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Biosynthesis of silver nanoparticles with antioxidant and bactericidal activities using *Capsicum annuum* fruit extract

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Metallic nanostructures, especially silver nanoparticles (AgNPs) have already found multiple applications in modern industry, science and medicine. Still, the production of nano-sized compounds often leads to the formation of toxic byproducts and possesses substantial environmental hazard. One of the promising solutions for the ecofriendly creation of nanomaterials predicts the use of a “green chemistry” approach using organisms, their parts or natural compounds to act as safe and effective producers of nanomaterials. Plant-mediated biosynthesis of silver nanoparticles includes the reduction of Ag^+ to Ag^0 by natural compounds, usually secondary metabolites which can be found in roots, leaves, cortex, fruits, flowers and seeds of various species. Aqueous extract of *Capsicum annuum* var. cv. (cultivar) Teja (S-17) fruits was used as a bioreducer for the reduction of AgNO_3 solution to AgNPs. The formation of the AgNPs was confirmed by the presence of the Tyndall effect of light scattering combined with colour change of the solutions. The properties of the nanoparticles were assessed with UV-visible spectroscopy and scanning electron microscopy. In the present study, we report the experimental optimization of operating parameters needed for silver biotransformation by *C. annuum*. Biosynthesized nanoparticles were 13–22 nm in size and spherical in shape. Colloidal solutions of AgNPs were also confirmed to show antioxidant activity in vitro as analyzed by the reduction of DPPH radicals. Characterization and application of AgNPs as bactericidal agents on two Gram-positive (*Micrococcus luteus*, *Staphylococcus aureus*) and two Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) prokaryotic microorganisms demonstrated the prevalent influence on Gram-positive strains.

Keywords: AgNPs; green synthesis; *Capsicum annuum*; antioxidant activity; bactericidal activity.

Introduction

Due to their prominent physical, chemical and biological properties, nanostructures of different composition have already become a significant part of human life, as they are readily used in fabrics, cosmetics, food production and medicine (Jorge de Souza et al., 2019; Arroyo et al., 2020). This functional diversity of nanomaterials arises from their strong dependence on size, shape, composition and functionalization of particular nanounits. Over the last decades, silver nanoparticles (AgNPs) have become one of the most widely used and thoroughly studied type of nanostructures. Even before the beginning of the “nano” era, silver was highly appreciated for its biological activity, physical and chemical properties, which appeared to be significantly enhanced when downscaled to the “nano” level. It has already been shown that AgNPs possess profound fungicidal, bactericidal and virucidal properties; they can be used in various approaches for cancer treatment; facilitate closure of wounds and promote regeneration of damaged tissues (Patra et al., 2015; Sujitha et al., 2015). Besides the direct application in therapeutic protocols, AgNPs have found their use in bioimaging, creation of biosensors and optimization of different types of spectroscopy (Dzhanagan et al., 2022).

Metallic nanoparticles can be synthesized using various methods, which include direct chemical reduction of salts, pyrolysis, electrochemical and photochemical techniques, laser ablation, vapour condensation, and biological approaches (Wei et al., 2015). Disregarding the compara-

tive effectiveness of physical and chemical pathways, their application can lead to reduced biocompatibility and increased cytotoxicity of nanostructures, combined with relative economic ineffectiveness. Simultaneously, the substrates and byproducts of such methods can pose a substantial environmental hazard (Anastas & Eghbali, 2010). The combination of these factors lead to emergence and rapid development of “green synthesis” – the production of nanoparticles by various biological substances. As metallic nanoparticles are generally created through the reduction of corresponding salts and compounds, any reducing biomolecule can serve as a potential agent for effective nanosynthesis. Thus, it has already been shown that different types of prokaryotic and eukaryotic microorganisms, fungi, plants or even their parts can be used for such green synthesis (Duan et al., 2015). Plant-mediated production of nanoparticles is a promising field of modern nanotechnology, as it provides the possibility to intensify the biological activity of nanostructures through their interaction with plant-derived substances, which include polysaccharides, vitamins, amino acids, alkaloids, flavonoids, phenolics, terpenoids, ketones, aldehydes and carboxylic acids (Rafique et al., 2017). The substantial benefit of green synthesis is that bioreducing compounds may act as coating agents, improving the stability of nanocolloids. Additionally, in case of medicinal plants, these molecules may preserve their pharmaceutical activity, endowing nanoparticles with improved therapeutic properties (Roy et al., 2019). Plant-mediated green synthesis has been successfully demonstrated on various species, while the choice of a particular producer depends on

the easiness of cultivations, effectiveness of bioreduction and predicted biological activity (Ovais et al., 2016). From this point of view, different types of pepper, *Capsicum* sp., appear to be promising agent for green synthesis, due to their ability to accumulate high amounts of capsaicinoids and phenolic compounds, combined with environmental unpretentiousness (Kim et al., 2016a).

Metallic nanoparticles are known to exert substantial ubiquitous bactericidal effects, influencing on Gram-negative and Gram-positive species. These properties are significantly enhanced in case of AgNPs due to release of silver ions from the surface of nanostructures (Carrillo-González et al., 2016). The antibacterial activity of AgNPs is executed through various mechanisms, which include direct and indirect DNA damage, disruption of protein biosynthesis and production of cell wall, deterioration of ion transport, dysregulation of enzymes and increased production of reactive oxygen species (Dakal et al., 2016). Although comparable effects can also be seen on eukaryotic targets, the application of green synthesis leads to substantial decrease in unwanted cytotoxicity, enabling the widespread use of AgNPs in modern medical techniques (Jadhav et al., 2018).

