

Green Synthesis of Silver Nanoparticles Using Green Tea: Characterisation and Antibacterial Properties

D. Barbir,^{a*} P. Dabić,^a I. Weber,^b and A. Rakić^{c,d}

^aUniversity of Split, Faculty of Chemistry and Technology, R. Boškovića 35, 21 000 Split, Croatia

^bUniversity of Split, Faculty of Science, R. Boškovića 33, 21 000 Split, Croatia

^cTeaching Institute for Public Health, Split - Dalmatia County, Vukovarska 46, 21 000 Split, Croatia

^dUniversity of Split, University Department of Health Studies, R. Boškovića 35, 21 000 Split, Croatia

This work is licensed under a
Creative Commons Attribution 4.0
International License



Abstract

In this study, silver nanoparticles were synthesised through an eco-friendly process without the use of organic or toxic solvents. The synthesis was carried out with green tea serving as both the reducing and stabilising agent, using 1 mM silver nitrate solution and distilled water as solvent. Catechins present in green tea were involved in silver ion reduction. Characterisation of the colloidal silver was conducted using UV-Vis spectrophotometry, FTIR spectroscopy, dynamic light scattering (DLS) and scanning electron microscopy (SEM). UV-Vis spectrophotometer confirmed the presence of silver nanoparticles through the observation of surface plasmon resonance, while DLS and SEM analyses were employed to assess nanoparticle size, shape, and sample uniformity. DLS analysis revealed particle distribution in colloidal silver with nanoparticle sizes ranging between 32 and 95 nm. FTIR analysis confirmed the presence of polyphenols, particularly catechins, in green tea, emphasising the role of OH groups in the reduction of silver ions and nanoparticle synthesis. SEM analysis showed that the silver nanoparticles were spherical, with an average size of 62 nm. The antibacterial efficacy of the silver nanoparticles was tested against *Escherichia coli* NCTC 13216 and *Staphylococcus aureus* ATCC 25923. The results demonstrated an antimicrobial effect of green tea-synthesised silver nanoparticles against both bacteria at specific concentrations.

Keywords

Green synthesis, silver nanoparticles, green tea, *Staphylococcus aureus*, *Escherichia coli*

1 Introduction

The green synthesis of silver nanoparticles (AgNPs) has emerged as a sustainable and eco-friendly alternative to conventional chemical and physical methods.¹ This approach leverages natural resources such as plant extracts, microorganisms, and biopolymers to reduce silver ions into nanoparticles while minimising the use of hazardous chemicals and energy-intensive processes.²

In recent years, significant progress has been made in the field of green synthesis of AgNPs. Researchers have explored a variety of biological sources, including plant leaves, fruits, roots, and even microorganisms like bacteria and fungi, to synthesise silver nanoparticles. These biological entities act as both reducing and stabilising agents, facilitating the formation of nanoparticles with desirable properties.^{3–6}

One notable advancement is the use of plant extracts, which are rich in bioactive compounds such as flavonoids, alkaloids, and terpenoids. These compounds not only reduce silver ions but also act as capping agents, improving nanoparticle stability and functionality. For instance, several studies have demonstrated the successful synthesis of AgNPs using extracts from plants like *Azadirachta indica*

(neem),⁷ *Ocimum tenuiflorum* (holy basil),⁸ and *Citrus limon* (lemon).⁹

The green synthesis of colloidal silver nanoparticles (AgNPs) has gained considerable attention in recent decades due to its eco-friendliness and sustainability. Unlike conventional methods that often involve toxic chemicals and generate hazardous waste, green synthesis relies on natural resources such as plant extracts, microorganisms, or biopolymers. These biological materials are renewable, biodegradable, and environmentally benign, making the process more sustainable and reducing its environmental footprint. Additionally, green synthesis is cost-effective, requiring lower energy consumption and utilising inexpensive, readily available raw materials. The process is also simpler and does not require specialised equipment or expertise, making it accessible for a wide range of applications. Another major advantage of green-synthesised AgNPs is their non-toxic and biocompatible nature, making them suitable for medical and biomedical applications such as drug delivery, wound healing, and antimicrobial coatings.¹⁰ However, despite these advantages, green synthesis of colloidal silver also presents challenges. One of the main challenges is the variability in the quality of the synthesised nanoparticles. The composition of plant extracts or biological materials can vary depending on factors such as species, growing conditions, and extraction methods, leading to inconsistencies in the size, shape, and properties of the nanoparticles, making standardisation difficult for industrial applications. Additionally, compared to conventional methods, green synthesis offers less precise control over

* Corresponding authors: Assoc. Prof. Damir Barbir, PhD

Email: damir.barbir@ktf-split.hr

Note: The investigations presented in this paper were part of the international conference "20th Ružička Days, Today Science – Tomorrow Industry", held on September 18–20, 2024, in Vukovar, Croatia.

