPs were characterized. According to XRD and TEM analysis results, it was observed that the average diameter of the synthesized Ag NPs was 16 ± 6 nm and its shape was a face-centered cubic (fcc). In addition, the toxic effects of biologically synthesized Ag NPs against a human healthy cell line (L929) were investigated in the study and the half-maximum inhibitory concentration (IC_{50}) was found to be 2.909 µL/mL.

Keywords: Green synthesis, Catharanthus roseus, Ag nanoparticles, Toxicity

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ÖZET

Bitki ekstraktları kullanılarak nanoparçacıkların biosentezi, diğer sentez yöntemlerine kıyasla daha uygun maliyetli, çevre dostu ve büyük ölçekli üretim için uygun olması sebebiyle büyük ilgi görmektedir. Bu çalışmada indirgeyici ve stabilize edici ajan olarak *Catharantus roseus* (*C. roseus*) ekstraktı kullanılarak Ag nanopartiküllerin (Ag NPs) sentezinin ve karakterizasyonunun gerçekleştirilmesi ve sağlıklı insan hücresine karşı toksik etkilerinin incelenmesi hedeflenmiştir. Ag NPs sentezi *C. roseus* ekstraktı kullanılarak başarılı bir şekilde gerçekleştirilmiştir. Sentezlenen Ag NPs FTIR, XRD, UV-Vis ve TEM analizleri ile karakterize edilmiştir. XRD ve TEM analiz sonuçlarına göre, sentezlenen Ag NPs ortalama partikül boyutunun 16 ± 6 nm ve şeklinin yüzey merkezli kübik olduğu gözlenmiştir. Ayrıca çalışmada biyolojik olarak sentezlenmiş olan Ag NPs sağlıklı insan hücre hattına (L929) karşı toksik etkileri araştırılmış ve yarı maksimum inhibitör konsantrasyonu (IC_{50}) 2.909 µL/mL olarak bulunmuştur.

Anahtar Kelimeler: Yeşil kimya, Catharanthus roseus, Ag nanopartikülü, Toksisite

1. Introduction

In recent years, Ag NPs have been the focus of attention of researchers due to their superior and unique properties compared to bulk counterparts. Ag NPs synthesis methods can be divided into three groups physical, chemical, and biological methods [1]. Chemical and physical methods have disadvantages such as the use of toxic substances, expensive synthesis, and harmful to the environment [2]. There has been an increased interest in biological methods recently to overcome these disadvantages. In biological methods, species such as plants, fungi, enzymes, and bacteria are used to synthesize nanoparticles [3,4].

Thanks to the superior properties of Ag NPs such as antibacterial [5], antifungal [6], and antimicrobial [7], it has many applications such as bioengineering, medicine, textile, and electronics. However, as the use of Ag NP-containing products increases, it causes various problems in human and environmental health. Some studies are showing that Ag nanoparticles reduce viability in cells such as rat liver cells and human fibroblasts [8,9]. In addition, it has been proven in some studies to be toxic to mouse germ cells as it inhibits mitochondrial function and increases lactate dehydrogenase leakage [10]. Due to its antibacterial properties, nanosilver can prevent the growth of various "friendly" bacteria in the soil. Silver can stop the denitrification process, which entails turning nitrates into nitrogen gas that is necessary for the plants, by having harmful effects on the denitrifying bacteria. Eutrophication of rivers, lakes, and marine ecosystems can result from the loss of environmental denitrification due to decreased plant productivity, which will ultimately lead to ecosystem collapse [11]. To further understand Ag NP toxicity, more research is required.

C. roseus L. is a summer seasonal herbaceous plant used in landscaping areas. Since its flowers resemble a propeller shape, its most commonly used name among the public is propeller flower. It is also known by different names such as rosette flower or vinca. It is used as an outdoor ornamental plant in many countries, including our country. Additionally, this plant is a source of pharmaceutical compounds such as terpenoid indole alkaloids, including vincristine and vinblastine, which have valuable antitumor properties [12].