In the present study, AgNPs were obtained through a green synthesis approach by using aqueous extract of *Capsicum annuum* fruits. Synthesized nanoparticles were then described through scanning electron microscopy (SEM) and UV-Vis spectrophotometry. The *in vitro* antioxidant potential and bactericidal activity of biosynthesized AgNPs was also evaluated.

Materials and methods

Healthy and fresh fruits of *Capsicum annuum* cv. Teja (S-17) were thoroughly washed with deionized water to remove any possible pollution. To eliminate the residual moisture, plant parts were air dried at 60 °C. Dry fruits with removed placenta were milled into the powder and were stored for subsequent experiments at 4 °C.

Two grams of milled fruits tissues were put in a flat bottomed flask, covered with 100 mL of deionized water and boiled for 20 minutes. The obtained extract was allowed to cool at room temperature with subsequent filtering through Whatman No. 1 filter paper. The qualitative phytochemical analysis of the *C. annuum* fruits aqueous extract was carried out to estimate the presence of different bioactive secondary metabolites (tannins, saponins, flavonoids, phenols, steroids, alkaloids, and terpenoids). Results of the qualitative phytochemical screening were expressed as (+) abundance, (+/-) moderate, and (-) absence.

To evaluate the presence of tannins, the ferric chloride test was performed. Five drops of a 10% solution of ferric chloride (FeCl₃) were added to 2 mL aqueous extract of *C. annuum* fruits. The distribution of blackish-green or blackish-blue markings indicates the presence of tannins in experimental extract (Kumaresan et al., 2019). The amount of saponins in extracts was estimated with the honeycomb test. 4 mL of the aqueous *C. annuum* fruits extract solution were added to a test tube containing 10 mL of distilled water. The test tube with mixture was vortexed intensively for 2 min and left for sedimentation for 10 min, and observed for honeycomb froth, which indicated the presence of saponins in the experimental extract (Auwal et al., 2014). Qualitative flavonoid content was evaluated through Shibusita's test. 5 mL of sodium hydroxide (NaOH) were added to 5 mL of the extract in a test tube. The formation of a yellow colour indicated the presence of flavonoids in the experimental extract (Kumaresan et al., 2019). Presence of phenols in the obtained extract was revealed with the ferric chloride test. 2 mL of the extract were added to 2 mL of distilled water. Then, 10% solution of FeCl₃ was added to the mixture. The presence of phenols in the experimental extract was indicated by the distribution of a bluish black colour (Shah & Yadav, 2015). Qualitative assessment of alkaloid content was performed via Dragendorff's test. 1 mL of Dragendorff's reagent was added to a test tube containing 5 mL of the aqueous *C. annuum* fruits' extract. The formation of an orange red precipitate showed the presence of alkaloids in the experimental extract (Joshi et al., 2013). Terpenoid compounds were discovered through the sulphuric test. 3 mL of the extract were evaporated to dryness, and 2 mL of concentrated sulphuric acid (H₂SO₄) were added and heated for about 3 min. A reddish brown colour indicated the presence of terpenoids in the experimental solution (Mustapha et al., 2023).

Silver nanoparticles were synthesized by the addition of 1 mM AgNO₃ solution to *C. annuum* cv. Teja (S-17) filtered extract (Smimov et al., 2021). To estimate the optimal proportion for green synthesis, three different mixtures were produced, with progressive increase in fruit extract content. The reduction of silver ions was performed through the addition of 10, 20 and 30 mL of fruit extract to 40, 30 and 20 mL of 1 mM silver nitrate solution (1:4, 2:3 and 3:2 solutions respectively). The obtained mixtures were developed at room temperature for two hours under 42 W, 6500 K LED lamp (Secret Jardin, Belgium). To verify the validity of our protocol, the nanoparticle synthesis was performed in triplicate, with no differences observed.

Optical properties of obtained AgNP solutions, with three different fruit extract: AgNO₃ ratios, were evaluated by UV-Vis spectrophotometry at resolution of 1 nm (Shimadzu UV-1800, Japan).

The morphology and size distribution of biosynthesized nanostructures was described by scanning electron microscopy (SEM, Tescan Mira 3 MLU). The investigation was performed after the purification via silver nanoparticles with subsequent desiccation at 60 °C.

The antioxidant activity of obtained AgNP solutions, fruit extract and AgNO₃ solution was estimated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability assay. Here, the original dark purple colour of DPPH is gradually lost through the transformation of this radical to its non-radical form. The change of colour of the reaction mixture from dark purple to yellow is manifested through the decrease of absorbance at 517 nm wavelength. This optical change was measured by UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). The antioxidant activity was represented as a percentage of inhibition that was calculated as the ratio of absorbance difference between blank and test solutions to the absorbance of test solution (Smimov et al., 2022).

