# One-step Green Preparation of Nano-silver Aqueous Solution, Characterization, and Its Antibacterial Potential for Cut Carnation Flowers

**Zhenpei Pang**
Zhongkai University of Agriculture and Engineering
**Hongmei Li**
Zhongkai University of Agriculture and Engineering
**Guidong Lin**
Zhongkai University of Agriculture and Engineering
**Yunhao Sun**
Zhongkai University of Agriculture and Engineering
**Hongjun Zhou**
Zhongkai University of Agriculture and Engineering
**Shenggen He** ( shenggenzhku@163.com )
Zhongkai University of Agriculture and Engineering

**Research Article**

**Keywords:** nano-silver (NS), green synthesis, antibacterial potential, cut carnation flowers, PVP

**Posted Date:** March 13th, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-284927/v1

# Abstract

The green synthesis of nano-silver (NS) has gained increasing attention owing to its cost-effectiveness and environmental friendliness. Herein, we have described a novel one-step green preparation method of stable NS aqueous solution using Tollens reagent as the silver ion source, D-fructose as the reduction agent, and polyvinyl pyrrolidone (PVP) as the stabilizing and capping agent. The optimum preparation parameters were 40 mL 10 g $L^{-1}$ PVP, 100 mL 0.116 mol $L^{-1}$ D-fructose, and 200 mL 0.001 mol $L^{-1}$ Tollens at a reaction temperature of 25 °C for 2 h. The prepared NS solution had a pH value of 7.10, with approximately spherical NS particles in the range of 13.95–87.36 nm. The resulting NS solution was stable under normal temperature, boiling water bath, and acidic environment; however, it did not exhibit stability under an alkaline environment. The NS solution had a remarkable absorption peak at 410 nm in its ultraviolet visible spectrum. Moreover, it exerted strong inhibitory effect on the growth of the bacteria isolated from the vase water of cut carnation 'Prince' flowers. These findings indicate that the obtained NS aqueous solution is potentially a practical and effective antibacterial agent for preserving cut carnations and other cut flowers.


# Introduction

Nano-silver (NS) is a novel material with a high surface-to-volume ratio. Owing to its unique physicochemical, biological, and antimicrobial properties, it is widely used in textiles, medical equipment, nonlinear optical devices, wastewater treatment, building materials, preservation of horticultural products, and other fields. Currently, it is regarded as one of the most promising materials of the century[1–7].

After harvesting cut flowers and foliage, the microbial contamination of the vase solution is the most common factor that causes stem blockage and shortened vase life. Microorganisms in cut flowers and foliage vase solution cause physical stem blockage, release toxic metabolites and enzymes, and produce ethylene[8]. To address this problem, there have been reported on the effectivity of NS postharvest treatment in maintaining the freshness of cut flowers, such as snapdragon, carnation, gladiolus, and gardenia foliage[6,7,9–11]. Particularly, NS has been used as an antimicrobial agent and ethylene inhibitor in packaging to extend the vase life of cut flowers and foliage[12]. It could prevent bacterial growth and effectively alleviate bacterial blockage in the vase solution and cut flower stem ends, thereby extending vase life; thus, NS has good application prospects in the field of postharvest storage and transportation of cut flowers.

To date, NS can be prepared by physical, chemical, and biological reduction approaches. Among these, chemical approaches are the most commonly used method[13–15]. Although chemical processes are convenient, they have a high preparation cost and negative effects to the environment; thus, it is not preferred in several research and applications[16–20]. Bioreduction is a novel method in preparing NS using various reducing substances or products in plants and microorganisms. It has the advantages of cost-

effectiveness and environmental benignity; therefore, there has been increasing interest in its research and application[19–25].

In recent years, there have been reports on the green preparation of NS using various plant tissue extracts, plant extract products, and *in vitro* products of microorganisms. Das et al. reported that NS solution produced by biosynthesis has significant effect on the postharvest preservation of mulberry leaves[26]. Xia et al. reported a biological method in synthesizing NS using the callus extracts from *Artemisia annua* L. under sunlight at 25000 lx. This confirmed that NS pulse can markedly inhibit bacteria growth in the vase solution during the whole vase period, alleviate bacterial blockage at the stem ends, and eventually prolong the vase life of cut carnation flowers[27]. Eze and Nwabor synthesized NS with an average particle size of 44.78 nm from protein expression of *Pichia pastoris* spent waste medium, confirming that NS strongly inhibits common foodborne pathogenic microbes, such as tyrosinase and pathogenic bacteria, *B. cereus*, and *E. coli*[5]. Dutta et al. also showed that the green synthesis of NS using *Citrus limetta* peel extract has a clear antibiofilm efficacy against major topical bacterial pathogens[28]. Philip synthesized NS sized 4 nm at room temperature using an aqueous solution of $AgNO_3$ and honey at pH 8.5[29]. Ravendran et al. reported an environmentally friendly method of preparing Au and Ag nanoparticles with glucose as the reducing agent and starch as the protecting agent[30]. Peng et al. synthesized NS by microwave irradiation with bamboo hemicellulose as the stabilizing agent and glucose as the reducing agent in an aqueous medium[31]. There are also relevant reports on the application of honey in the synthesis of NS[29,32]. Honey is used in the preparation of NS by utilizing the reducibility of fructose and glucose in its ingredients. Furthermore, Filippo et al. reported the rapid synthesis of NS by treating silver nitrate aqueous solution with maltose and sucrose as reducing agents[33]. Researchers have also reported the production of monodispersed spherical NS using fructose in a continuous flow reaction-discharge system[34].

In this study, we successfully conducted the green synthesis of a stable neutral NS aqueous solution using D-fructose as the reducing agent and polyvinyl pyrrolidone (PVP) as the capping agent. The proposed synthesis is a simple, fast, economical, and environmentally benign method that uses sustainable materials. Additionally, the preparation conditions, parameters, and basic properties of the NS solution were investigated. The optical properties of the green synthesized NS were analyzed by ultraviolet visible (UV-Vis) spectrophotometry and their granulometric properties were characterized by transmission electron microscopy (TEM). Finally, the inhibitory effect of the resulting NS against bacteria isolated from the vase water of cut carnation flowers was studied to establish a foundation for its further use in postharvest treatment and preservation of cut flowers.

# Results

**Green preparation of the NS aqueous solution.** The green preparation of NS aqueous solution with D-fructose was confirmed by the color changes of the solution after 2 h of incubation. The color of the solution changes with the reaction time (Fig. 1a–d) from light yellow (Fig. 1a) to dark yellow (Fig. 1b and

1c), and finally to reddish brown (Fig. 1d). The synthesis of NS in the solution is preliminarily determined after the reaction.

**$Ag^+$ ion residue in the prepared NS solution.** Compared with the control group with Ultra-pure water (UPW), the NS stock solution with NaCl solution did not exhibit any color change, turbidity, or precipitation (Fig. 2a and 2b), indicating that the NS solution had little to no $Ag^+$ ion residue.

