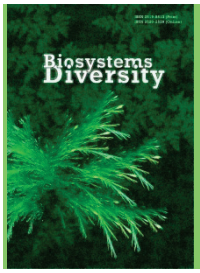

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Influence of silver nanoparticles synthesized from *Chaenomeles* leaf extracts on pathogenic microorganisms *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Fusarium culmorum*

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Herein, we report for the first time the biosynthesis of silver nanoparticles (AgNPs) using leaf extracts of *Chaenomeles* Lindl. (Rosaceae) plants and its spectral characteristics, as well as antifungal and antibacterial activity. Phytosynthesis of silver nanoparticles on the base of aqueous plant extracts and silver nitrate solution was carried out by an ecofriendly and cost-effective approach. UV-Vis spectroscopy was applied to validate the plant-mediated biosynthesis of AgNPs colloidal solutions by the Surface Plasmon Resonance (SPR) bands in the region of 450–500 nm, characteristic of polycrystalline silver nanoparticles. Scanning microscopy (SEM) revealed a wide variation in range 5–58 nm and a close to spherical shape of plant-derived AgNPs. Raman scattering spectroscopy revealed the suitability of biosynthesized silver nanoparticles as the substrates for surface-enhanced Raman scattering (SERS) spectroscopy with the highest efficiency of AgNPs, biosynthesized from leaf extract of *Ch.* × *superba*, which enhanced the Rhodamine 6G dye applied at a concentration of 10^{-7} M. Assay of antifungal activity performed by well diffusion method revealed the dose-dependent effect of all AgNPs against the phytopathogenic fungus *Fusarium culmorum*. The most effective AgNPs (*Ch. speciosa*-AgNPs, *Ch. cathayensis*-AgNPs, and *Ch. japonica*-AgNPs) achieved a 1.42–1.63 times greater zone of inhibition of the *F. culmorum* colonies' growth compared to the corresponding doses of the known chemical fungicide "Quadris". Micro preparations of the zones of incomplete growth inhibition presented changes in the mycelium morphology of *F. culmorum* due to the action of nanoparticles, such as deformation (curvature, expansion), and a decrease in the hyphae length and density compared to the control sample. Disc-diffusion assay showed notable species-specific antibacterial activity of AgNPs both against Gram-negative (*Klebsiella pneumoniae*) and Gram-positive (*Staphylococcus aureus*) strains. Summarizing, the results indicate the undeniable suitability of aqueous leaf extracts of the genus *Chaenomeles* species for the successful biosynthesis of silver nanoparticles with many useful properties, whose diverse applications require further research.

Keywords: plant extracts; green synthesis; metal nanoparticles; surface-enhanced Raman scattering; fungicide effects; antibacterial activity.

Introduction

Nanotechnology is one of progressive advances in recent times that has been utilized in various applied sciences, such as physics, biology, medical science, chemistry, and engineering (Wakeil et al., 2017), and now acquires a general purpose (Malik et al., 2023). Moreover, nanotechnology holds great potential to be implemented in every life aspect, including nanomaterial sciences, nanoelectronics, and nanomedicine (Singh, 2017). The fundamental component of nanotechnology is represented by nanoparticles (NPs), which are 1–100 nm in size, and can be made up of carbon, inorganic (metal, metal oxides) or organic matter (Anu & Saravanakumar, 2017; Vanlalveni et al., 2021; Joudeh & Linke, 2022). Nanoparticles frequently exhibit distinctive size-dependent features, mostly due to the large ratio of surface area to volume. Because of this, many of the physical characteristics of nanoparticles differ significantly from those of bulk materials, leading to a wide range of their novel uses (Chekman et al., 2014; Altammar, 2023).

The synthesis of nanoparticles can be performed by physical, chemical or biological methods (Iravani et al., 2014; Álvarez-Chimal & Ángel Arenas-Alatorre, 2023), of which the first two are usually expensive and potentially dangerous for the environment and human health, as they require significant doses of radiation and high concentrations of reducing agents and stabilizers (Sazak et al., 2023), and produce hazardous by-products (Ashique et al., 2022). Many adverse effects have been associated with chemical NPs synthesis due to the presence of some toxic chemical absorbed on their surface, which limits the prospects of their use (Hasan, 2015; Behravan et al., 2019). Ecofriendly alternatives to chemical and physical methods are biological ways of nanoparticles synthesis using bacteria (Hassan et al., 2016), fungi (Durán et al., 2007; Raheman et al., 2011; Smirnov et al., 2023), algae (Armagan et al., 2024; Savvidou et al., 2024), and plants (Logeswari et al., 2015; Bergal et al., 2022; Oliveira et al., 2022). Nanoparticles produced using non-toxic, biodegradable and renewable biological materials have a number of advantages, including biocompatibility, improved properties, cost-effectiveness, environmental sustainability (Blume et al., 2015; Parveen et al., 2016; Ashique et al., 2022).

In recent years, the development of environmentally friendly methods for the synthesis of nanoparticles has become an important field of nanotechnology, especially the biosynthesis of silver nanoparticles (AgNPs) (Hasan, 2015), which have many applications in microbiology, chemistry, food technology, cell biology, pharmacology and parasitology (Kavaz et al., 2018; Vanlalveni et al., 2021). The production of silver nanoparticles

includes the reducing of silver nitrate (Ag^+) to Ag^0 and stabilization of the synthesized AgNPs to prevent their aggregation. The biological sources used for the green synthesis of AgNPs can act simultaneously as catalyzing, reducing, stabilizing, or capping agents due to presence of the biologically active compounds (Behravan et al., 2019; Álvarez-Chimal & Ángel Arenas-Alatorre, 2023). In this regard, plant extracts containing enzymes, proteins, polyphenols, flavonoids, and terpenoids are capable of providing the processes necessary for the synthesis of nanoparticles. Therefore, the selection of plant material is an important search stage for identifying promising samples for successful green synthesis, in particular using extracts from different parts of plants (Chau et al., 2022; Naveed et al., 2023; Savvidou et al., 2024). Numerical studies have shown the effectiveness of the use of biosynthesized metal nanoparticles, including AgNPs, as antibacterial (Behravan et al., 2019; Bergal et al., 2022; Kamer et al., 2024) and antifungal (Kasprowicz et al., 2010; Jian et al., 2021; Li et al., 2022; Oliveira et al., 2022) agents. Despite the wide range of knowledge about the antimicrobial properties of AgNPs in general, their fungicidal ability remains less studied. Currently, AgNPs find a promising application within the Raman spectroscopy method as a substrate for improving the effect of surface-enhanced Raman scattering (SERS), in which an increase in the intensity of Raman scattering spectra by several orders of magnitude is observed (Dovbeshko et al., 2004; Chekman et al., 2014; Rusu et al., 2023). This method demonstrates high efficiency in the field of identification and determination of very small amounts of substances, which is important for chemical and biological sensors, biomedical diagnostics, and nanomedicine (Chekman et al., 2014).