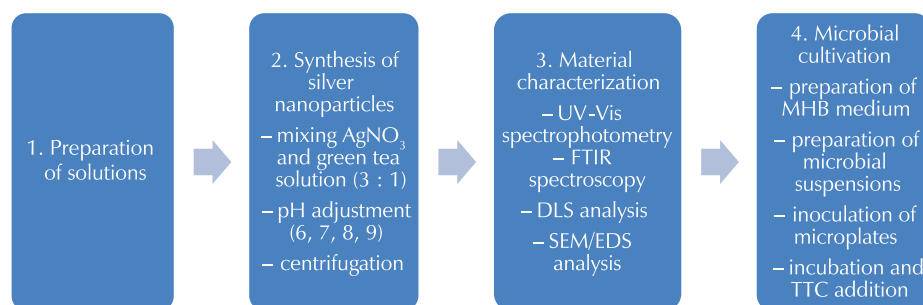


Fig. 1 – Schematic representation of the experimental procedure

Slika 1 – Shematski prikaz provedbe eksperimenta

nanoparticle size, shape, and crystallinity. Chemical methods, for example, allow for precise control over nanoparticle properties by using specific reagents and conditions, whereas green synthesis often produces polydisperse nanoparticles with a broader size distribution. Another drawback of green synthesis is the longer reaction time. While conventional methods enable rapid nanoparticle production, green synthesis typically requires more time, which can be a limitation for large-scale production.¹¹ Additionally, the process may introduce organic impurities from plant extracts or biological materials, which can interfere with nanoparticle synthesis or affect their properties. Removing these impurities can be challenging, potentially limiting the applicability of green-synthesised nanoparticles in certain fields. Furthermore, the mechanisms of silver ion reduction and nanoparticle stabilisation in green synthesis are not yet fully understood, making process optimisation difficult. While conventional methods have been extensively studied and optimised over the years, green synthesis remains a relatively new field that requires further research to fully explore its potential and limitations.¹²

Recent reviews highlight the growing interest in and advancements of green synthesis, emphasising the potential to produce nanoparticles with controlled size, shape, and functionality. These reviews also discuss the mechanisms of action, environmental benefits, and future prospects of green-synthesised AgNPs.^{12,13}

With the rise of antibiotic-resistant bacteria, alternative antimicrobial strategies must be developed. AgNPs have demonstrated significant potential¹⁴ due to their broad spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria. Their small size and large surface area enhance interactions with bacteria more effectively.¹⁵ Several mechanisms explain how AgNPs exert their antibacterial action, as follows: disruption of bacterial cell membranes – AgNPs damage bacterial membranes increasing their permeability and leading to cell death; generation of reactive oxygen species (ROS) – the presence of AgNPs in bacterial cells leads to the production of ROS, which damages DNA, proteins, and lipids; and interaction with biomolecules – the interaction of AgNPs with bacterial enzymes and proteins can cause essential cellular processes to be disrupted and even cell death.^{16–19} It remains

difficult, however, to fully understand AgNPs' antibacterial mechanisms. This study explores the feasibility of green synthesis of silver nanoparticles using green tea. The silver nanoparticles were characterised using UV-Vis spectrophotometry, FTIR spectroscopy, DLS, and SEM analyses. The antibacterial properties of the silver nanoparticles were tested against *Escherichia coli* NCTC 13216 and *Staphylococcus aureus* ATCC 25923.

2 Experimental

2.1 Materials

For the green synthesis of silver nanoparticles, the following solutions were prepared: 1 mM of silver nitrate (BDH Prolabo, UK), green tea (Darvitalis Zagreb, Croatia), and 0.1 M of sodium carbonate (Kemika Zagreb, Croatia) for pH adjustment of the solutions (6, 7, 8, 9). The green tea solution was prepared by mixing 10 g of dried organic green tea with 500 ml of boiling distilled water. The tea leaves were boiled at 90–95 °C for 20 min using a magnetic stirrer. The resulting hot solution was filtered through filter paper, and after cooling to room temperature, a second filtration was carried out using a vacuum filter pump with glass fibre filters (pore size: 1 µm). Fig. 1 shows a schematic representation of the experimental procedure.

2.2 Synthesis of silver nanoparticles

The synthesis was conducted by adding a silver nitrate solution dropwise to the green tea solution at a rate of one drop per second while stirring on a magnetic stirrer at a speed of 700 rpm. The pH of the solution was then adjusted using a sodium carbonate solution. For each sample, 45 ml of AgNO₃ solution and 15 ml of tea solution were used, maintaining a 3 : 1 ratio in favour of the AgNO₃ solution. Following nanoparticle synthesis and pH adjustment with a sodium carbonate solution, each synthesised sample was centrifuged at 16,000 rpm for 20 min. During centrifugation, the samples were washed twice with ultrapure water. After centrifugation and decantation, the silver nanoparticles were stored in dark containers and kept in a dark environment until characterisation.

2.3 Materials characterisation

The absorbance of the synthesised silver nanoparticles was determined using an Agilent Cary 5000 UV-Vis spectrophotometer (USA). This analysis confirmed the formation of silver nanoparticles, which typically absorb within the 380 and 440 nm range. Measurements were taken within the 300 to 600 nm range. The characterisation of functional groups present on the surface of AgNPs due to plant extracts was performed using FTIR spectroscopy. A Perkin-Elmer Spectrum One spectrophotometer (USA) was used, employing the HATR technique on a ZnS crystal (angle of incidence: 60°) within the wavenumber range of 4000–650 cm^{-1} . The IR spectra were recorded with a Perkin Elmer Spectrum One. To determine the size and size distribution of the silver nanoparticles, a DLS analysis was performed using a Litesizer 500 Anton Paar (Austria), which operates within a measuring range of 3.8 nm to 100 μm . SEM/EDS analyses of the silver samples were performed under low vacuum using a Jeol JEM-7610F Plus (Japan) equipped with an Oxford Ultim Max 65 SDD X-ray analyser.

2.4 Cultivation of microorganisms

The antibacterial efficacy of silver nanoparticles was tested against *Escherichia coli* NCTC 13216 and *Staphylococcus aureus* ATCC 25923. A double concentration of Mueller-Hinton Broth (MHB) (Biolife, Italy) was prepared in an Erlenmeyer flask by dissolving 42 g of sample in 1 l of distilled water. MHB was used both to prepare the culture of the test microorganisms *Escherichia coli* NCTC 13216 and *Staphylococcus aureus* ATCC 25923, and to dilute the initial microorganism suspension (Fig. 2). Microorganism suspensions were prepared at 0.5 McFarland, corresponding to 10^8 colony-forming units per millilitre (cfu ml^{-1}). The highest concentration of microorganisms was present in the

first well (10^8 cfu ml^{-1}). Dilution (microdilution) reached 10^3 cfu ml^{-1} . The zero well contained the positive control (100 μl of 0.5 McF (McFarland) suspension, 10^8 *E. coli* and *S. aureus* (check if the test strain works). The seventh well contained negative control, consisting of 100 μl of MHB. Wells 2–6 each received 100 μl of the same concentration of silver nanoparticles solution (30 ppm).