In this study, the biosynthesis of Ag NPs was carried out using *C. roseus* extract. In addition, the effects of synthesized Ag NPS against a human healthy cell line (L929) were investigated. This is the first study to examine the toxic effect of Ag nanoparticles synthesized using *C. roseus* extract. There are some studies in the literature about the synthesis of Ag nanoparticles using *C. roseus* extract [13–16], but no study has been found on its cytotoxic activity. This is the first study on the cytotoxic activity of synthesized Ag nanoparticles using *C. roseus* extract.

2. Material and Method

Preparation of *C. roseus* extract

C. roseus was collected from Antalya and its species were identified. *C. roseus*, was thoroughly washed with deionized water and removed from foreign wastes. The cleaned plant was dried in the shade under room conditions. The dried plant was ground and sieved with a particle size of less than 1.00 mm. 5 g of powdered *C. roseus* and 200 ml of deionized water were taken into a 500 mL flask and boiled under reflux for 10 minutes. Then, the extract was filtered after being cooled to room temperature. It was centrifuged at 9500 rpm for 5 minutes to remove the small residues remaining in the extract. The extract was stored at +4 ^{o}C to be used in further studies.

Biosynthesis of Ag NPs

$AgNO_3$ was used for the synthesis of Ag NPs. 90 mL of 1mM $AgNO_3$ solution was added to 10 mL of *C. roseus* extract and the mixture was stirred at 80 ^{o}C for 30 minutes in the dark.

Characterization of synthesized Ag NPs

By using FTIR, UV-Vis, TEM, and XRD analysis, the synthesized Ag NPs were characterized. The FTIR spectra of the synthesized Ag NPs and the plant extract were recorded using the JASCO FT/IR 4700 spectrometer at 4 cm^{-1} resolution at room temperature. UV-Vis spectra of Ag NPs were recorded in the wavelength range of 200 to 700 nm using a JASCO V-770 UV/Vis spectrometer operating at 1 nm resolution. The size and morphology of the synthesized Ag NPs were determined using a transmission electron microscope (JEOL JEM-1020). XRD analysis of Ag NPs was investigated using monochromatic Cu-Kα beams with a wavelength of 0.154056 nm at an angle of 2θ between 10^{o}-80^{o}.

Determination of toxic effects of synthesized AgNPs

The human healthy fibroblast cell line (L929) was kindly donated by Associate Professor Çiğdem Yücel from Erciyes University. L929 cells were grown in DMEM high-glucose, fetal bovine serum (10%), and glutamax (1%). Seeding of these normal human cells was done to a 96-well plate at a 4.5 x 10^3 cells/well density. L929 cells were exposed to AgNPs at concentrations of 5, 2.5, 1.25, 0.625, and 0.3125 µL/mL for 48 h. The 50 µL of the prepared MTT solution was added to the wells of a sterile plate and incubated for 2 h. Absorbance values were taken at 590 nm on the Epoch 2 Elisa plate reader. The IC_{50} values were calculated with GraphPad Prism Software 5.

3. Results

Characterization of synthesized Ag NPs

The most important and simplest way to confirm the formation of nanoparticles is the UV-Vis spectroscopy technique. The nanoparticles form a characteristic plasmon band at a certain wavelength in the UV-Vis spectrum. The UV-Vis spectrum of the synthesized AgNPs is given in Fig. 1. The absorption peak at 438 nm belongs to the characteristic SPR absorption band of synthesized Ag NPs. In the literature, it has been reported that the SPR band of Ag NPs can be obtained between 400-500 nm in UV-Vis analysis because of the excitation of surface plasmon vibrations.

