
MEDDOCS
International
IMPORTANCE & APPLICATIONS
OF NANOTECHNOLOGY

Low Cost Green Synthesis of Silver Nanoparticles using *Taiwan Oolong Tea* Extract

Cheng-You Tsou; Ruey-Jen Yang*

Department of Engineering Science, National Cheng Kung University, Tainan, Taiwan

Corresponding Author: Ruey-Jen Yang

Department of Engineering Science, National Cheng Kung University, Tainan, Taiwan

Tel: +886-6-2757575, Extn: 63343, Fax: +886-6-2766549

Email: rjyang@mail.ncku.edu.tw

Published Online: Aug 05, 2020

eBook: Importance & Applications of Nanotechnology

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Abstract

The organic compounds of *Taiwan Oolong tea* are used as low cost green reduction agents for the first time in this study to facilitate the biosynthesis of silver nanoparticles (AgNPs) using silver nitrate. The experiments commence by confirming the general feasibility of the proposed AgNP reaction process. A dilution method is then presented in which De-ionized (DI) water is added to the reaction solution to control the NP size and prevent metal oxide precipitation. It is shown that the synthesized AgNPs have a Polydispersity Index (PDI) of around 0.2 and an average particle size of less than 45 nm. They are thus of comparable quality to that of standard AgNPs produced using mainstream chemical synthesis methods. The results indicate that the proposed method provides a viable low-cost, low-energy and scalable route for the synthesis of AgNPs for a wide variety of applications.

Keywords: Green synthesis; Taiwan Oolong tea; Silver nanoparticles; Silver nitrate

Introduction

Biosynthesis methods have many advantages over traditional chemical synthesis routes, including a lower cost, minimal environmental impact, and the potential for one-pot synthesis. The literature contains many studies on the biosynthesis of silver nanoparticles (AgNPs) using plant extracts or tissue materials. For example, Rivera-Rangel et al. [1] used castor oil oily phase, Brij 96 V,1,2-hexanediol surfactants, and *Geranium* leaf aqueous extract to synthesize AgNPs with a size of 25~150 nm. Huang et al [2] synthesized AgNPs using *Cacumen Playcladi* extract. The results showed that the mean size and distribution of the AgNPs increased with an increasing silver nitrate precursor concentration. However, given a reaction temperature of 90°C, nanoparticles with a size of just 18.4 ± 4.6 mm could be obtained. Ahmed et al., [3] and Ahmed et al., [4] proposed green synthesis methods for the preparation of AgNPs using leaf extracts of *Skimmia laureola* and *Azadirachta indica*, respectively. Similarly, Baghizadeh et al [5] synthesized AgNPs using seed extract of *Calendula officinalis*. Dhand et al [6] proposed a zero-energy route for the preparation of AgNPs with a size of 20~30 nm using coffee seed extract as the reducing agent. Begum et al [7] used various aqueous solutions derived from black tea leaves to synthesize AgNPs. The results showed that stable nanoparticles of various shapes and sizes could be rapidly formed when using plain tea leaf broth or broth containing ethyl acetate as a precursor.

Gondwal and Joshi Nee Pant [8] synthesized AgNPs and CuNPs using aqueous leaf extract of *Cassia occidentalis*. The two NPs showed excellent antibacterial activity against *Escheri-*