**pH value, and thermal and acid-base stability of the prepared NS aqueous solution.** The prepared NS stock solution remained clear without noticeable agglomeration throughout the reaction process, with a neutral pH value of 7.10. Compared with the initial NS stock solution (Fig. 3a), there were no changes in the NS solution after 30 min of boiling water bath (Fig. 3b), indicating its good thermal stability. In addition, there were no evident changes in the NS solution after the addition of 1 mL 0.1 mM $HNO_3$ solution (Fig. 3c), demonstrating its good acidic stability. However, after adding 1 mL 0.1 mM NaOH solution, the color rapidly changed from reddish brown to grayish green (Fig. 3d), indicating the instability of the prepared NS solution under strong alkali condition.

**UV-Vis absorption spectrums.** The UV-Vis absorption spectra of the prepared NS solution NS solution exhibited a characteristic surface plasmon resonance band at 410 nm (Fig. 4) for the NS solution prepared with D-fructose, which is the characteristic absorption peak of the NS solution. This confirmed the formation of spherical NS particles. Furthermore, narrow absorption peaks and a smooth absorption spectrum were observed (Fig. 4), revealing the small particle size of the synthesized NS, and their monodispersion and non-aggregation. The appearance of the NS aqueous solution was stable after more than two months, as shown from the UV-Vis absorption spectrum of the NS.

**NS particle size.** TEM observation revealed the approximately spherical shape of the prepared NS particles and their distribution (Fig. 5a). The diameter of the NS particles varied from 13.95 to 87.36 nm with an average particle size of 61.39 nm. The size distribution histograms further showed the highest proportion (40.43%) of the particle size range of 61–80 nm, followed by 71–90 nm (35.11%) (Fig. 5b).

***In vitro* evaluation of antibacterial activity.** The antibacterial activity of the prepared NS aqueous solutions was tested against the bacteria isolated from the vase water of cut carnation flowers. Compared with the control (0.9% sterile saline), the circular filter paper wells exhibited distinct bacteriostatic rings (Fig. 6a–d). The results showed the notable inhibitory activity of the NS solution against the growth of the bacteria from the vase water of cut carnation flowers. Further observation of the zone size and visibility of the bacteriostatic ring of the circular filter paper wells showed that higher concentration of the NS solution resulted in a clearer bacteriostatic ring, more pronounced bacteriostatic effect (Fig. 6b, c, and d), and larger inhibition zone diameter. Additionally, the NS stock solution had the largest inhibition zone diameter of 10.87 mm, followed by the two-fold dilution and four-fold dilution of the NS solution, which were 9.86 and 7.86 mm, respectively. However, there was no bacteriostatic ring observed in the control group with circular filter paper wells dipped with sterile saline (Fig. 6a).

# Discussion

NS is a novel and excellent antibacterial material. In the recent years, its preparation and application has become a research hot spot in the field of nanomaterials[1,2,35]. However, at present, NS is mainly synthesized through chemical processes. Although the chemical preparation of NS is highly efficient, the chemicals used in the preparation process undoubtedly have adverse effects on the environment. With the development of environmentally benign technologies in material synthesis, there is increasing attention on the biosynthesis of nanoparticles[29]. The green synthesis of nanoparticles is a significant new research field of nanotechnology that has the advantages of simplicity, environmental benignity, and cost effectiveness, compared to chemical and physical approaches[8]. In the present study, neutral NS was successfully green prepared with D-fructose as the reducing agent and PVP as the capping agent.

The antibacterial properties of NS are related to the shape, size, dispersion, aggregation, and solubility of the nanoparticles[36]. The smaller size and better dispersion of NS result in a higher antibacterial activity. The antibacterial and dispersive properties of NS play an important role in the postharvest vase treatment and preservation of cut flowers to inhibit the growth of microorganisms in the vase solution and display an antagonizing ethylene effect as it is adsorbed on various parts of the cut flowers[6,7,12]. NS particles are prone to aggregation due to their strong intermolecular force, thereby influencing their antibacterial effect. Accordingly, it is critical to prevent the aggregation of NS particles and maintain their dispersion and uniform particle size. Capping agents are typically used to prevent the aggregation of NS particles. In this study, PVP was used as the capping agent to inhibit the aggregation of the NS particles during synthesis. Finally, an NS solution with good stability and uniform particle distribution (Fig. 5) was obtained, which are desirable properties for various applications of NS.

The synthesis of nanoparticles with antibacterial activities is an important direction in the development of new pharmaceutical products[37]. The green preparation of NS using pineapple peel extracts had evident antibacterial activity against pathogenic bacterial strains, *Pseudomonas aeruginosa* and *Bacillus subtilis*[38]. Dangi et al. reported that NS biosynthesis using *Berberis asiatica* root extract showed good antibacterial activity against *K. pneumoniae*, *E. coli*, *S. aureus*, and *S. typhimurium*. On the other hand, NS and antibiotics have a good synergistic activity that has high inhibition against *K. pneumoniae*[39]. In addition, several studies have shown that NS has outstanding ability to maintain the freshness of cut flowers, confirming its excellent germicidal effect and antagonizing effect against ethylene[6,7,10,11]. Four dominant bacteria (*Arthrobacter arilaitensis*, *Kocuria* sp., *Staphylococcus equorum*, and *Microbacterium oxydans*) that reduce vase life were isolated from the stem ends of cut carnation flowers, thereby proving that biosynthetic NS treatments significantly inhibit bacterial growth in the vase solution and cut stem ends of flowers and foliage during their vase life[12,26]. The green synthesized NS was used as the antimicrobial agent against three bacterial genera that were involved in decreasing the longevity of cut flower, showing the efficient antibacterial activity of NS against these bacteria[8]. In the present study, with the premise that the prepared NS solution did not have $Ag^+$ ion residue (Fig. 2), the bacteriostatic ring test showed that the green synthesis of NS using D-fructose had clear inhibitory effect on the growth of the

bacteria isolated from the vase water of cut carnation flowers with a dose-dependent effect (Fig. 6b–d). A neutral NS aqueous solution with good stability was obtained by a simple, convenient, and environmentally friendly one-step preparation, which can reduce the high cost and environmental pollution concerns of conventional synthesis methods.

# Conclusions

In the present work, our results demonstrated the feasibility of the green synthesis of stable neutral NS aqueous solution using D-fructose as the reducing agent and PVP as the capping agent. The proposed synthesis method has the advantages of simplicity, shorter time, and reduced use of toxic chemical reagents, compared to conventional chemical preparation methods. Thus, it could effectively reduce the preparation cost and adverse environmental impacts of NS synthesis. The resulting NS aqueous solution has good stability with well dispersed NS particles. Additionally, we investigated the optical properties of the NS solution using UV-Vis spectrophotometry to measure the particle size and shape of the NS particles using TEM and assess their antibacterial activity by bacteriostatic ring test. This study revealed the preparation conditions, parameters, basic characteristics, and characterization of the NS solution. Considering that the synthesized NS solution had strong inhibitory effect on the growth of the bacteria isolated from the vase water of cut carnations, it may serve as a practical and effective antibacterial agent for preserving cut carnations and other cut flowers. Future research objectives could include the optimization of the preparation conditions, and development of a new type of preservative and other products for the postharvest preservation and treatment of cut flowers.