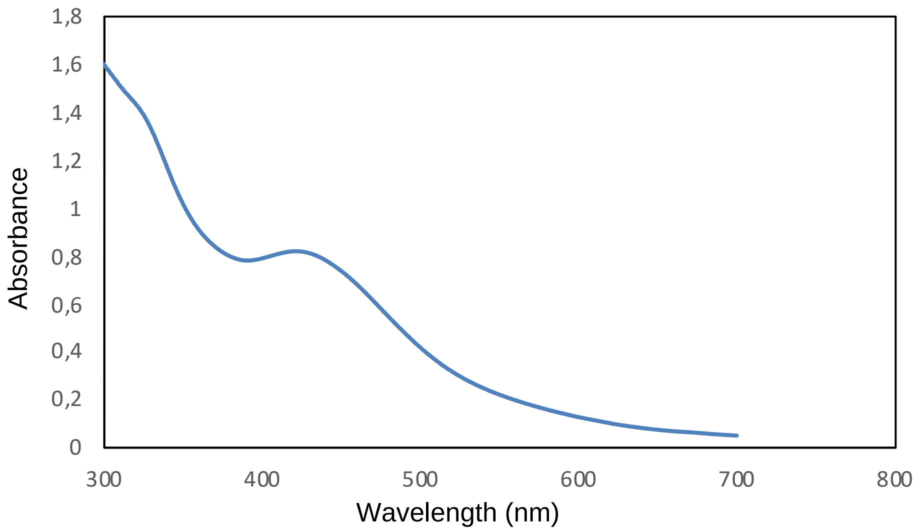

Figure 1: UV-Vis spectrum of synthesized Ag NPs

The crystal structure of Ag NPs synthesized by using *C. roseus* extract was identified using XRD analysis. Ag NPs showed four different diffraction regions of 38.1, 46.13, 67.4, and 77.3, representing (111), (200), (220), and (311) crystal surfaces, respectively. It was agreed that the XRD analysis data of Ag NPs were compatible with the data of the Joint Committee on Powder Diffraction Standards (04-0783). In addition, it was determined that it has a face-centered cubic crystal structure according to these standards.

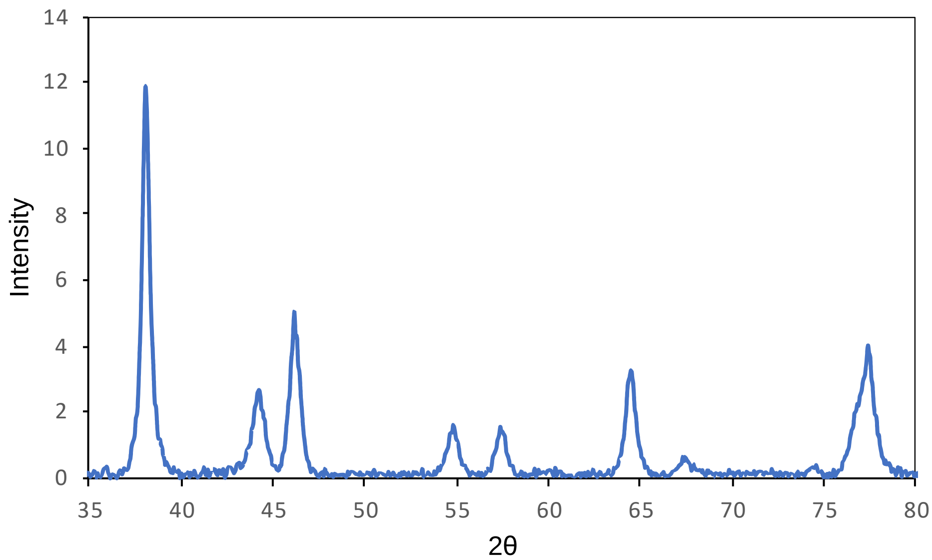

Figure 2: XRD spectrum of synthesized Ag NPs

TEM analysis helps us determine the sizes and morphologies of nanoparticles. The shape of the synthesized Ag NPs was determined to be roughly spherical as seen in Figure 3a. In addition, the average particle size was calculated by measuring the size of 100 nanoparticles from the TEM images using the image proplus 6 program. The results showed that the size of the synthesized Ag NPs ranged from 7 nm to 34 nm, with an average particle size of 16 ± 6 nm.

