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Article

Green Synthesis of Silver Nanoparticles Using *Annona diversifolia* Leaf Extract and Their Antimicrobial Application

Rogelio Solorzano-Toala[1], Daniel Gonzalez-Mendoza[2,*], Benjamin Valdez-Salas[3], Vianey Mendez-Trujillo[4], Federico Gutierrez-Miceli[1], Ernesto Beltran-Partida[3] and Olivia Tzintzun-Camacho[2]

[1]Tecnológico Nacional de México-Campus Tuxtla Gutiérrez, Tuxtla Gutiérrez, 03940, México
[2]Instituto de Ciencias Agrícolas de la Universidad Autónoma de Baja California, Mexicali, 21705, México
[3]Instituto de Ingeniería de la Universidad Autónoma de Baja California, Mexicali, 21100, México
[4]Facultad de Medicina de la Universidad Autónoma de Baja California, Mexicali, 21100, México
*Corresponding Author: Daniel Gonzalez-Mendoza. Email: danielg@uabc.edu.mx
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Abstract: The aim of this study was the synthesis of silver nanoparticle using *Annona diversifolia* Safford. The silver nanoparticles obtained were analyzed by spectroscopic methods and dynamic light scattering methods. The inhibition of AgNPs was evaluated against *Bacillus cereus*, *Klebsiella pneumoniae* and *Enterobacter aerogenes*. The results showed that AgNPs have high values at 3 keV and particle size between 45 to 58 nm with a homogenous morphology. The AgNPs showed growth inhibition against *Klebsiella pneumoniae* and *Enterobacter aerogenes*. Therefore studies are needed to confirm the potential antimicrobial of different AgNP from *A. diversifolia* in Gram negative and Gram positive bacteria.

Keywords: Green chemistry; AgNPs; plants; antimicrobial; biotechnology

1 Introduction

Currently, the nanotechnology opens the possibility for a wide variety of applications in agronomy sciences. Recent reports have shown that silver nanoparticles (AgNPs), due to their antimicrobial activity, could be considered as an alternative route for the design of a new generation of antimicrobial agents [1,2]. The nanoparticles have different mechanism of action against bacterial cells: (1) cell wall and membrane disruption; (2) AgNPs penetration and intracellular damage disrupting metabolic pathways; (3) biomolecules damage (e.g., DNA or proteins); and (4) free radicals production (e.g., reactive oxygen species generation) which may cause disruption in lipid membrane [3]. Generally, metallic nanoparticles are prepared using a great variety of chemical and physical methods which are quite expensive and potentially hazardous to the environment [4]. In contrast, green synthesis is a biotechnology optional to chemical and physical methods [5]. Today, the use of plants as source of metabolites for the reduction of different metals has been emphasized for the synthesis of metallic-nanoparticles [4,6]. Although there have been numerous studies about the toxicity of metallic nanoparticles obtained from chemical or physical methods on pathogenic bacteria, research on green synthesis of silver nanoparticles and their antimicrobial activities are insufficient [7]. In this sense, certain studies have proposed the synthesis of

AgNPs using aqueous extract of plants as bioreducing agents and their antimicrobial activity against Gram-positive and Gram-negative bacteria [4].

The genus *Annona* L. is the most important of the *Annonaceae* family due to its tasty flavor, high pulp content, and nutritional value and antioxidant properties. This genus is native from Southwestern Mexico and Central America. A previous research indicates that the region of Southeast Mexico and Guatemala is known as the center of origin of *A. diversifolia* [8]. This plant occupies a relevant position in Mexican fruticulture mainly Southeast region due to their significant content of total phenols, flavonoids and their antioxidant activity in leaves [9].