# Materials And Methods

**Materials.** Silver nitrate ($AgNO_3$), sodium chloride (NaCl), ammonia ($NH_3{\bullet}H_2O$, 25% aqueous solution), nitric acid ($HNO_3$), sodium hydroxide (NaOH), and PVP were purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China). D-fructose ($C_6H_{12}O_6$, MW = 180.16) was purchased from Aladdin (Shanghai, China). All reagents were of analytical grade. The bacteria isolated from the vase water of cut carnation ‘Prince’ flowers were provided by the Biotechnology Institute, Zhongkai University of Agriculture and Engineering. Ultra-pure water (UPW) was used for the experiments.

**Green preparation of NS aqueous solution.** First, 40 mL aqueous solution of 10 g $L^{-1}$ PVP, 100 mL 0.116 mol $L^{-1}$ D-fructose, and 200 mL 0.001 mol $L^{-1}$ Tollens reagent, which was produced by $AgNO_3$ and dilute ammonia water, was added to a 500 mL volumetric conical flask. The volume of the solution was increased to 500 mL with the addition of UPW. The conical flask was shaken while adding the previously mentioned liquid each time. The mouth of the conical flask was sealed with polyethylene film after adding all the solutions. The solution was then reacted at a room temperature of 25°C for approximately 2 h and shaken every 5 min. The block scheme of the 21.57 mg $L^{-1}$ NS stock solution used for the experiments is shown in Fig. 1.

**$Ag^+$ ion detection.** To detect $Ag^+$ ion residue in the prepared NS solution, 1 mL 0.1 mM NaCl solution was placed in a test tube and 1 mL NS stock solution was added and shaken until sufficient turbidity was observed. A control of 1 mL UPW added to 1 mL NS stock solution was also prepared. The two tubes were compared to observe the turbidity in each solution.

**pH value determination.** The prepared NS stock solution (10 mL) was added to a test tube and its pH value was measured with a portable pH meter (pH-902, EDKORS, Japan).

**Stability test in high-temperature boiling water bath.** The prepared NS stock solution (5 mL) was placed in a test tube and its physical changes were observed after a 30-min treatment in a high-temperature boiling water bath. Another 5 mL NS stock solution was transferred to another tube without high-temperature treatment for comparative observation.

**Stability test in acidic and alkaline conditions.** The prepared NS stock solution (5 mL) was added to test tubes with 1 mL 0.1 mM nitric acid solution and 1 mL 0.1 mM NaOH solution, respectively. The physical changes were observed after shaking the solutions and allowing it to stand for 10 min. Another 5 mL stock solution and 1 mL UPW were placed in another tube for comparative observation.

**UV-Vis spectrum analysis.** The prepared NS stock solution (4 mL) was placed in a quartz cuvette with 10 mm optical path after diluting the NS solution four times. UPW was used as the reference. Their UV-Vis absorption spectra were recorded in the range of 300–700 nm with 1 nm spectral bandwidth using a UV-Vis spectrophotometer (UV-2600, Shimadzu, Japan).

**Particle size observations.** The NS stock solution (100 µL) was absorbed and dried on carbon-coated copper grids. The size, size distribution, and morphology of the NS particles were observed by TEM (JEM-1400, JEOL Ltd., Japan) at a working voltage of 120 kV. The size of the NS particles was measured by cross method using the measurement software Image Pro Plus 6.0 (Media Cybernetics Inc., USA). More than 90 NS particles were noted.

**Assessment of antibacterial activity.** The antibacterial activity of the prepared NS was tested using the bacteria isolated from the vase water of cut carnation 'Prince' flowers by agar disk diffusion method. First, 20 mL sterilized nutrient agar was poured into petri dishes with 9.0 cm diameter while it was hot, and was allowed to cool and solidify at room temperature (25°C). The bacteria isolated from the vase water of cut carnation flowers were diluted to a concentration of $10^5$–$10^6$ CFU $mL^{-1}$ using sterile normal saline solution. Each 40 µL liquid bacterial germ was uniformly coated on the individual plates using a sterile coating rod. Using tweezers, three sterile circular filter paper wells of 5.5 mm diameter were placed on each plate. The NS stock solution (15 µL, initial concentration: 21.57 mg $L^{-1}$), two-fold dilution of the NS stock solution (concentration: 10.79 mg $L^{-1}$), and four-fold dilution of the NS stock solution (concentration: 5.39 mg $L^{-1}$) were added on the circular filter paper wells using micropipettes, respectively. In addition, 15 µL sterile saline was used as the control. Then, the plates were incubated at 37°C for 12 h and were inspected to determine their inhibition zone. The diameter of the inhibition zone

was measured by cross method using the measurement software Image Pro Plus 6.0 (Media Cybernetics Inc., USA).

# Declarations


### Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (Grant numbers 31672180 and 31972439), the Natural Science Foundation of Guangdong Province (Grant number 2019A1515011058), and Funds for Nanomaterial Innovation Team Construction of Guangdong Provincial Modern Agriculture Industry Technology System (2019KJ140).


### Author contributions

Z. P. conducted the experiments, analyzed the data, and wrote the draft manuscript. S. H. supervised the project and conceived the experiments. H. L. interpreted obtained data and revised the manuscript. G. L., Y. S. and H. Z. assisted with the writing of the manuscript and checked the methodology. All authors reviewed the manuscript.

### Competing interests

The authors declare no competing interests.

# References


1. Kumar, A. *et al*. In situ decoration of silver nanoparticles on single-walled carbon nanotubes by microwave irradiation for enhanced and durable anti-bacterial finishing on cotton fabric. *Ceram. Int.* **45,** 1011–1019. https://doi.org/10.1016/j.ceramint.2018.09.280 (2019).
2. Francis, S., Joseph, S., Koshy, E. P. & Mathew, B. Microwave assisted green synthesis of silver nanoparticles using leaf extract of *Elephantopus scaber* and its environmental and biological applications. *Artif. Cell Nanomed. Biotechnol.* **46,** 795–804. https://doi.org/10.1080/21691401.2017.1345921 (2018).
3. Xu, S. *et al*. Seed selection and storage with nano-silver and copper as potential antibacterial agents for the seagrass *Zostera marina*: implications for habitat restoration. *Sci. Rep.* **9,** 1–8. https://doi.org/10.1038/s41598-019-56376-0 (2019).
4. Vasudevan, K., Divyasree, M. C. & Chandrasekharan, K. Enhanced nonlinear optical properties of ZnS nanoparticles in 1D polymer photonic crystal cavity. *Opt. Laser. Technol.* **114,** 35–39. https://doi.org/10.1016/j.optlastec.2019.01.027 (2019).
5. Eze, F. N. & Nwabor, O. F. Valorization of *Pichia* spent medium via one-pot synthesis of biocompatible silver nanoparticles with potent antioxidant, antimicrobial, tyrosinase inhibitory and reusable catalytic activities. *Mat. Sci. Eng. C* 111104. https://doi.org/10.1016/j.msec.2020.111104 (2020).