Plants of the *Chaenomeles* Lindl. genus look like a suitable material for green synthesis of AgNPs because they are rich in bioactive compounds including high content of polyphenols, flavonoids and free phenolic acids, and are characterized by the substantial antimicrobial activity against some bacterial and fungal strains (Lykholat et al., 2021a, 2021b). At the same time, only the species *Chaenomeles sinensis* (Oh et al., 2018) has been used as a plant material for the biological synthesis of silver nanoparticles, as currently known. The purpose of our research is to characterize silver nanoparticles biosynthesized from *Chaenomeles* plant leaf extracts and to find out the effectiveness of their use as antifungal and antibacterial agents and as a substrate in Raman spectroscopy.

Material and methods

Plant material collection and plant extracts obtaining. Leaves of plants of the genus *Chaenomeles* Lindl. were used: *Ch. speciosa* (Sweet) Nakai, *Ch. cathayensis* (Hemsl.) C. K. Schneid., *Ch. japonica* (Thunb.) Lindl. ex Spach., *Ch.* × *superba* (Frahm) Rehder, and *Ch.* × *californica* Clarke ex Weber, *Ch. thibetica* T. T. Yu. Plant leaves were collected in May 2024 in the botanical garden of Oles Honchar Dnipro National University (48°26'07" N, 35°02'34" E, Dnipro, Ukraine), packed in slip-packs, labeled and delivered to the laboratory, where they were washed with distilled water, dried to a constant weight under laboratory conditions, crushed using a mortar and pestle.

Aqueous extracts of *Chaenomeles* leaves were prepared, for which 1.0 g of plant material was placed in a conical flask, 30 mL of distilled water was added, mixture was heated in a water bath to a temperature of 60 ºC and kept for 30 min. After cooling and settling for 2 hours, the extract was centrifuged for 15 minutes at 12,000 rpm.

Synthesis of silver nanoparticles. Biosynthesis of silver nanoparticles (AgNPs) was carried out under laboratory conditions of temperature and lighting by adding the 25 mM aqueous solution of silver nitrate ($AgNO_3$) to the freshly prepared plant extracts in a ratio of 5:1 (v/v). The formation of silver nanoparticles was first observed visually by changing the color of the solutions from yellow to brown within 3 hours.

The process of the color change in the *Chaenomeles* leaf extracts from light yellow to dark brown after the addition of silver nitrate solution occurred at different rates during three hours of incubation, which was a visual indication of the formation of silver nanoparticles (Fig. 1).

Spectroscopic characterization. UV-Vis spectroscopic analysis in the range of 400–900 nm was performed using a StellarNet Silver Nova 25 BWI6 (StellarNet Inc, 2015) spectrometer, which contains tungsten and deuterium lamps as excitation sources. No further instrumental or numerical correction of the spectra was performed. The biosynthesis of AgNPs was confirmed by the results of UV-Vis spectroscopy, which showed absorption maxima of all colloidal solutions of silver nanoparticles in the region of 450–500 nm, which corresponds to the Surface Plasmon Resonance (SPR) of polycrystalline silver nanoparticles (Fig. 2).

Raman spectra were excited with 457 or 532 nm solid-state lasers and acquired using a single-stage spectrometer MDR-23 (LOMO) equipped with a cooled CCD detector (Andor iDus 420, UK). The laser power density on the samples was less than 10^3 W/cm^2, to preclude any thermal or photo-induced modification of the samples. A spectral resolution of 4 cm^{-1} was determined from the Si phonon peak width of a single crystal Si substrate. The Si phonon peak position of 520.5 cm^{-1} was used as a reference for determining a position of the peaks in the Raman/SERS spectra of the analyte.

Raman spectroscopy was performed to detect the SERS effect of silver nanoparticles biosynthesized on the basis of *Chaenomeles* leaf extracts. Raman scattering spectra of the analyte molecule Rhodamine 6G (R6G) were obtained for AgNPs cleaned from organic matter in the colloidal and dry state at the excitation wavelength λ_{532} nm (Fig. 3a). A significant Raman enhancement effect (SERS effect) of Rhodamine 6G (R6G) scattering was observed in the case of AgNPs biosynthesized from *Ch.* × *superba* leaf extract at λ_{532} nm and λ_{457} nm (Fig. 3b).

Scanning electron microscopy. The morphology of the silver nanoparticles biosynthesized on the base of *Chaenomeles* leaf extracts was studied using scanning electron microscopy (SEM, Tescan Mira 3 MLU, Tescan Group, Czech Republic) according to standard protocol.

Study of antifungal activity. The well agar diffusion method was used to evaluate the silver nanoparticles biosynthesized using *Chaenomeles* leaf extracts against the phytopathogenic fungus *Fusarium culmorum* IMB-F-50716, which was provided by the Zabolotny Institute of Microbiology and Virology (Kyiv). Mycelial suspension of a 7-day-old culture of the phytopathogen was applied to the surface of the Chapek-Dox medium in Petri plates. The commercially available fungicide "Quadris" (250 g/L of azoxystrobin), recommended in a dose of 1.2 mL/L (equivalent to 0.3 μg/μL the active substance) for the protection of fruit and vegetable crops against late blight, alternariosis, fusarium wilt, etc., was used as a positive control. Distilled water served as a negative control. After inoculation of the microbial suspension, holes with a diameter of 5 mm were made in the nutrient medium using a sterile drill, into which 100 μL of test solutions of silver nanoparticles and "Quadris" were introduced. According to the design of the experiment, a series of dilutions of AgNPs colloidal solutions and fungicide in distilled water was made (Table 1). All Petri plates were incubated at a temperature of 28 ± 0.2 °C for 3 days. The antifungal activity of the test solutions was evaluated by the diameter of the zone of inhibition (mm) around the wells.