chia coli and *Salmonella typhi* bacteria, respectively. However, the AgNPs showed a lower toxicity than the CuNPs. Other similar studies confirming the favorable antibacterial properties of AgNPs fabricated through green synthesis routes (Logeswari et al. [9]; Shivakumar et al. [10]; Sondi and Salopek-Sondi [11]). Khatami et al [12] synthesized colloidal AgNPs with an average size of 12 nm using pine pollen as both a reducing agent and a capping agent. The antifungal properties of the NPs were evaluated *in vitro* and were shown to be highly effective in inhibiting and disrupting fungal growth. Kim et al [13] and Larue et al [14] showed that biosynthetic AgNPs not only have an excellent antibacterial and antifungal performance, but are also harmless to common plants, such as wheat and lettuce. As a result, they have significant potential as new anti-pathogenic agents in place of harmful pesticides. Kathiravan et al. [15] synthesized AgNPs using the plant extract of *Melia dubia*. The resulting NPs were tested on human breast cancer cells and were shown to exhibit both a remarkable cytotoxicity effect and a high therapeutic index. Recently, Valarmathi [16] successfully utilized a marine source of *S. filamentosa* to synthesize AgNPs for the first time. The produced AgNPs are sphere-shaped with the range between 20 and 30 nm in size. Sana and Dogiparthi [17] reported a simple, rapid, efficient and eco-friendly approach to produce AgNPs with bio-reduction of silver ions and aqueous extract of *G. Moluccana*. This method is also useful for large scale production of nanoparticles and could result in economic viability. Ahn et al [18] synthesized silver nanoparticles using thirty Chinese plant extracts via a green synthetic strategy. They showed that among the thirty extracts, seven extracts (*Cratoxylum formosum, Phoebe lanceolata, Scurrula parasitica, Ceratostigma minus, Mucuna birdwoodiana, Myrsine africana and Lindera strychnifolia*) exhibited the successful synthesis of silver nanoparticles. These results provide ample and systematic information for researchers on the green synthesis of silver nanoparticles using plant extracts.

Given the effective antibacterial and antifungal properties of biosynthetic AgNPs, together with their significant potential for pharmaceutical science and anti-cancer therapy, the synthesis of AgNPs through green, low-energy methods has attracted significant attention these days. However, very few low-energy or even zero-energy methods have thus far been proposed for controlling the biosynthesis process. Accordingly, the present study proposes a novel technique for controlling the growth of AgNPs produced using the leaf extract of *Taiwan oolong tea* by diluting the reaction mixture using carefully controlled amounts of De-Ionized (DI) water. The observation results show that the dilution procedure slows the reaction process and enables the synthesis of AgNPs with a quality comparable to that of NPs produced using a standard chemical synthesis route.

Material and methods

Silver nitrate was purchased from Panreac, Inc. (Taiwan), and used without further purification. *Oolong tea* leaf was purchased from Songxing Tea Factory in Changhua County, Taiwan. Finally, DI water was obtained from the Direct-Q5 system purchased from Merck (Germany).

2 grams of *oolong tea* leaves were added to 600 ml of DI water and left to stand for 24 hours. The tea broth was transferred to a covered 1000 ml beaker and heated. Once the broth reached boiling conditions, it was removed from the heat and allowed to cool naturally to room temperature. Finally, the broth was filtered through Waterman Paper #1 and stored in a refrigerator at 6°C to maintain a constant extract concentration throughout all of the experiments.

Initial concentration of silver nitrate

10 ml of silver nitrate with concentrations of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mM, respectively, was placed in six 100 ml clean beakers. 1 ml of tea extract was added to each beaker for 1 hour to synthesize AgNPs.

Volume of tea extract

0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 ml of tea extract was added to six 100 ml clean beakers. 10 ml of 1 mM silver nitrate solution was added to each beaker and left to stand for 1 hour to synthesize AgNPs.

Dilution method

10 ml of 1 mM silver nitrate solution was added to a 100 ml clean beaker. 1 ml of tea extract was added to the beaker and was allowed to stand for 15 minutes; resulting in a color change from light yellow to golden yellow. 90 ml of DI water was then added to the reaction solution and left to stand for 12 hours.

Nucleation time

10 ml of 1 mM silver nitrate was added to two 100 ml clean beakers. 1 ml of tea extract was added to each beaker and allowed to stand for 5 minutes and 20 minutes, respectively. 90 ml of DI water was then added to each beaker and left to stand for 12 hours.

Dilution ratio

10 ml of 1 mM silver nitrate was added to two 100 ml clean beakers. 1 ml of tea extract was added to each beaker and allowed to stand for 20 minutes. 90 ml and 190 ml of DI water was added to the two beakers, respectively, and left to stand for 24 hours.

Instruments

The absorbance spectra of the various reaction solutions were measured using a UV/vis spectrophotometer (GENESYS 10S, Thermo Fisher, USA). The size of the AgNPs was measured using a dynamic light scattering (DLS) instrument (Delsa Nano C, Beckman, USA).