6. Lin, X. *et al*. Improving the postharvest performance of cut spray 'Prince' carnations by vase treatments with nano-silver and sucrose. *J. Hortic. Sci. Biotech.* **94,** 513–521. https://doi.org/10.1080/14620316.2019.1572461 (2019).
7. Lin, S. *et al*. Nano-silver pretreatment delays wilting of cut gardenia foliage by inhibiting bacterial xylem blockage. *Sci. Hortic*. **246,** 791–796. https://doi.org/10.1016/j.scienta.2018.11.050 (2019).
8. Solgi, M. Evaluation of plant-mediated silver nanoparticles synthesis and its application in postharvest physiology of cut flowers. *Physiol. Mol. Biol. Plants.* **20,** 279–285. https://doi.org/10.1007/s12298-014-0237-3 (2014).
9. Rabiza-Świder, J., Skutnik, E., Jędrzejuk, A. & Rochala-Wojciechowska, J. Nanosilver and sucrose delay the senescence of cut snapdragon flowers. *Postharvest Biol. Technol.* **165,** 111165. https://doi.org/10.1016/j.postharvbio.2020.111165 (2020).
10. Liu, J. *et al*. Alleviation of effects of exogenous ethylene on cut 'Master' carnation flowers with nano-silver and silver thiosulfate. *Postharvest Biol. Technol.* **143,** 86–91. https://doi.org/10.1016/j.postharvbio.2018.04.017 (2018).
11. Li, H. *et al*. Nano-silver treatments reduced bacterial colonization and biofilm formation at the stem-ends of cut gladiolus 'Eerde' spikes. *Postharvest Biol. Technol.* **123,** 102–111. https://doi.org/10.1016/j.postharvbio.2016.08.014 (2017).
12. Naing, A. H. & Kim, C.K. Application of nano-silver particles to control the postharvest biology of cut flowers: A review. *Sci. Hortic.* **270,** 109463. https://doi.org/10.1016/j.scienta.2020.109463 (2020).
13. Bharti, S, Mukherji, S. & Mukherji, S. Extracellular synthesis of silver nanoparticles by *Thiosphaera pantotropha* and evaluation of their antibacterial and cytotoxic effects. *3 Biotech.* **10,** 237. https://doi.org/10.1007/s13205-020-02218-0 (2020).
14. Pirtarighat, S., Ghannadnia, M. & Baghshahi, S. Green synthesis of silver nanoparticles using the plant extract of *Salvia spinosa* grown in vitro and their antibacterial activity assessment. *J. Nanostructure Chem.* **9,** 1–9. https://doi.org/10.1007/s40097-018-0291-4 (2019).
15. Arshad, M. *et al*. Bioinspired synthesis of silver nanoparticles from *Morus alba* and their photocatalytic degradation and antibacterial activity assessment. *Pure Appl. Biol.* **7,** 1252–1260. https://doi.org/10.19045/bspab.2018.700145 (2018).
16. Alkhalaf, M. I., Hussein, R. H. & Hamza, A. Green synthesis of silver nanoparticles by *Nigella sativa* extract alleviates diabetic neuropathy through anti-inflammatory and antioxidant effects. *Saudi. J. Biol. Sci.* **27,** 2410–2419. https://doi.org/10.1016/j.sjbs.2020.05.005 (2020).
17. Yap, Y. H. *et al*. Green synthesis of silver nanoparticle using water extract of onion peel and application in the acetylation reaction. *Arab. J. Sci. Eng.* 1–11. https://doi.org/10.1007/s13369-020-04595-3 (2020).
18. Dehghanizade, S., Arasteh, J. & Mirzaie, A. Green synthesis of silver nanoparticles using *Anthemis atropatana* extract: characterization and in vitro biological activities. *Artif. Cell Nanomed. Biotechnol.* **46,** 160–168. https://doi.org/10.1080/21691401.2017.1304402 (2018).

19. Zhang, W., Xiao, B. & Fang, T. Chemical transformation of silver nanoparticles in aquatic environments: Mechanism, morphology and toxicity. *Chemosphere* **191,** 324–334. https://doi.org/10.1016/j.chemosphere.2017.10.016 (2018).
20. Osorio-Echavarría, J., Osorio-Echavarría, J., Ossa-Orozco, C. P. & Gómez-Vanegas, N. A. Synthesis of silver nanoparticles using white-rot fungus Anamorphous *Bjerkandera* sp. R1: influence of silver nitrate concentration and fungus growth time. *Sci. Rep.* **11,** 1-14. https://doi.org/10.1038/s41598-021-82514-8 (2021).
21. Sahoo, C. R. *et al*. Biogenic silver nanoparticle synthesis with cyanobacterium *Chroococcus minutus* isolated from Baliharachandi sea-mouth, Odisha, and *in vitro* antibacterial activity. *Saudi. J. Biol. Sci.* **27,** 1580–1586. https://doi.org/10.1016/j.sjbs.2020.03.020 (2020).
22. Wei, S. *et al*. A size-controlled green synthesis of silver nanoparticles by using the berry extract of *Sea Buckthorn* and their biological activities. *New J. Chem.* **44,** 9304–9312. https://doi.org/10.1039/d0nj01335h (2020).
23. Wei, L. *et al*. Silver nanoparticles: synthesis, properties, and therapeutic applications. *Drug Discov. Today.* **20,** 595–601. https://doi.org/10.1016/j.drudis.2014.11.014 (2015).
24. Rafique, M., Sadaf, I., Rafique, M. S. & Tahir, M. B. A review on green synthesis of silver nanoparticles and their applications. *Cell Nanomed. Biotechnol.* **45,** 1272–1291. https://doi.org/10.1080/21691401.2016.1241792 (2017).
25. Shaikh, A. E., Satardekar, K. V., Khan, R. R., Tarte, N. A. & Barve, S. S. Silver nanoparticles: green synthesis using Phoenix dactylifera fruit extract, characterization, and antioxidant and anti-microbial activities. *Appl. Nanosci.* **8,** 407–415. https://doi.org/10.1007/s13204-018-0682-3 (2018).
26. Das, D., Roy, S. S. & Mandal, P. Investigation of protein profile of nano-silver preserved mulberry leaves and silkworm larvae fed with the same leaves. *Appl. Nanosci.* **10,** 2383–2417. https://doi.org/10.1007/s13204-020-01416-9 (2020).
27. Xia, Q. H., Zheng, L. P., Zhao, P. F. & Wang, J. W. Biosynthesis of silver nanoparticles using Artemisia annua callus for inhibiting stem-end bacteria in cut carnation flowers. *IET. Nanobiotechnol.* **11,** 185–192. https://doi.org/10.1049/iet-nbt.2015.0125 (2016).
28. Dutta, T. *et al*. Green synthesis of antibacterial and antifungal silver nanoparticles using *Citrus limetta* peel extract: Experimental and Theoretical studies. *J. Environ. Chem. Eng.* **8,** 104019. https://doi.org/10.1016/j.jece.2020.104019 (2020).
29. Philip, D. Honey mediated green synthesis of silver nanoparticles. *Spectroc. Acta. Pt. A-Molec. Biomolec. Spectr.* **75,** 1078–1081. https://doi.org/10.1016/j.saa.2009.12.058 (2010).
30. Raveendran, P., Fu, J. & Wallen, S. L. A simple and “green” method for the synthesis of Au, Ag, and Au–Ag alloy nanoparticles. *Green Chem.* **8,** 34–38. https://doi.org/10.1039/b512540e (2006).
31. Peng, H., Yang. A. & Xiong, J. Green, microwave-assisted synthesis of silver nanoparticles using bamboo hemicelluloses and glucose in an aqueous medium. *Carbohydr. Polym.* **91,** 348–355. https://doi.org/10.1016/j.carbpol.2012.08.073 (2013).