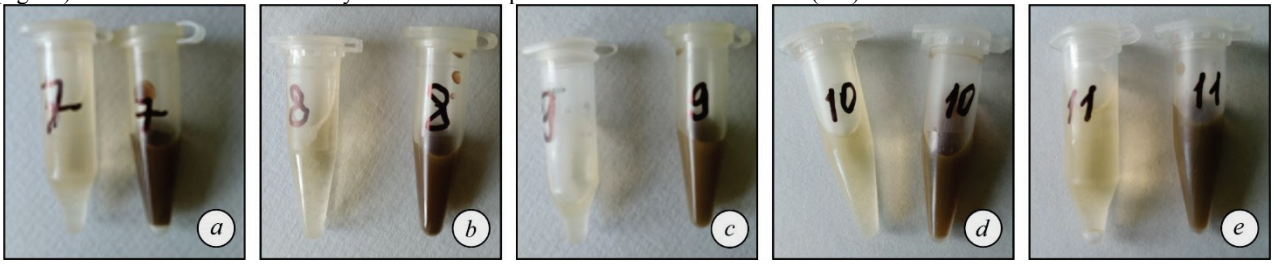


Fig. 1. Color change of *Chaenomeles* leaf extracts due to formation of AgNPs: (*a*) *Ch.* × *superba*-AgNPs, (*b*) *Ch. speciosa*-AgNPs, (*c*) *Ch.* × *californica*-AgNPs, (*d*) *Ch. cathayensis*-AgNPs, (*e*) *Ch. japonica*-AgNPs; on the left – plant extract before $AgNO_3$ adding, on the right – colloidal solution 3 h after $AgNO_3$ adding

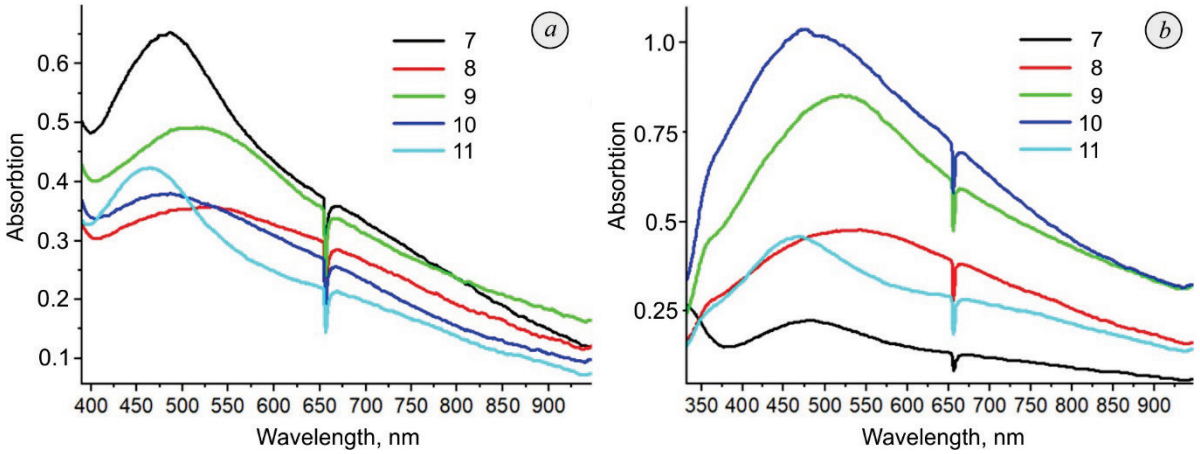
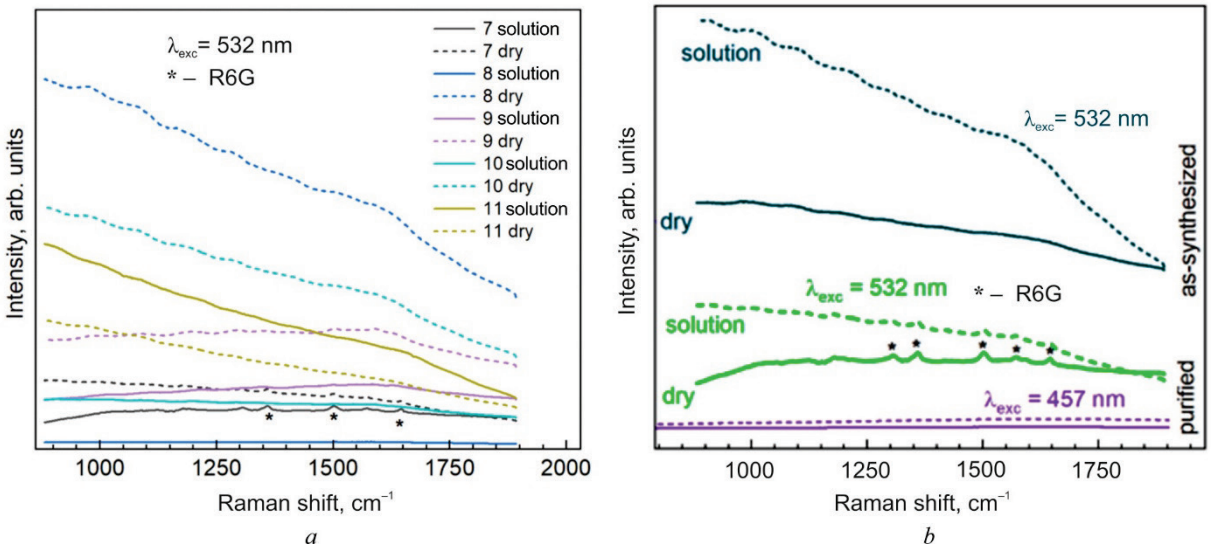


Fig. 2. UV-Vis spectra of (*a*) uncleaned and (*b*) cleaned AgNPs biosynthesized from *Chaenomeles* leaf extracts: *7* – *Ch.* × *superba*-AgNPs, *8* – *Ch. speciosa*-AgNPs, *9* – *Ch.* × *californica*-AgNPs, *10* – *Ch. cathayensis*-AgNPs, *11* – *Ch. japonica*-AgNPs



a *b*

Fig. 3. Raman spectra of 10^{-7} mol/L Rhodamine 6G dye added to AgNPs biosynthesized from *Chaenomeles* leaf extracts: (*a*) spectra of all cleaned samples under 532 nm excitation, obtained both for solutions (dashed curves) and after drying (solid curves); (*b*) spectrum of sample *Ch.* × *superba*-AgNPs before cleaning and after cleaning when excited by laser radiation with a wavelength of 532 nm and for cleaned when excited at 457 nm; *7* – *Ch.* × *superba*-AgNPs, *8* – *Ch. speciosa*-AgNPs, *9* – *Ch.* × *californica*-AgNPs, *10* – *Ch. cathayensis*-AgNPs, *11* – *Ch. japonica*-AgNPs