Results and discussion

Green synthesis principle of nanoparticles

Huang et al [2] measured the concentrations of four components of *Cacumen Platycladi* extract (i.e., reducing sugar, flavonoids, saccharide and protein) before and after the AgNP reaction process, respectively. The results indicated that the reaction process was dominated by the reducing sugars and flavonoids. Ajitha et al [19] prepared AgNPs using *Momordica charantia* leaf broth and found a significant change in the C-O-C bonds in the molecular structures of the glucose and flavonoid components of the extract following the reaction process (see **Figure 1**). Hence, it was again inferred that the glucose and flavonoids played a key role in the AgNP synthesis process. Based upon this principle, the organic compounds in Taiwan oolong tea leaf are used as green reduction agents to facilitate the biosynthesis of silver nanoparticles for the first time.

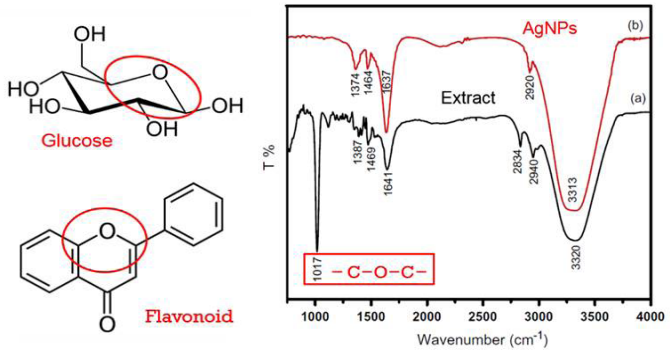

Figure 1: C-O-C-bond loss after AgNP reaction (Ajitha et al. [19]).

Effects of initial silver nitrate concentration

Figure 2 shows the UV/vis absorbance spectra of the reaction solutions prepared using silver nitrate solutions with concentrations of 0.1~1.0 mM. It is seen that for silver nitrate concentrations in the range of 0.4~1.0 mM, the absorption peak intensity increases approximately linearly with an increasing nitrate concentration. Moreover, a small blue-shift in the absorption spectrum occurs. However, for silver nitrate concentrations of 0.1 and 0.2 mM, the absorbance spectra are virtually the same and the absorption intensity has a low and approximately constant value for wavelengths of more than 400 nm. Hence, it is inferred that a low silver nitrate concentration results in a slow, or even stagnant, reaction process.

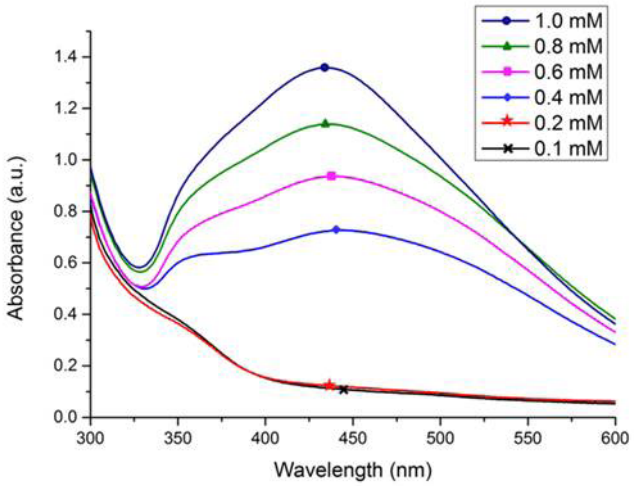

Figure 2: Absorbance spectra for reaction solutions produced using 1 ml of tea extract and silver nitrate concentrations of 0.1~1.0 mM.

Effects of tea extract volume

Figure 3(a) shows the UV/vis absorption analysis results for the reaction solutions produced with a constant silver nitrate concentration of 1 mM and tea extract volumes of 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 ml, respectively. For each sample, the peak absorption intensity occurs at a wavelength of 440 nm. Moreover, the magnitude of the peak intensity increases with an increasing extract volume. Hence, it is inferred that the amount of AgNPs synthesized in the reaction process increases with an increasing tea extract volume. An inspection of **Figure 3(b)** confirms that the quantity of the AgNPs increases linearly with an increasing extract volume with a correlation ratio of $R^2 = 0.9964$. In other words, the reaction process for extract volumes of 0.6 ~ 1.6 ml is predictable and stable.