The antimicrobial activity of test samples was evaluated against common Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and Gram-positive (*Micrococcus luteus*, *Staphylococcus aureus*) strains by agar diffusion test method (Suresh et al., 2016). The culture of the test samples was grown in nutrient broth (Himedia, USA) that was adjusted to turbidity standards of 0.5 McFarland. After the propagation of microorganisms, the solidified Mueller-Hilton agar was inoculated with 1 mL of a 24-hour culture suspension on the surface of Petri plates. To evaluate the antimicrobial activity of the obtained solutions, 5 wells were made at each agar plate agar with a cylindrical metal tube. The 100 µL samples of test solutions were then added to the wells. The control antibiotic influence was performed by aseptically placing of antibiotic disc impregnated with cefalexin CK30 (Himedia, USA) on the agar plates. All plates were left for 18–24 h at 37 °C for the development. The bactericidal effect was estimated as the diameter of zone of inhibition observed on cultures.

The obtained quantitative data was analyzed by the Shapiro-Wilk's normality test and Leven's test for dispersion homogeneity. As all the data allowed the application of parametric tests, ANOVA with post-hoc Duncan's multiple range test was performed using Statistica (StatSoft, USA). The observed differences were considered significant at P < 0.05. All numerical values were represented as mean ± standard deviation (x ± SD).

Results

The phytochemical screening of the aqueous extract of *C. annuum* fruits was conducted to determine the presence of bioactive agents with potential reducing and stabilizer properties. The revealed compounds – aromatic secondary metabolites are shown in Table 1. The results indicate the presence of variant secondary metabolites that include saponins, flavonoids, phenols, and alkaloids, whereas tannins and terpenoids were absent.

Table 1

Qualitative analysis of the *C. annuum* fruits aqueous extract (n = 5)

Phytochemicals	Tannins	Saponins	Flavonoids	Phenols	Alkaloids	Terpenoids
Status	-	+/-	+	+	+	-

To confirm the presence of AgNPs, we recorded UV-Vis spectra of Ag-extract mixtures, where distilled water was used as a blank. In all cases, we observed emergence of absorbance peaks at 454, 448 and 427 nm for 3:2, 2:3 and 1:4 solutions (*C. annuum* fruit extract : 1 mM AgNO₃) respectively that illustrate the manifestation of surface plasmon

resonance (SPR) in the mixture (Fig. 1). We also detected the change in colour of the experimental solution from light yellow at the initial point to different shades of red and brown, where darker colours corresponded to higher content of silver (Fig. 1, inset). All solutions remained stable and showed no colour changes within at least 24 h after the formation of nanoparticles. The presence of nanoparticles was additionally confirmed by the manifestation of Tyndall effect in experimental solutions (Fig. 1, inset), which represents light scattering at nanostructure.

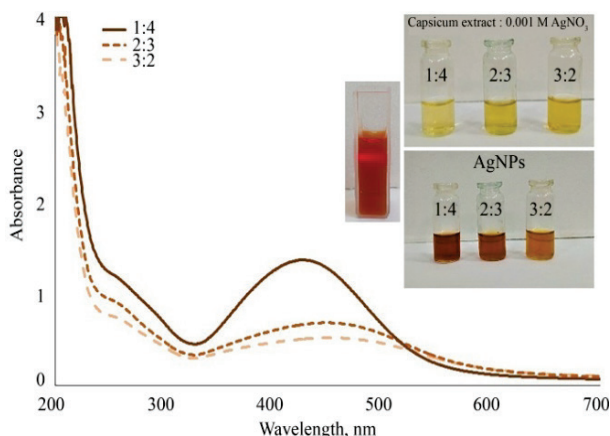


Fig. 1. Surface plasmon resonance of AgNPs observed at 427–454 nm confirms the biotransformation of colloidal AgNPs from Ag⁺ in experimental solutions with different fruit extracts : AgNO₃ ratios: the propagation of the SPR effect and Tyndall effect of light scattering is indicated by the colour change of solutions at inset

We further performed SEM analysis of resulting solutions to investigate morphology and size distribution of AgNPs. In all samples, nanoparticles were shown to be approximately spherical, with a size of 13–22 nm (Fig. 2).

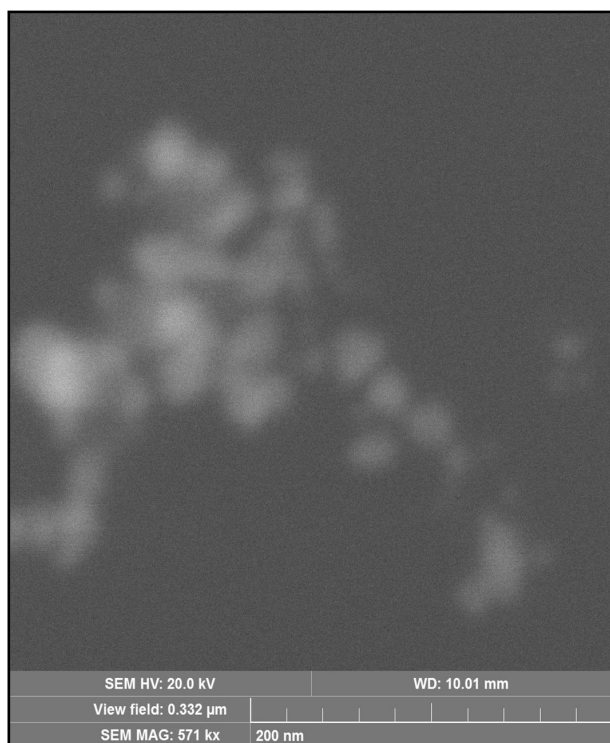


Fig. 2. Morphological analysis of AgNPs assessed by SEM revealed a spherical shape and range size from 13 to 22 nm

DPPH radical scavenging assay demonstrated the considerable antioxidant activity of *C. annuum* fruit extract. In contrary, reductive activity of 1 mM AgNO₃ solution was found to be almost 3-fold lower, clearly showing its weak antioxidant properties. Comparatively, all acquired solutions of AgNPs showed intermediate levels of DPPH inhibition (Fig. 3).