The prepared media with bacteria were incubated at 37 °C for 24 h. After 24 h of incubation, 40 μl of TTC (0.02 mg ml^{-1} solution of 2,3,5-triphenyltetrazolium chloride) was added to the wells (Fig. 3).²⁰

3 Results and discussion

UV-Vis analysis provided insight into the size of the nanoparticles in suspension. The basic principle of UV-Vis spectroscopy is that the analyte absorbs a portion of the electromagnetic radiation as it passes through the sample solution. Certain wavelengths of light can cause the conduction electrons in the metal to vibrate collectively, which is known as surface plasmon resonance. Smaller silver nanoparticles (10–20 nm) typically exhibit a small absorption peak with λ_{max} at 390–400 nm, whereas larger nanoparticles (100–220 nm) produce a broader peak, with λ_{max} shifted to longer wavelengths at 470–500 nm. In addition to the primary dipole resonance, the spectra of larger silver particles showed a secondary peak at a shorter wavelength, attributed quadrupole resonance. In addition to the size and shape of the particles, the concentration of nanoparticles also influences the observed colour and absorption at λ_{max} . Fig. 4 shows the UV-Vis spectra of the silver nanoparticle samples synthesised at different pH values.

The colour of the samples ranged from brown at pH = 6 to dark brown at pH = 9 (Fig. 5).

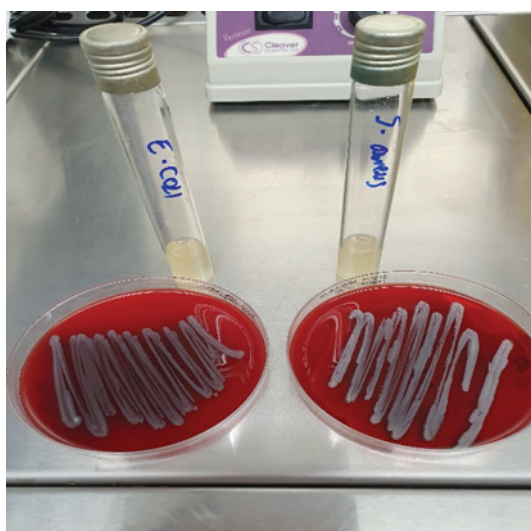


Fig. 2 – Cultures of test microorganisms
Slika 2 – Kulture testnih mikroorganizama

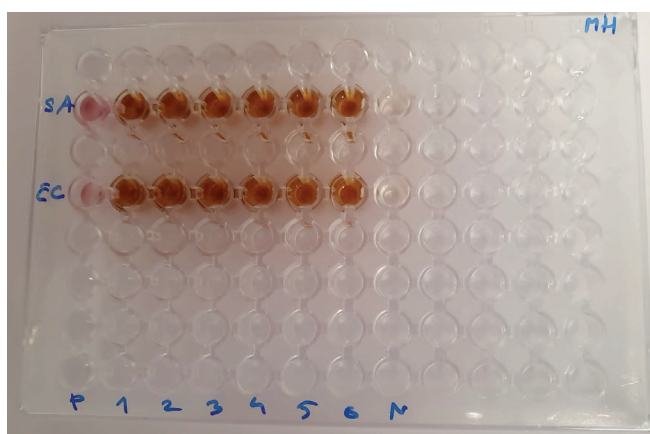


Fig. 3 – Microplate after addition of TTC
Slika 3 – Mikropločica nakon dodatka TTC-a

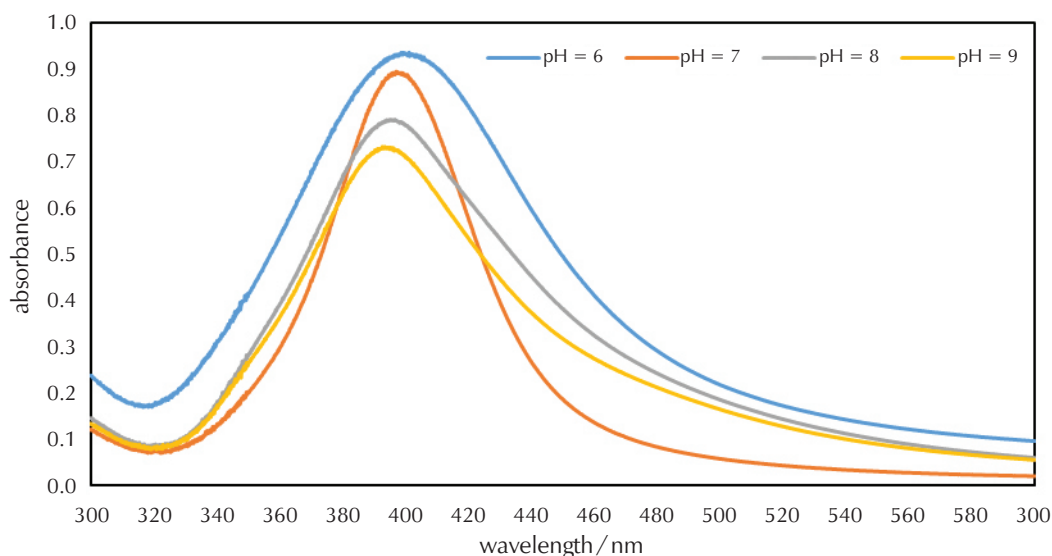


Fig. 4 – Absorption spectra of silver nanoparticles obtained at different pH
Slika 4 – Apsorpcijski spektri nanočestica srebra dobivenih pri različitom pH