Table 1
Variants of test solutions for antifungal activity against *F. culmorum*

Version of AgNPs solutions	Concentration of the AgNPs test solution, μg/μL			
	Ch. speciosa-AgNPs	*Ch. cathayensis*-AgNPs	*Ch. japonica*-AgNPs	"Quadris", μg/mL
Variant 1	5.0	15.0	10.0	2.5
Variant 2	1.0	3.0	2.0	0.5
Variant 3	0.2	0.6	0.4	0.1
Variant 4	0.04	0.12	0.08	0.02

Fungal preparations for light microscopy were made from the mycelium of *F. culmorum*, taken from the zones of incomplete growth inhibition, and stained with methylene blue. Microscopic analysis was performed at 200× magnification using a Ulab XY-B2T LED microscope (China), which is equipped with a trinocular attachment for connecting a digital Sigeta M3CMOS FMA050 16.0 camera (China).

Antibacterial activity research. The disc-diffusion method (Adil et al., 2019) was used to evaluate the antibacterial activity of biosynthesized AgNPs against Gram-negative (*Klebsiella pneumoniae* B920) and Gram-positive (*Staphylococcus aureus* B209) strains taken from the culture collection of Microbiology, Virology and Biotechnology Department of Oles Honchar Dnipro National University. A daily bacterial culture with CFU 10^9 mL^{-1} was sown on MPA medium in Petri plates. Biosynthesized AgNPs solutions were applied to sterile paper discs to obtain final doses of 20 μg per disc, following which the discs were placed on the agar surface. Plates were incubated at 27 ºC for 24 hours, after which the zone inhibition (mm) around the paper discs (except disc diameter) was measured, and results were compared with ofloxacin (5 μg per disc) effect.

Statistical analysis. All bioassays were carried out in quintuple replication. Results were expressed as mean ± standard deviation (x ± SD), and differences between samples were tested using Fisher's test and Tukey's multiple test. One-way ANOVA was used to determine the effect of nanoparticle concentration on the zone of microbial growth inhibition. All differences were considered statistically significant at $P < 0.05$.

Results

Scanning electron microscopy (SEM) analysis carried out to study the morphology of silver nanoparticles biosynthesized from *Chaenomeles* leaf extracts showed variability of AgNPs size and their aggregates in the range 5–58 nm and 60–236 respectively nm, and a close to spherical shape of AgNPs (Fig. 4).

Antifungal activity study performed by well diffusion method revealed the zones of incomplete growth inhibition of *F. culmorum* colonies by visual observation (Fig. 5).

With a decrease in the concentration of nanoparticles in the solutions, a decrease in the diameter of the inhibition zone was observed. The results of the study showed a dose-dependent activity of all biosynthesized silver

nanoparticles against *F. culmorum* (Table 2). Compared to the biosynthesized nanoparticles solutions, "Quadris" showed a lower inhibitory effect at concentrations of 2.5 and 0.5 μg/mL, whereas at concentrations 0.1 and 0.02 μg/mL there was no inhibition of *F. culmorum* growth.

The inhibitory effect of nanoparticles in different concentrations (variants 1, 2 and 3) had larger zones of inhibition of the growth of *F. Culmorum* colonies compared to the corresponding variants of "Quadris" dilutions. The largest inhibition zones under the influence of synthesized silver nanoparticles were observed in option 1 (initial concentration of the obtained nanoparticles) and option 2 (dilution of the original AgNPs colloidal solutions by 5 times), which was 42.2–62.7% and 39.5–49.2% higher compared to "Quadris", respectively.

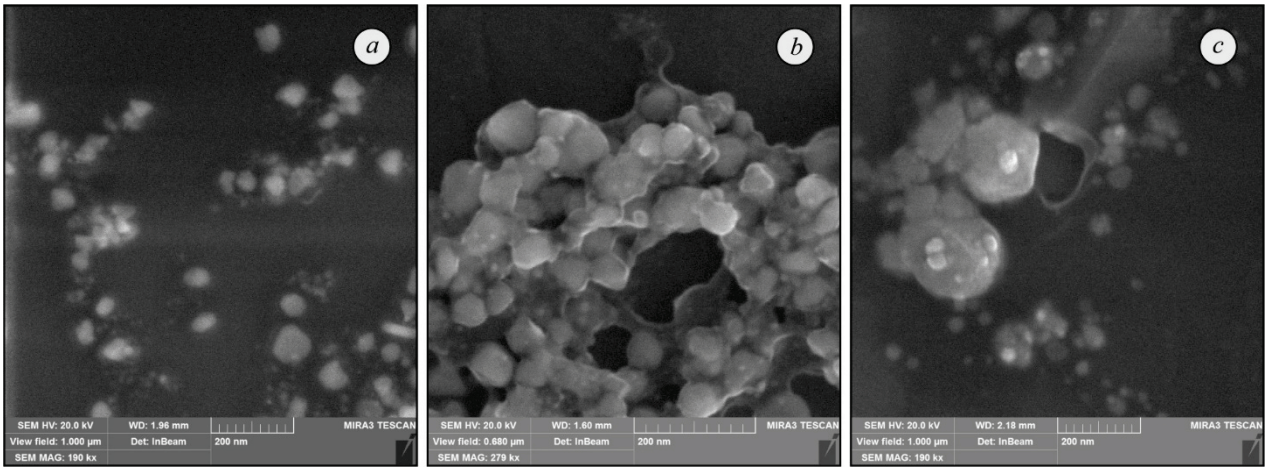


Fig. 4. Scanning electron microscopy micrograph (bar 200 nm) of silver nanoparticles derived from leaf extracts of *Chaenomeles* plants: (*a*) *Ch.* × *superba*-AgNPs, (*b*) *Ch. cathayensis*-AgNPs, (*c*) *Ch. japonica*-AgNPs