Bactericidal effects of AgNPs and AgNO₃ solutions against Gram-positive *M. luteus* and *S. aureus* were at the same level as that of cefalexin, which was represented in formation of clear zones (Fig. 4). We also demonstrated the decrease in antibacterial effectiveness of AgNPs against Gram-negative *E. coli* and *P. aeruginosa*, as compared to Gram-positive species (Fig. 5). However, both AgNPs and AgNO₃ solution showed bactericidal activity against cephalaxin-resistant *P. aeruginosa* (Fig. 5d).

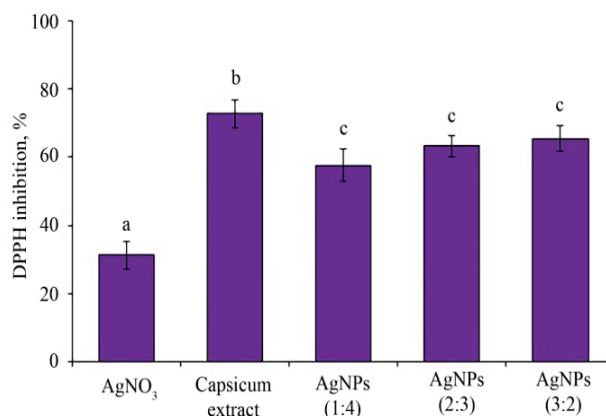


Fig. 3. Antioxidant activity of AgNPs as determined by DPPH assay showed the dose-dependent increase in antiradical capacity (n = 5; x ± SD): identical letter labels indicate absence of statistically significant difference at P < 0.05

Discussion

Aromatic secondary metabolites have received wide attention because of their various biological activities. Many of them have shown strong antioxidant properties. In our study we showed that *C. annuum* fruit aqueous extracts contained important biomolecules that can effectively reduce silver ions into silver nanoparticles. These results indicate that the obtained extract contained high amounts of aromatic secondary metabolites that can act as effective antioxidants, which is an important trait for nanoparticle biosynthesis (Gayathri et al., 2016).

A green approach in the synthesis of nanoparticles provides not only ecological friendliness, but also the possibility to easily observe the formation of nanostructures. For instance, accumulation of nanosized objects leads to change in spectral properties of a mixture. The formation of AgNPs is greatly facilitated under different types of illumination, i.e. natural sunlight or LED light, which is expressed in quicker colour changes (Bhaduri et al., 2013). The increased absorbance in the region of 450 nm is a characteristic representation of surface plasmon resonance of AgNPs. The shape and exact localization of SPR peaks depend on many factors, such as composition of nanostructures, their size and shape, as well as the presence of stabilizing agents, but amplitude directly corresponds to the concentration of nanoparticles (Pandey et al., 2012). Therefore, we have confirmed that shifting proportions of experimental mixtures towards Ag⁺ ions results in increased accumulation of AgNPs. The exact physical properties of green-synthesized nanomaterials are orchestrated by the combined action of various agents present in reaction mixture. Our synthesis protocol allowed us to obtain spherical AgNPs of a 13–22 nm size range. Similar size distribution was reported by Otunola, who synthesized 10.86 nm cuboidal AgNPs with *C. frutescens* extract (Otunola et al., 2017). However, Reda showed that green synthesis with *Capsicum* sp. extract might result in formation of large 60.9 nm particles (Reda et al., 2019). The observed irregularities might be the result of different accumulation of specific bioreducers by different plant species. Thus, we can conclude that the concentration of *C. annuum* fruit extract does not have a major impact on the physical properties of AgNPs and the differences in recorded absorption spectra arose mainly due to the concentrations of nanostructures.

Free-radical scavenging activity assays provide direct assessment of bioreductant potential for plant extracts. In particular, it has been shown that DPPH production quenching tightly correlates with the total amount

of phenolic compounds (Moon & Shibamoto, 2009). The observed effects indicate accumulation of phenolic compounds, predominantly capsaicin and dihydrocapsaicin by pepper fruits (Sora et al., 2015). All of green synthesized AgNP solutions showed significant decrease in DPPH

production, thus demonstrating the residual presence of phenolic compounds after the reduction of Ag^+ ions. The profound antioxidant properties of synthesized AgNPs may significantly attenuate their cytotoxicity, thus widening the therapeutic potential of nanoparticles (Barbasz et al., 2021).