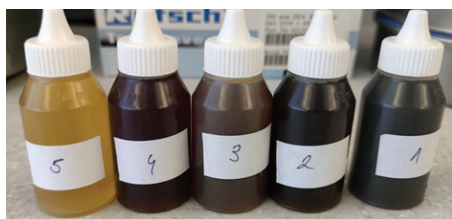


Fig. 5 – Solutions after synthesis, from left to right: green tea, silver nanoparticles at pH 9, 8, 7, and 6

Slika 5 – Otopine nakon provedene sinteze redom s lijeva na desno: zeleni čaj, nanočestice srebra pri pH 9, 8, 7 i 6

The UV-Vis spectra reveal that the absorption wavelength decreased as pH increased. The peak width at an absorption value of 0.5 (50 % absorption intensity) is closely related to particle size. Consequently, colloidal silver synthesised at pH 7 exhibited the smallest nanoparticle sizes.²¹

FTIR analysis was conducted to monitor the functional groups in the structure of green tea before and after silver nanoparticle synthesis. Fig. 6 shows the combined FTIR absorption spectra of silver nanoparticles synthesised at different pH values and in green tea solution. The characteristic peaks of the absorption spectra for all prepared samples are presented in Table 1.

Fig. 6 and Table 2 show that the green tea solution exhibited characteristic peaks at the wavelengths 3361.33 cm^{-1} , 2128.42 cm^{-1} , and 1638.75 cm^{-1} . The peak at 3361.33 cm^{-1} corresponds to the stretching vibrations of O–H groups bound to aromatic rings, which are characteristic of polyphenols. Polyphenols, such as catechins, are the primary bioactive compounds in green tea and are responsible for its antioxidant and reducing properties. The presence of this peak confirms the abundance of polyphenolic compounds in the green tea solution, which act as

reducing agents in the synthesis of silver nanoparticles. The peak at 2128.42 cm^{-1} is associated with the stretching vibrations of the C–O bond in carboxyl groups. Carboxyl groups are often found in organic acids and polyphenolic compounds, which can participate in the reduction of metal ions and stabilisation of nanoparticles. This peak further supports the presence of organic compounds in green tea that contribute to its reducing capabilities. The peak at 1638.75 cm^{-1} indicates the stretching vibrations of the C=O bond in carbonyl groups, typically found in catechin compounds. Catechins, such as epigallocatechin gallate (EGCG), are the most abundant polyphenols in green tea and are known for their strong reducing and antioxidant properties. The presence of this peak confirms the involvement of catechins in the green tea solution, which are likely responsible for the reduction of metal ions during nanoparticle synthesis.²² After synthesis, the peak characteristic of the OH group shifts to longer wavelengths at all pH values. A shift of the peak from a lower to a higher wavelength indicates that the mass of this molecule has decreased, as the vibrational frequency is inversely proportional to the mass of the vibrating molecule. The lighter the molecule, the higher the oscillation frequency and the higher the wavenumbers.²³ The shift in the OH peak indicates that the OH groups in polyphenolic compounds are actively involved in the reduction process regardless of pH, as expected, given that these groups are the primary functional groups responsible for the reducing properties of green tea polyphenols. The C–O stretching peak of green tea shifted to longer wavelengths only at pH = 6 and pH = 7, which can be attributed to the lower stability of catechins in alkaline media. Catechins are highly stable in acidic solutions (pH < 4), but their stability decreases progressively with increasing pH from 4 to 8, with extreme instability in alkaline solutions above pH = 8.²⁴ The stability of the C=O stretching peak further supports the notion that the reduction of metal ions and the stabilisation of nanoparticles are primarily driven by the OH groups in

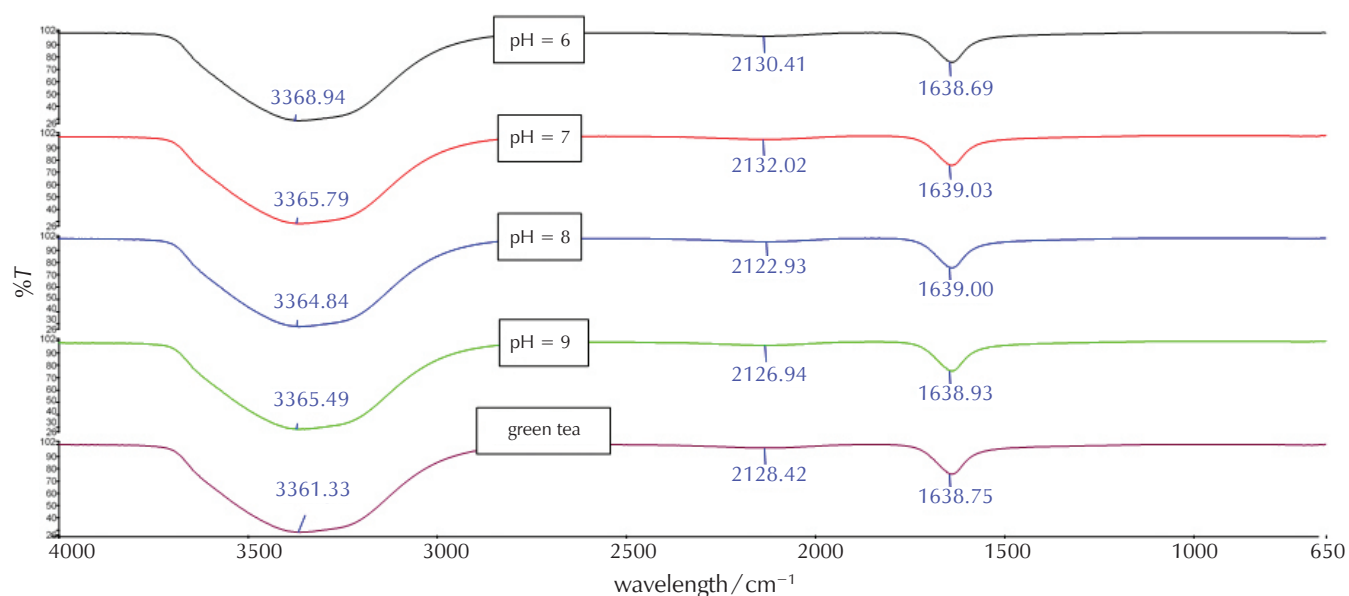


Fig. 6 – FTIR absorption spectra of green tea solution and synthesised samples at different pH values