However, even though the biochemical and medicinal properties effects of *A. diversifolia* leaves have been previously studied the use of *A. diversifolia* to synthesize silver nanoparticles has been scarcely evaluated. It is important for the aqueous extract of the leaves of *A. diversifolia* to be studied, especially since the information can be used for its properties that can be employed as a disinfectant solution for vegetables which can be transformed into solution for sanitizers. Therefore, in the present study, we report the synthesis of silver nanoparticles using leaves of *Annona diversifolia* and their antimicrobial activity against bacteria (*Bacillus cereus*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*) causing of diseases in vegetables.

2 Experimental

2.1 Materials

Samples of fresh and healthy leaves of *Annona diversifolia* were obtain from a native population in Comitan, Mexico. The aqueous extract of leaves was prepared by taking 30 g of each plant leaves and mixed with 300 mL of distilled water. Then, the mixture was kept in agitation to 2.5 g for 24 h at constant temperature. The samples were then centrifuged at 5000 g for 10 min to remove particulate matter and to get clear solutions, which were then stored under refrigeration at 4°C until use.

2.2 Biosynthesis of Silver Nanoparticles (AgNPs)

For biosynthesis of AgNPs an aqueous solution of silver nitrate (10 mM) was prepared and mixed with the leaf extract of *A. diversifolia* at a ratio of 4:1 (v/v). This solution was placed on a shaker in a dark chamber to minimize photo-activation of silver nitrate at room temperature with constant rotation at 40 ± 2°C for 30 min. The bio-reduction of Ag^{+} to Ag^{0} was confirmed by the color changing of the solution, which went from colorless to a brown shade. Its formation was also confirmed by measuring the absorbance of the AgNPs in solution from *A. diversifolia* leaf extract at 300–700 nm in a UV/V is spectrophotometer. The AgNPs were purified by centrifugation at 6000 g for 10 min and the precipitate was lyophilized for their used in antibacterial activity.

2.3 Characterizations of Biosynthesized AgNPs

Dynamic Light Scattering (DLS) for characterization of size and zeta potential of AgNPs in solution from *A. diversifolia*, was performed using a nanotrac wave instrument (Microtrac) [10].

2.4 Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS)

To identify the size, morphology and composite homogeneity of AgNPs from *A. diversifolia* a scanning electron microscope (SEM) JEOL 6010 was employed according to Abdelmoteleb et al. [2]. For EDS analysis, the AgNPs were drop-coated on to a carbon film and analyzed using instrument Bruker Quantax 400.

2.5 Antimicrobial Analysis

The antimicrobial activity of AgNPs was analyzed with disk diffusion method according to Gonzalez-Mendoza et al. [11]. 50 µl of aliquots of *Bacillus cereus*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* at a concentration of 10×10^{5} cells/mL were inoculated on sterile nutrient agar petri dishes. Then 20 µl of

synthesized AgNPs solution at 25%, 50%, 75% and 100% were prepared in distilled water and dropped on 7 mm sterile filter paper discs. Dried filter paper disks containing the different concentrations of AgNPs were placed in petri dishes and an absolute control (only leaf extract), negative control (discs with only water) and positive control ($AgNO_3$ solution) were separately prepared. Finalized, the period of incubation of each treatment (30°C ± 2°C for 24), the inhibition zones were visualized.

2.6 Total Phenol and Flavonoids Contents

The content of total phenolic compounds and flavonoids contents of the leaf extract and AgNPs from *A. diversifolia* were determined by colorimetric methods, according to proposed by Cervantes-Garcia et al. [12]. The content of phenolic compounds was expressed as mg GAE (equivalent of gallic acid)/gram of dry extract (DE) at 760 nm against blank. On the other hand, the flavonoids were calculated as mg quercetin equivalents (QE) per gram of DE at 510 nm.

2.7 Statistical Analysis

Differences between the treatments were compared using Tukey's test ($p \leq 0.05$), and Statistica Version 9.0 was used. Significant differences were accepted if $p \leq 0.05$. Results are indicated with the average of the values determined ± standard deviation (SD).