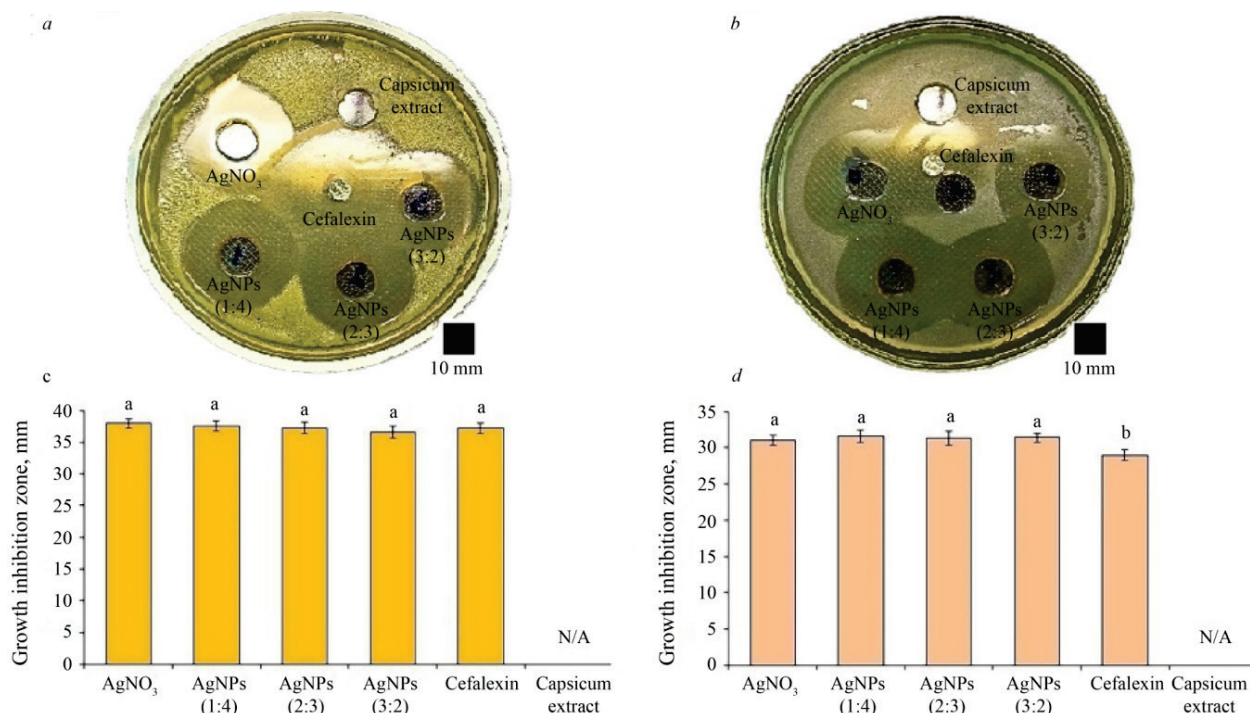


Fig. 4. Bactericidal activity of AgNPs visualized by agar diffusion method against Gram-positive microorganisms: *Micrococcus luteus* (a, c) and *Staphylococcus aureus* (b, d); N/A – not available (n = 15; $\bar{x} \pm SD$); identical letter labels indicate absence of statistically significant difference at $P < 0.05$