Slika 6 – Zajednički FTIR apsorpcijski spektri otopine zelenog čaja i sintetiziranih uzoraka pri različitim pH vrijednostima

Table 1 – Characteristic peaks of the absorption spectra of obtained silver nanoparticle suspensions and green tea solution

Tablica 1 – Karakteristični pikovi apsorpcijskih spektara dobivenih suspenzija nanočestica srebra i otopine zelenog čaja

Characteristic peaks Karakteristični pikovi / cm ⁻¹	Green tea Zeleni čaj	pH = 6	pH = 7	pH = 8	pH = 9
Stretching of the OH group Istezanje OH-skupine	3361.33	3368.94	3365.79	3364.84	3365.49
C–O stretching C–O istezanje	2128.42	2130.41	2132.02	2122.93	2126.94
C=O stretching C=O istezanje	1638,75	1638.69	1639.03	1639.00	1638.93

polyphenolic compounds, rather than by carbonyl groups. This is consistent with the known chemistry of green tea catechins, where the OH groups are the active sites for redox reactions. The FTIR results are consistent with previous studies that have characterised green tea extracts and their role in nanoparticle synthesis. Huang *et al.* reported similar FTIR peaks for green tea extracts, highlighting the presence of O–H, C–O, and C=O groups associated with polyphenolic compounds.²⁵ These groups were identified as key contributors to the reduction of metal ions and stabilisation of nanoparticles. Moulton *et al.* also observed that the O–H and C=O stretching vibrations in green tea extracts are indicative of catechins, which play a critical role in the green synthesis of nanoparticles.²⁶ The polydispersity index (PDI) and the hydrodynamic diameter were determined using the DLS method and are listed in Table 2. The PDI value ranged from 0 to 1. It was used to describe the width of the particle size distribution and provided information about the polydispersity of the sample. A PDI value of more than 0.400 indicates a polydisperse system. Non-spherical samples may not be suitable for DLS measurements and the data provided may be unreliable.²⁷

Table 2 – Hydrodynamic diameter and polydispersity index (PDI) of silver nanoparticle samples synthesised at different pH values

Tablica 2 – Hidrodinamički promjer i indeks polidisperznosti (PDI) uzoraka nanočestica srebra sintetiziranih pri različitim pH vrijednostima

Sample Uzorak	Hydrodynamic diameter Hidrodinamički promjer / nm	PDI
pH = 6	50	0.243
pH = 7	32	0.277
pH = 8	95	0.177
pH = 9	52	0.108

Table 2 shows that the PDI value of the synthesised silver nanoparticle samples decreases with increasing pH. In the analysed solutions, the PDI value remains below 0.400, indicating that the silver particles are relatively uniform, and can be considered a monodisperse system. The sample at

pH = 7 exhibits nanoparticles with the smallest diameter, while at pH = 8 and 9 more uniform and larger particles appear, which can be attributed to the instability of catechins in alkaline media.²⁸

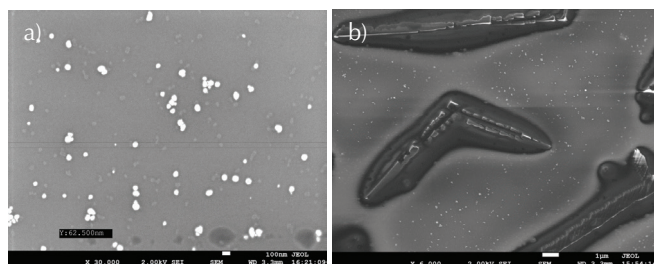


Fig. 7 – SEM analysis of (a) silver nanoparticles at pH = 6, and (b) display of green tea organic molecules in synthesised samples

Slika 7 – SEM analiza nanočestica srebra pri pH = 6 (a) i prikaz organskih molekula zelenog čaja u sintetiziranim uzorcima (b)

In Fig. 7a, the white dots representing silver nanoparticles are clearly visible. However, crystals can also be observed, which are actually impurities from the green tea (Fig. 7b). Although the samples underwent centrifugation and purification after synthesis, some organic molecules from the green tea extracts remained present in the sample. The silver particles are of regular (spherical) shape and reasonable size, which is consistent with the DLS analysis. The average size of silver nanoparticles was 62 nm. Table 3 shows the effect of silver nanoparticles synthesised at pH = 6 on bacteria at different concentrations. The silver concentration remained constant, while the bacterial concentrations varied. Negative values (–) indicate that bacteria are no longer present, meaning that the silver nanoparticles successfully eradicated the bacteria at these concentrations. This approach is innovative, as it investigates the effect of a fixed concentration of AgNPs on different concentrations of bacteria. This can offer new insights into the antibacterial activity of AgNPs. Given that few studies have adopted a similar approach, this research represents a valuable contribution to the field.