3 Results and Discussion

3.1 UV-VIS Spectrometry

In the present study, the synthesis of AgNPs was primarily confirmed by the formation of pale yellow color suggests the presence of silver nanoparticles due to the interaction of phytochemicals of aqueous extract of *A. diversifolia* that reduces silver ions into Ag-nanoparticles (Fig. 1). The formation of AgNPs by reduction of aqueous Ag during exposure to *A. diversifolia* extract was characterized by UV-Visible spectroscopy.

As shown in Fig. 2, the surface plasmon resonance (SPR) of the AgNPs was centered at approximately 460 nm.

Figure 1: Green synthesis of AgNPs using extracts of *A. diversifolia*

3.2 SEM and EDS

The morphology and size of AgNPs from *A. diversifolia* was analyzed using SEM, as shown in Fig. 3a. The SEM analysis clearly show AgNPs agglomerated, forming spherical-shaped particles with smaller size (45 to 58 nm). On the other hand, the EDS of AgNPs (Fig. 3b) revealed the presence of pure silver (45.92%) followed by peaks of silicon (55.70%) and carbon (11.58%) atoms, that suggested the interaction of

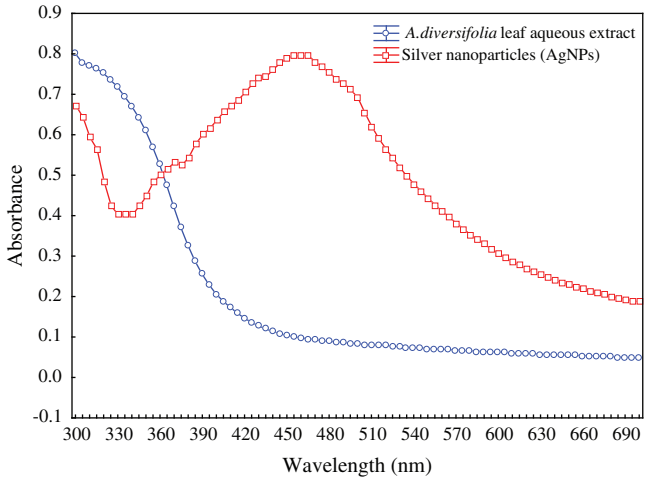

Figure 2: UV-Vis absorption spectrum of AgNPs and aqueous extract of *A. diversifolia*

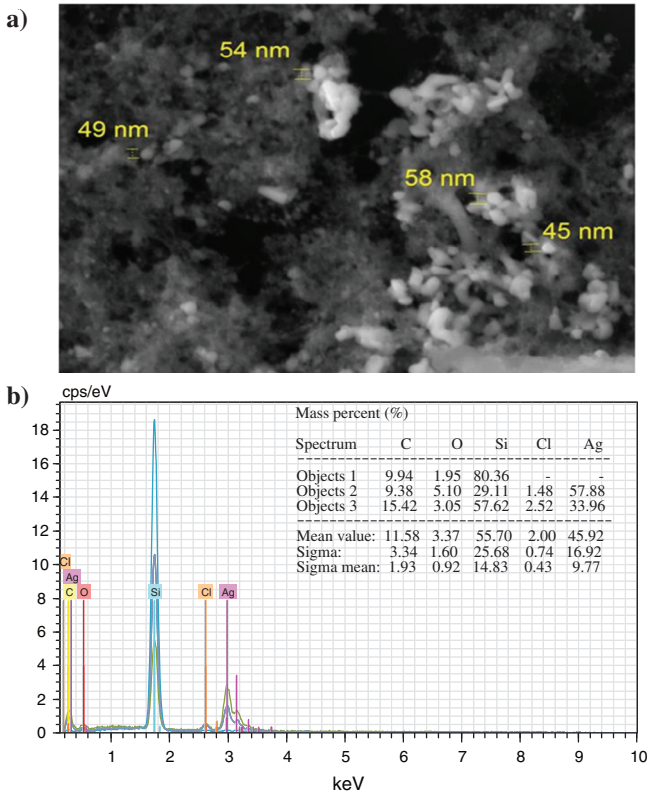

Figure 3: Scanning electron microscopy (a) and Energy dispersive X-ray spectrometer image of AgNPs produced from *A. diversifolia* (b)