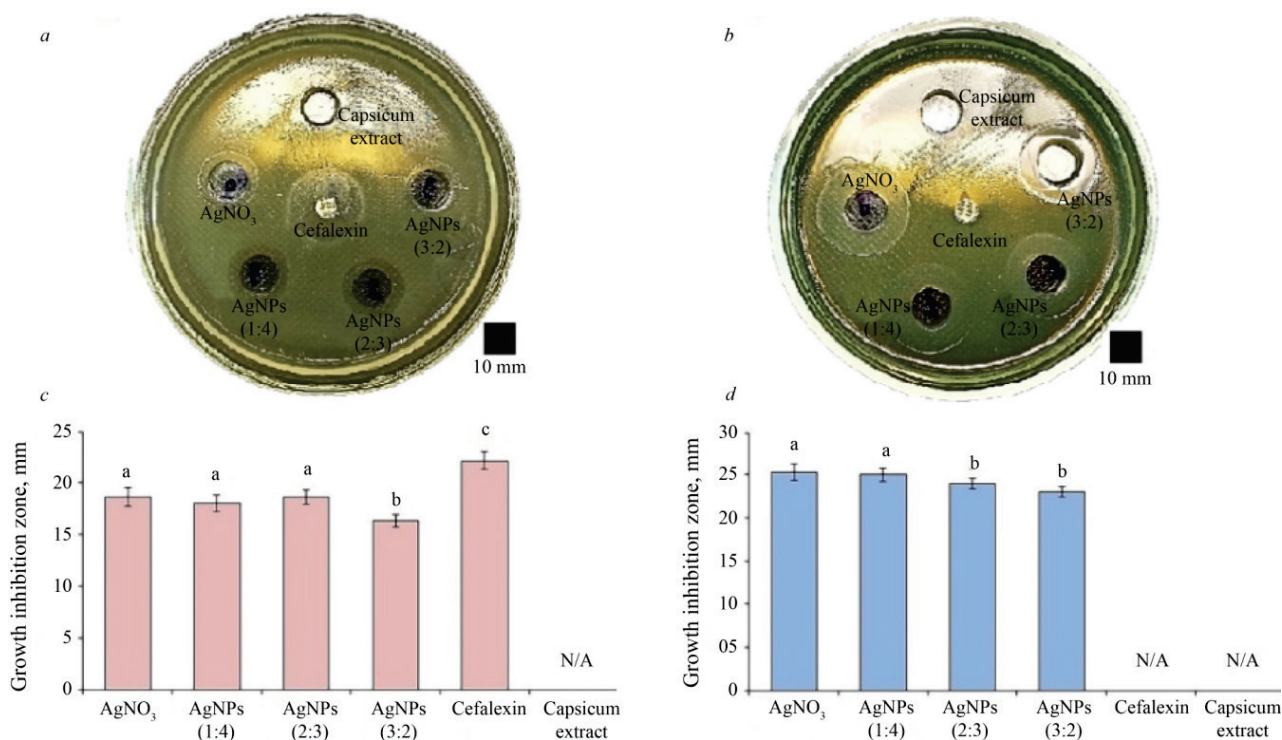


Fig. 5. Bactericidal activity of AgNPs visualized by the agar diffusion method against Gram-negative microorganisms: *Escherichia coli* (a, c) and *Pseudomonas aeruginosa* (b, d); N/A – not available (n = 15; $\bar{x} \pm SD$); identical letter labels indicate absence of statistically significant difference at $P < 0.05$

Antibacterial activity of nanoparticles of different composition and origin is one of the most valuable and actively studied properties of such structures. In our study, we compared bactericidal effects of widely used

antibiotic cephalaxin to AgNP solutions with various proportions of silver and *C. annuum* fruit extracts. Due to the specific features of cytoarchitecture, mostly structure of cell walls, Gram-positive and Gram-negative

strains might show differential susceptibility to different recognized and potential antibacterial substances (Franci et al., 2015). *M. luteus* and *S. aureus* were chosen as Gram-positive testing objects due to their common pathogenicity and profound drug resistant properties (Pantosti et al., 2007; Dutta et al., 2020). The absence of zones of inhibition after the application of *C. annuum* fruit extract indicated the exclusive role of silver as a source of bactericidal effects of AgNPs. Interestingly, antibacterial properties of silver compositions towards *S. aureus* exceeded the effect of cefalexin (Fig. 4d), making them a promising candidate for treatment of various pathological conditions, caused by drug-resistant strains of pathogenic *S. aureus* (de Jesús Ruiz-Baltazar et al., 2017). Solutions of AgNPs were also tested against Gram-negative *E. coli* and *P. aeruginosa*, where the former is a classical testing object and indicator of environmental pollution, and the latter is an etiological agent for multiple respiratory infections, associated with increased drug resistance (Radzig et al., 2013; Horcajada et al., 2019). Although the observed effects were less developed, compared to the results obtained on Gram-positive strains, we registered the dependence of the size of inhibition zones on Ag content in tested solutions. The differential sensitivity of Gram-positive and Gram-negative microorganisms to AgNPs has already been reported in numerous studies. For instance, Kim et al. (2016b) demonstrated AgNPs to be more effective against Gram-negative bacteria, while Cyril reached the opposite results (Cyril et al., 2019). These contradictions are usually explained by the binding properties of AgNPs, inherited from their bioreducers: the ability of nanoparticles to interact with lipid layers leads to disruption of Gram-negative outer plasma membranes, while protein binding is required for the deterioration of peptidoglycan layer of Gram-positive organisms (Cavassin et al., 2015). The increased effectiveness of AgNP solutions synthesized with *C. annuum* fruit extracts against *M. luteus* and *S. aureus*, as compared to *E. coli* and *P. aeruginosa*, might indicate the prevalent interaction of AgNPs with protein components as a result of surface functionalization.

Conclusion

We demonstrated a cost-effective and environmental-friendly method of AgNP synthesis using the extracts of *Capsicum annuum* cv. Teja (S-17). Plant extracts were shown to be highly efficient bioreducers, as the lowest relative amount of pepper extracts produced the AgNP solution with the most prominent activity. The resulting solutions showed both bactericidal and antioxidant properties, thus constituting a substantial ground for the green synthesis approach as a source of nanomaterials for different biomedical applications.