32. González Fá, A. J., Juan, A. & Di Nezio, M. S. Synthesis and characterization of silver nanoparticles prepared with honey: the role of carbohydrates. *Anal. Lett.* **50,** 877–888. https://doi.org/10.1080/00032719.2016.1199558 (2017).
33. Filippo, E., Serra, A., Buccolieri, A. & Manno, D. Green synthesis of silver nanoparticles with sucrose and maltose: morphological and structural characterization. *J. Non-Cryst. Solids.* **356,** 344–350. https://doi.org/10.1016/j.jnoncrysol.2009.11.021 (2010).
34. Dzimitrowicz, A. *et al*. Antibacterial activity of fructose-stabilized silver nanoparticles produced by direct current atmospheric pressure glow discharge towards quarantine pests. *Nanomaterials* **8,** 751. https://doi.org/10.3390/nano8100751 (2018).
35. Nootsuwan, N. *et al*. Development of novel hybrid materials from polylactic acid and nano-silver coated carbon black with distinct antimicrobial and electrical properties. *J. Polym. Res.* **25,** 90. https://doi.org/10.1007/s10965-018-1484-8 (2018).
36. Handayani, W., Listyarini, A. & Imawan, C. A simple synthesis of silver nanoparticles on cellulose paper for antimicrobial applications. *Mater. Sci. Forum.* **990,** 191–196. https://doi.org/10.4028/www.scientific.net/msf.990.191 (2020).
37. Olugbemi, I. T. Biosynthesis of silver nanoparticles from aqueous extract of unripe plantain peel and its antibacterial assay: a novel biological approach. *Int. J. Pharm. Sci. Health.* **5,** 9–16. https://doi.org/10.26808/rs.ph.i9v5.02 (2019).
38. Agnihotri, S., Sillu, D., Sharma, G. & Arya, R. K. Photocatalytic and antibacterial potential of silver nanoparticles derived from pineapple waste: process optimization and modeling kinetics for dye removal. *Appl. Nanosci.* **8,** 2077–2092. https://doi.org/10.1007/s13204-018-0883-9 (2018).
39. Dangi, S., Gupta, A., Gupta, D. K., Singh, S. & Parajuli, N. Green synthesis of silver nanoparticles using aqueous root extract of *Berberis asiatica* and evaluation of their antibacterial activity. *Chem. Data Collect.* **28,** 100411. https://doi.org/10.1016/j.cdc.2020.100411 (2020).