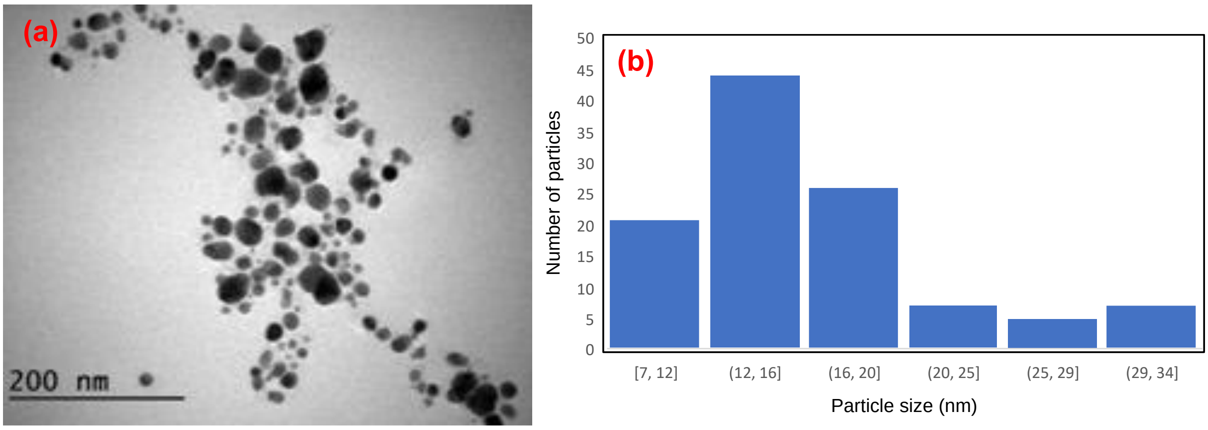

Figure 3: a) TEM images and **b)** TEM size distribution of synthesized Ag NPs

To find the water-soluble chemical components from the *C. roseus* plant extract that may be effective in the synthesis and stabilization of Ag NPs, the FTIR spectrum from the plant extract containing Ag NPs and the pure plant extract was investigated. In the FT-IR results of pure and nanoparticle-containing powder samples of the plant extract, significant peaks were found at 3250, 2923, 1580, 1400, 1026, and 594 cm^{-1} as seen in **Fig. 4**. The broad peak resulting from O-H stretching and N-H stretching, characterized by the presence of phenolic compounds and proteins, is seen at 3250 cm^{-1}. The peak resulting from C-H stretching is seen at 2923 cm^{-1}. The peak at 1580 cm^{-1} corresponds to the C=O stretch, indicating the presence of major components of flavonoids and terpenoids. The peak at 1400 cm^{-1} corresponds to the C=C stretch, indicating the presence of alkenes and aromatic compounds. The peak at 1026 cm^{-1} corresponds to the C-N stretch indicating the presence of polyphenols, aliphatic amines, alcohol, or phenols. The peak at 594 cm^{-1} corresponds to the C-Br stretch indicating the presence of alkyl halides. As can be observed from the FITR result, the extract contains a variety of functional groups that are responsible for the reduction and stabilization of nanoparticles.