E. coli (Gram-negative bacteria) shows greater sensitivity to AgNPs compared to *S. aureus* (Gram-positive bacteria). This can be seen from the fact that AgNPs completely inhibited

the growth of *E. coli* up to a concentration of 10^5 cfu ml⁻¹, while for *S. aureus*, inhibition was recorded up to a concentration of 10^6 cfu ml⁻¹. This difference in sensitivity can be explained by the different structure of the cell membrane between Gram-negative and Gram-positive bacteria. Gram-negative bacteria have a thin peptidoglycan layer and an outer membrane rich in lipopolysaccharides, which makes them more susceptible to damage caused by AgNPs. On the other hand, Gram-positive bacteria have a thick peptidoglycan layer that can provide additional protection. Several studies have reported that AgNPs exhibit stronger antibacterial effects against Gram-negative bacteria compared to Gram-positive bacteria, which is consistent with the findings presented in Table 3. For instance, Rai et al. demonstrated that AgNPs are more effective against *E. coli* due to the structural differences in the cell walls of Gram-negative and Gram-positive bacteria.²⁹ AgNPs showed a stronger inhibitory effect at lower bacterial concentrations (e.g., 10^3 – 10^5 cfu ml⁻¹) for both types of bacteria. This indicates that the antibacterial effect of AgNPs is dependent on the initial concentration of bacteria. At higher bacterial concentrations (e.g., 10^7 – 10^8 cfu ml⁻¹), AgNPs were insufficiently effective to completely inhibit growth, which may indicate that a higher concentration of AgNPs is required to achieve an antibacterial effect at high bacterial concentrations. The results show that the concentration of AgNPs must be adjusted to the initial concentration of bacteria in order to achieve an optimal antibacterial effect. This is particularly important in clinical or industrial applications where bacterial concentrations can vary significantly. The concentration-dependent antibacterial activity observed in this study is supported by the work of Sondi and Salopek-Sondi, who found that the efficacy of AgNPs is highly dependent on both the nanoparticle concentration and the initial bacterial concentration.³⁰ They reported that lower bacterial concentrations (e.g., 10^3 – 10^5 cfu ml⁻¹) are more susceptible to complete inhibition by AgNPs, while higher bacterial concentrations (e.g., 10^7 – 10^8 cfu ml⁻¹) require higher doses of AgNPs to achieve similar effects.

4 Conclusion

The green synthesis of silver nanoparticles can be successfully achieved using green tea, which is a natural reducing and stabilising agent. The appearance of absorption spectra at wavelengths around 400 nm was observed in all samples, confirming the successful synthesis of silver nanoparticles. DLS analysis revealed a particle distribution in colloidal silver with nanoparticle sizes between 32 and

Table 3 – Antibacterial activity of silver nanoparticles synthesised at pH 6
Tablica 3 – Antibakterijska aktivnost nanočestica srebra sintetiziranih pri pH 6

Microorganism suspension Spenzije mikroorganizama	MHB / cfu ml ⁻¹								
	Control Kontrola +	10 ⁸ 1	10 ⁷ 2	10 ⁶ 3	10 ⁵ 4	10 ⁴ 5	10 ³ 6	Control Kontrola –	
Silver nanoparticles Nanočestice srebra	+	+	+	–	–	–	–	–	<i>S. aureus</i> ATCC 25922
	+	+	+	+	–	–	–	–	<i>E. coli</i> NCTC 13216

95 nm. A high uniformity of the sample is characteristic of samples in an alkaline environment, while the smallest particles were obtained at pH 7 with a broader distribution of nanoparticle sizes in the sample. All colloidal silver samples were found to be monodisperse. FTIR analysis confirmed the presence of polyphenols, particularly catechins, in green tea, emphasising the role of OH groups in the reduction of silver ions and nanoparticle synthesis. Catechins are stable in acidic conditions but unstable in alkaline conditions, which affects their reducing ability. The C=O groups remained stable, further confirming that OH groups are the primary functional groups involved in the reduction process, and neutral pH proved to be the best for synthesising the smallest nanoparticles with a diameter of 32 nm. SEM analysis showed that the silver nanoparticles were spherical with an average size of 62 nm. The same concentration of AgNPs showed a greater antibacterial effect on *E. coli* compared to *S. aureus*, indicating differences in susceptibility between Gram-negative and Gram-positive bacteria. The effect of AgNPs depends on the initial bacterial concentration, with lower bacterial concentrations showing greater susceptibility. These results confirm the potential of AgNPs as an effective antibacterial agent, but highlight the need to optimise the concentration of AgNPs depending on the target bacterial species and the initial bacterial concentration.

List of abbreviations

Popis kratica

DLS	– dynamic light scattering – dinamičko raspršenje svetla
FTIR	– infrared spectroscopy with Fourier transformation – infracrvena spektroskopija s Fourierovom transformacijom
SEM	– scanning electron microscopy – skenirajući elektronski mikroskop
AgNPs	– silver nanoparticles – nanočestice srebra
ROS	– reactive oxygen species – reaktivne vrste kisika
PDI	– polydispersity index – indeks polidisperznosti