# Figures

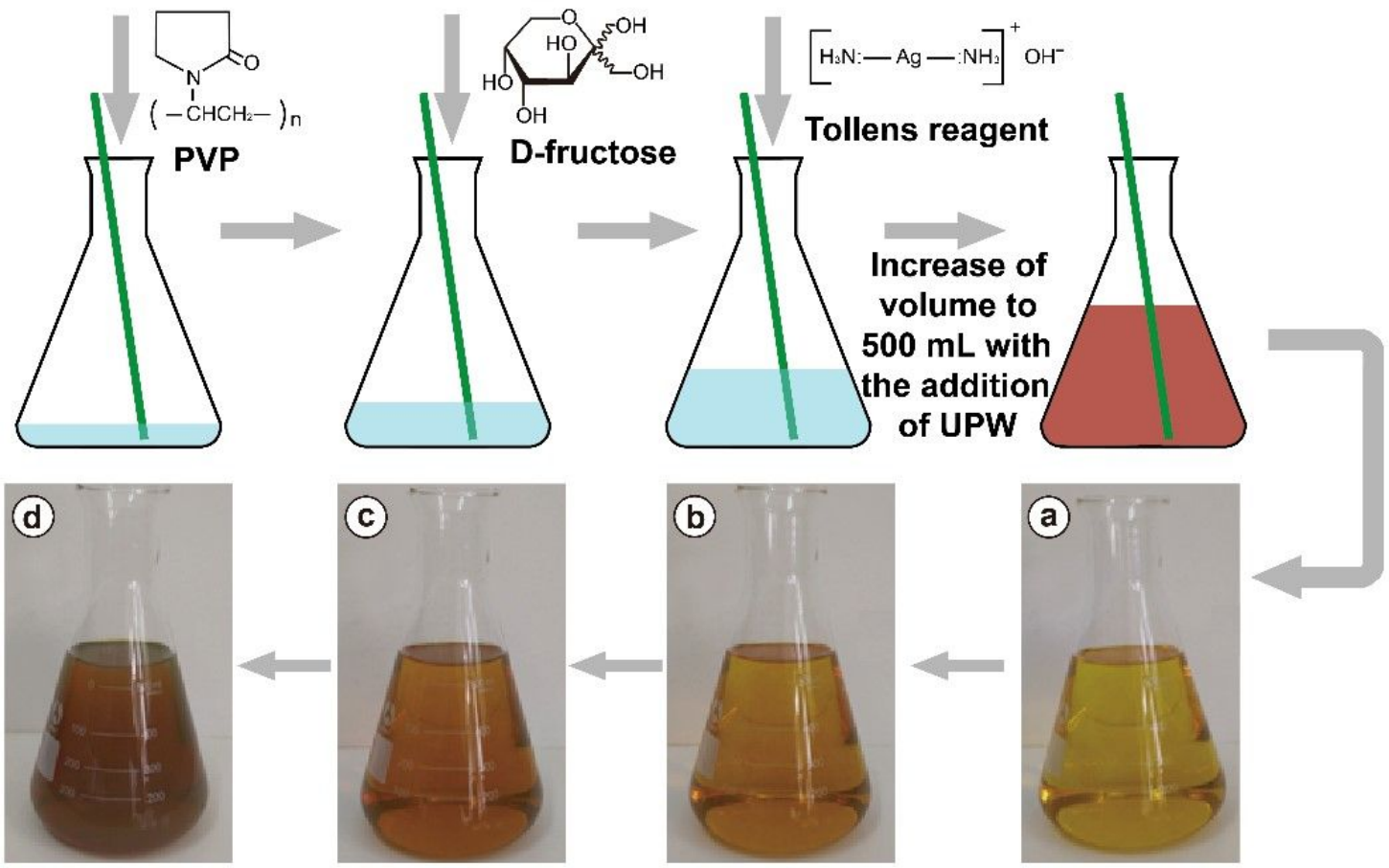



Figure 1

Schematic presentation of the green-prepared NS with the reaction times: (a) 30 min, (b) 60 min, (c) 90 min, and (d) 120 min (full reaction).

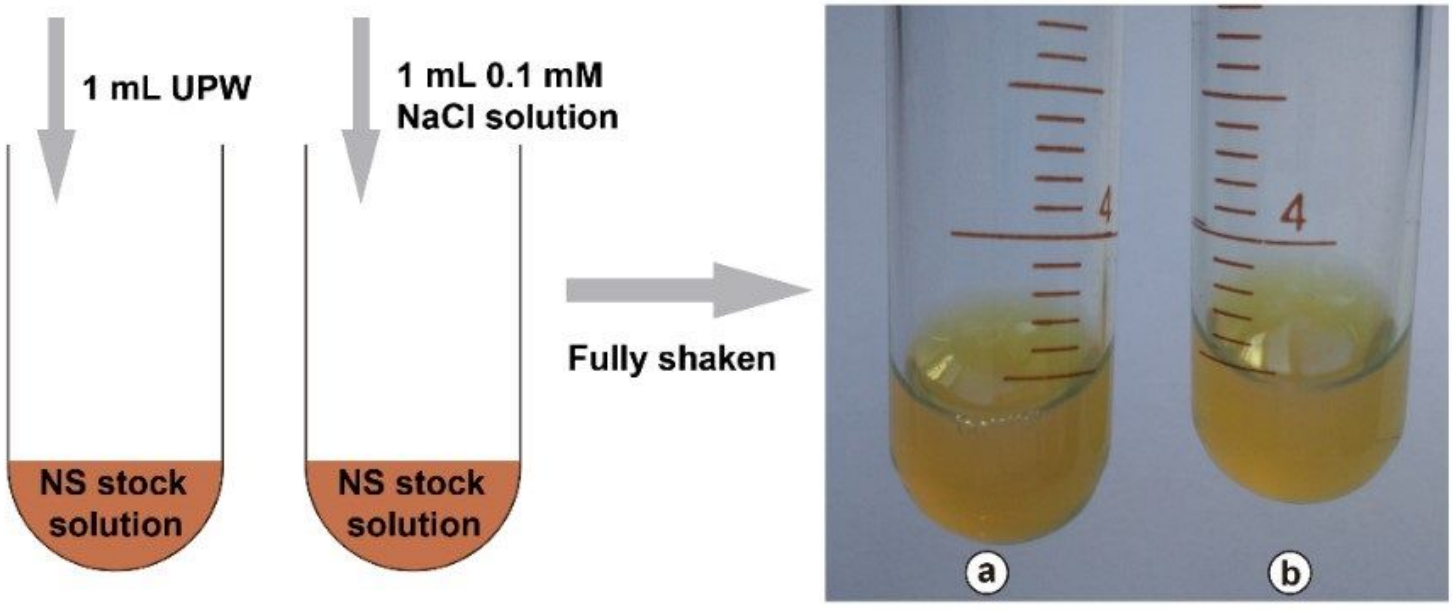
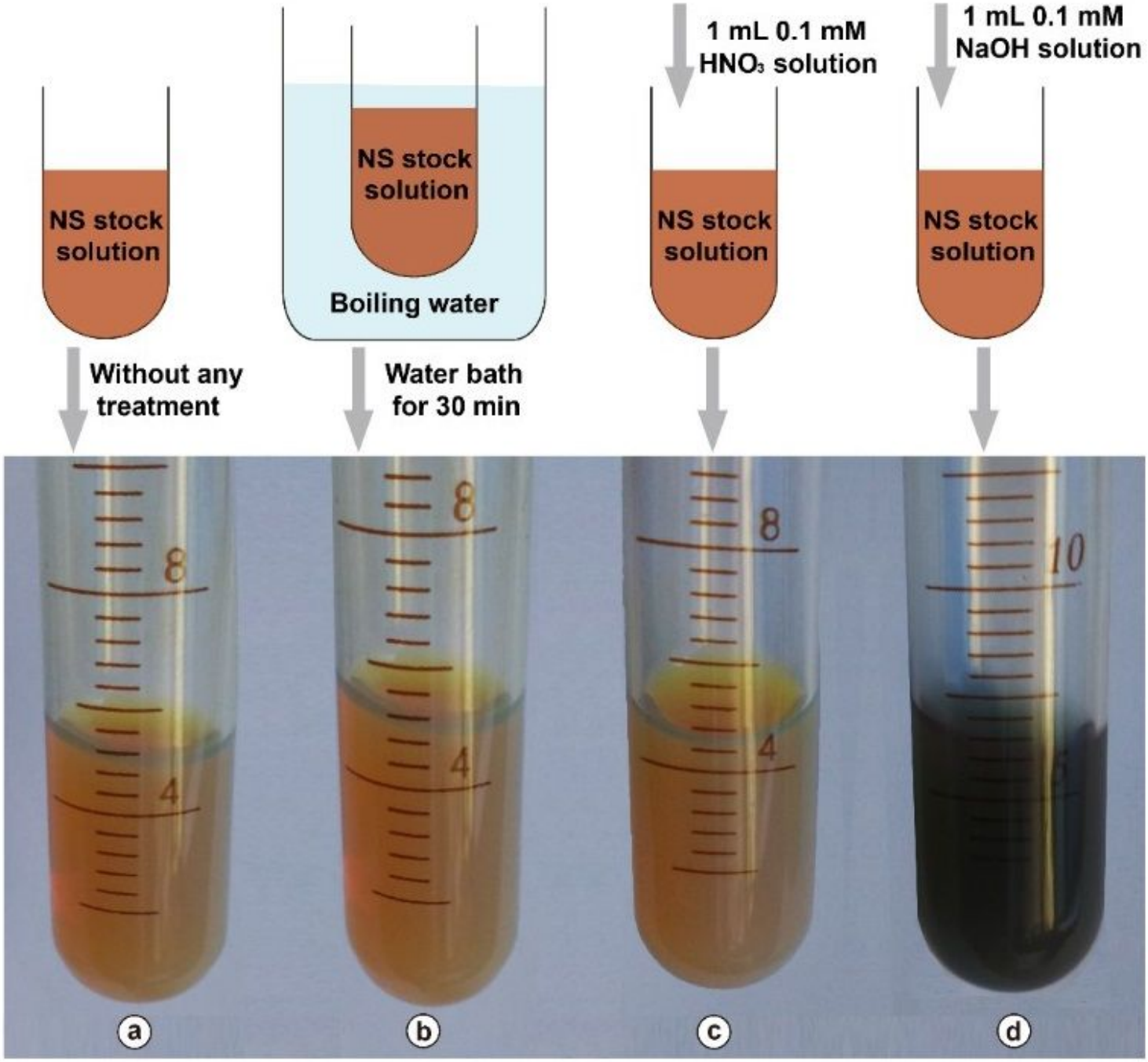