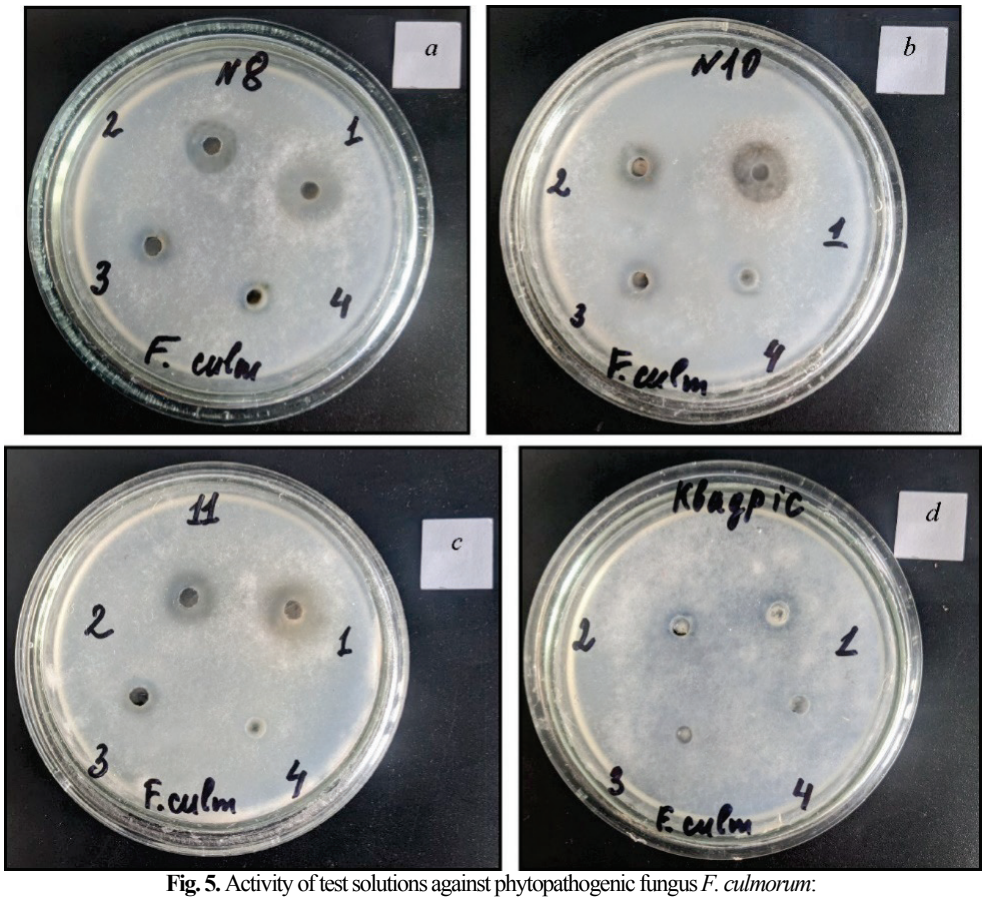


Fig. 5. Activity of test solutions against phytopathogenic fungus *F. culmorum*: (*a*) *Ch. speciosa*-AgNPs, (*b*) *Ch. cathayensis*-AgNPs, (*c*) *Ch. japonica*-AgNPs, (*d*) fungicide "Quadris"

Table 2

Diameter (mm) of zones of inhibition of the colony's growth of *F. culmorum* by test solutions (x ± SD, n = 5)

Version of AgNPs solutions	*Ch. speciosa*-AgNPs	*Ch. cathayensis*-AgNPs	*Ch. japonica*-AgNPs	"Quadris"
Variant 1	18.45 ± 0.55^{a}	18.55 ± 0.98^{a}	16.12 ± 0.49^{b}	11.34 ± 0.75^{c}
Variant 2	15.08 ± 0.81^{a}	11.73 ± 0.56^{b}	14.10 ± 0.68^{a}	10.11 ± 0.36^{c}
Variant 3	12.35 ± 0.71^{a}	10.70 ± 0.44^{b}	10.19 ± 0.51^{b}	0.00 ± 0.00^{c}
Variant 4	10.08 ± 0.76^{a}	9.93 ± 0.40^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}

Note: different letters in a row indicate a significant difference in means (by Tukey's test, $P \leq 0.05$); variants 1–4 correspond to the variants in Table 1.

The dose-dependent antifungal activity of the nanoparticles was confirmed by ANOVA analysis, which show the decomposition of the variance formed from the size of the growth inhibition zone of the phytopathogen into two components – intergroup (mean square is equal 49.995) and intragroup (mean square is equal 0.424) components with a predominance of intergroup variance. The F-ratio, which is 117.86, is the ratio of the between-group score to the within-group score with P being $7.26 \cdot 10^{-8}$. Since the P-value of the F-criterion is less than 0.05, there is a statistically significant difference between the average value of the zone of inhibition of the growth of *F. culmorum* by the test solutions of AgNPs depending on their concentration level.

In the course of a microscopic examination of preparations made from the zones of incomplete growth inhibition of *F. culmorum*, changes in the morphology of the mycelium and the density of the arrangement of hyphae were established. Under the influence of nanoparticles, deformation (curvature, expansion) and a decrease in the length of hyphae and mycelium density of the phytopathogen were observed compared to the control sample, which had a normal intact morphology in the form of long strands (Fig. 6).

Research on antibacterial activity performed by disc-diffusion method demonstrated prominent effects of all silver nanoparticles biosynthesized from *Chaenomeles* leaf extracts at a dose of 20 μg per disc against both Gram-negative (*Klebsiella pneumoniae* B920) and Gram-positive (*Staphylococcus aureus* B209) strains (Fig. 7). The visual observation of the Petri plates after incubation indicated the higher susceptibility of *St. aureus* strain to the action of all studied silver nanoparticles as compared to the inhibiting effects of AgNPs on the growth of *K. pneumoniae* strain. At the same time, the inhibiting effectiveness of the reference antibiotic ofloxacin against *K. pneumoniae* was much higher than that against *S. aureus*.

The diameters measurement of zones of bacterial growth inhibition showed low to moderate activity of silver nanoparticles against both bacterial strains relative to ofloxacin (Table 3).