biomolecules and $AgNP_S$ which might have come from the plant leaf extract [2]. In this sense, the reduction studies in other plants have reported the participation of carbonyl, phenolic and flavonoids in the stabilization of NPs [10,11]. The results suggested that the biomolecules in leaves extract may be responsible for the reduction of $AgNO_3$ and stabilization of nanoparticles. Alegria et al. [13] reported that presence of phenolic compounds from tea extracts may play an important role in the reduction and stability of AgNPs.

In this sense, total phenols and flavonoids present in *A. diversifolia* could be considered as an important reducing and stabilizing agent for AgNPs production [14]. Similar results were observed in *Sargassun vulgare* and *Yucca schidigera* after the applications of silver to aqueous extract of leaves [11,15]. Therefore, experiments about role of metabolites in AgNPs synthesis from *A. diversifolia* will be needed to know the exact mechanism of their formation. Some studies reported that the negative surface charge of AgNPs could be due to the adsorption of bioactive components present in the aqueous extract onto the nanoparticles surface [2]. Similar results were found by Salvioni et al. [16] who showed that the negative surface charge could be attributed to the interaction of metabolites with the NPs.

3.3 DLS and Zeta Potential

The DLS analyses showed a major particle size distribution peak at 34 nm and a second peak at 134 nm, which represented the existence of interaction between Ag ions and biomolecules from *A. diversifolia* and their aggregation (Fig. 4). On the other hand, zeta potential was found to be –20.2 mV for synthesized AgNPs indicating stability and tendency to form short particles. The average particle diameter determined by SEM was found to be 45–58 nm and 34–134 nm by DLS, this difference is according to reported by Ruiz-Romero et al. [15] who observed that hydrodynamic diameter determined by DLS was smaller than the SEM diameter.

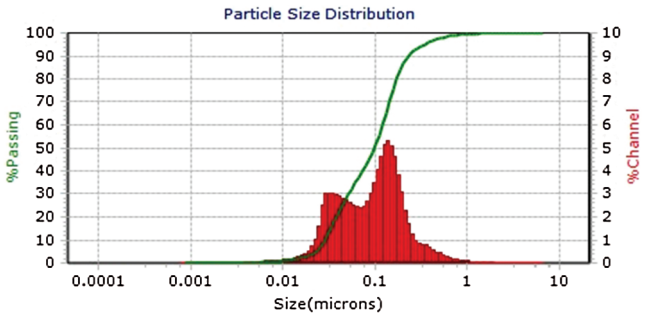

Figure 4: Particle size distribution of AgNPs from dynamic light scattering measurements

3.4 Antibacterial Activities of AgNPs

The results showed that different concentrations of AgNPs were significantly more effective against *Klebsiella pneumoniae*, and *Enterobacter aerogenes* (Gram negative microorganisms) compared with the positive control (Tab. 1). The mean growth inhibition zone diameters for *K. pneumoniae* with 25%, 50%, 75% and 100% of AgNPs were 10.5, 11.1, 11.5 and 11.5 mm, respectively; these diameters for *E. aerogenes* were 11.4, 11.0, 12.0 and 11.5 mm, respectively, while for the positive control group was 9.5 and 8.5 mm. Although the antimicrobial activity of AgNPs started at 25% for both microorganisms, there was not an increase in the inhibitory effect on both bacterial species with an increase in the concentration of AgNPs (Tab. 1 and Fig. 5).