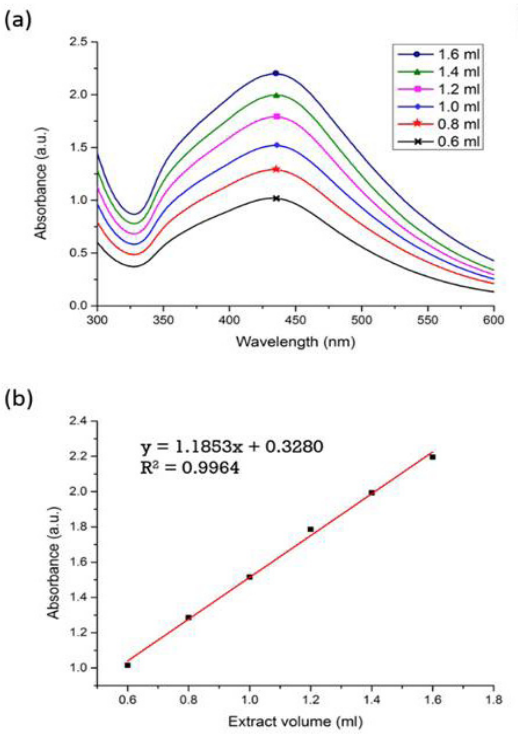

Figure 3: **(a)** Absorbance spectra for reaction solutions produced using silver nitrate concentration of 1 mM and tea extract volumes of 0.6 ~ 1.6 ml. **(b)** Regression analysis results for relationship between absorbance peak intensity and tea extract volume.

Effects of dilution control method

As described above in relation to **Figure 2**, for low concentrations of silver nitrate (i.e., 0.1 mM or 0.2 mM), a slow and almost stagnant reaction process is induced. In the present study, this phenomenon is deliberately exploited to prevent the excessive growth of the AgNP particles in the reaction process. In particular, 10 ml of 1 mM silver nitrate solution is initially reacted with 1 ml of tea extract for 15 minutes to synthesize AgNPs as seed crystals, and 90 ml of DI water is then added to the reaction solution. The solution is left to stand for 12 hours, during which time the seed crystals continue to slowly catalyze the metal ions not previously reacted in the solution to produce further AgNPs. **Figures 4(a) and 4(b)** show the initial reaction solution after 15 minutes and the diluted solution after 12 hours, respectively. **Figure 5** presents the DLS analysis results for the AgNP particle size after 12 hours. An inspection of the DLS results shows that the AgNPs have an average particle size of 45 nm and a Polydispersity index (PDI) of 0.264.

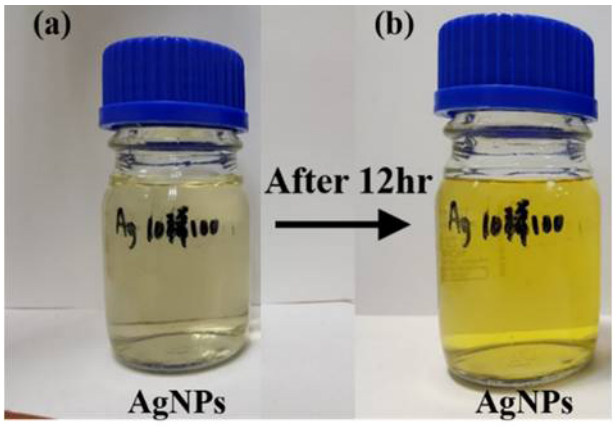

Figure 4: **(a)** Initial reaction solution with 1 mM silver nitrate. **(b)** Diluted reaction solution after 12 hours.

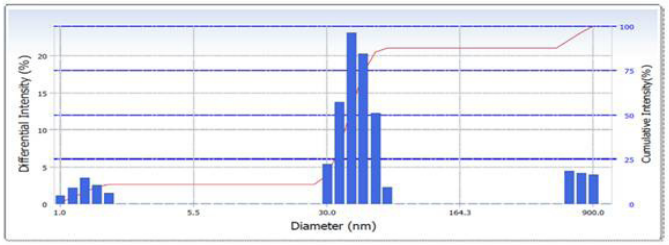

Figure 5: DLS analysis results for AgNP size distribution after 12 hours.

Effects of dilution ratio and nucleation time

Figure 6(a) shows the absorbance spectra of the diluted reaction solutions prepared with 90 ml and 190 ml of DI water, respectively. As expected, the peak absorption intensity of the solution diluted with 90 ml of DI water is higher than that of the solution diluted with 190 ml of DI water due to the greater AgNP concentration. Moreover, the DLS results show that the AgNPs prepared with 90 ml of DI water have a mean size of 37.5 nm, whereas those prepared with 190 ml of DI water have a slightly larger size of 39.5 nm.