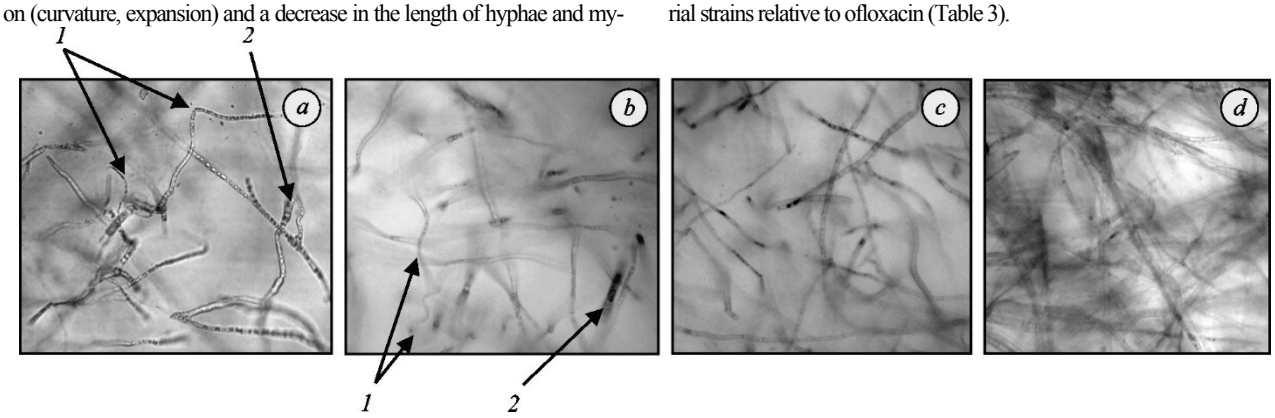


Fig. 6. Change in the morphology of the mycelium of *F. culmorum* in the zone of incomplete inhibition of colony growth under the influence of test solutions: (*a*) *Ch. speciosa*-AgNPs, (*b*) *Ch. cathayensis*-AgNPs, (*c*) "Quadris", (*d*) negative control (H_2O); deformation of the mycelium: *1* – curvature, *2* – expansion

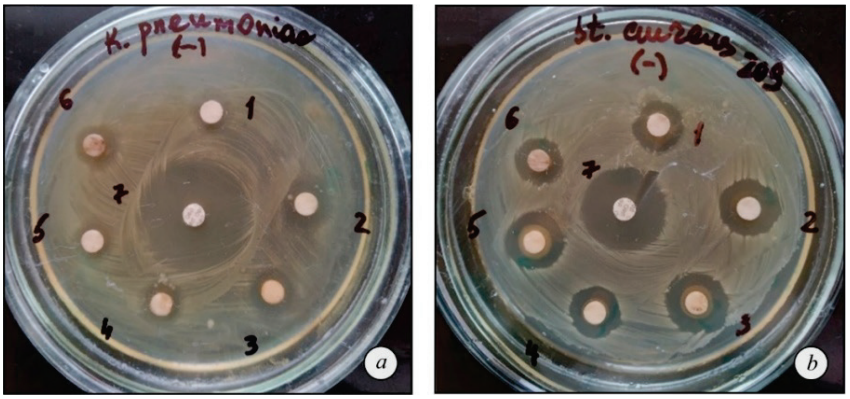


Fig. 7. Antibacterial activity of AgNPs biosynthesized from *Chaenomeles* leaf extracts against (*a*) *Klebsiella pneumoniae* and (*b*) *Staphylococcus aureus*: *1* – *Ch. thibetica*-AgNPs, *2* – *Ch.* × *superba*-AgNPs, *3* – *Ch. speciosa*-AgNPs, *4* – *Ch.* × *californica*-AgNPs, *5* – *Ch. cathayensis*-AgNPs, *6* – *Ch. japonica*-AgNPs, *7* – ofloxacin

Table 3
Diameter of the zone of inhibition (mm) of bacterial strains by AgNPs biosynthesised from *Chaenomeles* leaf extracts in doses 20 μg per disc (x ± SD, n = 5)

Bacterial culture	*Ch. thibetica*-AgNPs	*Ch.* × *superba*-AgNPs	*Ch. speciosa*-AgNPs	*Ch.* × *californica*-AgNPs	*Ch. cathayensis*-AgNPs	*Ch. japonica*-AgNPs	Ofloxacin, 5 μg per disc
K. pneumoniae B920	4.28 ± 0.32^{a}	7.21 ± 0.41^{b}	5.70 ± 0.44^{c}	4.25 ± 0.27^{a}	3.23 ± 0.30^{d}	5.37 ± 0.38^{c}	24.4 ± 1.04^{e}
St. aureus B209	7.53 ± 0.27^{a}	9.41 ± 0.49^{b}	10.96 ± 0.41^{c}	8.10 ± 0.36^{a}	9.32 ± 0.42^{b}	7.01 ± 0.20^{a}	16.2 ± 0.69^{d}

Note: different letters in a row indicate a significant difference in means by Tukey's test ($P \leq 0.05$).

The highest antibacterial activity against the Gram-negative bacterium *K. pneumoniae* was achieved by AgNPs biosynthesized from *Ch.* × *superba* leaf extracts (29.6% of the ofloxacin activity), whereas the lowest antibacterial effect was shown by AgNPs biosynthesized from leaf extracts of *Ch. cathayensis* (13.2% of the ofloxacin activity). In the inhibition of Gram-positive *S. aureus* strain, the highest activity was shown by AgNPs biosynthesized from *Ch. speciosa* leaf extracts (67.7% of the ofloxacin activity), while the AgNPs biosynthesized from leaf extracts of *Ch. japonica* demonstrated the lowest antibacterial effect (43.3% of the ofloxacin activity).

Discussion

This study represents an ecofriendly and cost-effective approach for the biosynthesis of silver nanoparticles on the base of leaf extracts of the genus *Chaenomeles* plants, which are reported for the first time as a base for nanoparticles synthesis. Visual observation of color change and UV-Vis spectroscopy of the colloidal solutions confirmed the successful processes of silver nitrate (Ag^{+}) reduction to Ag^{0}, and the production of silver nanoparticles and their stabilization, which was which was provided by phytocomponents of plant extracts. UV-Vis spectroscopy is a well-known

simple analytical technique for monitoring the formation of AgNPs (Kora et al., 2010; Remya et al., 2017). The peaks of surface plasmon resonance (SPR) of AgNPs biosynthesized from leaf extracts of different *Chaenomeles* plants in the region of 450–500 nm coincide with the known data about SPR maximum absorption by the silver nanoparticles derived from other plant extracts. For example, SPR with maximum absorbance at 435 nm confirmed the formation of AgNPs using *Argyreia nervosa* leaf extract (Saratale et al., 2017). The green synthesis of AgNPs using *Duabanga grandiflora* leaf extract was confirmed by the surface plasmon resonance maximum absorption at 453 nm (Das et al., 2024).