References

Literatura

1. M. Fahim, A. Shahzaib, N. Nishat, A. Jahan, T. A. Bhat, A. Inam, Green synthesis of silver nanoparticles: A comprehensive review of methods, influencing factors, and applications, *JCIS Open* **16** (2024) 100125, doi: <https://doi.org/10.1016/j.jciso.2024.100125>.
2. B. Senthil Rath, P. Senthil Kumar, S. Sanjay, V. Parthasarathy, R. Gayathri, N. V. Dai-Viet, Innovative eco-friendly silver nanoparticles: various synthesis methods, characterization and prospective applications, *Chem. Eng. Commun.* **212** (2025) 472–507, doi: <https://doi.org/10.1080/00986445.2024.2403117>.
3. A. Dhaka, S. C. Mali, S. Sharma, R. Trivedi, A review on biological synthesis of silver nanoparticles and their potential applications, *Results Chem.* **6** (2023) 101108, doi: <https://doi.org/10.1016/j.rechem.2023.101108>.
4. D. Rajput, S. Paul, A. Durve-Gupta, Green Synthesis of Silver Nanoparticles Using Waste Tea Leaves, *Adv. Nano Res.* **3** (2020) 1–14, doi: <https://doi.org/10.21467/anr.3.1.1-14>.
5. C. Vanlalveni, S. Lallianrawna, A. Biswas, M. Selvaraj, B. Changmai, S. Lalthazuala Rokhum, Green synthesis of silver nanoparticles using plant extracts and their antimicrobial activities: a review of recent literature, *RSC Adv.* **11** (2021) 2804–2837, doi: <https://doi.org/10.1039/D0RA09941D>.
6. S. Kaabipour, S. Hemmati, A review on the green and sustainable synthesis of silver nanoparticles and one-dimensional silver nanostructures, *Beilstein J. Nanotechnol.* **12** (2021) 102–136, doi: <https://doi.org/10.3762/bjnano.12.9>.
7. A. Verma, M. S. Mehata, Controllable synthesis of silver nanoparticles using Neem leaves and their antimicrobial activity, *J. Radiat. Res. Appl. Sci.* **9** (2016) 109–115, doi: <https://doi.org/10.1016/j.jrras.2015.11.001>.
8. A. M. Alex, S. Subburaman, S. Chauhan, V. Ahuja, G. Abdi, M. Abbasi Tarighat, Green synthesis of silver nanoparticle prepared with Ocimum species and assessment of anticancer potential, *Sci. Rep.* **14** (2024) 11707, doi: <https://doi.org/10.1038/s41598-024-61946-y>.
9. M. M. Alkhulaifi, J. H. Alshehri, M. A. Alwehaibi, M. A. Awad, N. M. Al-Enazi, N. S. Aldosari, A. A. Hatamleh, N. Abdel-Raouf, Green synthesis of silver nanoparticles using Citrus limon peels and evaluation of their antibacterial and cytotoxic properties, *Saudi J. Biol. Sci.* **27** (2020) 3434–3441, doi: [10.1016/j.sjbs.2020.09.031](https://doi.org/10.1016/j.sjbs.2020.09.031).
10. B. K. Dejene, Eco-friendly synthesis of metallic nanoparticles from agri-food waste extracts: Applications in food packaging and healthcare—A critical review, *Mater. Today Chem.* **45** (2025) 102619, doi: <https://doi.org/10.1016/j.mtchem.2025.102619>.
11. M. A. Dheyab, A. A. Aziz, S. H. Nowfal, F. S. Braim, W. Abdullatif, W. H. Kasasbeh, M. S. Jameel, S. T. Alanezi, M. Alrosan, N. Oladzadabbasabadi, Sustainable green synthesis of silver nanoparticles for safer biomedical application, *J. Environ. Chem. Eng.* **13** (2025) 115998, doi: <https://doi.org/10.1016/j.jece.2025.115998>.
12. S. Kazemi, A. Hosseingholian, S. D. Gohari, F. Feirahi, F. Moammeri, G. Mesbahian, Z. S. Moghaddam, Q. Ren, Recent advances in green synthesized nanoparticles: from production to application, *Mater. Today Sustain.* **24** (2023) 100500, doi: <https://doi.org/10.1016/j.mtsust.2023.100500>.
13. D. Gupta, A. Boora, A. Thakur, T. K. Gupta, Green and sustainable synthesis of nanomaterials: Recent advancements and limitations, *Environ. Res.* **231** (2023) 116316, doi: <https://doi.org/10.1016/j.envres.2023.116316>.
14. T. Bruna, F. Maldonado-Bravo, P. Jara, N. Caro, Silver nanoparticles and their antibacterial applications, *Int. J. Mol. Sci.* **22** (2021) 7202, doi: <https://doi.org/10.3390/ijms22137202>.
15. P. R. More, S. Pandit, A. De Filippis, G. Franci, I. Mijakovic, M. Galdiero, Silver nanoparticles: Bactericidal and mechanistic approach against drug resistant pathogens, *Microorganisms* **11** (2023) 369, doi: <https://doi.org/10.3390/microorganisms11020369>.
16. I. R. Santos, D. G. Ribeiro, P. da N. Mendes, W. Fontes, I. S. Luz, L. P. Silva, A. Mehta, Biotechnological potential of silver nanoparticles synthesized by green method to control phytopathogenic bacteria: contributions from a proteomic analysis, *Braz. J. Microbiol.* **55** (2024) 3239–3250, doi: <https://doi.org/10.1007/s42770-024-01538-0>.
17. C. Marambio-Jones, E. M. V. Hoek, A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment, *J. Nanopart.*