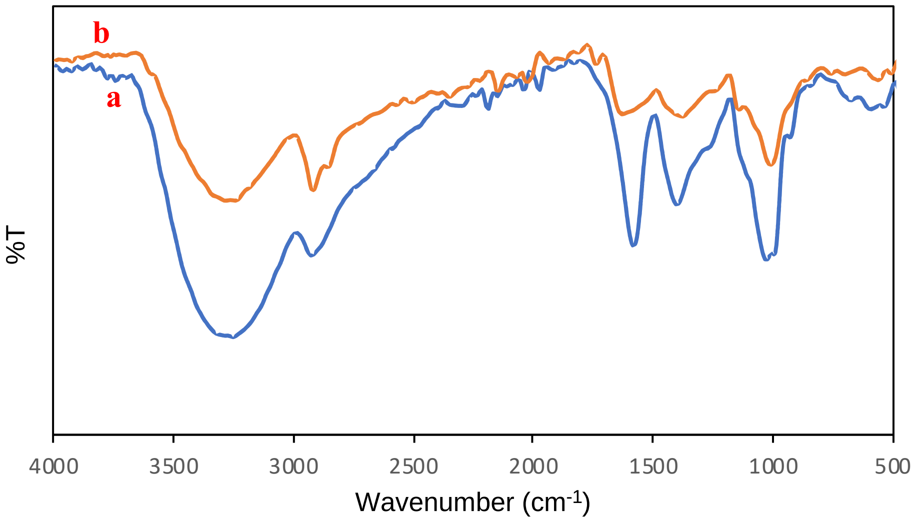

Figure 4: a) FTIR spectrum of *C. roseus* extract and **b)** Ag NPs in the *C. roseus* extract

Determination of toxic effects of synthesized AgNPs

The toxic effect of synthesized Ag NPs was tested *in vitro* in a healthy human cell line (L929). The obtained results are given in Table 1.

Table 1: Half-maximum inhibitory concentration of Ag NPs in L929 cell lines

Sample	IC_{50} (µL/mL)
AgNP	2.909

In the toxicity study, it was found that the synthesized nanoparticles had a toxic effect against L929 cells and the IC_{50} value was found to be 2.909 µL/mL as can be seen in Table 1.

4. Discussion and Conclusion

In this study, the green chemistry method, which is cheap, eco-friendly, and suitable for large-scale production, was used for the synthesis of Ag NPs. Ag NPs were successfully synthesized using *C. roseus* extract. No reducing or stabilizing chemicals were used, except plant extract, during the synthesis phase. Moreover, high temperature, pressure, and additional ambient conditions were not required during the synthesis.

The synthesized Ag NPs were characterized by UV-Vis, TEM, XRD, and FT-IR analysis. According to the TEM and XRD analysis results, it was observed that nanoparticles in a fcc structure with a size of 16±6 nm were successfully synthesized.

Ag NPs are widely used due to their unique anti-inflammatory and antimicrobial properties compared to their bulk structures. However, in addition to these superior properties, it has been reported in some studies that it has negative effects on humans and the environment. For this reason, researchers are working on the toxicity of Ag NPs. The toxicity of Ag NPs depends on their shape, size, and surface modifications [17,18]. For example, Carlson et al. reported that exposure to 15 nm or 30 nm Ag nanoparticles significantly reduced cell viability, whereas 55 nm Ag nanoparticles had little effect [19]. In another study, Liu et all examined the cytotoxicity of Ag NPs of different sizes (5nm, 20nm, and 50 nm) against four cell lines: A549, HePG2, MCF-7, and SGC-7901, and found that 5nm sized Ag NPs were more toxic than the others [20]. It is believed that the cytotoxicity of Ag NPs is produced through reactive oxygen species (ROS), resulting in a decrease in glutathione level and an increase in ROS level. Kim and Ryu reported that they observed an increase in oxidative stress, apoptosis, and genotoxicity when exposed to silver nanoparticles in their in vitro study on animal tissue and culture cells [21]. In this study, we examined the toxic effect of Ag NPs with an average particle size of 16nm, which we synthesized in the plant extract, on the human health cell line and found that it had a toxic effect with an IC_{50} value of 2.909 µL/mL.

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Declaration of Ethical Code

In this study, we undertake that all the rules required to be followed within the scope of the "Higher Education Institutions Scientific Research and Publication Ethics Directive" are complied with, and that none of the actions stated under the heading "Actions Against Scientific Research and Publication Ethics" are not carried out.