The absorption maximum at the UV-Vis spectrum of AgNPs synthesized from *Ch. japonica* extract (450 nm) may indicate the smaller size of these nanoparticles, compared to *Ch. speciosa*-AgNPs (absorption maximum at 525 nm) and *Ch. cathayensis*-AgNPs (maximum at 475 nm). The larger width of the absorption peaks in the UV-Vis spectra indicates a larger size dispersion of AgNPs biosynthesized from leaf extracts of *Ch. speciosa* and *Ch. cathayensis*, compared to *Ch. japonica*-AgNPs. UV spectra also provide tentative information about the shape of the synthesized nanoparticles, since spherical nanoparticles show only one surface plasmon resonance band in the absorption spectra, while two or more shape-dependent bands are observed for anisotropic particles (Durán et al., 2010). Among the absorption bands of AgNPs biosynthesized from leaf extracts of different *Chaenomeles* plants the most pronounced peaks were observed for *Ch.* × *superba*-AgNPs and *Ch. japonica*-AgNPs. The shape and spectral position of this absorption bands correlated with spectra obtained for nanoparticles synthesized using other plant extracts (Dzhagan et al., 2022; Smirnov et al., 2023). The obtained data are consistent with the results of spectral analysis (Behravan et al., 2019; Anandalakshmi, 2021; Savvidou et al., 2024) regarding the presence of a peak in the 450 nm region in the UV-Vis spectra of the synthesized nanoparticles, which is associated with the formation of silver nanoparticles (Amendola et al., 2010). According to the characteristics of the band, the analyzed silver nanoparticles have a spherical shape.

Formation, morphology and wide range of size variation of silver nanoparticles, biosynthesized from leaf extracts of *Chaenomeles* plants, were further confirmed by SEM microphotographs. SEM micrographs showed the spherical and near-spherical shape of individual AgNPs derived from the extracts of different *Chaenomeles* plants, as well as the presence of aggregates with smooth edges. In addition, SEM images showed that some silver nanoparticles were not observed directly, possibly because they could be coated with polymers from the *Chaenomeles* leaf extracts. This assumption is consistent with the data (Hossain, 2019) that silver nanoparticles, biosynthesized from the stem extract of *Andrographis paniculata*, were surrounded by polymers and were not directly observed on STEM images. The formation of AgNPs of different sizes synthesized from *Chaenomeles* leaf extracts does not show a discrepancy with the data of other researchers on the size of silver nanoparticles from leaf extracts of another plant species (Islam et al., 2025). Authors (Elangovan et al., 2015; Behravan et al., 2019; Savvidou et al., 2024) showed close sizes of AgNPs derived from plant leaf extracts.

One of the most promising applications of silver nanoparticles is the amplification of the Raman scattering signal of various molecules of ultra-low concentration (Dovbeshko et al., 2004; Rusu et al., 2023) due to Surface Enhanced Raman Scattering (SERS) effect. SERS analysis differs from many other methods due to its rich vibrational spectroscopic information, which has led to applications in several different fields, including electrochemistry, catalysis, biology, medicine, and others (Banaei et al., 2019). In order to obtain the maximum amplification, and, accordingly, to detect the lowest possible concentration of a certain substance (or several compounds at the same time), numerous research groups have developed various methods of obtaining plasmonic silver NPs of different shapes, sizes and surface functionalization (Borovaya et al., 2021; Kuntyi et al., 2021). In our study, this effect was observed for the silver nanoparticles biosynthesized from *Ch.* × *superba* leaf extract. It should be noted that it was possible to register the concentration 10^{-7} M of the specified analyte Rhodamine 6G, which is several orders of magnitude lower than in other experiments using nanoparticles synthesized on the basis of other plant extracts (Dzhagan et al., 2022). In addition to the actual application of the detection effect itself, the observation of the amplification effect is a confirmation that the stabilizing molecules do not form bulky shells, and foreign molecules have access to the surface of the nanoparticles. It is worth noting that achieving a significant SERS effect was possible on *Ch.* × *superba*-AgNPs subjected to purification from excess extract, which probably interferes with the effective contact of analyte molecules with the surface of silver nanoparticles. Another factor that improves the amplifying properties of purified colloids can be their partial aggregation, which is indirectly evidenced by some expansion and shift to the long-wavelength region of the SPR peak spectrum. As a result of the aggregation of two or more nanoparticles, the formation of so-called "hot spots" will take place – an even greater intensity of the electric field than on individual nanoparticles, and, accordingly, a greater amplification of the Raman signal at these points. It is known that the contribution of such "hot spots" to the Raman signal is decisive, and the absorption spectrum of such interacting nanoparticles is shifted to the long-wavelength region (Dzhagan et al 2024), which can explain in our case the presence of amplification at λ_{exc} = 532 nm and the absence at λ_{exc} = 457 nm. It is also worth noting that probably the composition of *Ch.* × *superba* leaf extract also has a significant impact on achieving a significant SERS effect, since for the series of samples/extracts investigated in this work, it was clearly observed only for that sample.

Research by a wide range of scientists has shown the positive contribution of green nanoparticles to antifungal ability. Thus, the antifungal effect of green copper nanoparticles synthesized from the juice of *Citrus medica* fruits against *Fusarium culmorum*, *F. graminearum*, and *F. oxysporum* was determined (Shende et al., 2015). Shende et al. (2024) found antifungal activity of AgNPs from *Citrus medica* fruit juice against *Aspergillus niger*, *A. flavus* and *Alternaria alternata*. Chowdhury et al. (2024) found that CuNPs + AgNPs composites formed from rice leaf extracts had significant antifungal activity (77%) compared to copper nanoparticles (62%, respectively). Khan et al. (2024) showed the inhibitory effect of silver nanoparticles against *Trichoderma* sp. and *Aspergillus* sp. with inhibition zones of 13.5 ± 0.9 and 13.0 ± 0.7 mm, respectively. Our study revealed that silver nanoparticles synthesized from *Chaenomeles* leaf extracts have pronounced antifungal activity. Univariate variance analysis revealed a direct positive dependence of the zone of inhibition of *Fusarium culmorum* on the concentration of the solution of silver nanoparticles derived from the extracts of different plant species ($F = 117.86$, $P = 7.26 \cdot 10^{-8}$). Statistical sample comparison confirmed that nanoparticles of *Ch. speciosa*-AgNPs, *Ch. cathayensis*-AgNPs and *Ch. japonica*-AgNPs at different concentrations have a larger zone of inhibition of the growth of *F. culmorum* colonies compared to the corresponding variants of the "Quadris" which was applied in eight-fold higher concentration compared to the manufacturer's protocol.