- Res. **12** (2010) 1531–1551, doi: <https://doi.org/10.1007/s11051-010-9900-y>.
18. N. Durán, M. Durán, M. B. de Jesus, A. B. Seabra, W. J. Fávaro, G. Nakazato, Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity, *Nanomedicine* **12** (2016) 789–799, doi: <https://doi.org/10.1016/j.nano.2015.11.016>.
 19. Y. N. Slavin, J. Asnis, U. O. Häfeli, H. Bach, Metal nanoparticles: understanding the mechanisms behind antibacterial activity, *J. Nanobiotechnol.* **15** (2017) 65, doi: <https://doi.org/10.1186/s12951-017-0308-z>.
 20. M. Balouiri, M. Sadiki, S. Koraichi Ibnsoouda, Methods for in vitro evaluating antimicrobial activity: A review, *J. Pharm. Anal.* **6** (2016) 71–79, doi: <https://doi.org/10.1016/j.jpha.2015.11.005>.
 21. D. Barbir, P. Dabić, M. Meheš, The use of PWHM and Mie methods in estimation of colloidal silver particle size obtained by chemical precipitation with sodium borohydride, *Hem. Ind.* **73** (2019) 397–404, doi: <https://doi.org/10.2298/HEMIND190719031B>.
 22. L. Wang, E. Santos, D. Schenk, M. Rabago-Smith, Kinetics and mechanistic studies on the reaction between cytochrome c and tea catechins, *Antioxidants* **3** (2014) 559–568, doi: <https://doi.org/10.3390/antiox3030559>.
 23. A. Kharabi Masooleh, A. Ahmadikhah, A. Saidi, Green synthesis of stable silver nanoparticles by the main reduction component of green tea (*Camellia sinensis* L.), *IET Nanobiotechnol.* **13** (2018) 183–188, doi: [10.1049/iet-nbt.2018.5141](https://doi.org/10.1049/iet-nbt.2018.5141).
 24. Q. Vuong, C. E. Stathopoulos, M. H. Nguyen, J. B. Golding, P. D. Roach, Isolation of green tea catechins and their utilization in the food industry, *Food Rev. Int.* **27** (2011) 227–247, doi: <https://doi.org/10.1080/87559129.2011.563397>.
 25. I. De Leersnyder, L. De Gelder, I. Van Driessche, P. Vermeir, Revealing the importance of aging, environment, size and stabilization mechanisms on the stability of metal nanoparticles: A case study for silver nanoparticles in a minimally defined and complex undefined bacterial growth medium, *Nanomaterials* **9** (2019) 1684, doi: <https://doi.org/10.3390/nano9121684>.
 26. J. Huang, Q. Li, D. Sun, Y. Lu, Y. Su, X. Yang, X. Chen, Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf, *Nanotechnology* **18** (2007) 105104, doi: <https://doi.org/10.1088/0957-4484/18/10/105104>.
 27. M. C. Moulton, L. K. Braydich-Stolle, M. N. Nadagouda, S. Kunzelman, S. M. Hussain, R. S. Varma, Synthesis, characterization and biocompatibility of “green” synthesized silver nanoparticles using tea polyphenols, *Nanoscale* **2** (2010) 763–770, doi: <https://doi.org/10.1039/C0NR00046A>.
 28. Y. Jiang, Z. Jiang, L. Ma, Q. Huang, Advances in nanodelivery of green tea catechins to enhance the anticancer activity, *Molecules* **26** (2021) 3301, doi: <https://doi.org/10.3390/molecules26113301>.
 29. M. Rai, A. Yadav, A. Gade, Silver nanoparticles as a new generation of antimicrobials, *Biotechnol. Adv.* **27** (2009) 76–83, doi: <https://doi.org/10.1016/j.biotechadv.2008.09.002>.
 30. I. SonDI, B. Salopek-Sondi, Silver nanoparticles as antimicrobial agent: A case study on *E. coli* as a model for Gram-negative bacteria, *J. Colloid Interface Sci.* **275** (2004) 177–182, doi: <https://doi.org/10.1016/j.jcis.2004.02.012>.

SAŽETAK

Zelena sinteza nanočestica srebra pomoću zelenog čaja: Karakterizacija i antibakterijska svojstva

Damir Barbir,^{a*} Pero Dabić,^a Ivana Weber^b i Anita Rakić^{c,d}

U ovom radu nanočestice srebra sintetizirane su na ekološki prihvatljiv način i bez upotrebe organskih ili toksičnih otapala. Sinteza je provedena sa zelenim čajem kao redukcijskim i stabilizirajućim sredstvom uz uporabu 1 mM otopine srebrnog nitrata i destilirane vode kao otapala. Znatna količina katehina u zelenom čaju uključena je u redukciju srebrovih iona. Karakterizacija koloidnog srebra provedena je UV-Vis spektrofotometrom, FTIR-om, DLS-om (engl. *Dynamic Light Scattering*) i SEM-om (engl. *Scanning Electron Microscopy*). UV-Vis spektrofotometrom dokazana je prisutnost nanočestica srebra pojavom površinske plazmonske rezonancije, dok su DLS i SEM analize primijenjene za procjenu veličine i oblika nastalih nanočestica srebra te ujednačenost dobivenog uzorka nanočestica. DLS analiza pokazala je raspodjelu veličine nanočestica srebra u području od 32 do 95 nm. FTIR analiza potvrđuje prisutnost polifenola, posebice katehina, u zelenom čaju, naglašavajući ulogu OH skupina u redukciji iona srebra i sintezi nanočestica. SEM analiza pokazala je da su nanočestice srebra sferične s prosječnom veličinom od 62 nm. Antibakterijska učinkovitost nanočestica srebra ispitana je na bakterijama *Escherichia coli* NCTC 13216 i *Staphylococcus aureus* ATCC 25923. Antimikrobni učinak nanočestica srebra dobivenih sa zelenim čajem dokazan je za te dvije bakterije do određenih koncentracija.

Ključne riječi

Zelena sinteza, nanočestice srebra, zeleni čaj, *Staphylococcus aureus*, *Escherichia coli*

^aSveučilište u Splitu, Kemijsko-tehnološki fakultet,
R. Boškovića 35, 21 000 Split

^bSveučilište u Splitu, Prirodoslovno-matematički fakultet,
R. Boškovića 33, 21 000 Split

^cNastavni zavod za javno zdravstvo Splitsko-dalmatinske
županije, Vukovarska 46, 21 000 Split

^dSveučilište u Splitu, Sveučilišni odjel zdravstvenih
studija, R. Boškovića 35, 21 000 Split

Izvorni znanstveni rad
Prispjelo 28. siječnja 2025.
Prihvaćeno 21. ožujka 2025.