References

1 Beyene, H.D., Werkneh, A.A., Bezabh, H.K. and Ambaye, T.G. (2017) Synthesis Paradigm and Applications of Silver Nanoparticles (AgNPs), a Review. *Sustainable Materials and Technologies*, **13**, 18–23. https://doi.org/10.1016/j.susmat.2017.08.001.

2 Aydin Acar, Ç. and Pehlivanoğlu, S. (2019) Gümüş Nanopartiküllerin Biberiye Özütü Ile Biyosentezi ve MCF-7 Meme Kanseri Hücrelerinde Sitotoksik Etkisi. *Süleyman Demirel Üniversitesi Sağlık Bilimleri Dergisi*, **10**, 172–176. https://doi.org/10.22312/sdusbed.543053.

3 Philip, D. (2009) Biosynthesis of Au, Ag and Au–Ag Nanoparticles Using Edible Mushroom Extract. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **73**, 374–381. https://doi.org/10.1016/j.saa.2009.02.037.

4 Hemlata, Meena, P.R., Singh, A.P. and Tejavath, K.K. (2020) Biosynthesis of Silver Nanoparticles Using *Cucumis Prophetarum* Aqueous Leaf Extract and Their Antibacterial and Antiproliferative Activity Against Cancer Cell Lines. *ACS Omega*, **5**, 5520–5528. https://doi.org/10.1021/acsomega.0c00155.

5 Patil, A.P., Kapadnis, K.H. and Elangovan, S. (2021) Antibacterial Applications of Biosynthesized AgNPs: A Short Review (2015-2020). *Material Science Research India*, **18**, 143–153.

6 Mallmann, E.J.J., Cunha, F.A., Castro, B.N.M.F., Maciel, A.M., Menezes, E.A. and Fechine, P.B.A. (2015) Antifungal Activity of Silver Nanoparticles Obtained by Green Synthesis. *Revista do Instituto de Medicina Tropical de São Paulo*, **57**, 165–167. https://doi.org/10.1590/S0036-46652015000200011.

7 Sharma, V.K., Yngard, R.A. and Lin, Y. (2009) Silver Nanoparticles: Green Synthesis and Their Antimicrobial Activities. *Advances in Colloid and Interface Science*, **145**, 83–96. https://doi.org/10.1016/j.cis.2008.09.002.

8 Akter, M., Sikder, Md.T., Rahman, Md.M., Ullah, A.K.M.A., Hossain, K.F.B., Banik, S., Hosokawa, T., Saito, T. and Kurasaki, M. (2018) A Systematic Review on Silver Nanoparticles-Induced Cytotoxicity: Physicochemical Properties and Perspectives. *Journal of Advanced Research*, **9**, 1–16. https://doi.org/10.1016/j.jare.2017.10.008.

9 Suliman Y, A.O., Ali, D., Alarifi, S., Harrath, A.H., Mansour, L. and Alwasel, S.H. (2015) Evaluation of Cytotoxic, Oxidative Stress, Proinflammatory and Genotoxic Effect of Silver Nanoparticles in Human Lung Epithelial Cells: Effects of Silver Nanoparticles in Human Lung Epithelial Cells. *Environmental Toxicology*, **30**, 149–160. https://doi.org/10.1002/tox.21880.

10 Oh, S.-J., Kim, H., Liu, Y., Han, H.-K., Kwon, K., Chang, K.-H., Park, K., Kim, Y., Shim, K., An, S.S.A. and Lee, M.-Y. (2014) Incompatibility of Silver Nanoparticles with Lactate Dehydrogenase Leakage Assay for Cellular Viability Test Is Attributed to Protein Binding and Reactive Oxygen Species Generation. *Toxicology Letters*, **225**, 422–432. https://doi.org/10.1016/j.toxlet.2014.01.015.

11 Prabhu, S. and Poulose, E.K. (2012) Silver Nanoparticles: Mechanism of Antimicrobial Action, Synthesis, Medical Applications, and Toxicity Effects. *International Nano Letters*, **2**, 32. https://doi.org/10.1186/2228-5326-2-32.