In contrast, the results showed that means of growth inhibition for all the concentrations of AgNPs were less than those in the positive control group in *B. cereus* ($p < 0.05$). In diverse studies, the AgNPs have demonstrated their broad spectra of inhibition against Gram-positive and Gram-negative bacteria [17]. Kędziora et al. [18] suggest that positive particle surface charge is essential to increase the antibacterial efficacy. However, this effect contrasted with our results, which showed a significantly inhibitory effect of AgNPs against Gram negative microorganism but not with Gram positive with respect to positive control ($AgNO_3$) (Fig. 5). In the present study, dose dependent effect of AgNPs on the inhibition of bacteria was not observed. In this sense, Achaira et al. [18] reveled that bacterial sensitivity to AgNPs was found to vary depending on microbial species and emphasized the dependence on morphology and distribution

Table 1: Inhibition of microorganisms by AgNPs from *A. diversifolia*

Microorganisms	Zone of inhibition (mm)					
	AgNPs from *A. diversifolia*			*A. diversifolia*		$AgNO_3$
	25%	50%	75%	100%	100%	10 mM
Bacillus cereus	8.5 ± 0.07^a	9 ± 0.24^a	9 ± 0.07^a	9 ± 0.06^a	0	9.2 ± 0.54^a
Klebsiella pneumoniae	10.5 ± 0.05^b	11 ± 0.10^b	11.5 ± 0.05^b	11.5 ± 0.07^b	0	8.5 ± 0.12^a
Enterobacter aerogenes	11 ± 0.35^c	11 ± 0.24^b	12.0 ± 0.42^c	11.5 ± 0.15^b	0	9.5 ± 0.36^a

Results are expressed as mean ± standard deviation of values from triplicate experiments. Values with the same letter (a, b or c) within each line are equal according to the Tukey test at $p \leq 0.05$.

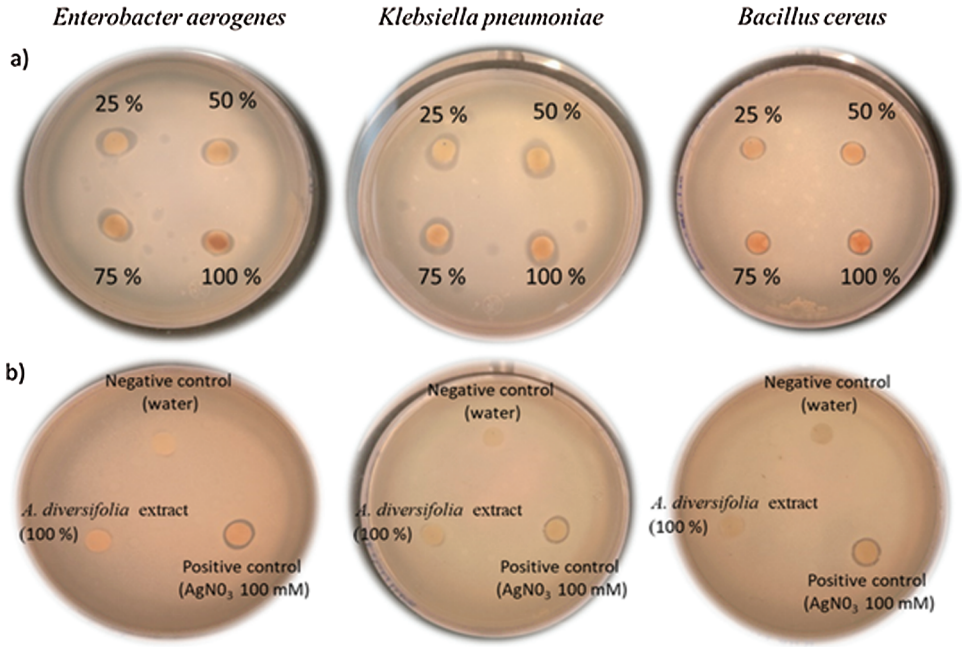

Figure 5: Inhibition of microorganisms by AgNPs from *A. diversifolia*: (a) microorganisms with different doses of Ag-NPs; (b) microorganism with *A. diversifolia* extract and control negative (water) and positive (10 mM $AgNO_3$)