Figure 2

Detection of Ag+ ions in the prepared NS stock solution: (a) NS stock solution mixed with UPW, and (b) NS stock solution mixed with NaCl solution.




## Figure 3

Visual observation of the stability of the green synthesized NS stock solution: (a) without any treatment (control), (b) after boiling water bath treatment for 30 min, (c) after adding 1 mL 0.1 mM HNO3 solution, and (d) after adding 1 mL 0.1 mM NaOH solution.

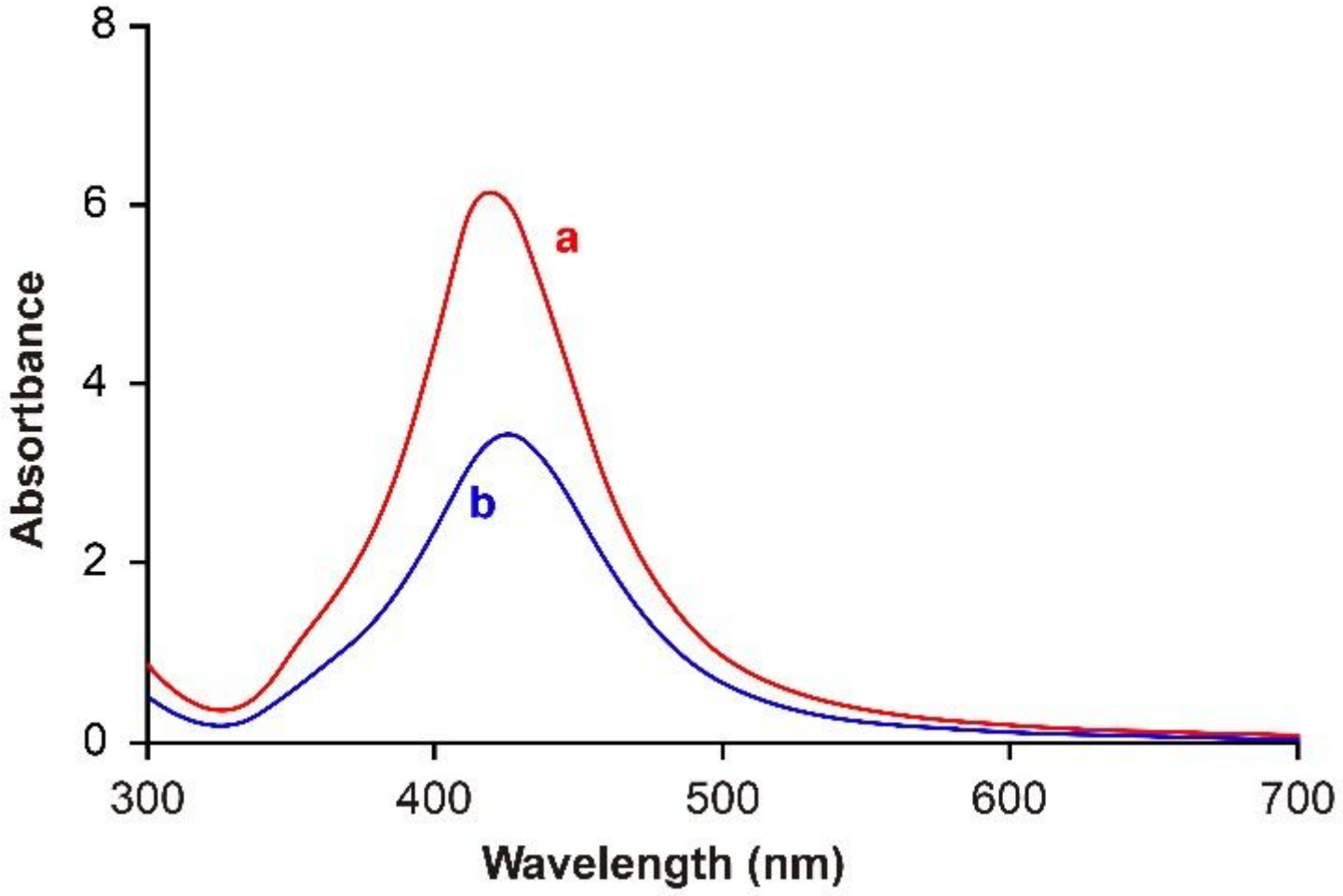
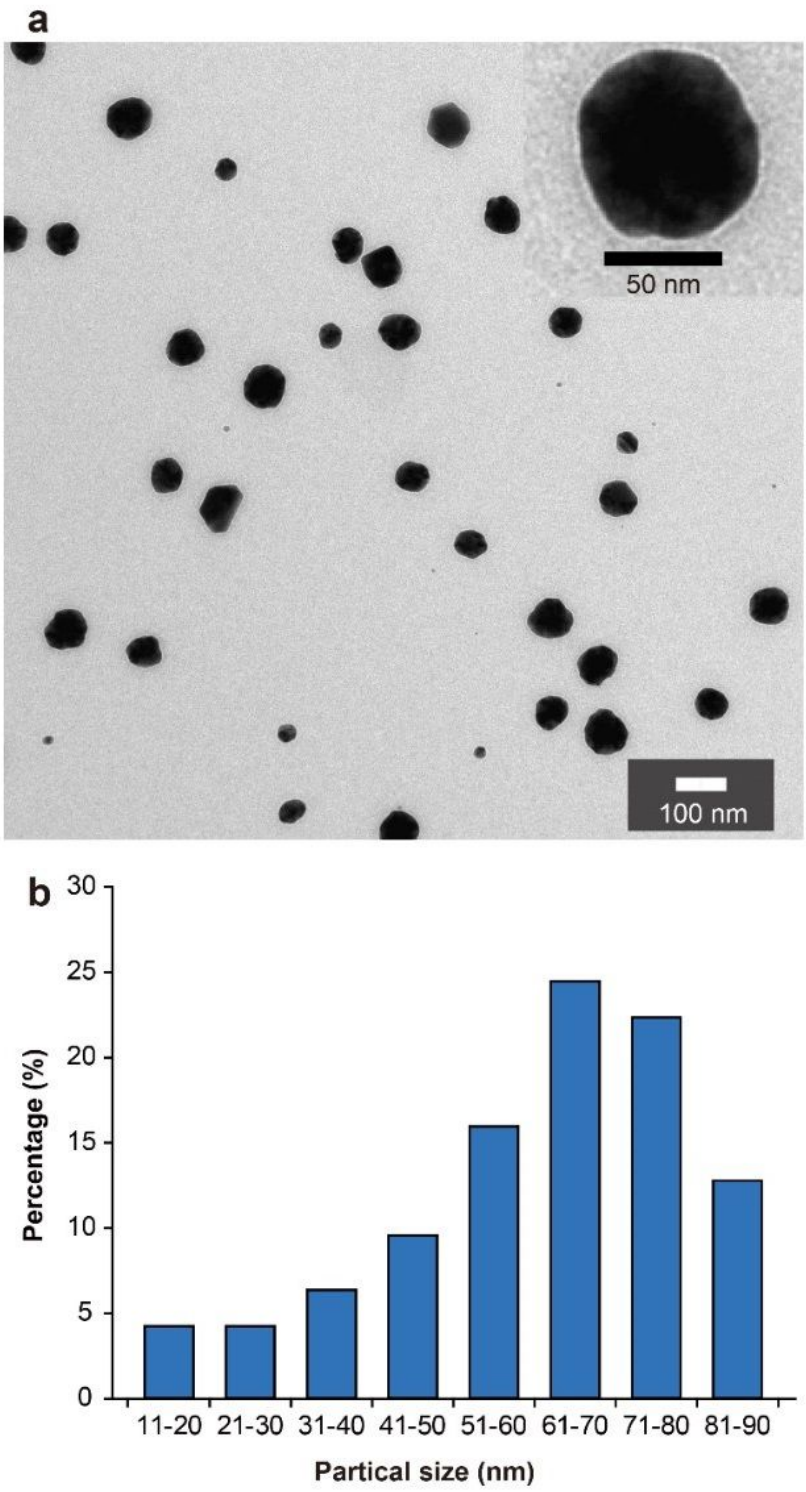