12 Aslam, J., Khan, S.H., Siddiqui, Z.H., Fatima, Z., Maqsood, M., Bhat, M.A., Nasim, S.A., Ilah, A., Ahmad, I.Z. and Khan, S.A. (2010) Catharanthus Roseus (L.) G. Don. An Important Drug: It's Applications and Production. *Pharmacie Globale (IJCP)*, **4**, 1–16.

13 Osibe, D.A., Chiejina, N.V., Ogawa, K. and Aoyagi, H. (2018) Stable Antibacterial Silver Nanoparticles Produced with Seed-Derived Callus Extract of *Catharanthus Roseus*. *Artificial Cells, Nanomedicine, and Biotechnology*, **46**, 1266–1273. https://doi.org/10.1080/21691401.2017.1367927.

14 Al-Shmgani, H.S.A., Mohammed, W.H., Sulaiman, G.M. and Saadoon, A.H. (2017) Biosynthesis of Silver Nanoparticles from *Catharanthus Roseus* Leaf Extract and Assessing Their Antioxidant, Antimicrobial, and Wound-Healing Activities. *Artificial Cells, Nanomedicine, and Biotechnology*, **45**, 1234–1240. https://doi.org/10.1080/21691401.2016.1220950.

15 Kotakadi, V.S., Rao, Y.S., Gaddam, S.A., Prasad, T.N.V.K.V., Reddy, A.V. and Gopal, D.V.R.S. (2013) Simple and Rapid Biosynthesis of Stable Silver Nanoparticles Using Dried Leaves of Catharanthus Roseus. Linn. G. Donn and Its Anti Microbial Activity. *Colloids and Surfaces B: Biointerfaces*, **105**, 194–198. https://doi.org/10.1016/j.colsurfb.2013.01.003.

16 Ponarulselvam, S., Panneerselvam, C., Murugan, K., Aarthi, N., Kalimuthu, K. and Thangamani, S. (2012) Synthesis of Silver Nanoparticles Using Leaves of Catharanthus Roseus Linn. G. Don and Their Antiplasmodial Activities. *Asian Pacific Journal of Tropical Biomedicine*, **2**, 574–580. https://doi.org/10.1016/S2221-1691(12)60100-2.

17 Siddiqi, K.S., Husen, A. and Rao, R.A.K. (2018) A Review on Biosynthesis of Silver Nanoparticles and Their

Biocidal Properties. *Journal of Nanobiotechnology*, **16**, 14. https://doi.org/10.1186/s12951-018-0334-5.

18 Tashi, T., Gupta, N.V. and Mbuya, V.B. (2016) Silver Nanoparticles: Synthesis, Mechanism of Antimicrobial Action, Characterization, Medical Applications, and Toxicity Effects. *J. Chem. Pharm. Res*, **8**, 526–537.

19 Carlson, C., Hussain, S.M., Schrand, A.M., K. Braydich-Stolle, L., Hess, K.L., Jones, R.L. and Schlager, J.J. (2008) Unique Cellular Interaction of Silver Nanoparticles: Size-Dependent Generation of Reactive Oxygen Species. *The Journal of Physical Chemistry B*, **112**, 13608–13619. https://doi.org/10.1021/jp712087m.

20 Liu, W., Wu, Y., Wang, C., Li, H.C., Wang, T., Liao, C.Y., Cui, L., Zhou, Q.F., Yan, B. and Jiang, G.B. (2010) Impact of Silver Nanoparticles on Human Cells: Effect of Particle Size. *Nanotoxicology*, Taylor & Francis, **4**, 319–330.

21 Kim, S., Choi, J.E., Choi, J., Chung, K.-H., Park, K., Yi, J. and Ryu, D.-Y. (2009) Oxidative Stress-Dependent Toxicity of Silver Nanoparticles in Human Hepatoma Cells. *Toxicology in Vitro*, **23**, 1076–1084. https://doi.org/10.1016/j.tiv.2009.06.001.