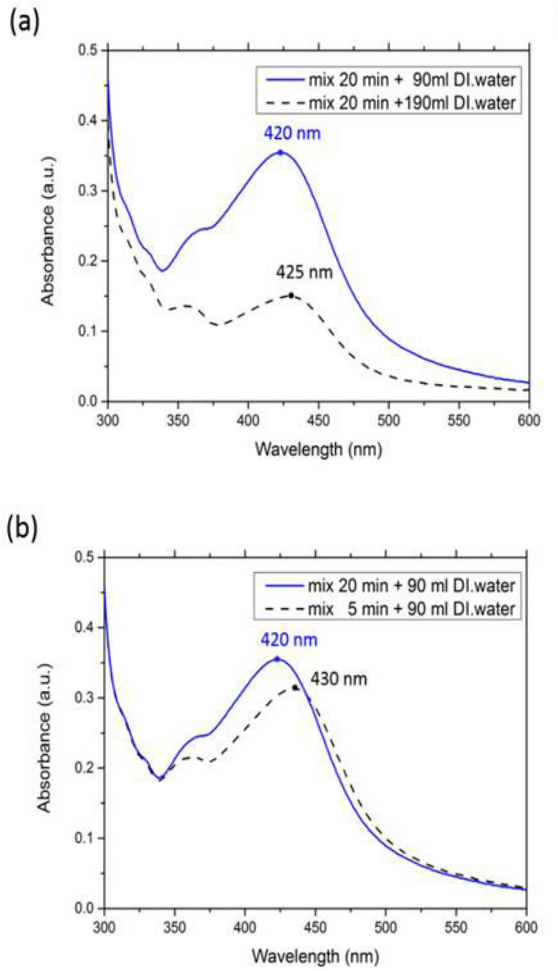

Figure 6: **(a)** Absorbance spectra of AgNP solutions diluted with 90 ml and 190 ml of DI water, respectively. **(b)** Absorbance spectra of diluted AgNP solutions with initial reaction times of 5 minutes and 20 minutes, respectively.

Figure 6(b) shows the absorbance spectra of the diluted AgNP solutions prepared with initial reaction times of 5 minutes and 20 minutes, respectively. As expected, the absorption peak intensity of the solution prepared with an initial reaction time of 20 minutes is greater than that of the solution prepared with a reaction time of 5 minutes due to the corresponding increase in the AgNP size. The difference between the reaction solutions is obvious even to the naked eye; with the solution prepared using a reaction time of 5 minutes having a dim yellow appearance and that with a reaction time of 20 minutes having a far darker yellow hue. Furthermore, the DLS analysis results confirm that the AgNPs with a 20-minute reaction time have an average size of 37.5 mm, while those prepared with a 5-minute reaction time have a slightly smaller size of 36 nm.

Conclusion

The present study has proposed a method for the synthesis of AgNPs using the leaf extract from *Taiwan Oolong tea* for the first time as the reducing and capping agent and silver nitrate as the precursor. The results have confirmed that the intensity of the AgNP reaction process increases with both an increasing leaf extract volume and an increasing silver nitrate concentration. However, for a low silver nitrate concentration (less than 0.2 mM), a stagnant reaction process occurs. This phenomenon has been exploited in the present study as a low-cost and low-energy control method for preventing excessive AgNP growth and metal oxide precipitation. The DLS results have shown that the AgNPs have a mean size of 45 nm and a polydispersity index of 0.264. It is noted that these properties are consistent with those of AgNPs fabricated through traditional chemical routes. Overall, the results indicate that the proposed method provides a viable low-cost, low-energy and scalable route for the synthesis of AgNPs for a wide variety of applications.

References

1. Rivera-Rangel RD, González-Muñoz MP, Avila-Rodriguez M, Razo-Lazcano TA, Solans C. Green synthesis of silver nanoparticles in oil-in-water microemulsion and nano-emulsion using geranium leaf aqueous extract as a reducing agent. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2018; 536: 60-67.