with reactive faces of the AgNPs for antibacterial activity. These authors reported that dose dependent effect of AgNPs on the Gram-positive and Gram-negative bacteria on the basis of disc diffusion method, suggested, that at low concentrations of NPs, the interaction of particles with the cell wall of bacteria decreases, while at the high concentrations, aggregation probability of the particle increases causes less interactions with bacteria and NPs. On other hand, Bankier et al. [20] compared effects of multiple metallic nanoparticles against *Staphylococcus aureus* and *Pseudomonas. aeruginosa*. They concluded that the inhibitory effect of nanoparticles could be due to a difference in cell wall structure between the two pathogens and factors of nanoparticle, such as size, shape and charge. Although this study revealed that AgNPs are a good antibacterial candidate. The variation in antimicrobial activity of AgNPs of plants extract obtain in different investigation may be attributed to their metabolites present in the plants as well as microorganism strains used and synthesis of NPs [2,10]. Therefore studies are needed to confirm the

potential antimicrobial of different doses of AgNP from *A. diversifolia* in Gram negative and Gram positive bacteria.

Salvioni et al. [16] show that AgNPs with a 20 nm diameter and a negative zeta potential have a high antibacterial activity in Gram negative bacteria compared to colloidal silver. In this sense, the phenolic compounds could act as capping agents of NPs and this may affect the growth, considering the divergence in basic structural design of cell walls between the bacterial groups. In this sense, the effect of phenolic compounds could be explained by adsorption to cell membranes, interaction with enzymes, substrate and metal ion deprivation [21,22]. As shown in Tab. 2, a significant difference was observed among the respective values obtained. The total phenolic content was significantly different ($p < 0.05$) among aqueous extract and AgNPs from *A. diversifolia*. As shown in the case of total phenolics, the concentration of flavonoids in the extracts and AgNPs from *A. diversifolia* was significantly different. In this case, the AgNPs from *A. diversifolia* similarly showed a higher ($p < 0.05$) flavonoid content than the aqueous extracts (Tab. 2).

Table 2: Total phenolic and flavonoid content in extract and AgNPs from *A. diversifolia*

Polyphenols	Leaf extract-*A. diversifolia* (mg GAE/g)	AgNPs from *A. diversifolia* (mg QE/g)
Total Phenolics	27 ± 0.05^{a}	31 ± 0.04^{b}
Total Flavonoids	0.26 ± 0.12^{a}	3 ± 0.06^{b}

Results are expressed as mean ± standard deviation of values from triplicate experiments. Values with the same letter (a or b) within each line are equal according to the Tukey test at $p \leq 0.05$.

Then, the differences in the inhibition of microorganisms by AgNPs and *A. diversifolia* extract may be results of a major concentration of phenolic compounds in AgNPs with respect to only *A. diversifolia* extract. Therefore, the increasing content of polyphenols after the reaction can be attributed to organic complexes present in the extract and not involved in the Folin–Ciocalteu reaction [13,23].

4 Conclusion

In the present study, our results showed the capacity of aqueous extract of *Annona diversifolia* as efficient and environment-friendly reducing agent in the synthesis of AgNPs. Characterization of green AgNPs revealed that the particles were spheroidal in shape with a particle size distribution range of 45–58 nm along with 45.92% silver content. These findings revealed that green AgNPs exhibited a significantly inhibitory effect against Gram negative but not with Gram positive bacteria. These results suggest that green AgNPs could be used for antibacterial treatment of vegetables. Although, future studies are necessary to reveal the exact mechanism of toxicity of green silver nanoparticles in Gram negative bacteria.