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References

- Anastas, P., & Eghbali, N. (2010). Green chemistry: Principles and practice. *Chemical Society Reviews*, 39(1), 301–312.
- Arroyo, G. V., Madrid, A. T., Gavilanes, A. F., Naranjo, B., Debut, A., Arias, M. T., & Angulo, Y. (2020). Green synthesis of silver nanoparticles for application in cosmetics. *Journal of Environmental Science and Health, Part A*, 55(11), 1304–1320.
- Auwal, M. S., Saka, S., Mairiga, I. A., Sanda, K. A., Shuaibu, A., & Ibrahim, A. (2014). Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (Thom mimosa). *Veterinary Research Forum*, 5(2), 95–100.
- Barbasz, A., Oćwieja, M., Piergies, N., Duraczyńska, D., & Nowak, A. (2021). Antioxidant-modulated cytotoxicity of silver nanoparticles. *Journal of Applied Toxicology*, 41(11), 1863–1878.
- Bhaduri, G. A., Little, R., Khomane, R. B., Lokhande, S. U., Kulkarni, B. D., Mendis, B. G., & Šiller, L. (2013). Green synthesis of silver nanoparticles using sunlight. *Journal of Photochemistry and Photobiology A: Chemistry*, 258, 1–9.
- Carrillo-González, R., Martínez-Gómez, M. A., González-Chávez, M. del C. A., & Mendoza-Hernández, J. C. (2016). Inhibition of microorganisms involved in deterioration of an archaeological site by silver nanoparticles produced by a green synthesis method. *Science of the Total Environment*, 565, 872–881.
- Cavassin, E. D., de Figueiredo, L. F. P., Otoch, J. P., Seckler, M. M., de Oliveira, R. A., Franco, F. F., Marangoni, V. S., Zucolotto, V., Levin, A. S. S., & Costa, S. F. (2015). Comparison of methods to detect the in vitro activity of silver nanoparticles (AgNP) against multidrug resistant bacteria. *Journal of Nanobiotechnology*, 13(1), 64.
- Cyril, N., George, J. B., Joseph, L., Raghavamenon, A. C., & Syllas, V. P. (2019). Assessment of antioxidant, antibacterial and anti-proliferative (lung cancer cell line A549) activities of green synthesized silver nanoparticles from *Derris trifoliata*. *Toxicology Research*, 8(2), 297–308.
- Dakal, T. C., Kumar, A., Majumdar, R. S., & Yadav, V. (2016). Mechanistic basis of antimicrobial actions of silver nanoparticles. *Frontiers in Microbiology*, 7, 1831.
- de Jesús Ruiz-Baltazar, A., Reyes-López, S. Y., Larrañaga, D., Estévez, M., & Pérez, R. (2017). Green synthesis of silver nanoparticles using a *Melissa officinalis* leaf extract with antibacterial properties. *Results in Physics*, 7, 2639–2643.
- Duan, H., Wang, D., & Li, Y. (2015). Green chemistry for nanoparticle synthesis. *Chemical Society Reviews*, 44(16), 5778–5792.
- Dutta, T., Ghosh, N. N., Das, M., Adhikary, R., Mandal, V., & Chattopadhyay, A. P. (2020). Green synthesis of antibacterial and antifungal silver nanoparticles using *Citrus limetta* peel extract: Experimental and theoretical studies. *Journal of Environmental Chemical Engineering*, 8(4), 104019.
- Dzhagan, V., Smimov, O., Kovalenko, M., Mazur, N., Hreshchuk, O., Taran, N., Plokhovska, S., Pirko, Y., Yemets, A., Yukhymchuk, V., & Zahn, D. R. T. (2022). Spectroscopic study of phytosynthesized Ag nanoparticles and their activity as SERS substrate. *Chemosensors*, 10(4), 129.
- Franci, G., Falanga, A., Galdiero, S., Palomba, L., Rai, M., Morelli, G., & Galdiero, M. (2015). Silver nanoparticles as potential antibacterial agents. *Molecules*, 20(5), 8856.
- Gayathri, N., Gopalakrishnan, M., & Sekar, T. (2016). Phytochemical screening and antimicrobial activity of *Capsicum chinense* Jacq. *International Journal of Advances in Pharmaceutics*, 5(1), 12–20.
- Horcajada, J. P., Montero, M., Oliver, A., Sorli, L., Luque, S., Gómez-Zorrilla, S., Benito, N., & Grau, S. (2019). Epidemiology and treatment of multidrug-resistant and extensively drug-resistant *Pseudomonas aeruginosa* infections. *Clinical Microbiology Reviews*, 32(4), e00031-19.
- Jadhav, K., Deore, S., Dhamecha, D., Rajeshwari, H. R., Jagwani, S., Jalalpure, S., & Bohara, R. (2018). Phytosynthesis of silver nanoparticles: Characterization, biocompatibility studies, and anticancer activity. *ACS Biomaterials Science and Engineering*, 4(3), 892–899.
- Jorge de Souza, T. A., Rosa Souza, L. R., & Franchi, L. P. (2019). Silver nanoparticles: An integrated view of green synthesis methods, transformation in the environment, and toxicity. *Ecotoxicology and Environmental Safety*, 171, 691–700.
- Joshi, A., Bhoje, M., & Sattarkar, A. (2013). Phytochemical investigation of the roots of *Grewia microcos* Linn. *Journal of Chemical and Pharmaceutical Research*, 5(7), 80–87.
- Kim, J.-S., An, C. G., Park, J.-S., Lim, Y. P., & Kim, S. (2016a). Carotenoid profiling from 27 types of paprika (*Capsicum annuum* L.) with different colors, shapes, and cultivation methods. *Food Chemistry*, 201, 64–71.
- Kim, T. Y., Cha, S.-H., Cho, S., & Park, Y. (2016b). Tannic acid-mediated green synthesis of antibacterial silver nanoparticles. *Archives of Pharmacal Research*, 39(4), 465–473.
- Kumaresan, M., Kannan, M., Chandrasekhar, C., & Vasanthi, D. (2019). Phytochemical screening and antioxidant activity of *Jasminum multiflorum* (pink kakkada) leaves and flowers. *Journal of Pharmacognosy and Phytochemistry*, 8(3), 1168–1173.
- Moon, J.-K., & Shibamoto, T. (2009). Antioxidant assays for plant and food components. *Journal of Agricultural and Food Chemistry*, 57(5), 1655–1666.
- Mustapha, T., Ithnin, N. R., Othman, H., Abu Hasan, Z.-I., & Misni, N. (2023). Bio-fabrication of silver nanoparticles using *Citrus aurantifolia* fruit peel extract (CAFPE) and the role of plant extract in the synthesis. *Plants*, 12(8), 1648.
- Otunola, G., Afolayan, A., Ajayi, E., & Odeyemi, S. (2017). Characterization, antibacterial and antioxidant properties of silver nanoparticles synthesized from aqueous extracts of *Allium sativum*, *Zingiber officinale*, and *Capsicum frutescens*. *Pharmacognosy Magazine*, 13(50), 201–208.
- Ovais, M., Khalil, A. T., Raza, A., Khan, M. A., Ahmad, I., Islam, N. U., Saravanan, M., Ubaid, M. F., Ali, M., & Shinwari, Z. K. (2016). Green synthesis of silver nanoparticles via plant extracts: Beginning a new era in cancer theranostics. *Nanomedicine*, 11(23), 3157–3177.
- Pandey, S., Goswami, G. K., & Nanda, K. K. (2012). Green synthesis of biopolymer-silver nanoparticle nanocomposite: An optical sensor for ammonia detection. *International Journal of Biological Macromolecules*, 51(4), 583–589.