2. Huang J, Zhan G, Zheng B, Sun D, Lu F, et al. Biogenic silver nanoparticles by Cacumen Platycladi extract: Synthesis, formation mechanism, and antibacterial activity. Industrial & Engineering Chemistry Research. 2011; 50: 9095-9106.

3. Ahmed MJ, Murtaza G, Mehmood A, Bhatti TM. Green synthesis of silver nanoparticles using leaves extract of Skimmia laureola: Characterization and antibacterial activity. Materials Letters. 2015; 153: 10-13.

4. Ahmed S, Saifullah MA, Swami BL, Ikram S. Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. Journal of Radiation Research and Applied Sciences. 2016; 9: 1-7.

5. Baghizadeh A, Ranjbar S, Gupta VK, Asif M, Pourseyedi S, et al. Green synthesis of silver nanoparticles using seed extract of Calendula officinalis in liquid phase. Journal of Molecular Liquids. 2015; 207: 159-163.

6. Dhand V, Soumy L, Bharadwaj S, Chakra S, Bhatt D, et al. Green synthesis of silver nanoparticles using Coffea arabica seed extract and its antibacterial activity. Mater Sci Eng C Mater Biol Appl. 2016; 58: 36-43.

7. Begum NA, Mondal S, Basu S, Laskar RA, Mandal D. Biogenic synthesis of Au and Ag nanoparticles using aqueous solutions of

Black Tea leaf extracts. Colloids Surf B Biointerfaces. 2009; 71: 113-8.

8. Gondwal M, Pant GJN. Synthesis and catalytic and biological activities of silver and copper nanoparticles using Cassia occidentalis. Int J Biomater. 2018;6735426.

9. Logeswari P, Silambarasan S, Abraham J. Ecofriendly synthesis of silver nanoparticles from commercially available plant powders and their antibacterial properties. Scientia Iranica. 2013; 20: 1049-1054.

10. Shivakumar M, Nagashree KL, Yallappa S, Manjappa S, Manjunath KS, et al. Biosynthesis of silver nanoparticles using pre-hydrolysis liquor of Eucalyptus wood and its effective antimicrobial activity. Enzyme Microb Technol. 2017; 97: 55-62.

11. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: A case study on E. coli as a model for Gram-negative bacteria. J Colloid Interface Sci. 2004; 275: 177-182.

12. Khatami M, Mortazavi SM, Kishani-Farahani Z, Amini A, Amini E, et al. Biosynthesis of silver nanoparticles using Pine Pollen and evaluation of the antifungal efficiency. Iran J Biotechnol. 2017; 15: 95-101.

13. Kim DY, Saratale RG, Shinde S, Syed A, Ameen F, et al. Green synthesis of silver nanoparticles using Laminaria japonica extract: Characterization and seedling growth assessment. Journal of Cleaner Production. 2018; 172: 2910-2918.

14. Larue C, Castillo-Michel H, Sobanska S, Cecillon L, Bureau S, et al. Foliar exposure of the crop Lactuca sativa to silver nanoparticles: Evidence for internalization and changes in Ag speciation. J Hazard Mater. 2014; 264: 98-106.

15. Kathiravan V, Ravi S, Ashokkumar S. Synthesis of silver nanoparticles from Melia dubia leaf extract and their in vitro anticancer activity. Spectrochim Acta A Mol Biomol Spectrosc. 2014; 130: 116-21.

16. Valarmathi N, Ameen F, Almansob A, Kumar P, Arunprakash S, et al. Utilization of marine seaweed Spyridia filamentosa for silver nanoparticles synthesis and its clinical applications. Materials Letters. 2020; 263: 15 127244.

17. Sana SS, Dogiparthi LK. Green synthesis of silver nanoparticles using Givotia moluccana leaf extract and evaluation of their antimicrobial activity. Materials Letters. 2018;226: 47-51

18. Ahn EY, Jin H, Park Y. Assessing the antioxidant, cytotoxic, apoptotic and wound healing properties of silver nanoparticles green-synthesized by plant extracts. Mater Sci Eng C. 2019; 101: 204-216

19. Ajitha B, Reddy YA,Reddy PS. Biosynthesis of silver nanoparticles using Momordica charantia leaf broth: Evaluation of their innate antimicrobial and catalytic activities. J Photochem Photobiol B. 2015; 146: 1-9.