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Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

References

1. Lamsal, K., Kim, S. W., Jung, J. H., Kim, Y. S., Su, K. K. et al. (2011). Inhibition effects of silver nanoparticles against powdery mildews on cucumber and pumpkin. *Mycobiology, 39(1),* 26–32. DOI 10.4489/MYCO.2011.39.1.026.

2. Abdelmoteleb, A., Valdez-Salas, B., Ceceña-Duran, C., Tzintzun-Camacho, O., Gutierrez-Miceli, F. et al. (2017). Silver nanoparticles from *Prosopis glandulosa* and their potential application as biocontrol of *Acinetobacter calcoaceticus* and *Bacillus cereus*. *Chemical Speciation and Bioavailability, 29(1),* 1–5. DOI 10.1080/09542299.2016.1252693.
3. Vazquez-Muñoz, R., Meza-Villezcas, A., Fournier, P. G. J., Soria-Castro, E., Juarez-Moreno, K. et al. (2019). Enhancement of antibiotics antimicrobial activity due to the silver nanoparticles impact on the cell membrane. *PLoS One, 14(11),* e0224904. DOI 10.1371/journal.pone.0224904.
4. Bernardo-Mazariegos, E., Valdez-Salas, B., González-Mendoza, D., Abdelmoteleb, A., Camacho, O. T. et al. (2019). Silver nanoparticles from *Justicia spicigera* and their antimicrobial potentialities in the biocontrol of foodborne bacteria and phytopathogenic fungi. *Revista Argentina de Microbiologia, 51(2),* 103–109. DOI 10.1016/j.ram.2018.05.002.
5. Manik, U. P., Nande, A., Raut, S., Dhoble, S. J. (2020). Green synthesis of silver nanoparticles using plant leaf extraction of *Artocarpus heterophylus* and *Azadirachta indica*. *Results in Materials, 6,* 100086. DOI 10.1016/j.rinma.2020.100086.
6. Vaseghi, G., Rafiee, L., Javanmard, S. H. (2017). Non-viral delivery systems for breast cancer gene therapy. *Current Gene Therapy, 17(2),* 147–153. DOI 10.2174/1566523217666170606124131.
7. Jeong, S. H., Yeo, S. Y., Yi, S. C. (2005). The effect of filler particle size on the antibacterial properties of compounded polymer/silver fibers. *Journal of Materials Science, 40(20),* 5407–5411. DOI 10.1007/s10853-005-4339-8.
8. Lobo, M., Delgado, O., Cartagena, J., Fernandez, E., Medina, C. (2007). Categorización de la germinación y la latencia en semillas de chirimoya (*Annona cherimola* L.) y guanábana (*Annona muricata* L.), como apoyo a programas de conservación de germoplasma. *Agronomía Colombiana, 25,* 231–244.
9. Luna-Cazáres, L. M., González-Esquinca, A. R. (2008). Actividad antibacteriana de extractos de *Annona diversifolia* Safford y *Annona purpurea* Mociño & Sessé ex Dunal. *Polibotánica, 25,* 120–125.
10. Eric, C. A., Benjamín, V., Monica, C., Mario, A. C., Francisco, D. M. et al. (2017). Silver nanoparticles biosynthesized by secondary metabolites from *Moringa oleifera* stem and their antimicrobial properties. *African Journal Biotechnology, 16(9),* 400–407. DOI 10.5897/AJB2016.15840.
11. González-Mendoza, D., Valdez-Salas, B., Carrillo-Beltran, M., Castro-Lopez, S., Mendez-Trujillo, V. et al. (2018). Antimicrobial effects of silver-phyconanoparticles from *Sargassun vulgare* against spoilage of fresh vegetables caused by *Bacillus cereus*, *Fusarium solani* and *Alternaria alternate*. *Internatioal Journal Agriculture and Biology, 20,* 1230–1234.
12. Cervantes-Garcia, D., Troncoso-Rojas, R., Sanchez-Estrada, A., Gonzalez-Mendoza, D., Gituerrz-Miceli, F. et al. (2016). Effects of cadmium on total phenolic compounds and flavonoids in *Euglena gracilis*. *Gayana (Concepción), 80(1),* 1–5. DOI 10.4067/S0717-65382016000100001.
13. Alegria, E., Ribeiro, A., Mendes, M., Ferraria, A. M., Botelho do Rego, A. et al. (2018). Effect of phenolic compounds on the synthesis of gold nanoparticles and its catalytic activity in the reduction of nitro compounds. *Nanomaterials, 8(5),* 320. DOI 10.3390/nano8050320.
14. Julian-Loaeza, A. P., Santos-Sanchez, N. F., Valadez-Blanco, R., Sanchez-Guzman, B. S., Salas-coronado, R. (2011). Chemical composition, color and antioxidant activity of three varieties of *Annona diversifolia* Safford fruits. *Industrial Crops and Products, 31(2),* 1262–1268. DOI 10.1016/j.indcrop.2010.06.012.
15. Ruiz-Romero, P., Valdez-Salas, B., González-Mendoza, D., Mendez-Trujillo, V. (2018). Antifungal effects of silver phytonanoparticles from *Yucca shilerifera* against strawberry soil-borne pathogens: *Fusarium solani* and *Macrophomina phaseolina*. *Mycobiology, 46(1),* 47–51. DOI 10.1080/12298093.2018.1454011.
16. Salvioni, L., Galbiati, E., Collico, V., Alessio, G., Avvakumova, S. et al. (2017). Negatively charged silver nanoparticles with potent antibacterial activity and reduced toxicity for pharmaceutical preparations. *International Journal Nanomedicine, 12,* 2517–2530. DOI 10.2147/IJN.S127799.
17. Franci, G., Falanga, A., Galdiero, S., Palomba, L., Mahendra, R. et al. (2015). Silver nanoparticles as potential antibacterial agents. *Molecules, 20(5),* 8856–8874. DOI 10.3390/molecules20058856.