- Pantosti, A., Sanchini, A., & Monaco, M. (2007). Mechanisms of antibiotic resistance in *Staphylococcus aureus*. *Future Microbiology*, 2(3), 323–334.
- Patra, S., Mukherjee, S., Barui, A. K., Ganguly, A., Sreedhar, B., & Patra, C. R. (2015). Green synthesis, characterization of gold and silver nanoparticles and their potential application for cancer therapeutics. *Materials Science and Engineering: C*, 53, 298–309.
- Radzig, M. A., Nadochenko, V. A., Koksharova, O. A., Kiwi, J., Lipasova, V. A., & Khmel, I. A. (2013). Antibacterial effects of silver nanoparticles on gram-negative bacteria: Influence on the growth and biofilms formation, mechanisms of action. *Colloids and Surfaces B: Biointerfaces*, 102, 300–306.
- Rafique, M., Sadaf, I., Rafique, M. S., & Tahir, M. B. (2017). A review on green synthesis of silver nanoparticles and their applications. *Artificial Cells, Nanomedicine, and Biotechnology*, 45(7), 1272–1291.
- Reda, M., Ashames, A., Edis, Z., Bloukh, S., Bhandare, R., & Abu Sara, H. (2019). Green synthesis of potent antimicrobial silver nanoparticles using different plant extracts and their mixtures. *Processes*, 7(8), 510.
- Roy, A., Bulut, O., Some, S., Mandal, A. K., & Yilmaz, M. D. (2019). Green synthesis of silver nanoparticles: Biomolecule-nanoparticle organizations targeting antimicrobial activity. *RSC Advances*, 9(5), 2673–2702.
- Shah, R. K., & Yadav, R. N. S. (2015). Qualitative phytochemical analysis and estimation of total phenols and flavonoids in leaf extract of *Sarcochlamys pulcherrima*. *Global Journal of Bio-Science and Biotechnology*, 4(1), 81–84.
- Smimov, O. E., Kalynovskyi, V. Y., Yumyna, Y. M., Zelena, P. P., Skoryk, M. A., Dzhan, V. M., & Taran, N. Y. (2021). Green synthesis of silver nanoparticles using aqueous extract of hot chili pepper fruits and its antimicrobial activity against *Pseudomonas aeruginosa*. *The Ukrainian Biochemical Journal*, 93(5), 102–110.
- Smimov, O., Kalynovskyi, V., Yumyna, Y., Zelena, P., Levenets, T., Kovalenko, M., Dzhan, V., & Skoryk, M. (2022). Potency of phytosynthesized silver nanoparticles from *Lathraea squamaria* as anticandidal agent and wheat seeds germination enhancer. *Biologia*, 77, 2715–2724.
- Sora, G. T. S., Haminiuk, C. W. I., da Silva, M. V., Zielinski, A. A. F., Gonçalves, G. A., Bracht, A., & Peralta, R. M. (2015). A comparative study of the capsaicinoid and phenolic contents and *in vitro* antioxidant activities of the peppers of the genus *Capsicum*: An application of chemometrics. *Journal of Food Science and Technology*, 52(12), 8086–8094.
- Sujitha, V., Murugan, K., Paulpandi, M., Panneerselvam, C., Suresh, U., Roni, M., Nicoletti, M., Higuchi, A., Madhiyazhagan, P., Subramaniam, J., Dinesh, D., Vadivalagan, C., Chandramohan, B., Alarfaj, A. A., Munusamy, M. A., Barnard, D. R., & Benelli, G. (2015). Green-synthesized silver nanoparticles as a novel control tool against dengue virus (DEN-2) and its primary vector *Aedes aegypti*. *Parasitology Research*, 114(9), 3315–3325.
- Suresh, S., Karthikeyan, S., Saravanan, P., Jayamoorthy, K., & Dhanalekshmi, K. I. (2016). Comparison of antibacterial and antifungal activity of 5-amino-2-mercapto benzimidazole and functionalized Ag₃O₄ nanoparticles. *Karbala International Journal of Modern Science*, 2(2), 129–137.
- Wei, L., Lu, J., Xu, H., Patel, A., Chen, Z.-S., & Chen, G. (2015). Silver nanoparticles: Synthesis, properties, and therapeutic applications. *Drug Discovery Today*, 20(5), 595–601.