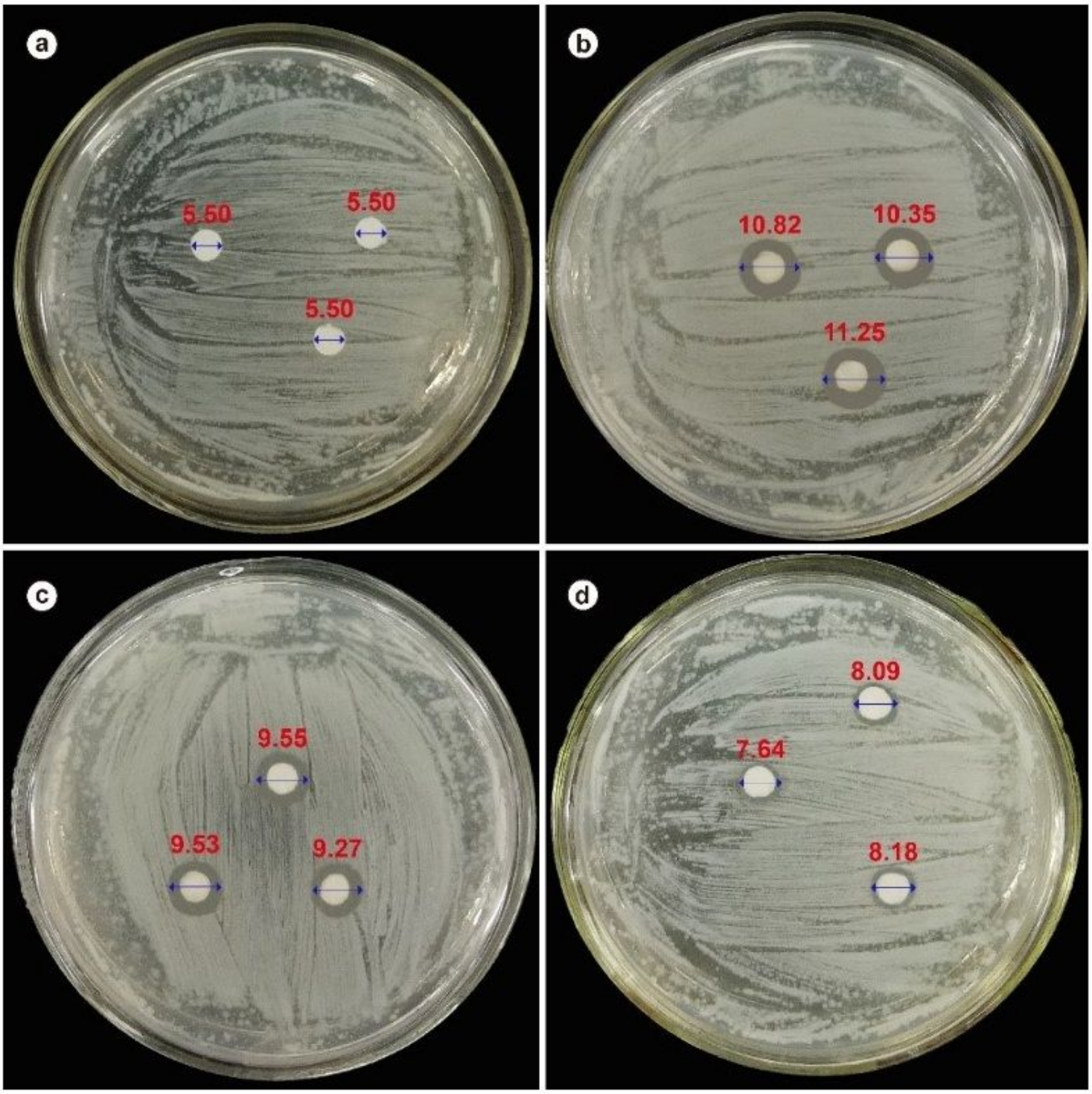

**Figure 4**

UV-Vis absorption spectra of the green synthesized NS stock solution: (a) on the day of preparation, and (b) two months after preparation.




## Figure 5

TEM images (a) and size distribution (b) histograms of the green synthesized NS.




## Figure 6

Inhibitory activity of the prepared NS solution on the growth of bacteria isolated from the vase water of cut carnation ‘Prince’ flowers: (a) control with 0.9% sterile saline, (b) NS stock solution, (c) two-fold dilution of NS solution, and (d) four-fold dilution of NS solution. The inhibition zone diameter is indicated by arrows and the values are in millimeters.