18. Kędziora, A., Speruda, M. K., Krzyżewska, E., Rybka, J., Rajkumari, J. et al. (2018). Similarities and differences between silver ions and silver in nanoforms as antibacterial agents. *International Journal Molecular Sciences, 19 (2),* 444. DOI 10.3390/ijms19020444.
19. Acharya, D., Singha, K. M., Pandey, P., Mohanta, B., Rajkumari, J. et al. (2018). Shape dependent physical mutilation and lethal effects of silver nanoparticles on bacteria. *Scientific Reports, 8(1),* 280. DOI 10.1038/s41598-017-18590-6.
20. Bankier, C., Matharu, R. K., Cheong, Y. K., Ren, G. G., Cloutman, E. et al. (2019). Synergistic antibacterial effects of metallic nanoparticle combinations. *Scientific Reports, 9(1),* 16074. DOI 10.1038/s41598-019-52473-2.
21. Puupponen, P. R., Nohynek, L., Meier, C., Kähkönen, M., Heinonem, M. et al. (2011). Antimicrobial properties of phenolic compounds in berries. *Journal of Applied Microbiology, 90(4),* 494–507. DOI 10.1046/j.1365-2672.2001.01271.x.
22. Taguri, T., Tanaka, T., Kouno, I. (2004). Antimicrobial activity of 10 different plant polyphenols against bacteria causing food borne disease. *Biological and Pharmaceutical Bulletin, 27(12),* 1965–1969. DOI 10.1248/bpb.27.1965.
23. González-Mendoza, D., Troncoso-Rojas, R., Gonzalez-Soto, T., Grimaldo-Juarez, O., Ceceña-Duran, C. et al. (2018). Changes in the phenylalanine ammonia lyase activity, total phenolic compounds, and flavonoids in *Prosopis glandulosa* treated with cadmium and copper. *Annals of the Brazilian Academy of Sciences, 90(2),* 1465–1472. DOI 10.1590/0001-3765201820170622.