In our study, changes in the morphology of the mycelium of *F. culmorum* were also observed in the zone of incomplete growth inhibition, compared to the control. In particular, biosynthesized silver nanoparticles caused the deformation of *F. culmorum* hyphae in the form of curvatures and expansions, and also reduced the density of their arrangement. Therefore, silver nanoparticles biosynthesized from *Chaenomeles* leaf extracts had a significant negative effect on the morphology and ultrastructure of the phytopathogenic fungus. The similar data were reported (Li et al., 2022) that silver nanoparticles were able to cause abnormal morphological changes in fungal hyphae, which became twisted and wrinkled, while mycelia of fungi untreated with silver nanoparticles had a normal morphology with a uniform thickness and a smooth surface. Abnormal morphology of the mycelium, studied with the help of scanning and transmission electron microscopes, is associated with a change in the permeability of the mycelium cell membrane. AgNPs caused significant cell damage, including obvious vacuolization and cell structure loosening. Narayanan & Park (2014) during the study of antifungal activity of silver nanoparticles showed the deformation of the mycelium of *Gloeophyllum abietinum*, *G. trabeum*, *Chaetomium globosum*, and *Phanerochaete sordida*, while the mycelium of the fungus from the control sample had a branched, intact morphology.

Antimicrobial properties of nanoparticles give great hopes to researchers in connection with the ability to overcome the resistance of pathogenic microbes to antibiotics (Mubeen et al., 2021; Adil et al., 2023). The greatest attention is paid to the use of silver nanoparticles, which are

characterized by a large ratio of surface area to volume, which increases their biological activity and causes excellent antimicrobial properties. In our study, the AgNPs biosynthesized from leaf extracts of different *Chaenomeles* plants, exhibited notable and species-specific inhibition of both *K. pneumoniae* (13–30% of the ofloxacin activity, respectively due to *Ch. cathayensis*-AgNPs and *Ch.* × *superba*-AgNPs action) and *S. aureus* (43–68% of the ofloxacin activity, respectively due to the action of *Ch. japonica*-AgNPs and *Ch. speciosa*-AgNPs). That is, the study results indicated the higher antibacterial effect of silver nanoparticles derived from *Chaenomeles* leaf extracts against Gram-positive bacterium as compared to Gram-negative. Our finding coincides with the data (Hossain et al., 2019) regarding the highest antibacterial activity against *S. aureus* achieved by the silver nanoparticles biosynthesized on the base of *Andrographis paniculata* stem extract. However, other research showed the opposite results of the antibacterial activity of silver nanoparticles. For example, Gram-negative strains *K. pneumonia* and *E. coli* were most sensitive to AgNPs biosynthesized from bacterial cultural medium silver nanoparticles (Hassan et al., 2016), as well as to the silver nanoparticles biosynthesized using *Xylocopa virginica* wings' extract (Ewunkem et al., 2023). The mechanism of antibacterial action of silver nanoparticles can be implemented in different ways: adhesion of AgNPs on the surface of the cell wall and membrane of bacteria, penetration of AgNPs into the bacterial cell and damage to cellular structures (mitochondria, vacuoles, ribosomes) and biomolecules (proteins, lipids and DNA), oxidative stress, caused by the formation of reactive oxygen species (ROS) and free radicals, modulation of bacterial signaling pathways, as well as releasing silver ions (Ag^+) by AgNPs contributes to antibacterial activity (Sánchez-López et al., 2020). Considering the use of very different biological substrates for the biosynthesis of silver nanoparticles, differences in their antibacterial effects may be related to the characteristics of the nanoparticles themselves, due to uneven conditions of biosynthesis, as well as to the implementation of certain ways of inhibiting bacteria by AgNPs, which requires further research.

Conclusion

By the method of "green" synthesis using an aqueous solution of silver nitrate and aqueous leaf extracts of genus *Chaenomeles* plants under laboratory conditions of temperature and lighting, colloidal solutions of silver nanoparticles were obtained: *Ch. speciosa*-AgNPs, *Ch. cathayensis*-AgNPs, *Ch. japonica*-AgNPs, *Ch.* × *superba*-AgNPs, and *Ch.* × *californica*-AgNPs.

The presence of nanoparticles was confirmed and characterized by the methods of UV-vis spectroscopy and SEM microscopy, which indicate that all synthesized nanoparticles exhibit the property of localized surface plasmon resonance and have a close to spherical shape and size variability in the range of 5–58 nm. Raman scattering spectroscopy studies proved the high ability of *Ch. speciosa*-AgNPs to enhance the Rhodamine 6G scattering signal, which indicates the promising use of these nanoparticles as a substrate for Surface Enhanced Raman Scattering (SERS).

The results of studying the fungistatic properties of biosynthesized nanoparticles show that *Ch. speciosa*-AgNPs, *Ch. cathayensis*-AgNPs and *Ch. japonica*-AgNPs had the most significant activity against the phytopathogenic fungus *Fusarium culmorum* compared to the commercially available fungicide "Quadris". The fungicidal activity of all three active silver nanoparticles caused the deformation of the hyphae of the mycelium of *F. culmorum* in the form of curvatures and expansions, in addition to which a decrease in the density of the arrangement of hyphae was observed under the influence of *Ch. speciosa*-AgNPs and *Ch. cathayensis*-AgNPs.

The AgNPs biosynthesized from leaf extracts of all *Chaenomeles* plants demonstrated the species-specific inhibition of both Gram-negative (*K. pneumoniae*) and Gram-positive (*S. aureus*) bacterial strains. The highest activity against *K. pneumoniae* was shown by *Ch.* × *superba*-AgNPs (29.6% of the ofloxacin activity), while *Ch. speciosa*-AgNPs achieved the greatest inhibition of *S. aureus* strain (67.7% of the ofloxacin activity). The study results showed that silver nanoparticles produced by the phytosynthesis approach can be efficiently used as antimicrobial agents, and confirmed the perspective of further studies of possible applications of plant-derived nanoparticles.

The authors declare no conflict of interest.

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